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Research Article

Host Plant and Leaf-Age Preference of *Luprops tristis* (Coleoptera: Tenebrionidae: Lagriinae: Lupropini): A Home Invading Nuisance Pest in Rubber Plantation Belts

Sabu K. Thomas, Mohandas Greeshma, and P. Aswathi

Post Graduate & Research Department of Zoology, St. Joseph's College, Devagiri, Calicut, Kerala 673008, India

Correspondence should be addressed to Sabu K. Thomas, sabukthomas@gmail.com

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Massive seasonal invasion by the litter-dwelling beetle *Luprops tristis*, into residential buildings prior to monsoon rains, and their prolonged state of dormancy render them a very serious nuisance pest in rubber plantations in the Western Ghats in southern India. Feeding preferences of *L. tristis* towards leaf litter of seven trees co-occurring in rubber plantations, cashew (*Anacardium occidentale*), mango (*Mangifera indica*), jackfruit (*Artocarpus heterophyllus*), wild jack (*Artocarpus hirsutus*), cocoa (*Theobroma cacao*), cassia (*Cassia fistula*), sapota (*Manilkara zapota*) and rubber (*Hevea brasiliensis*) were analyzed with no-choice and multiple-choice leaf disc tests. Results showed that *L. tristis* is a generalist feeder with a defined pattern of preference, with the leaf litter of rubber being the most preferred followed by those of jackfruit and cocoa. Tender leaves were preferred over mature leaves except for cocoa and sapota. Equal preference towards tender and mature cocoa leaves, presence of patches of cocoa plantations and the scarce distribution of other host plants in rubber plantation belts leads to the proposal that in the absence of tender and mature rubber leaves, cocoa becomes the major host plant of *L. tristis*.

1. Introduction

Seasonal mass invasion of a litter-dwelling detritivorous beetle, *Luprops tristis* (Fabricius, 1801) (Coleoptera: Tenebrionidae: Lagriinae: Lupropini), numbering 0.5–4 million per residential buildings prior to the onset of south west monsoon showers and subsequent aggregation in prolonged state of dormancy (Figure 1) render them a very serious nuisance pest in rubber plantation tracts in the Western Ghats in southern India [1]. Litter stands of rubber tree [*Hevea brasiliensis,* (Willd. ex Adr. De Jus) Müll. Arg. 1865] are the breeding and feeding habitat for *L. tristis*, with prematurely abscised leaves as the most preferred food resource, and a synchronized life cycle with the leaf phenology of rubber [2]. Their near absence in natural forests in contrast to exceptionally high abundance in rubber plantation litter established that rubber is the major host plant of the insect in the region [3–5]. Prevention of premature leaf fall of rubber may regulate the population build up of *L. tristis* in rubber plantations [2, 5]. However, their presence in the leaf litter of trees namely, cashew (*Anacardium occidentale,* Linnaeus 1753), mango (*Mangifera indica,* Linnaeus 1753), jackfruit (*Artocarpus heterophyllus,* Lamarck 1789), wild jack (*Artocarpus hirsutus,* Lamarck 1789), cocoa (*Theobroma cacao,* Linnaeus 1753), cassia (*Cassia fistula,* Linnaeus 1753), and sapota (*Manilkara zapota,* Linnaeus 1753) co-occurring in rubber belts (personal observations, first author) led to the hypothesis that *L. tristis* may also feed on the leaf litter of these plants. Hence, it is essential to determine the feeding preference of *L. tristis* on these potential alternate host plants before attempting control by prevention of premature leaf fall in rubber plantations.

We propose that (i) *L. tristis* is a specialist feeder on rubber litter, and (ii) does not feed on the leaf litter of cashew, mango, jackfruit, wild jack, cocoa, cassia and sapota plants. Data generated is expected to contribute towards adoption of control strategies to prevent the possible spread of *L. tristis* to nonrubber plantation belts where plantations of potential host plants are prevalent.

2. Materials and Methods

2.1. Study Organism. *Luprops tristis* (Fabricius, 1801) is generally regarded as an inconspicuous litter-dwelling detritivore but for their exceptionally high abundance in the rubber plantation litter stands across the moist south Western Ghats [1]. They are regionally referred to as "*Mupli vandu*" in Central and South Kerala and "*Ola prani,*" "*Ola chathan,*" or "*Otteruma*" in North Kerala in South India. No data exists on its ancestral host or about alternate host plants as it remained as a minor darkling beetle species of least importance, till it became a nuisance pest in residential buildings with the spread of rubber plantations in the moist western slopes of the Western Ghats during 1960–1970 period [6].

2.2. Host Plants. Plants with which *L. tristis* associated are as follows.

Mango (*Mangifera indica*: *Anacardiaceae*: Sapindales) is an evergreen tree, indigenous to the Indian subcontinent. Although an evergreen tree, large quantities of old leaves are shed during summer vegetative flush. The leaf-flushing period can have one to five flushing events with the whole canopy flushing in synchrony or in patches [7]. The most common native variety, *Nattumavu*, in the rubber belts was selected for the study.

Cashew (*Anacardium occidentale*: *Anacardiaceae:* Sapindales) was originally spread from Brazil by the Portuguese and is widely grown for cashew kernels popularly known as "cashew nuts" [8, 9]. Although an evergreen tree, large quantities of old leaves are shed during presummer period prior to flowering (first author, personal observations).

Jackfruit (*Artocarpus heterophyllus: Moraceae*: Rosales) is a tall evergreen tree with spreading canopy. Although an evergreen tree, large quantities of old leaves are shed during summer vegetative flush. It is a common tree in the rubber belts as farmers use its fruits and seeds as a food item, leaves for fodder, and stem for timber [10].

Wild jack (*Artocarpus hirsutus*: *Moraceae*: Rosales) is a tall evergreen tree species that is endemic to the Western Ghats [11]. Large quantities of old leaves are shed during summer vegetative flush. It is a common tree in the rubber belts as planters allow a few trees to grow in the midst of rubber plantations due to the high commercial value of its wood and its taller canopy which do not interfere with growth of rubber plants.

Rubber (*Hevea brasiliensis*: *Euphorbiaceae*: Malpighiales) is a deciduous tree with a major annual leaf shedding during December, leaf flush in January, and flowering in February. Rubber plantations of about half a million hectares are present along the western slopes of the Western Ghats in the South Indian state of Kerala [2].

Cocoa (*Theobroma cacao*: *Malvaceae*: Malvales) is an evergreen tree native to Central America and South America. It was introduced as a crop plant into many tropical African and Asian countries for cocoa seeds which are used to make cocoa powder and chocolate [12]. It is a common tree in the rubber plantation belts as it grows well in the under storey of rubber in the region.

Sapota (*Manilkara zapota*: *Zapotaceae*: Ericales) is an evergreen tree native to Southern Mexico, Central America and the Caribbean [13]. It is grown in the front yards of residential buildings in the rubber belts for fruits as well as a shade tree.

Cassia (*Cassia fistula*: *Fabaceae*: Fabales) is a deciduous tree of deciduous forests ranging from tropical thorn to moist through subtropical thorn to moist forest zones and is a native of India. It produces yellow flowers in drooping racemes, making it an extremely showy tree in bloom with only flowers and no leaves [14]. It is a common ornamental tree in the surroundings of residential buildings as its flowers are considered as an auspicious first sight at the crack of dawn on the day of *Vishu*, a new year festival celebrated in the region [15]. Leaf shedding occurs during December–February period and flowering during March-April period.

2.3. Experiment Setup. The present investigation was carried out during March 2010 using test insects and host plant leaves collected from the vicinity of a rubber plantation in the Devagiri College campus, located 6 km east from the Malabar Coast at Calicut (11°15_N, 75°50_E), in the Kerala state of India.

To ensure uniformity of age at the beginning of the experiment, teneral adults were collected based on their brownish white body color [1] from rubber plantation litter in the college hostel premises. Collected beetles were reared in clay vessels placed in an environmental chamber and fed with a mixture of diced tender and mature leaves of all eight leaf types for 10 days to reduce the possible effect of leaf quality variations on growth rate and feeding preference. Beetles were deprived of food for 24 hrs before starting the feeding experiments.

Food preferences were analyzed with multiple choice and no choice leaf disc tests in the second week of March 2010 on successive days. Tender leaves of the eight potential plants were collected from the branches of the same height during February-March 2010 and senescent leaves during November 2009 to February 2010 period. Freshly sprouted leaves of five days of age were categorized as tender and the leaves turning yellowish brown prior to the onset of annual leaf shedding as senescent. Collected leaves were kept frozen in plastic bags, and undamaged leaves were used for analysis.

Leaf discs (400 mm^2) of each leaf type were cut and were individually marked with stapler pins (one stapler pin on leaf type 1; 2 pins parallel to each other on leaf type 2; 2 pins crosswise on leaf type 3; 3 pins parallel to each other on leaf type 4, and so on) that enabled their identification. One leaf disc of each leaf type was placed inside a clay vessel (9 cm diameter × 5 cm height) with a distance of 5 mm between the leaf discs for multiple-choice leaf disc tests, and a single leaf disc for no-choice leaf disc tests. Fifteen replicates of each experiment were conducted for tender and senescent leaves separately. In both, no-choice and multiple-choice experiments, three teneral beetles were introduced into the centre of the vessel and were allowed to feed for 24 hrs, (8 am to 8 am). Leaf area consumed was estimated using a 1 mm^2 mesh size-reticulated paper glued on a glass slide.

Table 1: Quantity (mm^2) of leaves consumed (mean ± SD) by *L. tristis* in multiple and no-choice experiment tests.

Leaf type	Tender leaves		Senescent leaves	
	No choice	Multiple choice	No choice	Multiple choice
Cashew	9.60 ± 7.84	1.93 ± 2.80	2.37 ± 3.94	0.31 ± 0.51
Cassia	6.37 ± 10.84	1.05 ± 2.59	0.92 ± 1.50	0.13 ± 023
Cocoa	76.93 ± 56.68	20.56 ± 43.95	4.13 ± 5.47	4.00 ± 4.80
Jackfruit	125.33 ± 83.61	41.66 ± 67.32	7.37 ± 7.72	0.47 ± 0.49
Mango	3.93 ± 4.91	0.13 ± 0.3	1.77 ± 2.15	0.38 ± 0.47
Rubber	103.03 ± 85.41	84.13 ± 66.78	48.40 ± 42.54	44.43 ± 34.59
Sapota	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Wild jack	13.13 ± 8.80	6.83 ± 5.86	1.30 ± 0.96	0.65 ± 0.67

Table 2: Two-way ANOVA for feeding preference of *L. tristis* with respect to the leaf type and leaf age in no-choice and multiple-choice experiment tests.

	No-choice			
	SS	df	MS	*F*	*P* value
Leaf type	1,4733.44	7	210.49	45.79	<.05
Leaf age	269.63	1	269.63	58.66	<.05
Leaf type × Leaf age	340.26	7	48.61	10.57	<.05
Error	1,029.62	224.00	4.60		
	Multiple-choice				
	SS	df	MS	*F*	*P* value
Leaf type	1,182.14	7	168.88	42.09	<.05
Leaf age	71.62	1	71.62	17.85	<.05
Leaf type × Leaf age	134.28	7	19.18	4.78	<.05
Error	898.73	224.00	4.01		

Table 3: One-way ANOVA for the quantity of tender and senescent leaves consumed by *L. tristis* in no-choice and multiple-choice experiment tests.

Leaf type	No-choice test		Multiple-choice test	
	F	*P*	*F*	*P*
Cashew	14.45	0	5.16	.03
Cassia	5.15	.03	7.55	.01
Cocoa	0.4	.53	2.23	.15
Jack fruit	34.35	0	9.09	.01
Mango	3.62	.05	3.55	.05
Rubber	3.64	.05	4.45	.04
Wild jack	46.17	0	23.33	0

Amount of leaf disc consumed during the tests was estimated by subtracting the unconsumed area from the initial area of 400 mm^2.

2.4. Data Analysis. Significance levels of the variation in the quantity of leaf consumed among the leaf types and leaf ages were assessed with two-way ANOVA and pairwise differences among leaf types with Tukey-Kramer post hoc tests (*t*-tests). Significance level of the variation in the quantity of leaf consumed between the tender and mature leaves of each leaf type were assessed with one-way ANOVA. The preference hierarchy was reached by ranking the eight leaf types based on the significance level of the pair wise treatments for each host plant. All analyses were done following square root transformation of the data [16]. Significance was determined at $P < .05$. All statistical analyses were performed by using Minitab 16 Academic Software for windows [17].

3. Results

Variation in the quantity consumed by *L. tristis* among the eight leaf types and between the leaf ages, in both no-choice and multiple-choice experiments, was recorded (Tables 1, 2, 3 and 4).

3.1. Senescent Leaves. L. tristis consumed more senescent rubber leaves than all other leaf types in both no-choice and multiple-choice experiments (Tables 1, 4 and 5). No feeding was recorded on sapota.

3.2. Tender Leaves

No-Choice Experiments. Equal quantity of tender leaves of rubber and jackfruit were consumed (Tables 1, 4 and 5). Rubber and jackfruit were preferred over other six leaf types (cocoa, cashew, mango, wild jack, cassia and sapota). No feeding was recorded on sapota.

Table 4: Tukey multiple comparisons (t-test) of the variation in the feeding preference of *L. tristis* towards tender and senescent leaves of different leaf types in no-choice and multiple-choice experiment tests.

Leaf types	Tender leaves		Senescent leaves	
	No choice	Multiple choice	No choice	Multiple choice
Rubber/Cashew	0	0	0	0
Rubber/Mango	0	0	0	0
Rubber/Jackfruit	0.95	0	0	0
Rubber/Wild jack	0	0	0	0
Rubber/Cocoa	0	0	0	0
Rubber/Cassia	0	0	0	0
Rubber/Sapota	0	0	0	0
Cashew/Mango	0.94	0.98	1	1
Cashew/Jackfruit	0	0.01	0.41	1
Cashew/Wild jack	1	0.84	1	1
Cashew/Cocoa	0.97	0.34	0.97	0.2
Cashew/Cassia	0.97	1	1	0.2
Cashew/Sapota	0.08	0.95	0.56	0.99
Mango/Jackfruit	0	0	0.37	1
Mango/Wildjack	0.63	0.27	1	1
Mango/Cocoa	1	0.04	0.96	0.25
Mango/Cassia	1	1	1	0.25
Mango/Sapota	0.65	1	0.6	0.99
Jackfruit/Wild jack	0	0.33	0.39	1
Jackfruit/Cocoa	0	0.83	0.95	0.33
Jackfruit/Cassia	0	0	0.11	0.33
Jackfruit/Sapota	0	0	0	0.97
Wild jack/Cocoa	0.71	0.99	0.97	0.45
Wild jack/Cassia	0.71	0.54	1	0.45
Wild jack/Sapota	0.01	0.19	0.58	0.92
Cocoa/Cassia	1	0.13	0.72	1
Cocoa/Sapota	0.56	0.02	0.08	0.03
Cassia/Sapota	0.56	1	0.92	0.03

Multiple-Choice Experiments. More quantity of tender rubber leaves were consumed than all other seven leaf types (cashew, mango, jackfruit, wild jack, cocoa, cassia and sapota) (Tables 1, 4 and 5). Jack fruit leaves were preferred over four leaf types (cashew, mango, golden shower and sapota) and cocoa over two leaf types (mango and sapota). No feeding was recorded on sapota.

3.3. Comparison of the Quantity of Tender and Senescent Leaves Consumed

No-Choice Experiments. Comparison of the tender and senescent leaves consumed revealed that no difference for cocoa and sapota leaves. More tender leaves were consumed for rubber, jackfruit, cashew, mango, wild jack and cassia (Tables 1 and 3).

Table 5: Preference hierarchy of *L. tristis* to various leaf types and leaf ages.

Leaf type	Tender		Senescent	
	No-choice	Multiple-choice	No-choice	Multiple-choice
Cashew	2	5	2	2
Cassia	2	5	2	2
Cocoa	2	3	2	2
Jackfruit	1	2	2	2
Mango	2	6	2	2
Rubber	1	1	1	1
Sapota	3	7	2	2
Wild Jack	2	4	2	2

Multiple-Choice Experiments. More quantity of tender leaves of rubber, jackfruit, wild jack, cashew, mango and cassia leaves were consumed, and no difference was noticeable for cocoa and sapota (Tables 1 and 3).

4. Discussion

4.1. Feeding Preference towards Tender and Senescent Leaves. Preference for tender leaves of most host plants except cocoa highlights the importance of leaf age in determining the food selection and food preference of *L. tristis*. Reasons for the preference towards tender leaves of most host plants, preference hierarchy in its food selection and non-differentiation of mature and tender leaves of cocoa, and non-feeding on sapota are not understood. High nutritional value could be the reason for the high preference towards tender leaves [18, 19]. Analysis of leaf physical and chemical traits is necessary to reach conclusions. Seasonal availability of tender rubber leaves by way of leaf disease-mediated premature fall and the distinct feeding preference of *L. tristis* on tender rubber leaves is cited as the reason for the high abundance of *L. tristis* in rubber plantations [5], and it is expected that control of premature leaf fall may lead to decline in the population buildup of *L. tristis* in rubber plantation belts. However, feeding on the tender leaves of other trees common in rubber plantation belts indicates that *L. tristis* has alternate leaf resources in the absence of rubber leaves. Analysis of reproductive performance of *L. tristis* on alternate host plants, survival experiments, oviposition, and larval feeding preferences are necessary to reach conclusions about the implications of present findings. Among the various alternate host plants, feeding pattern on cocoa requires special attention. In addition to the equal preference of *L. tristis* towards its mature and tender leaves, periodical pruning off tender shoots by the farmers leads to frequent tender leaf availability in cocoa plantations. How tender leaf availability of cocoa facilitates the population buildup of *L. tristis* needs to be ascertained. Spotting of *L. tristis* in cocoa plantations, equal preference towards tender and mature cocoa leaves and presence of patches of cocoa plantations and the scarce distribution of other host plants in rubber belts leads to the proposal that in the absence of

Figure 1: Aggregated beetles on the wall of a residential building.

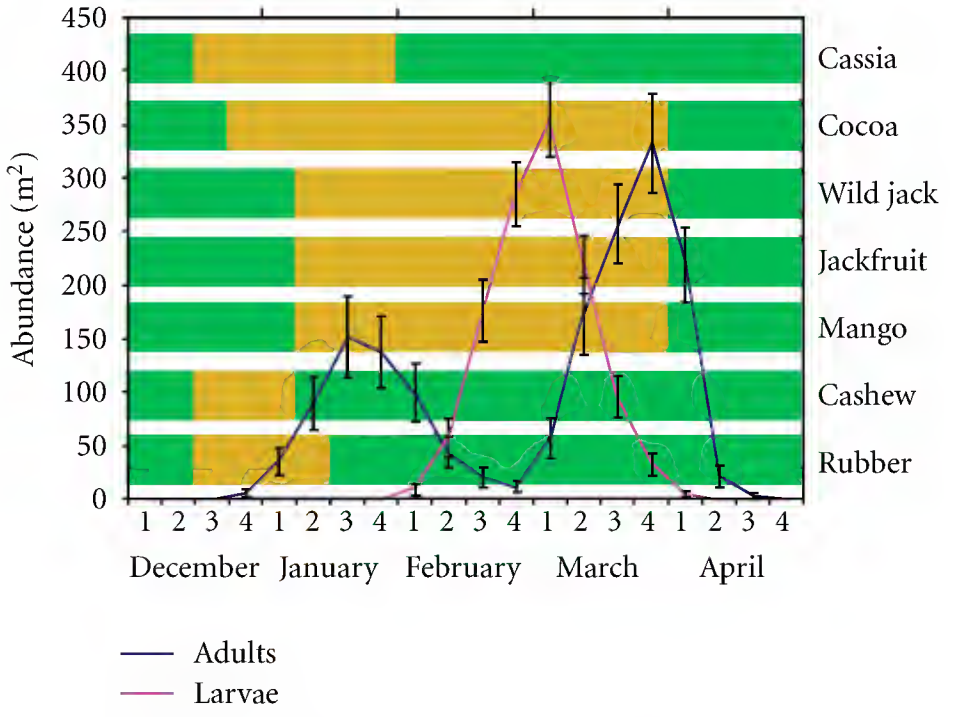

Figure 2: Foliage phenology of the host plants and population buildup of *L. tristis* in litter habitat during post-rainy breeding and feeding period (abundance data of *L. tristis* based on [2]; yellow colour depicts peak leaf fall period of evergreen plants and annual leaf shedding period of deciduous plants; blue coloured line depicts adults and pink coloured line larvae).

tender and mature rubber leaves, cocoa becomes the major host plant of *L. tristis* more than other host plants.

4.2. Host Plants of L. tristis and Implications. First experimental confirmation that *Luprops tristis* is a generalist and not a specialist on rubber and that it has distinct preference hierarchy in its food selection with rubber becoming the most preferred host plant species followed by jackfruit and cocoa is reached at. It raises the question whether showing inclination to feed up on three plants is enough to consider the beetle as a generalist? However, record of *L. tristis* in non-rubber belts in South East India before the introduction of rubber plantations in the moist south west India [20–22] indicates that rubber is not the sole host plant or ancestral host plant of *L. tristis*.

Selection of jackfruit and cocoa as the second most preferred food choice indicates that rubber plantation litter-centered efforts to control *L. tristis* such as premature leaf fall prevention in rubber plantations alone may not be effective as *L. tristis* may switch over to alternate host plants. Presence of *L. tristis* in the litter stands of cocoa, jackfruit, wild jack, cashew, mango and cassia (first author, personal observations) indicates that postdormancy beetles that arise from the 8-9 month long dormancy with the onset of post monsoon dry conditions ([2]; Figure 2) might be taking a transitory shelter and sustain on the tender and senescent leaves of cocoa and tender leaves of other host plants till annual litter fall followed by premature leaf fall occurs in rubber plantations.

Based on the host plant selection behavior of polyphagous insects [19, 23] and the phenology of *L. tristis* in rubber belts [5], preference towards rubber is attributed to two possibilities. Rubber is the most high-quality host plant species among the listed host plants. Feeding on other leaf types, even when rubber leaves were available during multiple-choice tests, logically raises question about the possible reasons for feeding on mixed diets and the advantages it provides. These observations reiterate the need for survival experiments and analysis of reproductive performance of *L. tristis* on each one of these host plants to reach conclusions. Secondly, literature on host plant selection of phytophagous insects revealed that concentrating on a particular species enables information about the environment to be processed more efficiently, increases the rate of host plant location/utilization [24–28], processes more information about that species by the females, and detects the variation in the quality of individual plants more efficiently [19, 29]. However, with overspecialization of *L. tristis* on the seasonally available leaves of the deciduous host plant, rubber would have affected its survival chances whereas generalist feeding behavior provides access to a greater resource base, a more nutritionally balanced diet [18, 30–32]. Hence, feeding on mixed diets of different evergreen host plants (generalist feeding behavior) may be an adaptive strategy of *L. tristis* to avoid overspecialization on a deciduous host plant (rubber) with highly seasonal leaf shedding behavior. However, evidence from plant-feeding insects showed that early experience in feeding on one type of resource may make it easier to exploit the same resource later in life or influence host choices later in development [33–35]. Hence, it is possible that the high degree of preference expressed for rubber could partly reflect pre-experiment feeding on this plant and associated induction of host preferences.

4.3. Predictions. High preference of *L. tristis* towards rubber leaves, presence of alternate host plants in rubber belts and earlier data on its wide prevalence in rubber plantations, high reproductive potential of *L. tristis,* and the possession of defensive gland secretions that deters the natural predators in litter habitat and aggregation sites [36, 37] indicate that prospects of further increase of *L. tristis* across the rubber belts is a certainty. Although *L. tristis* is recorded from Sri Lanka, South India, and Nepal [22], it is not recorded as a nuisance pest in these regions. Selection of rubber followed by jackfruit and cocoa as the most favored host plant and non-differentiation between tender and mature cocoa leaves indicates that *L. tristis* has high potential to

become a nuisance pest in the new rubber plantation belts coming up in non-traditional rubber belts in the Indian subcontinent as well as in the cocoa plantation belts in South India.

4.4. Implications for Pest Management. Findings from the study have important bearing on the pest management strategies to be adopted for *L. tristis*.

(1) Present study establishes that *L. tristis* is a generalist feeder and not a specialist on rubber, and it has distinct preference hierarchy in its food selection with rubber becoming the most preferred host plant species followed by jackfruit and cocoa.

(2) Selection of jackfruit and cocoa as the preferred food choice after rubber, and its feeding on alternate host plants indicates that field stands-based efforts for control of *L. tristis* should consider the leaf litter accumulated around the alternate host plants.

(3) Non-differentiation of tender and mature cocoa leaves, shows that in the absence of rubber leaves cocoa plants become the major host plant of *L. tristis* than the less abundant jackfruit trees whose fallen tender leaves may not be available in sufficient quantity. Hence, it is likely that availability of cocoa litter might have contributed towards population build up of *L. tristis* in regions where intercropping of cocoa and rubber is practiced.

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Research Article

Distribution and Diversity of the Cryptic Ant Genus *Oxyepoecus* (Hymenoptera: Formicidae: Myrmicinae) in Paraguay with Descriptions of Two New Species

T. Delsinne,[1] W. Mackay,[2] A. Wild,[3] Y. Roisin,[4] and M. Leponce[1]

[1] *Biological Evaluation Section, Royal Belgian Institute of Natural Sciences, 29 street Vautier, 1000 Brussels, Belgium*
[2] *Laboratory for Environmental Biology, The University of Texas, El Paso, TX 79968, USA*
[3] *Department of Entomology, University of Illinois, 505 South Goodwin Avenue, Urbana, IL 61801, USA*
[4] *Department of Evolutionary Biology and Ecology, Université Libre de Bruxelles, 50 Avenue Roosevelt, 1050 Brussels, Belgium*

Correspondence should be addressed to T. Delsinne, thibaut.delsinne@sciencesnaturelles.be

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We discuss the diversity and distribution of the ant genus *Oxyepoecus* in Paraguay. *Oxyepoecus inquilinus* is recorded for the first time, and new distribution data are given for *O. rastratus* and *O. vezenyii*. Published data for *O. bruchi*, *O. rastratus*, *O. reticulatus*, and *O. vezenyii* are summarized. Two new species are described (*O. bidentatus* n. sp. and *O. striatus* n. sp.), and a key to the workers of the seven Paraguayan *Oxyepoecus* species is provided. At Teniente Enciso National Park, four species cooccur. This locality appears as a promising site for studies documenting the biology of this poorly known ant genus, and because of the IUCN "vulnerable" Red List classification of *O. inquilinus*, the importance of the Teniente Enciso National Park for biological conservation is clearly established.

1. Introduction

Oxyepoecus [1] is a genus of cryptobiotic ants collected infrequently from Colombia to Chile [2–5]. The genus is a member of the tribe Solenopsidini in the subfamily Myrmicinae [6]. It currently includes 20 species [3, 4, 7], including two species described as new in this paper. Four *Oxyepoecus* species were previously recorded in Paraguay: *O. bruchi* [1], *O. rastratus* [8], *O. reticulatus* [7], and *O. vezenyii* [9, 10].

The genus is differentiated from other Solenopsidini by the 11-segmented antennae with a three-segmented apical club, the clypeus with four teeth, and the dentate propodeum [3]. In addition, the petiole and postpetiole nodes are high and often anteroposteriorly compressed [3].

The biology of the genus is poorly known, but three species (*O. inquilinus* [11], *O. daguerrei* [12], and *O. bruchi* [1]) are suspected to be inquilines of *Pheidole* or *Solenopsis* [1, 7, 11], although the exact nature of the relationship is unclear [3]. These three species are considered as "Vulnerable D2" [13], meaning they are suspected to be "facing a high risk of extinction in the wild in the medium-term future" because "their populations are characterized by an acute restriction in their area of occupancy (typically less than 100 km^2) or in the number of locations (typically fewer than five)." Due to the current rapid loss of biodiversity and uncertainty concerning the conservation status of social insects [14], data to increase our knowledge of threatened species are desperately needed.

Here, we report *Oxyepoecus inquilinus* for the first time from Paraguay and provide new distribution data for *O. vezenyii* and *O. rastratus*. Two new species are described.

2. Materials and Methods

The sampling of ant assemblages in the Chaco region of Paraguay was based on 560 Winkler and 720 pitfall samples collected between 2001 and 2004 in 11 localities along a 410 km transect beginning at Río Verde and ending at Fortín Mister Long close to the Bolivian border (Figure 1) [15, 16]. Sampling was always carried out at the end of the dry season (September–November). For Winkler extractions, the leaf litter present inside a one m^2 quadrat was collected and sifted

and its fauna was extracted for 24 h. Pitfall traps consisted of 70 mm diameter drinking cups, containing water and a drop of detergent, operating for at least 24 hours. Vegetation corresponds to relatively well-preserved xeromorphic forests [17]. Elevation, mean annual rainfall and temperature, mean maximal temperature of the warmest month, and mean minimal temperature of the coldest month are provided for each locality [16].

Specimens from the Paraguayan oriental region were collected following previous techniques [10]. Data were supplemented by the examination of existing museum material [10]. Finally, data from the literature and the online specimen database http://www.antweb.org/ were added in order to provide a complete overview of the diversity and distribution of *Oxyepoecus* in Paraguay.

3. Measurements and Indices

All measurements are in millimeters. The abbreviations are as follows.

TL: Total length from the anterior margin of the head (in vertical position) to the posterior edge of the gaster measured in lateral view.

HL: Head length, measured in full face view, from the anterior margin of the medial lobe of the clypeus to the posterior border of the head (excluding the mandibles).

HW: Head width, the maximum width of the head measured in full face view, excluding the eyes.

EL: Eye length, the maximum diameter of eye.

SL: Scape length, excluding the basal condyle.

PL: Petiole length, the maximum length of the node measured in dorsal view, starting at the base of the anterior face and ending at the base of the posterior edge.

PW: Petiole width, the maximum width of the node measured in dorsal view.

PPL: Postpetiole length, the maximum length of the node measured in dorsal view, as above.

PPW: Postpetiole width, the maximum width of the node measured in dorsal view.

WL: Weber's length, measured from the anterior edge of the pronotum to the posterior edge of the metapleural lobe.

CI: Cephalic index, HW/HL $\times$ 100.

SI: Scape index, SL/HL $\times$ 100.

The terminology is based on [18].

Holotypes, paratypes, and voucher specimens have been deposited at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium, (RBINS), the University of Texas at El Paso (CWEM), the Alexander L. Wild personal collection (ALWC), the Museum of Comparative Zoology, Harvard University (MCZC), the Los Angeles Country Museum, Los Angeles, California, USA (LACM) and the "Museo Nacional de Historia Natural del Paraguay", San Lorenzo, Paraguay (INBP).

4. Results

The following species of *Oxyepoecus* were collected in Paraguay (Figure 1).

4.1. Oxyepoecus bidentatus. Delsinne and Mackay, n. sp. (Figures 2 and 3).

Diagnosis. Its worker morphology places this species within the *rastratus* species-group [4]. The reticulate-costulate dorsal surface of the head (Figures 2(a) and 2(d)) and the well-defined subpostpetiolar process, forming a pair of prominent blunt teeth (Figure 3), separate *O. bidentatus* from all the other species of *Oxyepoecus*. The gyne and male are unknown.

Description of the Worker. Measurements of holotype, paratypes ($n = 2$) between parentheses: TL 1.94 (1.86–1.90), HL 0.52 (0.51–0.55), HW 0.44 (0.42–0.46), EL 0.07 (0.06-0.07), SL 0.32 (0.32–0.35), PL 0.07 (0.07–0.11), PW 0.17 (0.19–0.23), PPL 0.09 (0.08–0.12), PPW 0.22 (0.25–0.28), WL 0.63 (0.60–0.64), CI 85 (83-84), and SI 62 (63-64).

Lateral clypeal teeth are well developed, directed anteriorly; eye small, about 16–18 ommatidia, five ommatidia in greatest diameter; scape in repose failing to reach posterior border of head by about two maximum widths; sides of head nearly straight, parallel; frontovertexal margin slightly convex; pronotal shoulder gently angulate, marked with striae; notopropodeal (=metanotal) groove indistinct; propodeal angles developed, with two medium-sized acute teeth; subpetiolar process well-developed, lobe-like, directed ventrally; subpostpetiolar process well-developed, forming pair of blunt teeth, directed ventrally; nodes of petiole and postpetiole high and dorsally rounded, compressed anteroposteriorly; in lateral view, petiolar node higher than postpetiolar node; as seen from above, postpetiole much broader than petiole.

Long erect hairs abundant on clypeus, vertex, dorsum of mesosoma, petiole, postpetiole, all surfaces of gaster; mandibles, antennae, legs, and dorsal surface of head with abundant shorter semierect hairs.

Mandibles smooth and shiny, with few scattered punctures; head dorsum reticulate-costulate, lateral costulae attain compound eye and posteriorly vertexal margin; dorsopronotum and mesonotum longitudinally costate; dorsopropodeum transversely costate (about 10–12 costae on dorsal face), anterior half of the lateropronotum mostly smooth and glossy, sometimes with faint longitudinal costae; posterior half of the lateropronotum, mesopleuron and lateropropodeum covered by sparse longitudinal costae; nodes of petiole and postpetiole transversely costate; gaster smooth and glossy with sparse punctures.

Body Color. Concolorous Reddish Brown.

Etymology. From Latin, *bidens*, referring to the subpostpetiolar process forming a pair of well-defined teeth.

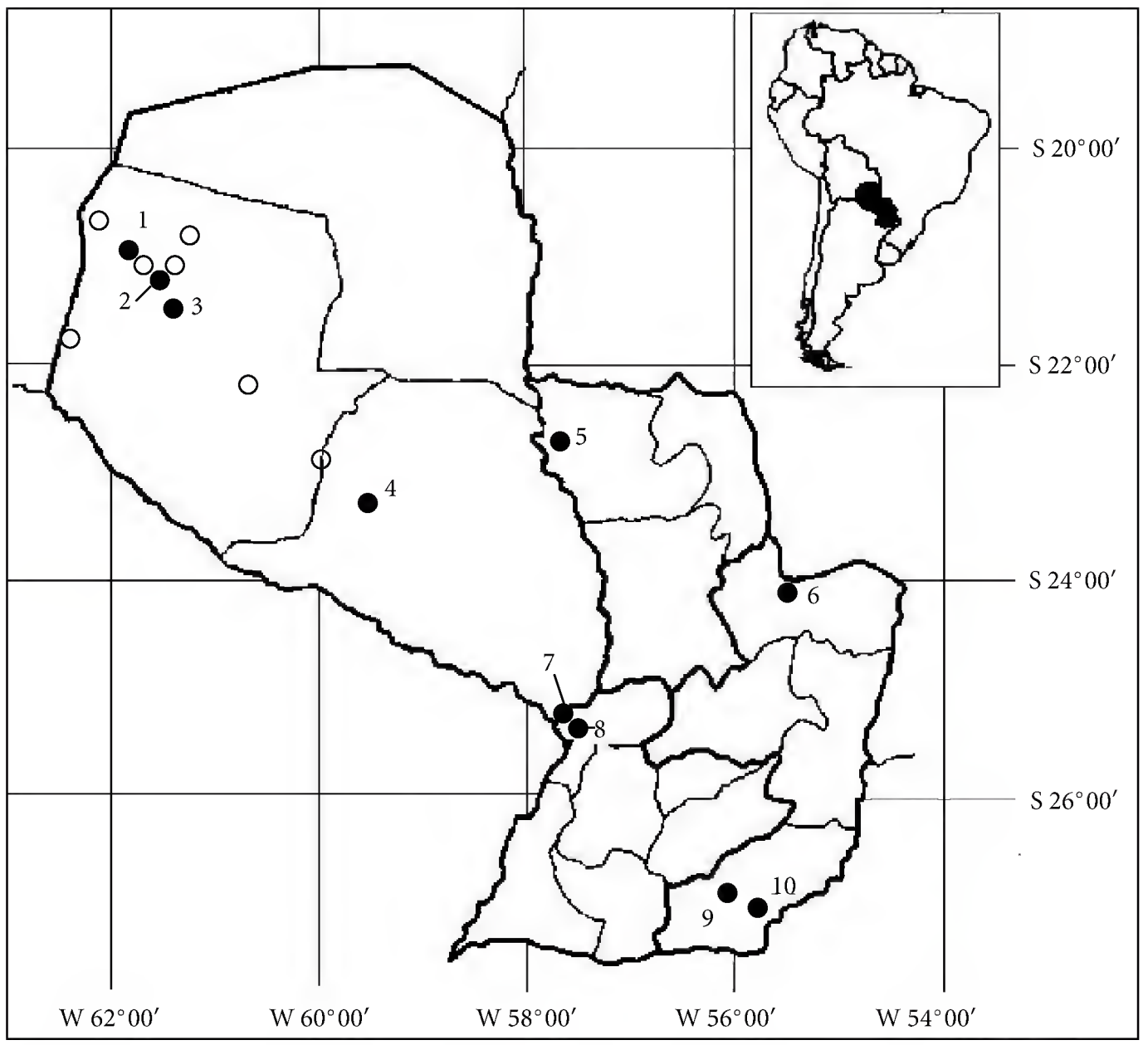

Figure 1: Distribution of *Oxyepoecus* in Paraguay. In this country, seven species have been reported: *O. bidentatus* n. sp. (collected at localities 2, 3, 4), *O. bruchi* (7), *O. inquilinus* (1, 2), *O. rastratus* (2, 3, 6, 10), *O. reticulatus* (9), *O. striatus* n. sp. (4), and *O. vezenyii* (2, 3, 5, 8). Localities are (1) Nueva Asunción, (2) Teniente Enciso National Park, (3) Garrapatal, (4) Río Verde, (5) Puerto Max, (6) Reserva Natural del Bosque Mbaracayú, (7) San Lorenzo, (8) Luque, (9) Santa María, and (10) Pastoreo. For information, localities of the Paraguayan dry Chaco sampled by Delsinne et al. [15, 16] but where no *Oxyepoecus* species were collected are also shown on the picture (empty symbols).

Distribution. *Oxyepoecus bidentatus* was found in three localities of the Paraguayan dry Chaco (Figure 1). Because the maximal distance between localities was 340 km, *O. bidentatus* is suspected to be widely distributed in xeromorphic Chacoan forests, even if rarely found.

Type Series

Holotype worker. Paraguay: Presidente Hayes: Río Verde, Lat: S 23.22, Long: W 59.20, 15-16.X.2003, Delsinne T., 24-hour pitfall sample (specimen number 29272, SIDbase [19], RBINS). Images of the holotype are available at http://projects.biodiversity.be/ants.

Paratype workers. Paraguay: Presidente Hayes: Río Verde, Lat: S 23.22, Long: W 59.20, 15-16.X.2003, Delsinne T., one worker, 24-hour pitfall trap, specimen number 32013, MCZC; Boquerón: T. Enciso N.P., Lat: S 21.21, Long: W 61.66, 03–05.XI.2001, Leponce M., five workers in three Winkler samples, RBINS, INBP, specimen numbers 7598, 7683, 7684, 7698, and 32605 (scanning electron microscope (SEM) pictures of the specimen number 7684 are available at http://projects.biodiversity.be/ants); Boquerón: Garrapatal, Lat: S 21.45, Long: W 61.49, 05-06.XI.2001, Leponce M., one worker, Winkler sample, specimen number 24606, RBINS.

Comparison. *Oxyepoecus bidentatus* is the only species of the genus to have both the dorsal surface of the head entirely covered by sculpture and a bidentate subpostpetiolar process. The anterior subpostpetiolar process of *O. bruchi* of the *vezenyii* species-group is also prominent and bidentate [1, 3], but the dorsal surface of the head is mainly smooth and shining except for two patches of fine, longitudinal rugulae which do not reach posteriorly to the vertex margin nor laterally to the compound eye. Criteria separating *O. bidentatus* from other species of the *rastratus* species-group are the mesopleuron and lateropropodeum covered by longitudinal costae (and not reticulate as for *O. myops, O. rosai,* and *O. reticulatus*), and the presence of a reticulate-costulate sculpture on the dorsal surface of the head reaching posteriorly to the vertexal margin and laterally to the compound eye.

Biology. The fact that workers were extracted from leaf litter (Winkler method) or were collected in pitfall samples, while

(a) (b)

(c) (d)

FIGURE 2: *Oxyepoecus bidentatus* Delsinne and Mackay, n. sp.; holotype worker (number 29272): in frontal (a), lateral, (b) and dorsal views (c); paratype worker (number 7684): detail of the vertex sculpture (d). Note the dorsal surface of the head entirely reticulate-costulate (a, d) and the subpostpetiolar process bidentate (b).

no gynes were found, suggests that this species nests in the soil, but workers forage in the leaf litter when abiotic conditions are favorable. Localities where the species was found have a mean annual rainfall and temperature ranging from 593 to 887 mm and from 23 to 25°C, respectively [16].

4.2. Oxyepoecus bruchi [1]. Paraguay: Central: San Lorenzo, Lat: S 25.33, Long: W 57.55, 4.X.1979, Vaucher C., one worker, specimen code CASENT0178098, ALWC. The specimen has been imaged and is available on AntWeb (http://www.antweb.org/).

4.3. Oxyepoecus inquilinus [11]. Paraguay: Boquerón: T. Enciso N.P., Lat: S 21.21, Long: W 61.66, 03–05.XI.2001, Leponce M., two workers from one Winkler sample, specimen numbers 7617, and 7619, RBINS (SEM photographs of the specimen number 7617 are available at http://projects.biodiversity.be/ants); Boquerón: Nueva Asunción, Lat: S 20.70, Long: W 61.93, 02–06.XI.2001, Leponce M., two workers in two four-day pitfall traps, specimen numbers 30660, 30678, RBINS.

4.4. Oxyepoecus rastratus [8]. Paraguay: Canindeyú: Reserva Natural del Bosque Mbaracayú, Jejuimi, Lat: S 24.1, Long: W 55.53, 02.V.1996, Wild A., seven workers and one dealate queen, collection code AW0129, ALWC, INBP, LACM (nest in red rotting log; wood was too hard for a full excavation; one chamber uncovered with gyne and brood), humid subtropical tall forest, one worker and the dealate gyne have been imaged and are available on AntWeb (http://www.antweb.org/), specimen codes CASENT0178099 and CASENT0178100, respectively; Boquerón: Teniente Enciso National Park, Lat: S 21.21, Long: W 61.66, 03–05.XI.2001, Leponce M., 28 workers and one gyne in eight Winkler samples, worker numbers 7302, 7303, 7746, 7633, 7634, 32602, 7646, 7647, 7674, 23778, 7669, 25221, 405701, RBINS, INBP, CWEM, gyne number 7662, RBINS (SEM photographs of the specimen number

23778 are available at http://projects.biodiversity.be/ants); Boquerón: Garrapatal, Lat: S 21.45, Long: W 61.49, 05-06.XI.2001, Leponce M., one worker, Winkler sample, specimen number 25221, RBINS.

In addition, data from the literature [4] include: Paraguay: Itapúa: Pastoreo, Lat: S 25.38, Long: W 55.83, 03.X. 1974, Duelli P., collection code 399, three workers.

4.5. Oxyepoecus reticulatus [7]. Paraguay: Itapúa: Santa María, no specific location information, 25.X.1982, Baud F., three workers, ALWC, one worker has been imaged and is available on AntWeb (http://www.antweb.org/), specimen number CASENT0178101.

4.6. Oxyepoecus striatus. Mackay and Delsinne, n. sp. (Figure 4).

Diagnosis. Its worker morphology places this species within the *vezenyii* species-group [3]. The worker is a small specimen with longitudinal striae covering the promesonotum (Figures 4(c) and 4(d)) and transverse striae on the dorsopropodeum. The clypeal teeth are well defined and sharp. The gyne and male are unknown.

Description of the Worker. Measurements of holotype; paratypes ($n = 2$) between parentheses: TL 1.58 (1.6-1.7), HL 0.46 (0.46–0.48), HW 0.35 (0.35-0.36), EL 0.05 (0.07-0.07), SL 0.29 (0.29–0.31), PL 0.06 (0.06-0.06), PW 0.16 (0.17-0.18), PPL 0.08 (0.09-0.09), PPW 0.19 (0.21-0.22), WL 0.51 (0.49–0.51); CI 76 (75-76), SI 63 (62–65).

Mandible with four teeth, with diastema (gap) between basal and subbasal teeth; lateral clypeal teeth well-developed but small, not lobe-like, directed anteriorly; eye small, with about 18 ommatidia, five ommatidia in greatest diameter; scape in repose failing to reach posterior border of head by about two maximum widths; sides of head nearly straight, parallel, frontovertexal margin slightly convex; pronotal shoulder slightly marked with stria; inferior pronotal process well developed; notopropodeal groove poorly developed; propodeal angles developed, acute; subpetiolar process well developed, lobe-like, directed ventrally, anterior and posterior faces of petiole nearly parallel; two subpostpetiolar angles present, anterior and posterior faces of postpetiole nearly parallel; as seen from above, postpetiole much broader than petiole, postpetiole slightly angulate laterally.

Erect hairs abundant on mandibles, clypeus, dorsal surface of head, dorsum of mesosoma, petiole, postpetiole, all surfaces of gaster, legs with semierect hairs; appressed pubescence sparse, few hairs noticeable on head and gaster.

Mandibles smooth and shiny, with few scattered punctures, most of dorsum of head with scattered coarse punctures, medial area smooth and glossy, mesonotum with longitudinal parallel striae, dorsopropodeum with fine transverse striae, lateropronotum mostly smooth and glossy, mesopleuron and lateropropodeum striolate, nodes of petiole and postpetiole smooth and glossy, posterior face of postpetiole transversely striolate, gaster smooth and glossy.

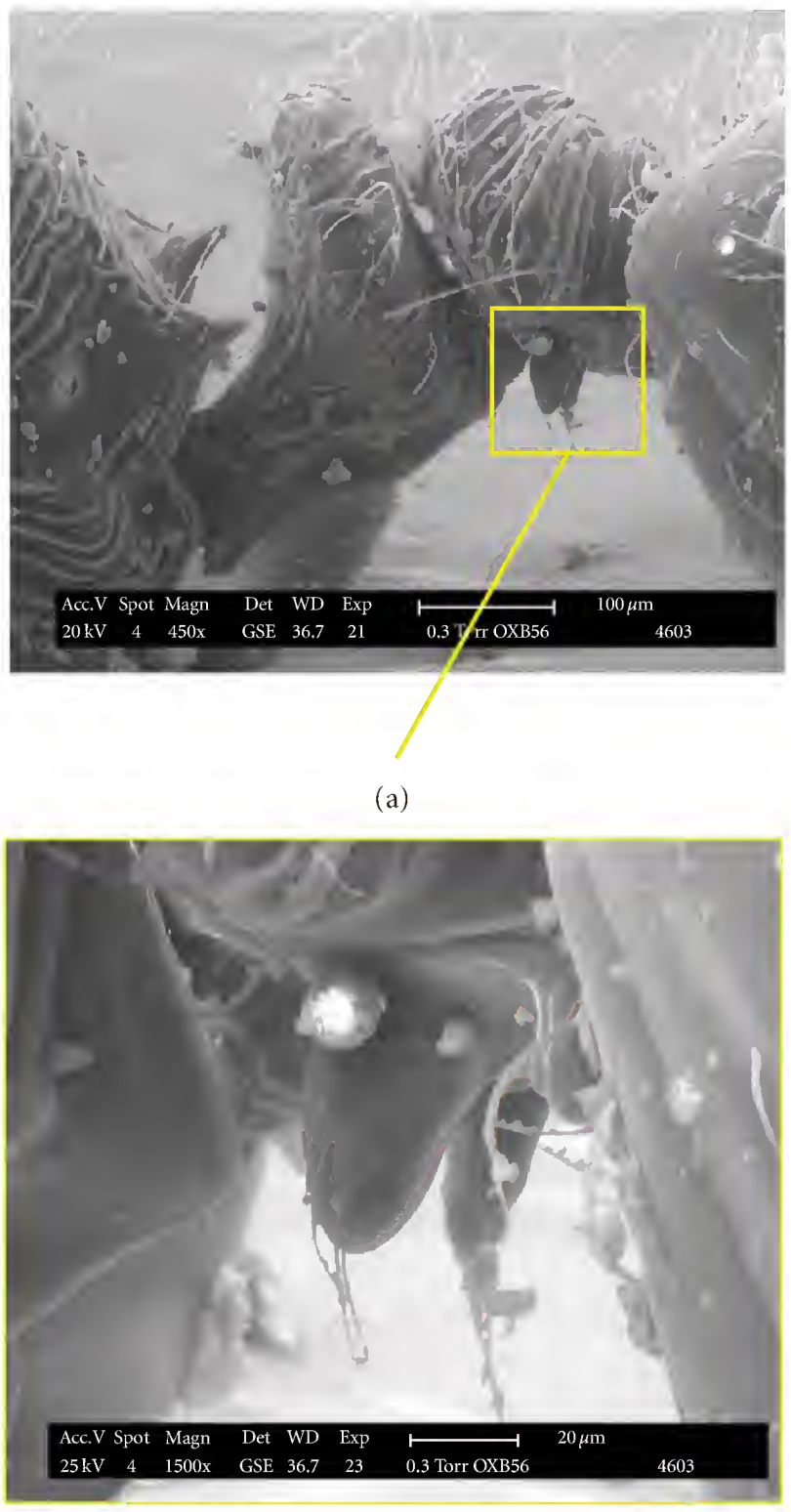

FIGURE 3: *Oxyepoecus bidentatus* Delsinne and Mackay, n. sp.; paratype worker (number 7684): detail of petiole and postpetiole (a) and detail of the bidentate subpostpetiolar process (b).

Body color. Concolorous Medium Reddish Brown.

Etymology. From Latin, *stria*, referring to the striae covering the dorsum of the mesosoma.

Distribution. Known only from the type locality.

Type Series

Holotype worker. Paraguay: Presidente Hayes: Río Verde, Lat: S 23.22, Long: W 59.20, 15-16.X.2003, Delsinne T., 24-hour pitfall sample, specimen number 32606, MCZC.

Paratypes. Same data as holotype, three workers, in three 24-h pitfall samples, specimen numbers 29523, 29531, 29667, RBINS, INBP. Images of the specimen number 29531 are available at http://projects.biodiversity.be/ants.

(a) (b)

(c) (d)

FIGURE 4: *Oxyepoecus striatus* Mackay and Delsinne, n. sp.; paratype worker (number 29531): in frontal (a), lateral, (b) and dorsal views (c, d). Note the longitudinal striae covering the promesonotum (c, d).

Comparison. This species is a member of the *vezenyii* species-group [3] defined principally in having a predominantly smooth and glossy dorsum of the head. It is very similar to the relatively common *O. vezenyii*, but can be easily distinguished as the promesonotum of *O. vezenyii* is nearly completely smooth and glossy (the dorsopropodeum of *O. vezenyii* has transverse striae as in *O. striatus*).

Oxyepoecus striatus appears most similar to *O. browni*, which has a similar sculptured promesonotum, short posterior propodeal face, moderately well-developed costulae between the frontal carinae, and a large lobe-like subpetiolar process. *Oxyepoecus striatus* can be separated as being smaller; the clypeal teeth are well defined, sharp, and directed anteriorly (not lobe-like and directed inward). The frontal lobes are more widely spaced than those of *O. browni* (separated by 0.11 mm) and the head is covered by coarse punctures (except for the smooth medial area).

Biology. The specimens were collected in three separate pitfall samples. The mean annual rainfall and temperature of the locality where the species was found were 887 mm and 23°C, respectively [16].

4.7. Oxyepoecus vezenyii [10]. Paraguay: Boquerón: T. Enciso N.P., Lat: S 21.21, Long: W 61.66, 03–05.XI.2001, Leponce M., 23 workers and five gynes in 12 Winkler samples, worker numbers 7692, 7726, 7737, 7738, 7744, 7690, 7691, 7599, 32603, 32604, 7659, 7660, RBINS, INBP, CWEM, (SEM photographs of the specimen number 7737 are available at http://projects.biodiversity.be/ants), gyne numbers 7753, 7731, 22806, 7661, 7618, RBINS; Boquerón: Garrapatal, Lat: S 21.45, Long: W 61.49, 05-06.XI.2001, Leponce M., one worker, Winkler sample, specimen number 24598, RBINS; Central: Luque, Lat: S 25.27, Long: W 57.57, 11.VIII-6.X. 1982, Kochalka J., Pitfall trap, sample code IBN230, two workers, one of them has been imaged and is available on AntWeb (http://www.antweb.org/), specimen code CASENT0178102, ALWC.

In addition, the type specimen (worker) was collected in Paraguay (Concepción: Puerto Max Forel) [9].

5. Key to the Workers of Oxyepoecus in Paraguay

(1) Cephalic dorsum entirely sculptured (Figure 2(a); 2).

(1′) Cephalic dorsum completely smooth or with at least a smooth median frontal stripe (Figure 4(a); 4).

(2) Mesopleuron and lateropropodeum covered by longitudinal costae (Figure 4(b); 3).

(2′) Mesopleuron and lateropropodeum irregularly reticulate and punctuate *O. reticulatus*.

(3) Cephalic dorsum with dense costulae, subpostpetiolar process shaped as transverse crest, triangular in side view, not bidentate *O. rastratus*.

(3′) Cephalic dorsum with reticulated costulae, subpostpetiolar process prominent and bidentate (Figure 3) *O. bidentatus* n.sp.

(4) Eyes large, with more than 40 ommatidia in total *O. inquilinus*.

(4′) Eyes small, with about 20 ommatidia in total (Figure 4(b); 5).

(5) Subpostpetiolar process prominent and bidentate with anteriormost process much larger than posterior tooth *O. bruchi*.

(5′) Subpostpetiolar process with two subparallel crests of approximately equal size (Figure 4(b); 6).

(6) Promesonotum nearly entirely smooth and glossy *O. vezenyii*.

(6′) Promesonotum covered with longitudinal striae (Figures 4(c) and 4(d)) *O. striatus* n. sp.

6. Discussion

Seven *Oxyepoecus* species are recorded from Paraguay. Two of them are new species described in this paper: *O. bidentatus* and *O. striatus* from the *rastratus* and *vezenyii* species-groups, respectively. These species-groups now include eight and 12 species, respectively. *Oxyepoecus bidentatus* was found in three localities, 20 to 340 km away from each other, indicating that this species may be widely distributed within the Paraguayan dry Chaco. *Oxyepoecus striatus* is only known from the type locality.

Oxyepoecus rastratus was documented from South and South-East Brazil and from Eastern Paraguay [4]. Its presence at T. Enciso N.P. increases its range nearly 700 km to the West. In addition, samples in the dry Chaco and in the Paraná forest suggests that this species may be present in a variety of biomes.

Oxyepoecus reticulatus has been recorded in a dozen localities in South and Southeastern Brazil, mainly in relatively dry forests [4]. The Paraguayan data increase its distribution by nearly 360 km to the West.

Oxyepoecus bruchi was collected in 1948 and 1953 in Argentina (Córdoba and Tucumán provinces) and more recently (2003) in Brazil (Palhoça, Santa Catarina State) [3, 4, 7]. Only one specimen was collected in 1979 in Paraguay (Central). Although the species was rarely collected, its distribution seems potentially large. Nevertheless, *O. bruchi* appears locally rare, justifying its vulnerable status [13]. This species is suspected to be an inquiline of the ants *Pheidole rosae* (named *Ph. silvestrii* in [7]) and *Ph. obtusopilosa* [1, 7, 11], but these species were not present in our samples and, to our knowledge, have not been collected in Paraguay [10].

Oxyepoecus inquilinus was sampled in two localities of the Paraguayan dry Chaco. In the literature, *O. inquilinus* was reported from two savanna localities of the Brazilian Cerrado [7], one Brazilian pasture [4], one anthropogenic area (i.e., the "Jardin del Instituto Miguel Lillo") from the Argentinean Tucumán province [11], and one locality in the Bolivian Bení Department [7]. In addition, one worker closely related to *O. inquilinus* was collected in a savanna-morichal habitat from Colombia, but its specific status awaits further investigation [2]. Finally, *O. inquilinus* was recently sampled in a Valdivian forest of Chile [5]. If the identity of the Colombian specimen is confirmed, *O. inquilinus* is the most broadly distributed species of the genus. In fact, its Colombian and Chilean localities represent both the northernmost and southernmost limits of distribution for the entire genus. Although data are insufficient to determine the exact requirements of this species, *O. inquilinus* seems to be present both in open and closed habitats, in degraded and pristine ecosystems, and in dry and wet areas. The distribution of this species seems large but discontinuous, and *O. inquilinus* is apparently locally rare, justifying its vulnerable status [13]. *O. inquilinus* is suspected to be inquiline in *Pheidole radozskowskii* nests [11]. At T. Enciso N.P., the same Winkler sample collected two workers of *O. inquilinus* and 11 workers of *Ph. radozskowskii*. However, at Nueva Asunción the latter was not recorded in our 60 Winkler and 60 pitfall samples, suggesting that *O. inquilinus* is not restricted to this host species.

With the exception of the Paraguayan type specimen [9], *Oxyepoecus vezenyii* is exclusively known from Brazil where it was sampled in different ecosystems over a large spatial scale [3, 4]. The presence of this species in the Paraguayan dry Chaco increases its range nearly 400 km to the West.

In this study, *Oxyepoecus* individuals were mainly collected using the Winkler extraction method. This technique is highly effective for sampling minute and cryptic ant species which were previously suspected to be rare before the development of the method [20, 21]. This may be the case for *Oxyepoecus* species [3]. Nevertheless, in dry forests the Winkler sampling is strongly influenced by the rainfall regime, and a recent rainfall may increase its efficiency both in terms of species collected and species occurrences [15]. Teniente Enciso National Park was the single locality of the dry Chaco sampled a day after a rainfall. This bias may be the reason why a relatively large number of *Oxyepoecus* species and individuals were collected in this park and just a few in the other dry Chacoan localities. We hypothesize that using the Winkler method during the rainy season of the Paraguayan dry Chaco (December–April) [22] will increase the probability of collecting *Oxyepoecus*.

Oxyepoecus ants appear diversified and well established at Teniente Enciso National Park, where 58 individuals

representing four species were collected during a single sampling session (03–05.XI.2001). This locality may hence constitute a promising reference site to undertake studies concerning these poorly known myrmicinae. Moreover, the presence of *O. inquilinus* at T. Enciso N.P. emphasizes the biological conservation importance of this park.

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Research Article

First Record of *Lenomyrmex inusitatus* (Formicidae: Myrmicinae) in Ecuador and Description of the Queen

T. Delsinne[1] and F. Fernández[2]

[1] *Biological Evaluation Section, Royal Belgian Institute of Natural Sciences, 29 rue Vautier, 1000 Brussels, Belgium*
[2] *Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Apartado 7495, Bogotá D.C., Colombia*

Correspondence should be addressed to T. Delsinne, thibaut.delsinne@sciencesnaturelles.be

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The rarely collected ant *Lenomyrmex inusitatus* Fernández 2001 is recorded for the first time in Ecuador. The queen is described. The new record is the southernmost limit of distribution for the genus. A key to the workers of the six *Lenomyrmex* species and a key for the known queens are provided.

1. Introduction

The myrmicine ant genus *Lenomyrmex* Fernández and Palacio 1999 includes six species rarely collected from Costa Rica to Ecuador [1–3]. The genus is characterised by elongate mandibles bearing a series of minute peg-like denticles that arise behind the masticatory margin, by frontal lobes that are poorly expanded laterally, by large and deep antennal fossae, and by pedunculate petiole, with a poorly defined node [1]. The fact that *Lenomyrmex* possesses both primitive (e.g., promesonotal suture well developed) and derived (e.g., specialized morphology of the mandibles) characters makes ascertaining its correct phylogenetic position challenging [1, 2, 4]. The genus was tentatively placed in its own tribe, Lenomyrmecini [5], but its position within the Myrmicinae remains to be determined [5]. Preliminary results of a phylogenetic analysis (Ant-AToL project, http://www.antweb.org/atol.jsp) indicate that *Lenomyrmex* falls within a clade of predominantly New World ants that includes the tribes Attini, Cephalotini, Dacetini, and the genus *Pheidole* (T. Schultz and P. Ward, comm. pers.).

The worker of *Lenomyrmex inusitatus* Fernández 2001 is distinguished from other *Lenomyrmex* workers by smooth and shiny mesosoma with well-developed propodeal spines and by the foveolate-striate sculpture covering all the dorsal surface of its head [2]. *L. inusitatus* has an unusual distribution since it is the single *Lenomyrmex* species recorded east of the Andes [2]. Nevertheless, it was previously only known from the type locality ("Territorio Kofanes", Nariño, Colombia). Here, the species is recorded for the first time in the Eastern Cordillera of the South-Ecuadorian Andes.

Among *Lenomyrmex* species, the queen caste has been described only for *L. mandibularis* Fernández and Palacio 1999 and *L. wardi* Fernández and Palacio 1999. In this paper, we provide the first record and a description of the queen of *L. inusitatus*.

2. Materials and Methods

The sampling of *Lenomyrmex* in the Ecuadorian Andes is part of a rainfall exclusion experiment [6] and was based on the Winkler extraction method. The leaf litter inside a 0.25 or 0.5-m^2 quadrat was collected and sifted and its fauna was extracted during 48 h. All specimens were collected close to the Podocarpus National Park, within the "Copalinga" property, at 1420 m (Zamora-Chinchipe province, Ecuador). Vegetation corresponds to an evergreen lower montane forest [7]. Mean annual precipitation is about 2100 mm. Mean temperature in the leaf litter from December 2009 to May 2010 was 18.5°C (min–max: 15.7–22.2°C).

(a)

(b)

(c)

Figure 1: Worker (specimen number 4042619) of *Lenomyrmex inusitatus* Fernández 2001: in (a) frontal, (b) lateral, and (c) dorsal views. Note the predominantly smooth and shiny mesosoma, with no erect hairs (b, c) and the foveolate head, with median longitudinal striae (a).

A worker (no. 4042619, from sample no. 40426) and a queen (no. 4042602, from the same sample) have been imaged (Figures 1 and 2, resp.) and are available at http://projects.biodiversity.be/ants.

Measurements and Indices. All measurements are in millimeters. The abbreviations are as follows:

HL: Head length, measured in full face view, from the anterior margin of the medial lobe of the clypeus to the posterior border of the head (excluding the mandibles).

HW: Head width, the maximum width of the head measured in full face view, excluding the compound eyes.

ML: Mandible length, the maximum length of the mandible measured in dorsal view, from the anteriormost portion of the head to the apex of closed mandibles.

EL: Eye length, the maximum diameter of the eye in frontal view.

SL: Scape length, excluding the basal condyle and the neck.

WL: Weber's length, measured diagonally in lateral view from the anterior edge of the pronotum to the posterior edge of the propodeal lobe.

PL: Petiole length, the axial distance from the dorsal corner of the posterior peduncle to the nearest edge of the propodeal lobe.

PW: Petiole width, the maximum transverse distance across the node measured in dorsal view.

PPL: Postpetiole length, the axial distance from the base of the node in front to the tip of the posterior peduncle measured in lateral view.

PPW: Postpetiole width, the maximum transverse distance across the postpetiole in dorsal view.

GL: Gaster length, in lateral view, from the anterior edge of the first tergum to the posterior edge of the last visible tergum.

GW: Gaster width, in dorsal view, the maximum transverse distance across the gaster.

TL: Total length measured in lateral view (ML + HL + WL + PL + PPL + GL).

OI: Ocular index, EL/HW × 100.

CI: Cephalic index, HW/HL × 100.

SI: Scape index, SL/HL × 100.

Queens and workers have been deposited at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium, (RBINS), the Laboratorio de Entomología—Universidad Técnica Particular de Loja, Loja, Ecuador (UTPL), and the Museo de Insectos, Instituto de Ciencias Naturales—Museo de Historia Natural, Universidad Nacional de Colombia, Santafé de Bogotá D.C., Colombia (ICN).

3. Results (Tables 1 and 2)

3.1. Material Examined. A total of 34 workers and two dealated queens of *Lenomyrmex inusitatus* were collected. The worker (Figure 1) corresponds to the description of the holotype [2], except that it is slightly smaller.

Table 1: Key to the workers of the six described *Lenomyrmex* species.

1. Mesosoma predominantly smooth and shiny, with no erect hairs	2
–Mesosoma with conspicuous sculpture and at least a pair of erect hairs	3
2(1). Propodeum without spines; head only foveolate (SW Colombia)	*foveolatus*
–Propodeum with a pair of acute and well-defined spines; head foveolate, with median longitudinal striae (Cordillera Oriental of the Andes in S Colombia and S Ecuador)	*inusitatus*
3(1). Dorsum of head and petiole with longitudinal conspicuous costae; erect hairs of antennal scape as long as or longer than maximum diameter of scape; body ferruginous yellow (W Panama)	*costatus*
–Dorsum of head densely rugo-reticulate; sculpture of the petiole variable, rugulate to rugo-reticulate or longitudinally striate but never costate; erect hairs of antennal scape not longer than maximum diameter of the scape; body brownish black or dark red brown	4
4(3). Length of propodeal spines approximately equal to distance between their bases; mesopleuron with some irregular longitudinal striae, but mostly smooth and shiny; metapleuron with irregular longitudinal striae; HL > 0.80 mm; mesosoma with only two suberect hairs on the pronotum (SW Colombia)	*mandibularis*
–Length of propodeal spines variable, either shorter or longer than distance between their bases; metapleuron and subsequent portion of mesopleuron with fine transverse rugulae or rugo-reticulate, without smooth areas; HL < 0.80 mm; mesosoma with numerous erect to suberect hairs	5
5(4). Propodeal spines shorter than distance between their bases; eyes with six or seven facets in maximum diameter; petiolar node protruding over the peduncle and well defined; postpetiolar dorsum with longitudinal striae (NW Ecuador, SW Colombia)	*wardi*
–Propodeal spines longer than distance between their bases; eyes with about nine facets in maximum diameter; petiolar node undifferentiated from the peduncle; postpetiolar dorsum smooth and polished (Costa Rica)	*colwelli*

Table 2: Key for the known queens of *Lenomyrmex*.

1. Head foveolate, with median longitudinal striae; mesosoma predominantly smooth and shiny, with sparse punctures on pronotum, mesopleuron, metapleuron, and propodeum, scutellum and axillae foveolate, mesoscutum foveolate-striate, no erect hairs	*inusitatus*
–Head densely rugo-reticulate; mesosoma covered by sculpture, mesopleuron, scutellum, and propodeal dorsum with striae, axillae rugo-reticulate, mesoscutum rugulose, erect hairs	2
2(1). Propodeal spines approximately equal in length to distance between their bases; integument predominantly shiny; HL > 0.80	*mandibularis*
–Propodeal spines notably shorter than distance between their bases; integument predominantly opaque; HL < 0.80	*wardi*

Workers. ECUADOR: Zamora-Chinchipe province: Zamora: Bombuscaro: Copalinga property; Lat: −4.083; Long: −78.967; 26.IV-01.V.2010; collected by Delsinne T. and Arias Penna T.; 34 workers in 23 Winkler samples (number of specimens/Winkler sample: 1–4); sample codes: 40343, 40367, 40369, 40374, 40375, 40382, 40387, 40391, 40395, 40417, 40418, 40424, 40426, 40428, 40437, 40439, 40440, 40446, 40449, 40453, 40455, 40457, 40459, 40461; RBINS, UTPL, ICN.

Worker Measurements (no. 4042619). TL 4.23, HL 0.74, HW 0.64, ML 0.41, SL 0.60, EL 0.16, WL 1.15, PL 0.62, PW 0.20, PPL 0.30, PPW 0.24, GL 1.11, GW 0.76, CI 86, OI 24, SI 81.

Queens. ECUADOR: Same data as workers; two queens in two Winkler samples; sample codes: 40426 and 40343; RBINS, UTPL.

Queen Measurements (no. 4042602). TL 4.34, HL 0.75, HW 0.65, ML 0.41, SL 0.59, EL 0.20, WL 1.16, PL 0.64, PW 0.21, PPL 0.27, PPW 0.24, GL 1.11, GW 0.78, CI 86, OI 31, SI 79.

Queen Diagnosis (Figure 2). The queen is similar to the worker [2] but differing in the following characters: anterior margin of clypeus mostly convex, with a slight median notch or concavity; compound eyes bigger, with 11-12 facets in maximum diameter; three ocelli present; mesosoma robust; dorsum of pronotum smooth and shiny, with sparse punctures; mesoscutum foveolate, with longitudinal striae; scutellum and axillae foveolate, with smooth and shiny interspaces; dorsum of propodeum completely smooth and polished; propodeal spines long and stout but shorter than distance between their bases; mesopleuron with anepisternum clearly separated from katepisternum by a suture; lateral face of pronotum, anepisternum, katepisternum, metapleuron, and

(a)

(b)

(c)

Figure 2: Queen (specimen number 4042602) of *Lenomyrmex inusitatus* Fernández 2001: in (a) frontal, (b) lateral, and (c) dorsal views. Note the predominantly smooth and shiny mesosoma, with mesoscutum foveolate-striate and without erect hairs (b, c) and the foveolate head, with median longitudinal striae (a).

lateral face of propodeum mostly smooth and shiny, with some sparse punctures; punctures of lateral and dorsal faces of petiole and postpetiole more defined and deeper than in workers; short and appressed pilosity more abundant on mesosoma than in workers.

4. Discussion

Lenomyrmex inusitatus is, with *L. wardi* and *L. foveolatus*, the third *Lenomyrmex* species collected in Ecuador [1, 8]. To our knowledge, the new record represents only the tenth locality known for the entire genus and constitutes its southernmost limit of distribution. The range of the species and of the genus increases nearly 510 km and 415 km to the South, respectively. Although data remain insufficient to understand the biogeography of *Lenomyrmex*, it is interesting to note that the new record confirms the presence of *L. inusitatus* on the Eastern side of the Cordillera Oriental of the Andes.

Lenomyrmex species were collected from elevations close to sea level to 1800 m but seem to be mainly restricted to mid-elevations, that is, 1100–1500 m ([1–3], this study). The degree of queen-worker dimorphism is weak, suggesting small colony sizes and absence of claustral independent colony foundation [9]. *Lenomyrmex* ants seem always locally rare and it is in fact the first time that up to 34 workers have been collected within a relatively small area (400 m^2). A thorough inspection of the dead wood laying on the ground and of soil samples failed to uncover any nest of *L. inusitatus*. This and the fact that both workers and dealate queens were extracted from the leaf litter (Winkler method) may indicate that this species nests and forages in the leaf litter. The unusual morphology of the mandibles suggests that *Lenomyrmex* is a specialist predator on an unknown prey. This habit is possibly linked to its apparent rarity and restricted elevational distribution. More data are needed to accurately determine the biology and biogeography of these interesting ants.

N.B. After submitting the paper, two additional workers were found within a soil sample, at slightly higher elevation (1500 m), within the "Copalinga" property. The two workers were maintained alive during six days. They moved relatively slowly and feigned death when disturbed. They did not feed on any offered food items (alive and dead termites, millipedes, mites, various insect parts, sugar/water, tuna, biscuits). The information for these specimens are ECUADOR: Zamora-Chinchipe province: Zamora: Bombuscaro: Copalinga property; Lat: −4.082; Long: −78.968; 13.IV.2011; collected by Delsinne T. and Arias Penna T.; two workers in one soil sample (= a thorough visual search for ants for twenty person-minutes from a 15 × 15 × 15-cm core of soil); specimen codes: 4649901 and 4649902; RBINS.

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Research Article

Comparative Immature Morphology of Brazilian Fire Ants (Hymenoptera: Formicidae: *Solenopsis*)

Eduardo Gonçalves Paterson Fox,[1] Daniel Russ Solis,[2] Mônica Lanzoni Rossi,[3] Jacques Hubert Charles Delabie,[4] Rodrigo Fernando de Souza,[2] and Odair Correa Bueno[2]

[1] *Laboratório de Entomologia Médica, Centro de Ciências da Saúde, Federal University of Rio de Janeiro (UFRJ), 21.945-970 Ilha do Fundão, RN, Brazil*
[2] *Centro de Estudos de Insetos Sociais, Instituto de Biociências, Sao Paulo State University (UNESP), 13506-900 Rio Claro, SP, Brazil*
[3] *Laboratório de Histopatologia e Biologia Estrutural de Plantas, Centro de Energia Nuclear na Agricultura, University of São Paulo (USP), 13400-970 Piracicaba, SP, Brazil*
[4] *Laboratório de Mirmecologia, Centro de Pesquisa do Cacau, 45650-000 Itabuna, BA, Brazil*

Correspondence should be addressed to Eduardo Gonçalves Paterson Fox, ofoxofox@gmail.com

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Although common in Brazil, the biology of the fire ant *Solenopsis saevissima* (Smith) is still poorly studied. Larval descriptions are useful to genus-level ant systematics and sometimes to species-level taxonomy. This study presents a detailed description of juveniles of *S. saevissima* from Brazil, which were compared with Brazilian specimens of *Solenopsis invicta* Buren, *Solenopsis geminata* (Fabricius), and *Solenopsis altipunctata* Pitts. Different larval instars were separated by diagnostic morphological traits which were confirmed by observing moults. Reproductive larvae could be easily sorted by their distinctive body dimensions and shape. Contrary to previous reports on this species, the larvae of *S. saevissima* proved to be generally identical to those of *S. invicta*, while a few specimens resembled those of other close species, such as *Solenopsis megergates* Trager. Mature larvae thus presented considerable intraspecific variation in some characters recently proposed to aid fire ant species separation (morphology of head hairs).

1. Introduction

The importance of immature morphology to insect systematics and taxonomy was extensively discussed in previous studies [1–3]. The present investigation is part of a series of studies on ant larvae which attempt to remedy the limitations in the available morphological information on immature ant morphology.

Solenopsis (Hymenoptera: Formicidae) is a cosmopolitan ant genus that includes approximately 300 species, of which over 108 occur in the New World [4]. Some of the largest species are aggressive, polymorphic ants commonly known as "fire ants" that are usually harmful in both their native and invasive geographical ranges. The *Solenopsis saevissima* species group (sensu [5]) includes some 13 species of fire ants which are markedly difficult to identify because of the plasticity of morphological characters, and because of their strong polymorphism. In an attempt to propose a phylogenetic hypothesis for species within the complex, Pitts et al. [5] revisited the morphological characters as originally proposed by Trager [6] and added new ones, including the use of head setae of last-instar larvae. Of the fire ant species analysed in his study, there are published larval descriptions only for *S. invicta* and *S. geminata* [7–9].

The fire ant *Solenopsis saevissima* Smith is widespread and common in Brazil, however still remains a generally poorly studied species. Their larvae are undescribed. Recently, a broad genetic study [10] demonstrated that it comprises a number of cryptic species along the Brazilian coast. This prompted the question of whether or not larvae of geographically distant populations of *S. saevissima* would be morphologically identical. The goal of the present study

was to describe each immature stage of *S. saevissima* obtained from three distant localities, as well as to compare these larvae with specimens of *Solenopsis invicta* Buren, *Solenopsis geminata* (Fabricius), and *Solenopsis altipunctata* Pitts from Brazil.

2. Material and Methods

2.1. Collection of Samples. Whole nests of *S. saevissima* were obtained from three different localities in Brazil: (1) Pouso Alegre, Minas Gerais (MG) (22°13′S, 45°56′W), (2) Pedro do Rio, Rio de Janeiro (RJ) (22°20′S, 43°7′W), and (3) Ilhéus, Bahia (BA) (14°15′S, 39°13′W). In addition, whole nests of *S. geminata* were obtained at site (3); *S. invicta* was obtained from Rio Claro, São Paulo (SP) (22°23′S, 47°32′W) and at site (2); and *S. altipunctata* was obtained at site (2). Species identification was based on Trager [6] and Pitts et al. [5]; only "typical" morphs were used, that is, those clearly presenting the following set of characters. *Solenopsis saevissima*: a poorly developed central clypeal tooth, lack of a medial frontal streak, and absence of a frontal ocellus; *S. geminata*: all mentioned characters plus major workers with characteristically cordate enlarged heads, lacking the central clypeal tooth, and bearing shorter scapes and blunt mandibles; *S. invicta*: well-developed clypeal tooth and carinae, evident medial frontal streak, and absence of a frontal ocellus; *S. altipunctata*: similar to *S. invicta*, but with central clypeal tooth poorly developed, medial frontal streak feeble, and anterior wings of queens with medial cell open.

DNA was extracted and mtDNA sequencing followed the same methods described by Ross et al. [10], confirming species identifications. Species identification was corroborated using chemical characters (venom alkaloids and cuticular hydrocarbons) as described in a separate publication.

Voucher specimens of all immature and adult stages of the collected nests were deposited in the entomological collections of Instituto Biológico and Museu de Zoologia (MZUSP), SP, Brazil.

2.2. Determination of Larval Instars. The first instar and the last instar can be directly identified as hatching larvae and prepupae, and thus be used as reference to determine others. Other distinct instar characteristics used were based on Petralia and Vinson [9]. Larval instar characteristics were further validated during the descriptions from observing moulting larvae.

2.3. Differentiation of Larvae from Different Castes. Worker larvae only differed when mature in bodily dimensions, thus a size interval is given. Gyne and male larvae were considerably larger than worker larvae and presented typical body shapes of their own. These were directly confirmed as they moulted into male or female alate pupae.

2.4. Description of the Immature Forms. All collected samples were fixed in Dietrich's solution (900 mL distilled water, 450 mL 95% ethanol, 150 mL 40% formaldehyde, 30 mL acetic acid) for 24 h, and then preserved in 70% alcohol. Samples to be analysed under the scanning electron microscope were dehydrated in an alcohol graded series (80–100%; a 10-min-dip for each concentration), and critical-point dried (Balzers CPD/030). Dried specimens were then attached to aluminium stubs with double-faced conductive adhesive tape and gold-sputtered with a Balzers SCD/050 sputterer. Observations and images were obtained as soon as possible after sample preparation. Samples to be analysed under the compound microscope were warmed for 15 min in KOH 10% and placed in a small drop of glycerin on a microscope slide.

The morphological descriptions were based on over 10 larvae of each instar. The larvae were analyzed and described under a compound light microscope (Zeiss MC80 DX, with maximum magnification of 1000x), and illustrations were obtained with a scanning electron microscope (LEO 435 VP, at 20.0 kV). With a stereomicroscope (Zeiss Stemi SV11, with maximum magnification of 66x) equipped with a micrometric eyepiece, we obtained measures of every stage. All terminology used herein follows G. C. Wheeler and J. Wheeler [2], and measures are given either as approximate measures, size intervals, or mean ± SD followed by the number (n) of individuals analyzed, depending on sample size. Further specimens were later mounted on glass slides to rapidly check for intraspecific variations.

2.5. Comparison with Other Samples. Last instar larvae of the following species were also rapidly analyzed for intraspecific variations: *S. invicta* from SP and RJ, *S. altipunctata* from RJ, and *S. geminata* from BA.

3. Results

3.1. Identification of Cryptic Species within S. saevissima. Mitochondrial DNA sequences were compared with accessions from the National Center for Biotechnology Information (NCBI/GenBank). Two of the three samples of *S. saevissima* could be amplified—BA and RJ—no sequences were recovered from MG. Their haplotypes were, respectively, W11 and W51, thus belonging to two different cryptic populations (refer to NCBI for further details on the haplotypes), and displayed different sets of cuticular hydrocarbons (not shown). Larvae of the different populations of *S. saevissima* proved identical, and are jointly described below.

3.2. Immature Morphology of Solenopsis saevissima (Smith, 1855)

3.2.1. Egg (Figure 1(a)). Widely ovoid in shape, about 0.18 mm × 0.25 mm, with whitish embryo showing through the transparent chorion. No outer ornamentation or orifices (Figure 1(a)). Eclosion by medial transverse rupture (Figure 1(b)), when first-instar larva outgrows chorion.

3.2.2. First Larval Instar (Figures 1(c)–1(f)). Body profile attoid, defined in [2] as: very short, stout, and curved, with segmentation indistinct; "diameter approximately equal to distance from labium to anus; anus being terminal."

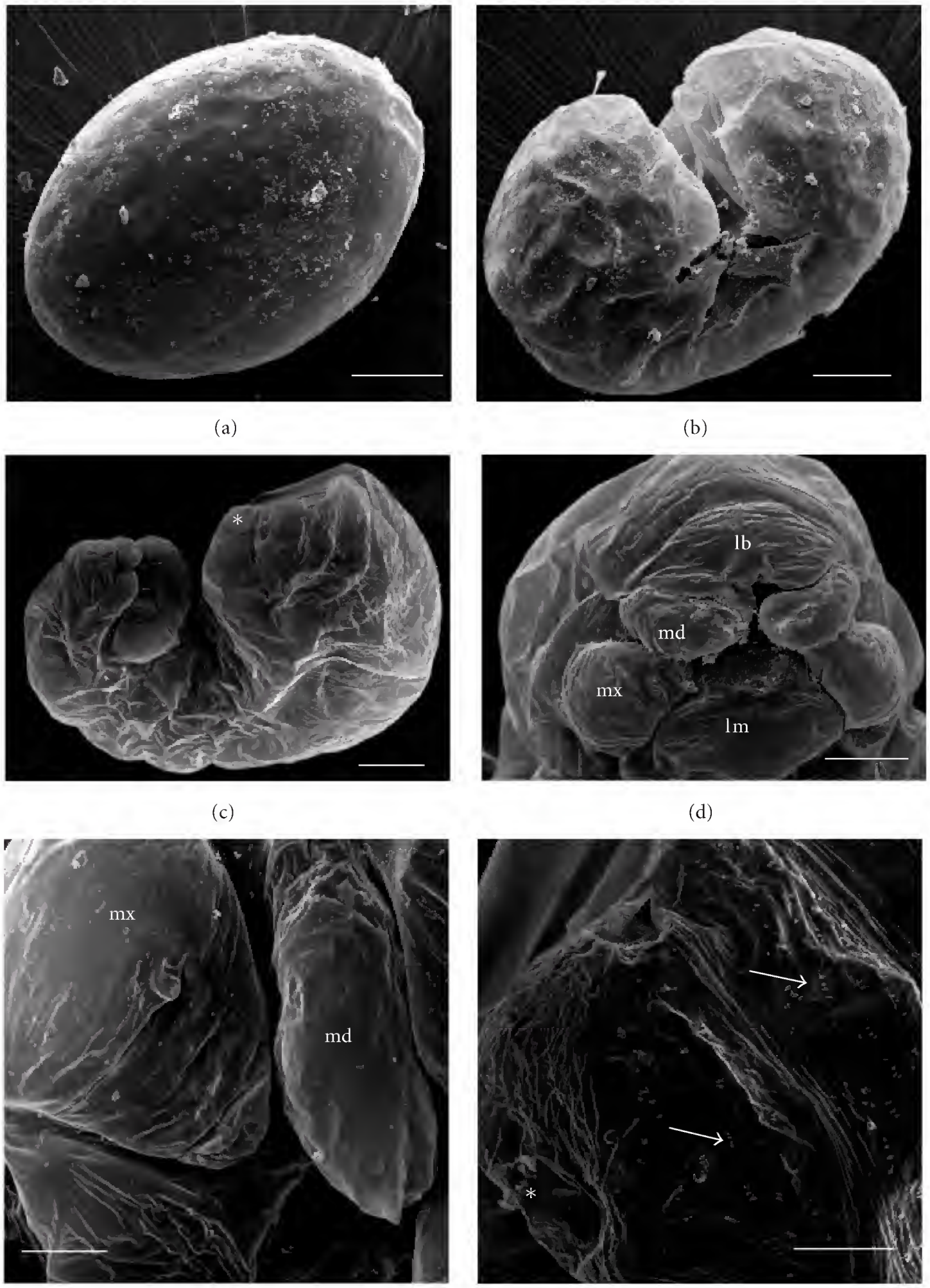
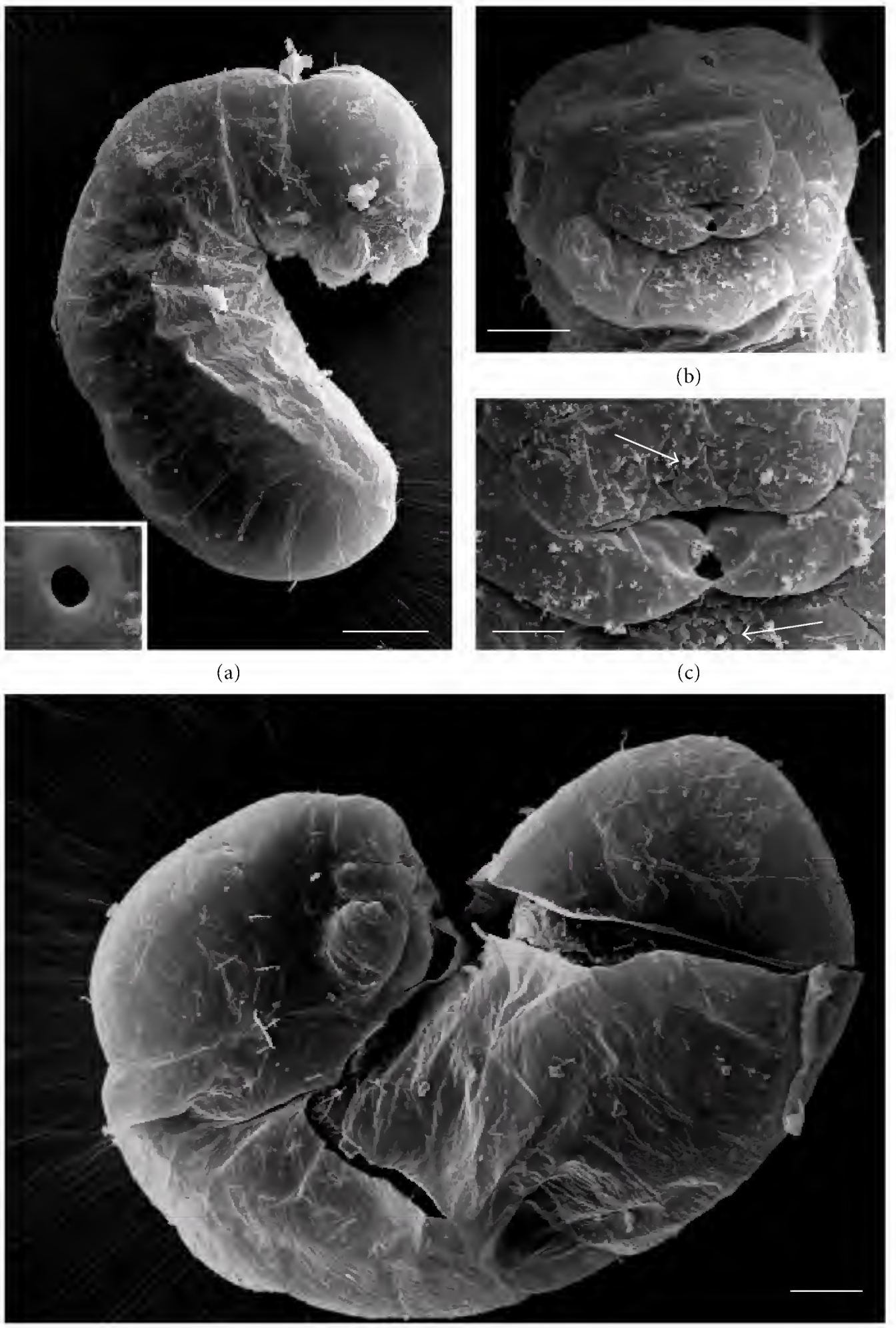


FIGURE 1: Egg and first instar larva of *Solenopsis saevissima*. (a) Egg. (b) Hatching larva. (c) Oblique view of first instar larva; inset: thoracic spiracle. (d) Head capsule and mouthparts; lb: labrum; md: mandible; mx: maxilla; lm: labium. (e) Detail of mouthparts, showing right maxilla and mandible. (f) Terminal region of body, showing anus (asterisk) and rows of spinules (setae). Respective sizes of scale bars (μm): 50, 25, 50, 20, 10, and 15.

Body about 0.290–0.340 mm long × 0.140–0.160 mm wide ($n = 5$); body length through spiracles 0.520 mm ($n = 1$) (Figure 1(c)). Ten inconspicuous pairs of spiracles, first one larger in diameter (0.002 mm) than others (0.001 mm). Integument surface smooth, without setation (Figure 1(c)), however with short spines over posterior abdominal region and around anus (Figure 1(f)). Head capsule subelliptical, 0.120–0.140 mm wide ($n = 5$), without setation or sensilla (Figure 1(d)). Clypeus and labrum fused to a single semicircular structure (0.035 mm wide) (Figure 1(d)); mandibles round and transparent, with two short apical teeth, about 0.025 mm long and 0.018 mm wide ($n = 2$; Figure 1(e)). Maxillae lobose about 0.020 mm long and 0.020 mm wide ($n = 1$); maxillary palps and galea indistinct (Figure 1(e)).



(a) (b) (c) (d)

FIGURE 2: Second instar larva of *Solenopsis saevissima*. (a) Larva on side view; inset: thoracic spiracle. (b) Head capsule. (c) Mouthparts; arrows: spines around mouth entrance. (d) Larva moulting to third instar. Respective sizes of scale bars (μm): 100, 40, 15, and 50.

Labium ovoid, about 0.030 mm wide ($n = 3$) (Figure 1(d)); labial palps indistinguishable.

3.2.3. Second Larval Instar (Figures 2(a)–2(d)). Body profile attoid, greatly curved, with anus terminal; 0.480 ± 0.010 mm long and 0.230 ± 0.010 mm wide at widest somites ($n = 9$); body length through spiracles 0.640 mm ($n = 1$) (Figure 2(a)). Body hairs scarce and always simple, 0.026–0.030 mm long ($n = 47$), concentrated on the dorsal area of the first thoracic somite and over the terminal region of the body (not shown). Ten pairs of unornamented spiracles (inset in Figure 2(a)), first one slightly larger (0.010 mm) than the rest (0.006 mm) ($n = 7$). Head capsule subelliptical, 0.150–0.180 mm wide ($n = 9$; Figure 2(b)). Head hairs distributed as follows: between six and eight over the occipital border, two or three on vertex, and five on each gena. Antennae difficult to spot and bearing three basiconic sensilla (not shown). Mouthparts: Clypeus fused

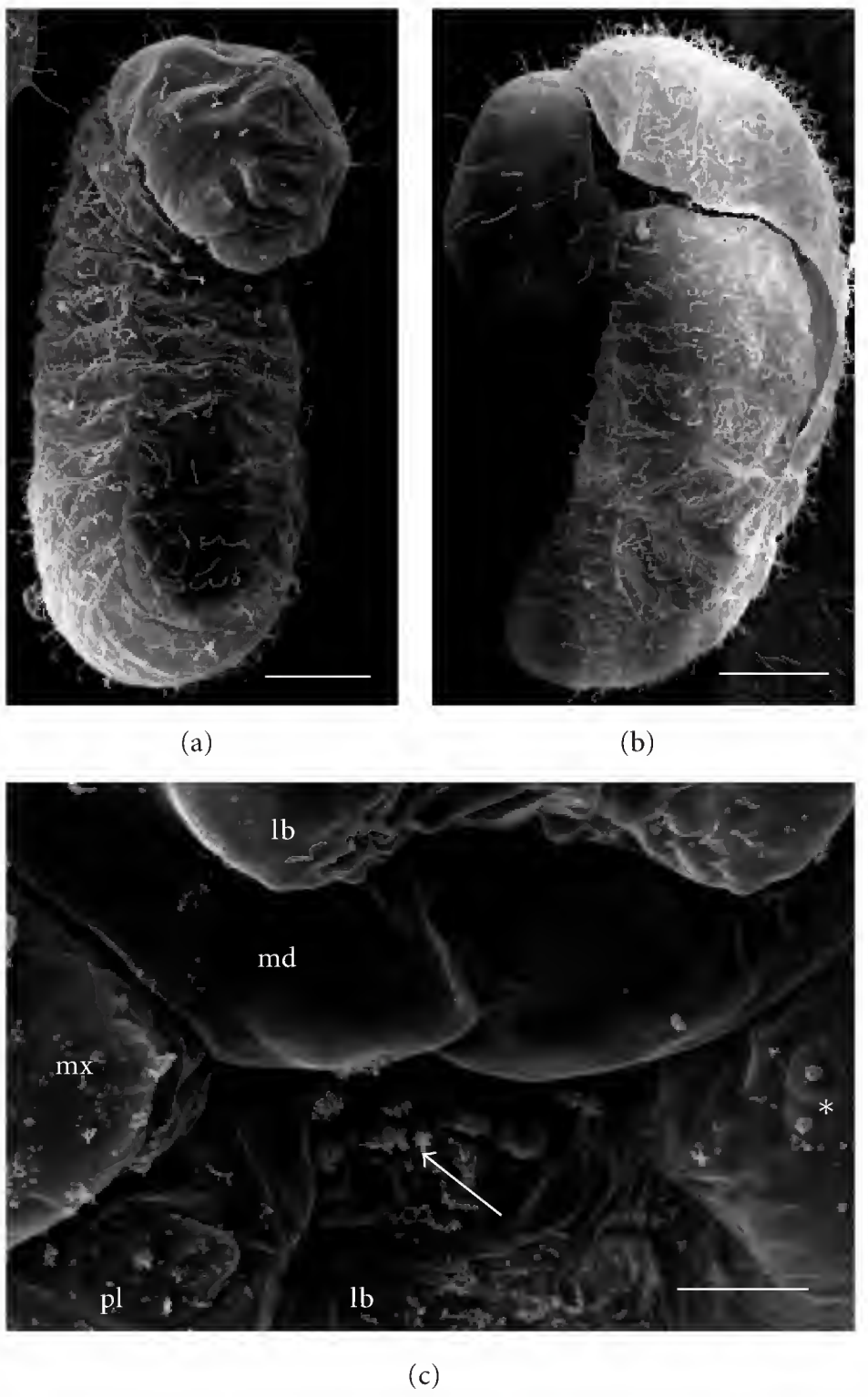


FIGURE 3: Third instar larva of *Solenopsis saevissima*. (a) Larva in frontal view. (b) Larva moulting to fourth instar. (c) Mouthparts; lb: labrum; md: mandible; mx: maxilla; lm: labium; arrow: spiny papillae at mouth entrance; asterisk: galea. Respective sizes of scale bars (μm): 100, 100, and 10.

with labrum into a single short trapezoidal structure about 0.080 mm wide and 0.090 mm long (n = 2), with a row of four simple hairs (Figures 2(b) and 2(d)); spiny papillae on dorsal surface near mouth entrance (Figure 2(c)). Maxillae lobose and 0.049 mm long and 0.050 mm wide (n = 2), with one simple hair at base (not shown). Mandibles unpigmented and roughly camponotus in shape, yet with pronounced apical tooth and one small subapical tooth; about 0.05 mm long and 0.033 mm wide at base (n = 5; Figure 2(c)). Labium a 0.06 mm-wide sphere, no visible palps or spinneret (not shown); densely spinulose near mouth entrance (Figure 2(c)). Moult occurs by apparently random rupture of tegument skin (Figure 2(d)).

3.2.4. Third Larval Instar (Figures 3(a)–3(c)). Body profile roughly dolichoderus, defined in [2] as "short, stout, plump (...) with both ends broadly rounded; anterior end formed by enlarged dorsum of prothorax; head ventral, near anterior end; no neck; somites indistinct." About 1.220 mm ± 0.010 mm long and 0.480 mm ± 0.010 mm wide (n = 172); length through spiracles about 1.300 mm (n = 2) (Figures 3(a) and 3(b)). Body hairs uniformly distributed and of three types: deeply bifid (0.020–0.03 mm long), bifid (0.030 mm long), and simple, with curved hook-like tips (0.010–0.050 mm long) (Figures 3(a) and 3(b)). Simple hairs predominant, except for ventral region of anterior somites ("food basket" area), which is naked and without spinules (not shown). Bifid hairs also found over most of body surface, but predominant on posterior body region. Ten pairs of spiracles, with the first slightly larger (0.100 mm) than the rest (0.070 mm) (n = 26). Head capsule 0.280 ± 0.010 mm wide (n = 172); subelliptical and presenting three types of hairs: simple with tip hooked (0.040 mm long), smooth and simple (0.007 mm long), and bifid (0.015–0.020 mm long) (not shown). Head hairs distributed as follows: six or seven hairs on occipital border, some (up to 1–3) bifurcated in some specimens, five hook-tipped simple hairs, and three or four bifid hairs on the vertex (some specimens had only simple hook-tipped hairs), two or three hook-tipped hairs on frons, five to eight simple hairs on each gena (bifid in some specimens, while one had one 3-branched hair). Antennae slight elevations with three basiconic sensilla (not shown). A conspicuous pair of enclosed sensilla on base of each mandible. Mouthparts (Figure 3(b)): clypeus and labrum fused into a single trapezoidal structure 0.087 mm wide (n = 6), slightly depressed mesad with a row of four simple hairs; four to six setaceous sensilla on anterior face of labrum, and six to seven basiconic sensilla on posterior face of labrum (not shown), the latter densely endowed with spinulose papillae (Figure 3(c)). Maxillae paraboloidal, about 0.050 mm long and 0.037 mm wide, with a hook-tipped hair near the base (some specimens with one additional short simple hair) and two setaceous sensilla; maxillary palpus a simple elevation with four basiconic sensilla, and galea represented by a pair of basiconic sensilla (Figure 3(c)). Mandibles poorly sclerotized, about 0.057 mm long and 0.037 mm wide at base (Figure 3(c)). Labium elliptical, about 0.1 mm wide, with one or two setaceous sensilla on the surface below the opening of the sericteries—not shown, an horizontal slit about 0.040 mm—and a conspicuous cluster of spiny papillae towards mouth entrance (Figure 3(c)).

3.2.5. Fourth Larval Instar of Worker (Figures 4(a)–4(d)). Body profile pheidole, defined in [2] as with "abdomen short, stout, and straight; head ventral near anterior end, mounted on short stout neck, which is the prothorax; ends rounded, one end more so than the other." Larvae varying from 1.350 to 2.850 mm long (n = 77) and 0.580 to 1.30 mm wide (n = 77) (Figure 4(a)). Dimensions of spiracle peritremes and mandibles of larvae of different sizes always about the same (n = 20 specimens of different sizes). All measurements given below were taken from a 3.00-mm-long worker larva, unless stated otherwise. Body length through spiracles 0.630–4.220 mm (n = 7 larvae of different sizes). Body hairs uniformly distributed, of three types: deeply

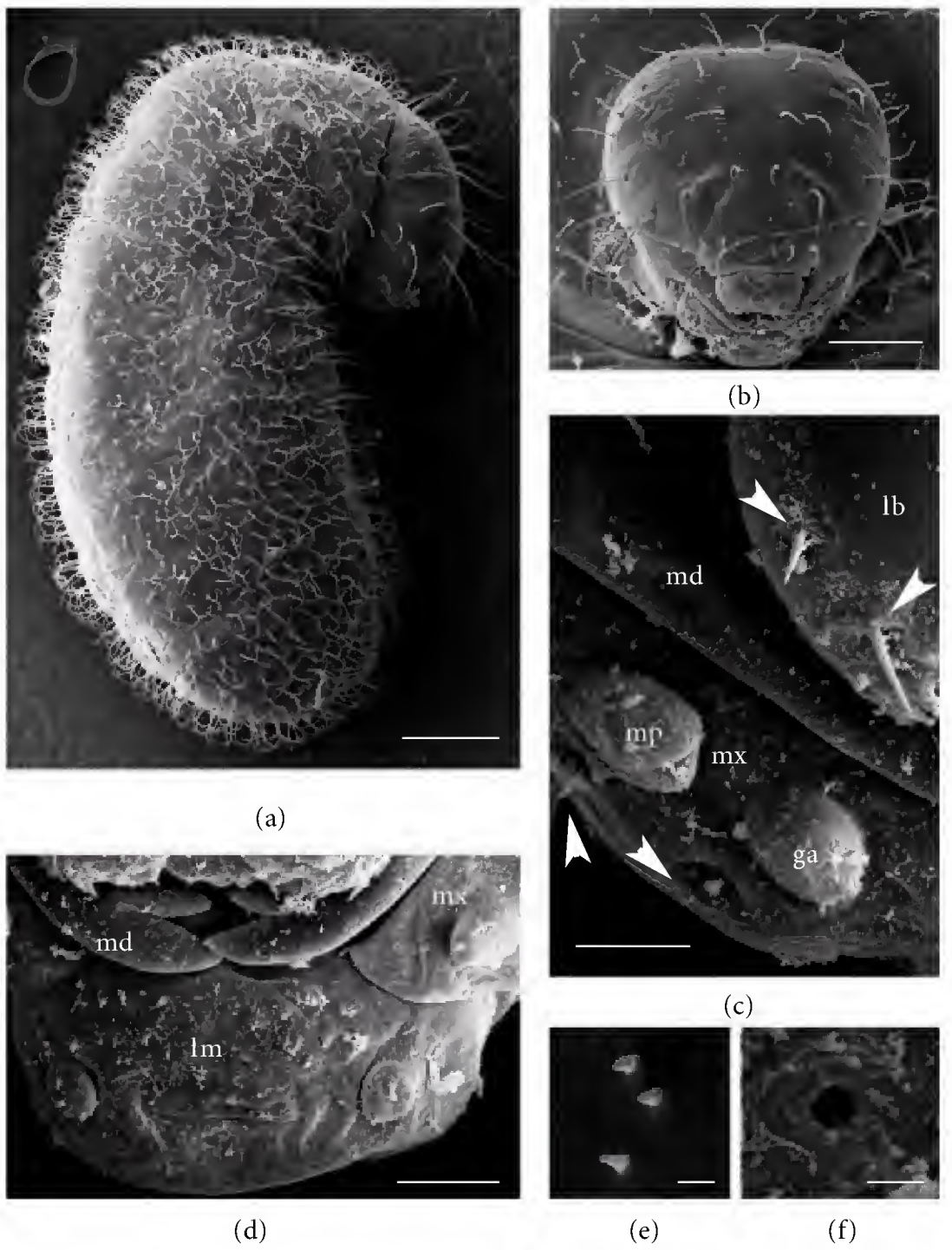


FIGURE 4: Fourth instar larva of *Solenopsis saevissima* worker. (a) Larva in side view. (b) Head capsule in full frontal view. (c) Frontal view of right mouthparts; lb: labrum; mx: maxilla; md: mandible; mp: maxillary palp; ga: galea; arrowheads: setaceous sensilla. (d) Lower mouthparts; md: mandibule; mx: maxilla; lm: labium. (e) Antennal sensilla (setaceous). (f) thoracic spiracle. Respective sizes of scale bars (μm): 200, 100, 10, 15, 5, and 3.

bifid (0.075 mm), bifid (0.700 mm), and simple (0.055 mm). Simple hairs predominate on ventral region of anterior somites, while bifid hairs predominate over the rest of the body (Figure 4(a)). "Food basket" area usually naked and without spines, except in larger specimens (not shown). Ten pairs of unornamented spiracles (Figure 4(f)), the first slightly larger (0.016 mm) than others (0.014 mm), and last pair smallest (0.100 mm). Head capsule 0.370 ± 0.020 mm wide (n = 13); subelliptical and with 20–30 hairs of two types: simple (0.100–0.120 mm) and bifid (0.570 mm), distributed as follows: seven or eight (rarely nine) hairs on occipital border, usually bifid (central hairs sometimes simple, see Figure 8), two or three hairs on each side of vertex (one usually bifid), two to four simple hairs on frons, five to seven simple hairs on each gena (Figure 4(b)). Antennae with three 1 μm-long setaceous (often basiconic) sensilla (Figure 4(e)). A pair of enclosed sensilla near base of mandibles (not shown). Clypeus poorly delimited from cranium and rectangular, with a row of four simple hairs at midheight (Figure 4(b)). Mouthparts: Labrum clearly delimited and roughly rectangular, slightly depressed mesad, 0.100–0.126 mm wide, with six basiconic sensilla and seven to eight setaceous sensilla on anterior face, ventral surface densely covered with rounded, spiny papillae (not shown). Maxilla roughly parabolic in shape, about 0.085 mm long and 0.047 mm wide, with two setaceous sensilla near base of palps (Figure 4(c)). Galea paxiliform and 0.015 mm long, and maxillary palpus digitiform and 0.22 mm long, the first tipped with two setaceous sensilla and the latter with four sensilla, two basiconic, one setaceous, and one enclosed (Figures 4(c) and 4(d)). Mandibles ectatommoid in shape, heavily sclerotized, and stout (0.100 mm long and 0.037 mm wide) with two apical teeth (Figure 4(d)) and two prominent subapical teeth followed by a long blade with two or three molar denticles (not shown). Labium rounded, about 0.8 mm wide; labial palps being simple elevations about 0.012 mm wide with four basiconic sensilla and one setaceous sensillum on top; labial surface below palps with two or three basiconic sensilla and one or two setaceous sensilla at varied positions; labial surface above the palps

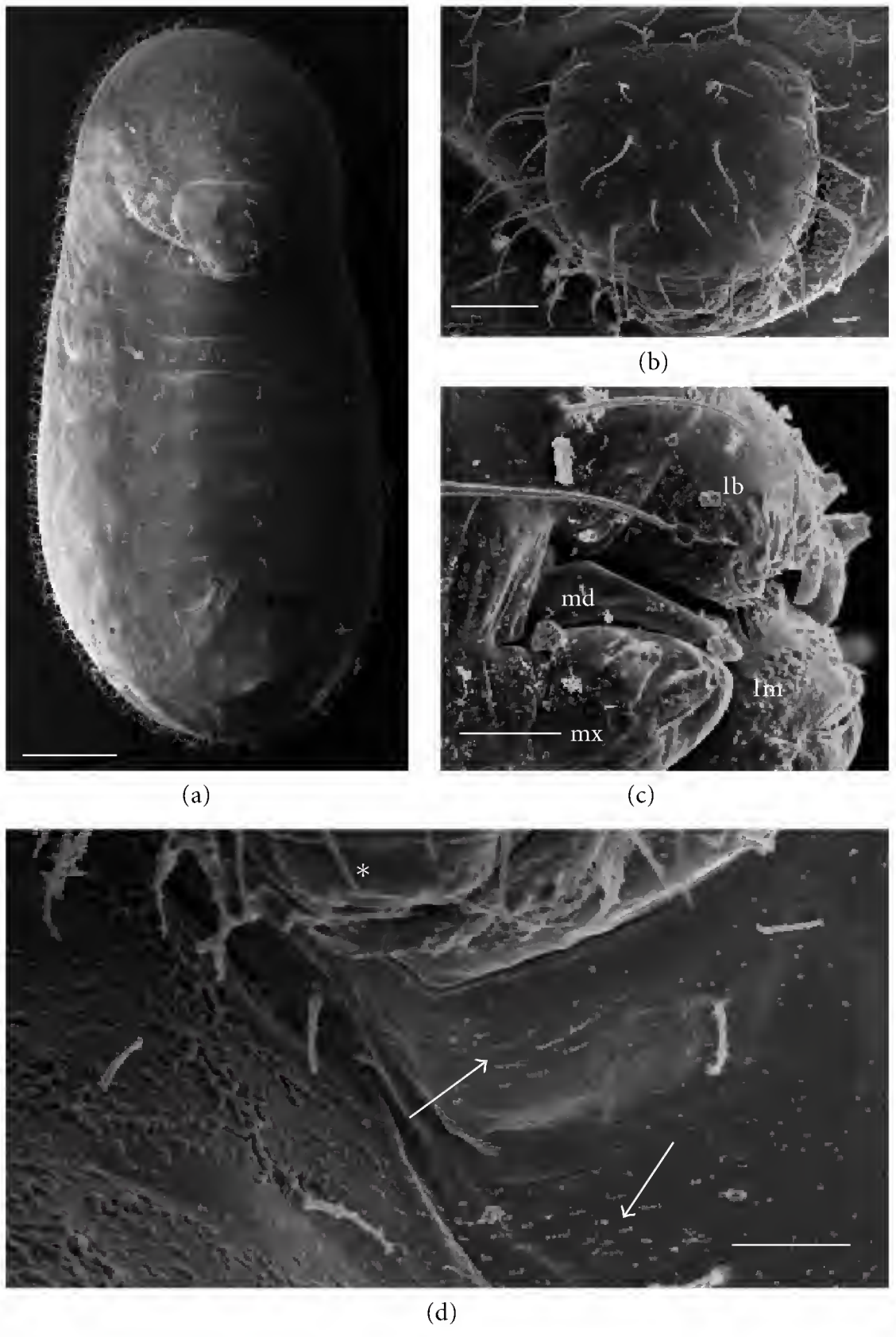


FIGURE 5: Last instar larva of *Solenopsis saevissima* gyne. (a) Body in frontal view. (b) Head capsule in full frontal view. (c) Mouthparts in oblique view; lb: labrum; md: mandibule; mx: maxilla; lm: labium. (d) Food basket area, asterisk: lower mouthparts; arrows: rows of spinules. Respective sizes of scale bars (μm): 400, 100, 50, and 50.

endowed with sparse spines directed to the mouth entrance (Figure 4(d)). Opening of sericteries a horizontal slit about 0.035 mm long with an inconspicuous, enclosed sensillum by the end of each extremity (Figure 4(d)).

3.2.6. Reproductive Larvae (Figures 5 and 6). The reproductive larvae differed from worker larvae only in the last instar by their greater size and unique shape (compare Figures 4(a), 5(a), and 6(a)). Mature larvae (prepupae) of males measured 3.80–4.50 mm ($n = 12$), with greatly engorged thoraxes (Figure 6(a)), and a whitish hue because of the development of a thicker integument (not shown). Mature larvae of gynes are longer (4.80–6.20 mm long) ($n = 7$) and swollen (Figure 5(a)). Also, greater body size results in a decrease in density of body hairs, thus reproductive larvae look distinctly less hairy than worker larvae.

A few morphological particularities were noted, probably deriving from their further increase in size, as described below.

3.2.7. Gyne Larvae (Figures 5(a)–5(d)). Antennal sensillae always setaceous, longer (not shown). Tentorial pits clearly discernible on cranium (Figure 5(b)). Labial surface under mouth entrance and posterior face of labrum densely spinulose (Figure 5(c)). Food basket area with rows of short spines (Figure 5(d)).

3.2.8. Male Larvae (Figures 6(a)–6(f)). First thoracic spiracle much larger than the remaining ones (inset of Figure 6(a)), peritreme opening with valve-like projections. Antennal sensillae always setaceous, longer; well-developed tentorial pits (Figures 6(b) and 6(d)). Maxillary palps slightly longer

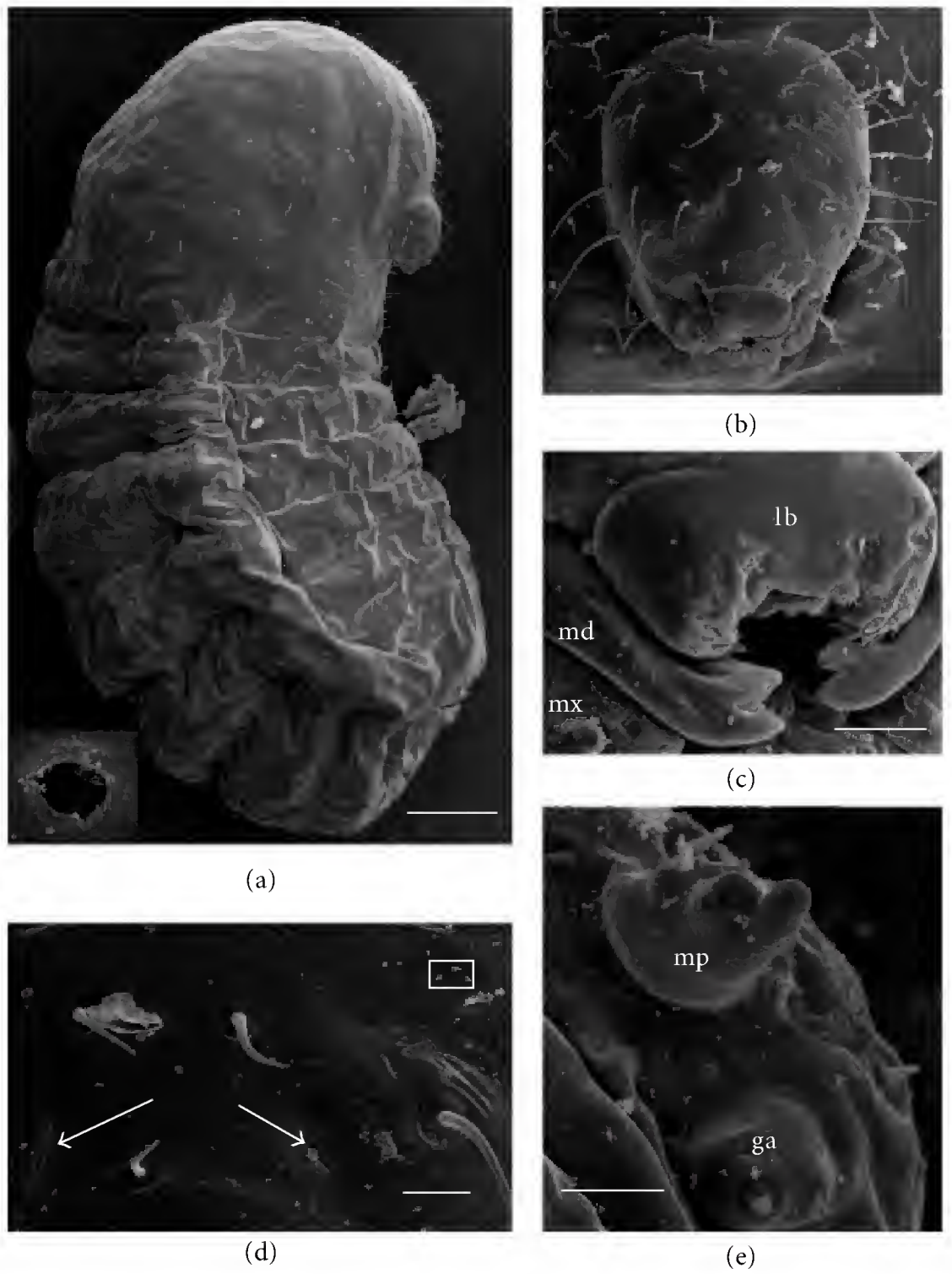


FIGURE 6: Last instar larva of male of *Solenopsis saevissima*. (a) Body in side view. (b) Head capsule in full frontal view. (c) Mouthparts in oblique view; lb: labrum; mx: maxilla; md: mandible. (d) Detail on frons of head capsule; arrows: tentorial pits; white box: left antenna. (e) Detail on right maxilla, showing maxillary palp (mp) and galea (ga). Respective sizes of scale bars (μm): 400, 100, 20, 30, and 10.

and paxilliform, due to the presence of a well-developed, enclosed apical sensillum (Figures 6(c) and 6(e)). Similarly, galea with a distinct shape due to enlarged apical sensilla (Figure 6(e)).

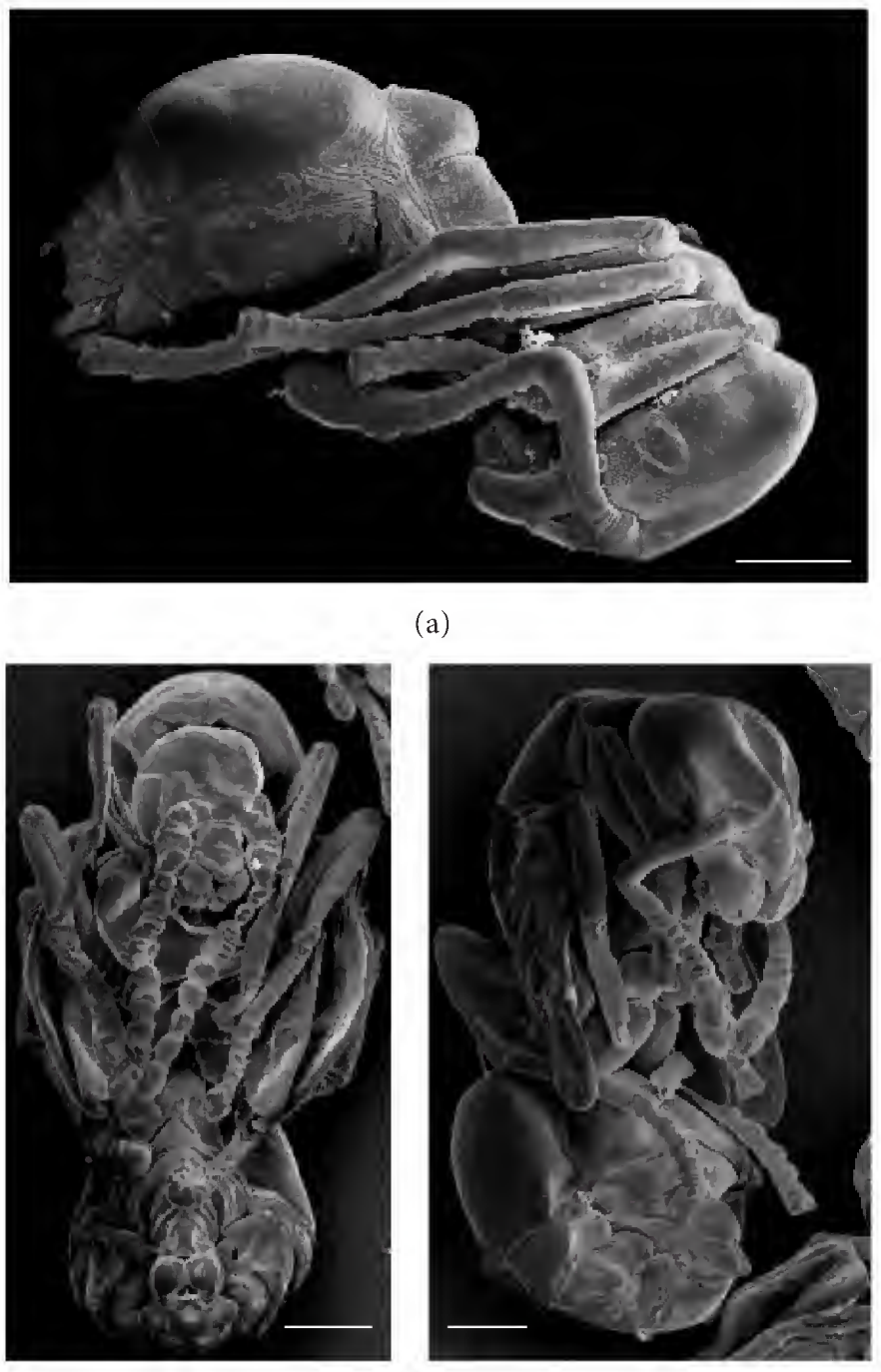
3.2.9. Pupae (Figures 7(a)–7(c)). Young pupae yellowish white, colour darkening with age as they mature into imagoes. Always exarate and without cocoons, yet pupal skin clearly discernible detached from the developing exoskeleton, particularly upon petiole (Figure 7(a)). Worker pupae (Figure 7(a)) varied 2.00–4.00 mm long ($n = 29$), while male pupae (Figure 7(b)) averaged 4.20 mm ($n = 4$), and gyne pupae (Figure 7(c)) measured 5.30–5.50 mm long ($n = 6$).

3.3. Comparisons with Other Species. From comparing numerous last instar larvae of *S. saevissima* with *S. invicta* and *S. altipunctata*, we were unable to pinpoint specific character states that could be used to differentiate between these species—that is, they are identical. All presented marked intraspecific variation in the morphology of head setae (i.e., "hairs" according with the terminology of the Wheelers), in which occipital and even vertexal hairs can be either simple, bifid, or at times 3-branched. Variations occurred among specimens from within the same nests and geographical locations. It is worth noting that a few specimens of *S. saevissima* and *S. invicta* had all head hairs simple, which is reminiscent of other species (read further notes in discussion).

Head hairs of specimens of *S. geminata* proved less variable than in other species, with occipital hairs usually bifid, sometimes 3-branched at random positions. Other characteristics were as described for *S. saevissima*.

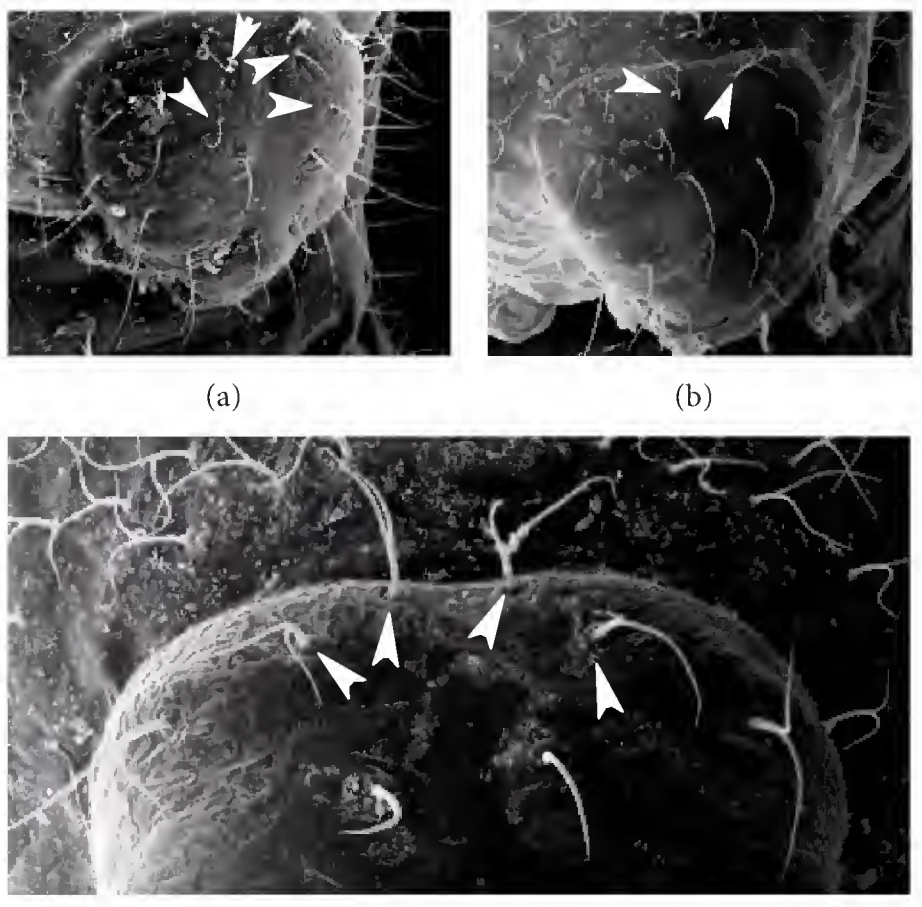
4. Discussion

This is the first description of juvenile stages in *S. saevissima*, and the first larval description of a fire ant to include specimens of different castes and geographical locations.



(a) (b) (c)

FIGURE 7: Pupae of major worker (a), male (b), and gyne (c) of *Solenopsis saevissima*. Respective sizes of scale bars (μm): 300, 500, and 500.



(a) (b) (c)

FIGURE 8: Head hairs (= setae) of fourth instar larvae from different fire ant species. Medial hairs of second row of vertex and occipital region are indicated with arrowheads. (a) *Solenopsis saevissima* with medial hairs simple (compare with Figures 4(b), 5(b), and 6(b), in which specimens had bifid hairs). (b) *Solenopsis invicta* with bifid hairs. (c) *Solenopsis geminata* with bifid hairs. Respective sizes of scale bars (μm): 80, 100, and 30.

Younger first- and second-instar larvae were always found in low frequencies in the collected nests, suggesting that they may last only for a few hours before moulting, or may reside in part of the nest not typically collected. This can only be determined by direct experimentation and observation of the duration of each larval instar.

The extensive similarities between reproductive and worker larvae were previously noted by G. C. Wheeler and J. Wheeler [2]. These authors reported only being able to distinguish reproductive from worker ant larvae using the last instar, as by this stage reproductive larvae are considerably larger. The distinct body shape acquired by the sexual larvae of males and gynes of *S. saevissima* makes sexual separation usually quite easy. It is certainly caused by the inner developing pupa. The greatly enlarged thoracic spiracle of male prepupae is probably related to intense metabolism in that somite (e.g., development of flight muscles?). This and the alterations in the integument of male larvae merit direct investigation.

The larval instars of *S. invicta* were previously described by O'Neil and Markin [8], who also presented descriptions of larvae of all castes, yet a later study by Petralia and Vinson [9] added SEM images of all juvenile stages along with detailed descriptions, correcting several flaws in the original description by O'Neil and Markin [8]. Moreover, O'Neil and Markin [8] claimed that head widths of larvae of different castes were significantly different, but our observations do not support this assertion. In fact, it is clear from our results that Dyar's rule should be applicable for instar separation in fire ants using not only head width, but also mandible length and spiracle diameter, despite the pronounced worker polymorphism. Direct demonstration of this fact is warranted.

In a recent revision of morphological characters and phylogenetic relationships within fire ants, Pitts et al. [5] proposed the use of different sets of character states of the head setae (= hairs) of fourth instar larvae to facilitate species separation. Proposed head hairs to be used were those above the antennal level, individualized in the "first and second rows on vertex" and the "occipital row." The series of larval characters described by Pitts et al. [5] indicates that 4th-instar larvae of *S. invicta* would differ from those of *S. saevissima* and *S. geminata* in the morphology of the medial head hairs of the occipital region: in *S. geminata* and *S. saevissima* such hairs would always be bifid, yet larvae of *S. invicta* would vary in having simple or bifid hairs (for further details, see Pitts et al. [5]). These three species can alternatively be easily recognized based on other traits of major workers. In *S. altipunctata*, mature larvae would have the occipital row with inner hairs always bifid, with others

simple; adult forms are, however, difficult to separate from *S. invicta* and *S. saevissima* without examining the queens.

However, in the present investigation we found considerable variation in the pattern of head hairs of mature larvae of *S. saevissima* from different nests from Rio de Janeiro, Minas Gerais and Bahia. As noted, some specimens even had all medial head hairs above antennal level simple, which should be diagnostic of *Solenopsis richteri* Forel [5]. By relying on the larval characters proposed by Pitts et al. [5], one would have mistaken the specimen of Figure 7(a) for *Solenopsis megergates*, many others for *S. invicta*, and a few for *S. richteri*. Our observations, thus, confirm the larval characters proposed by Pitts et al. [5] for *S. invicta* and *S. geminata*, but demonstrate that larvae of *S. saevissima* cannot be differentiated from other species because of extensive intraspecific (even intranest) variation. Also, similar intraspecific variation was observed with larvae of *S. altipunctata*. Similar marked intraspecific variation was also recently observed in larvae of *Paratrechina longicornis* Latreille [11], thus the phenomenon is most likely universal (among ants?).

As mentioned, subsequent to the study of Pitts et al. [5], Ross et al. [10] demonstrated the existence of cryptic species within *S. saevissima*. It should be noted that there is considerable molecular evidence that some of the other fire ant species might also include cryptic species [12]. It should be stressed that the present study was based on at least two different haplotypes of *S. saevissima* (samples from MG were not sequenced) and considerable intraspecific variation in this character state was detected in all of them, thus the morphology of head hairs is definitely not a reliable character for sorting between fire ant species given the present state of knowledge. We suspect that the samples examined by Pitts et al. [5] included only a few larvae of each species (the exact number of observations was not given), thus leading to biased conclusions.

In summary, the present description adds to the limited body of knowledge about juvenile stages of ants. Some of the observed traits found may have taxonomic importance (best suited for genus-level comparison), and probably reflect specializations in the life history of the group. We would not recommend the use of fire ant larvae for species identification in South America given the present state of knowledge, as one of the most common fire ant species, *S. saevissima*, exhibits considerable intraspecific variation that overlaps with other species, and there is significant evidence that this is also the case in closely related species.

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Research Article

Plant Killing by Mutualistic Ants Increases the Density of Host Species Seedlings in the Dry Forest of Costa Rica

Sabrina Amador-Vargas[1, 2]

[1] *Escuela de Biología, Universidad de Costa Rica, San Pedro, Costa Rica*
[2] *Integrative Biology, University of Texas at Austin, 1 University Station C0930, Austin, TX 78712, USA*

Correspondence should be addressed to Sabrina Amador-Vargas, samadorv@gmail.com

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Some species of plant-mutualistic ants kill the vegetation growing in the vicinities of their host plant, creating an area of bare ground (clearing). The reduced competition in the clearing may facilitate the establishment of host species sprouts (clones and seedlings), which in turn benefits the ants with additional food and shelter ("sprout-establishment hypothesis"). To test this hypothesis, the density and origin of *Acacia collinsii* sprouts growing inside clearings and in the vicinities of acacia plants without clearings were compared. Also, to assess the pruning selectivity of acacia ants (*Pseudomyrmex spinicola*), seedlings were transplanted into clearings. The reaction of ants towards unrewarding acacia seedlings (without food and shelter) was also tested. The density of acacia sprouts growing inside clearings was almost twice that in the vicinities of host plants without clearings, and sprouts were inhabited by nestmates of the colony that made the clearing. Clones and seedlings were found in similar proportions in the clearings, and ants did not kill unrewarding acacia seedlings or seedlings unrelated to their host. The benefit reported here for the ants could be in conflict with the host plant, especially when the plant has rhizomal reproduction.

1. Introduction

In obligatory ant-plant mutualisms, the ants obtain food and shelter from their host, and, in exchange, they defend the plant against herbivores [1, 2]. In addition to attacking insect or vertebrate herbivores, some ants (henceforth, resident ants) also kill plants in the vicinity of their host tree by biting or poisoning [1, 3–10], functioning as allelopathic agents [4]. By killing neighboring plants, the ants leave an area of bare ground around the host plant (henceforth, "clearing"; Figure 1).

Two main functions have been attributed to the clearings: (1) isolating the colony and (2) reducing competition for the host plant. The isolation that the clearings provide to the host tree may also prevent invasions from other ants by reducing or eliminating access venues to the colony that other invasive ants might use to enter the tree. Davidson et al. [11] noted that most ant species that kill nearby vegetation defend themselves by stinging and are unable to defend themselves against ants that are chemically defended (i.e., ants that are stingless and use chemical sprays). Empirical data showed contradictory results: *Triplaris* trees inhabited by *Pseudomyrmex* ants that were artificially connected to neighboring vegetation had more invading ants than isolated ones, but connected trees of *Cordia nodosa* associated with *Allomerus* ants did not have more invasions [11]. Also, *Crematogaster* ants living in nonwaxy species of *Macaranga* trees pruned more intensively than those living on trees with waxy barriers [9], presumably because ants in waxy trees are already isolated from potential intruder ants, which have difficulty walking on waxy surfaces.

Ants can also enhance growth of their host plant and obtain more resources for their colony by pruning nearby vegetation. Any plant growing close to the host acacia is a competitor for light, water, nutrients, or space. Therefore, killing neighboring plants or vines growing on the acacia reduces host plant competition [4]. Janzen suggested this hypothesis based on observations of acacias weakened or dying under the shadow of vines or trees. Besides the observations on *Acacia* by Janzen, it is known that *Tococa* trees inhabited by *Myrmelachista* ants die when the ants do

Figure 1: Clearing made by *Pseudomyrmex spinicola* ants around their host plant *Acacia collinsii* (center, diameter of 4 cm marked by the scale bar), showing three acacia sprouts (white arrows). A small portion of the clearing of a second acacia is also visible (top right corner). Growing outside the clearing is the grass species (*Oplismenus* sp., Poaceae) used as control in the experiments.

not kill the surrounding vegetation, apparently due to light attenuation resulting from overgrowth by neighboring plants [12].

Recently, a third function has been attributed to the clearings. If competition is reduced in the surroundings of the host tree, it may facilitate the establishment of host species sprouts (defined here as seedlings or shoots from rhizomes), a hypothesis called "sprout establishment hypothesis" [13]. Pruning ants can expand their colony to inhabit the recently established neighboring sprouts and benefit from the food and shelter provided by those sprouts growing in the clearing. To date, our knowledge about the seedlings growing inside the clearings is very limited. For several ant species, we know that they kill any plant inside the clearings except for seedlings of the mutualistic species [4, 12, 14], but little is known about the seedling's origin (dispersed seeds versus vegetative growth from the host plant), or how specific are ants in identifying or favoring host seedlings over seedlings of other plants. The ants kill any plant that is in contact with their host tree, apparently either to prevent the growth of encroaching vegetation [3], or to minimize invader entry points [11]. However, we do not know how they react when the contacting plant is an unrewarding seedling (i.e., a seedling that does not provide food or shelter), and whether they evaluate the long-term potential benefits of keeping a seedling that is not producing any reward against the short-term potential threat of having a seedling with leaves or branches in contact with the plant.

In this paper, I address several aspects of the sprout establishment hypothesis in the obligatory mutualism of acacia plants (*Acacia collinsii*) with *Pseudomyrmex spinicola* ants. I tested (1) whether the clearings favored the establishment of acacia sprouts, by comparing the density of sprouts near host plants with or without clearings; (2) whether the sprouts were inhabited by the same colony that made the clearing; (3) whether sprouts in a clearing were occupied by ants more often than sprouts growing near acacias without clearings. To understand whether the pruning behavior was favoring clones of the host acacia over seedlings coming from other plants, I evaluated the proportion of the sprouts established in the clearings that were growing from seeds or from rhizomes of the host plant. Additionally, I experimentally tested whether ants allowed saplings that were not clones or seedlings from their host tree to grow in the clearings. And finally, I evaluated whether ants kill or protect acacia seedlings that were not offering any immediate reward (nectar, protein bodies, or swollen thorns) but were functioning as bridges for potential intruders.

2. Methods and Materials

2.1. Study Site. The investigation was conducted in the dry forest of Palo Verde National Park (10° 21′ N, 85 21′ W) in Guanacaste, Costa Rica. Palo Verde has a mean annual rainfall of 1500 mm and elevation ranging from 0 to 100 m. This area has a well-defined dry season from November to May and a rainy season from June to October. *A. collinsii* plants inhabited by *P. spinicola* occur in secondary growth forest where the lianas and vines are very common. These ants defend the colony against predators or intruders by stinging, and they kill vegetation around their host by biting and cutting the leaves or stems, producing a circular clearing around the host plant (radii between ~30 cm up to 2 m; [3], this paper). In Palo Verde, it is possible to find acacias with clearings and acacias without clearings (normally inhabited by other *Pseudomyrmex* species, by the chemically defended *Crematogaster brevispinosa*, and few trees without ants). *A. collinsii* trees are able to reproduce sexually by seeds, or vegetatively by rhizomes that produce clones of the adult plant [3]. In the study site of the present investigation, the invasion threat for resident ants comes from both arboreal ants, which could displace them from the tree, and army ants that can predate on the brood. The sampling was carried out during the wet season of 2007 and 2008.

2.2. Density of Acacia Sprouts. In June 2007, I searched for solitary *Acacia collinsii* trees with clearings and inhabited by *P. spinicola* ants (Figure 1), and for acacia trees without clearings, to compare the density of acacia sprouts growing in the vicinity of the plant. A plant was considered to be solitary when it was separated by more than 4 m from another conspecific adult. Almost all of these clearings were approximately circular, so the radius was used as an estimate of the clearing's size. For each acacia, radii were measured (±0.5 cm) from the trunk of the acacia (hereafter, central acacia) to the edge of the cleared area in the four cardinal directions, and the mean was used to calculate the area of the corresponding circle. I estimated the size of the acacia ($n = 60$) by measuring the diameter of the acacia (±0.05 cm) at the ground level and counted all the acacia sprouts with swollen spines growing inside the clearing. For acacias without clearings ($n = 48$), I counted all sprouts growing within 1 m of the acacia. I used the estimated area of the clearing to calculate the density of acacia sprouts per square centimeter. Means ± standard deviations are presented. The density of acacia sprouts was compared with nonparametric ANCOVA test using ranks, following the procedure of McSweeney and Porter [15] and Conover and Iman [16],

because the response variable did not meet normality and homoscedasticity. This statistical method takes into account the within-group and total-group regression when adjusting the dependent variable to the covariate [17]. The clearing (presence or absence) was considered a fixed factor, and plant diameter was used as a covariate. The covariate was included because the size of the plant could be correlated with the number of saplings growing into de clearing, for example, older plants could have more sprouts.

2.3. Inhabitants of the Sprouts Growing in the Clearing or Near Acacias without Clearings. To test whether ants on the sprouts were from the same colony that made the clearing, the sprouts from the density measurements were observed for ants. If the sprout had ants, I identified the species and verified whether they belonged to the same colony of ants on the central acacia. Colony identity was deduced by observing whether the ants on the sprout walked in a line back and forth between the central acacia and the sprout. If I did not find ants walking on the ground at that moment, they were encouraged to walk onto a stick from the sprout, allowing them to walk onto the central acacia. I checked whether the resident ants let the intruder walk on the tree or attacked it by biting and stinging (these ants recognize nestmates by chemical cues [18]). As control, I induced ants from the central acacia to walk onto a stick and then reintroduced them to the central acacia, in different place from where I placed the ant coming from the nearby sprout. The frequency of sprouts with nestmates, ants from other species, or unoccupied were compared by chi-square tests separately for plants with clearings or without clearings.

Another prediction is that sprouts in a clearing should be more often occupied by ants, than sprouts growing close to acacias without clearings. The density of sprouts occupied by ants (response variable) and the density of unoccupied sprouts (response variable) were compared by separate ANCOVA tests using ranks, where the fixed factor and covariate were clearing (presence or absence) and the plant diameter, respectively.

2.4. Identity of the Acacia Sprouts Established in a Clearing. To test whether plant killing was favoring host clones over seedlings (i.e., that ants were favoring the vegetative growth of their host plant), I determined the proportion of sprouts corresponding to clones and seedlings, on the clearings of 22 acacias. On each clearing, I dug about 25 cm deep around the main acacia and around the sprouts, looking for rhizomes. When rhizomes were found, they were followed to elucidate connections between the main acacia and the sprouts. Sprouts were classified as "independent" when its main root entered vertically into the ground, and it was not attached to a rhizome. When a horizontal rhizome between the sprout and the central acacia was found, the sprout was classified as a "clone of the main acacia". I classified some sprouts as "having horizontal roots" when the sprout lacked a main root growing vertical into the ground, and it was attached to a horizontal rhizome making an inverted "*T*" or an "*L*" with the stem but was not connected to the central acacia. In each clearing, the main acacia's diameter and the distance (±0.5 cm) to the sprouts around it were measured. After corroborating that the diameter of the plant was not correlated with the proportion of clones (Spearman correlation, $R = -0.20$, $P = 0.37$), a Wilcoxon matched pairs test was used to compare the quantity of sprouts coming from seeds and those that were clones of the acacia. Means and standard deviations are presented.

Because the relation between established seedlings and the main acacia was impossible to determine with the previous observations, I performed field experiments where I placed acacia seedlings of known origin inside clearings and tested the reaction of the ants. I planted seeds of *A. collinsii* in bags with soil, and grew them on partially shaded tables protected from large herbivores (deer, horses, and cows). The *A. collinsii* seeds came from trees that were more than five kilometers away from the experimental trees. In similar bags, I also planted stolons of *Oplismenus* sp. (Poaceae) with 7-8 leaves, a grass native of the study area that is regularly killed by *Pseudomyrmex* ants. When the acacias had six or seven leaves (3-4 weeks after planting), they were introduced into the clearing of 12 solitary acacias (hereafter, experimental trees) that had less than three young acacias growing naturally in the clearing, to determine whether the ants will kill them or allow them to grow. In the clearing of each experimental tree, I placed two bags with *A. collinsii* seedlings at half of the clearings, and two bags with *Oplismenus* stolons as controls. Within each clearing, I placed bags with conspecific plants diametrically opposed from each other. Ants were able to walk on the vertical surface of the plastic bag without impediment. After placing bagged plants, I observed them for 10 days twice a day for 2 minutes, checking for *P. spinicola* ants pruning or chewing the plant. I checked the plants one more time after 20 days from the setup of the experiment. The experimental placement of plants in the clearings allowed me to evaluate the reaction of the ants to acacia seedlings that were not clones or seedlings from their host tree.

2.5. Unrewarding Acacia Seedlings in Contact with the Host Tree. To understand whether the decision of pruning a sprout changed when the acacia sprout also represented a potential bridge for intruders, I placed two plants in bags in contact with the trunk of solitary acacias inhabited by *P. spinicola* ants. One of the plants in the pair was an acacia seedling, and the other was either an *Oplismenus* grass stolon (7-8 developed leaves, $n = 22$) or a *Coursetia caribaea* shrub seedling ($n = 12$). I used the *C. caribaea* (Fabaceae) seedlings (aprox. 15 cm height) because it is a woody species with pinnate leaves like the acacia. Both plant species are common in the study area. Acacia seedlings used in the experiment did not have nectaries, food bodies or swollen spines, and, therefore, did not offer food or shelter to the ants. The diameter of the experimental acacias ranged from 2 to 2.5 cm at ground level. For all the plants, I placed the bag next to the trunk and arranged the leaves such that the second leaf touched the acacia. All colonies were sampled only once, and all bagged plants were used for a single colony. Similar observations to the previous experiment were done for 8 days.

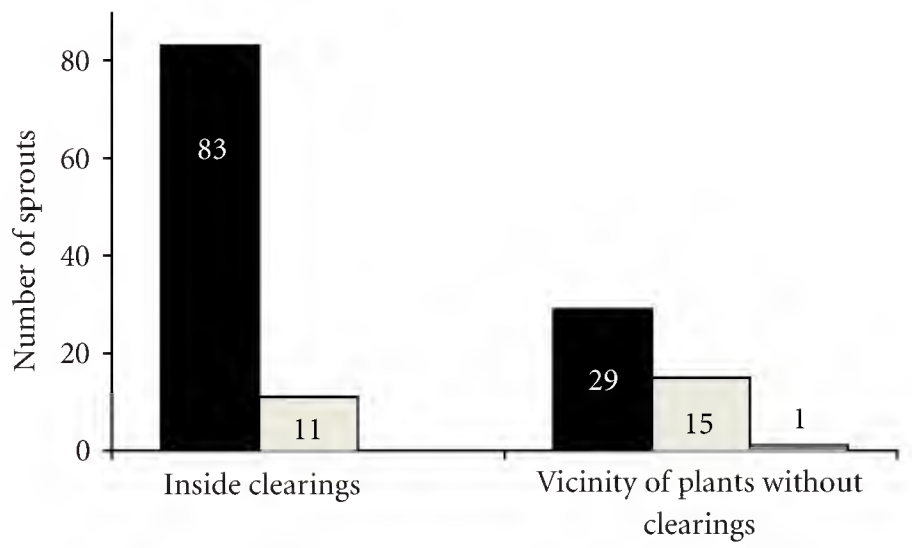

FIGURE 2: Absolute number of sprouts inhabited by workers from the same colony of the central acacia (black), unoccupied (white), or with ants from a different species (gray), for host plants with clearings ($n = 60$) or without them ($n = 48$).

3. Results

3.1. Density of the Sprouts Growing in the Clearing or Near Acacias without Clearings and Their Inhabitants. There was a higher sprout density in clearings (0.71 ± 0.15 sprouts/cm^2, $n = 69$) than in the vicinity of plants without clearings (0.40 ± 0.22 sprouts/cm^2, $n = 35$; nonparametric ANCOVA by ranks $F_{(1, 101)} = 3.73$, $P = 0.05$). When looking at occupied sprouts (i.e., with ants), they were also at higher densities in clearings than close to acacias without clearings (0.46 ± 0.96 sprouts/cm^2 versus 0.26 ± 0.54 sprouts/cm^2, respectively; non parametric ANCOVA by ranks, $F_{(1, 101)} = 4.09$, $P = 0.04$). On the other hand, there were similar numbers of unoccupied sprouts (without ants) per square meter within clearings and near acacias without clearings (0.23 ± 1.29 versus 0.13 ± 0.37 sprouts/cm^2, respectively; ANCOVA by ranks, $F_{(1, 101)} = 0.003$, $P = 0.95$).

The majority (88%) of the A. *collinsii* sprouts growing inside clearings were occupied by ants from the same colony of the experimental acacia (not treated aggressively by the resident colony); the remaining 12% were unoccupied (Figure 2). The sprouts near acacias without clearings showed different proportions of sprouts on each category ($\chi^2 = 11.8$, d.f. $= 2$, $P = 0.003$): 65% were occupied by ants that were not attacked by the resident colony, 33% were unoccupied, and 2% were occupied by another species of ant (*Crematogaster brevispinosa* ants inhabited the main acacia, but the sprouts had *P. spinicola* ants).

3.2. Identity of the Acacia Sprouts Established in a Clearing. Inside the clearings, there was an equal amount of sprouts arising from seeds (4.3 ± 5.3 sprouts per acacia, median = 3) as clones from the acacia (2.9 ± 4.1, median = 1; Wilcoxon paired test, $T = 81$, $P = 0.37$). The results were consistent when the sprouts classified as "having horizontal roots" were incorporated as "clones" in the analysis (Wilcoxon paired test, $T = 98$, $P = 0.54$). It is very likely that these saplings are clones from the acacia, especially when the rhizome they come from was directed towards the central acacia.

None of the acacia seedlings placed in the clearings were pruned or severed, but all *Oplismenus* grasses were pruned. The majority of the colonies started pruning the grass on the same day that I first observed them on the plant, but some did not do so until after 9 days from the day that ants were first seen on the plants. Two grasses remained uncut by the ants during the first 10 observation days. But, by day 20, the ants had completely severed the main stem of all 24 grasses, whereas all 24 acacias were intact.

3.3. Unrewarding Acacia Seedlings in Contact with the Host Tree. All *Oplismenus* and *C. caribaea* plants were pruned, whereas all acacia seedlings were not damaged but were defended by the ants. All experimentally placed acacia seedlings were found by the ants on the same day I set them next to the host acacia, except for one of them that did not have ants until the third day. All these colonies were active pruners, because ants began to prune the grasses and the woody seedlings (*C. caribaea*) on the same day (8 cases) or the day following introduction of these plants (4 cases).

4. Discussion

The sprout establishment hypothesis proposes that by actively clearing vegetation around the acacia, the ants reduce plant competition, allowing the establishment of host species sprouts, which in turn benefits the ants with additional food and shelter [13]. The study findings support this hypothesis. Fonseca [19] found that nesting space was the main factor limiting colony size for *Pseudomyrmex concolor* associated with *Tachigali* trees (Fabaceae), and for seven other ant species that are obligatory mutualists of plants [20]. Food and shelter provided by *A. collinsii* are crucial for survival and colony growth of *P. spinicola* [3] and could also limit the colony growth rate or size. Relatively pure stands of acacia trees are common in the dry forest of Central America [3], and they were probably produced because of mutualistic ants killing other vegetation. In these patches, one colony (i.e., offspring of one queen) could occupy all surrounding shoots, and usually two or three colonies occupy larger patches in areas of more than 3×6 m [21]. Even beyond pure stands of acacia, colonies established on two neighboring plants (one much larger than the other) are frequently found. Therefore, *P. spinicola* ants sometimes expand their nest to more than one tree, suggesting that plant resources are limiting the colony size. Concordantly, I observed a higher density of acacia sprouts inside the clearings when compared with the density without clearings. Additionally, the sprouts were occupied by the same ant colony inhabiting the central acacia. However, the sprout-establishment hypothesis alone cannot fully explain this ant behavior, because the number of occupied sprouts in clearings is similar to the number of seedlings available for colonization in plants without clearings. The competition hypothesis proposed by Janzen [4], and the isolation against potential intruders, may also provide other benefits to the colony.

Selective pruning of foreign seedlings but not acacia seedlings placed inside the clearings also concurs with predictions of the sprout-establishment hypothesis. Food

quantity has been shown to be a limiting factor for colony size of *Crematogaster* ants associated with *Macaranga* species [22], and Keeler [23] also found *Pseudomyrmex flavicornis* ants visiting the nectaries of *Ipomoea carnea* plants growing in contact with their host plantes and they did not sever it. *Myrmelachista* ants also selectively kill vegetation around their host tree, leaving alive saplings of the two plant species they inhabit [8]. Thus, it is likely that ants may allow other plants to grow close to their host as long as they obtain benefits from the intruder plant. For the acacia ants, Janzen [24] argued that ants would prune acacia sprouts only if they were not offering benefits to the ants, or if they were not clones from their host. However, the results from this investigation show that pruning behavior do not necessarily, or exclusively, benefit the ant's host plant, and that workers refrained from cutting acacias that were not sprouts from their host. There could be a potential conflict between the host acacia and its resident ants because the clones and seed of the host tree have to compete for the favorable conditions of the clearing with other acacia seedlings. The benefit for the ants could also be detrimental for the plants, because the aggregations of acacia sprouts may also be more vulnerable to predation (Janzen-Connell model [25, 26]), unless the defense of ants counteracts this effect. A study that follows the survival of acacia seedlings, both inside and outside clearings and solitary versus aggregated sprouts, is necessary to evaluate this potential conflict.

The ants still did not kill or prune saplings that were not providing immediate benefits, such as swollen thorns, nectarines, or food bodies, and these may be used as a bridge to get access to the host tree [11]. Potential intruder ants in the study area include workers of other colonies of *P. spinicola,* other acacia ants (*Pseudomyrmex flavicornis, Pseudomyrmex nigrocinctus,* and *Pseudomyrmex nigropilosus*), other arboreal species (*Pseudomyrmex gracilis* and *Crematogaster brevispinosa*) and predatory army ants (*Eciton burchelli parvispinum* and *Neivamyrmex pilosus mexicanus*). Even though other colonies of *Pseudomyrmex* are not predators, they represent potential threats because they use the same shelters (swollen thorns), and are attracted to both glucose-free nectar and Beltian bodies [27, 28]. Invasion of a colony by other *Pseudomyrmex* colonies is likely to occur via branches because of their arboreal habit, and, therefore, the clearings could deter these ants. In the experiments, ants did not kill acacia sprouts in contact with their plant, maybe because other *Pseudomyrmex* ants did not occupy them; hence the risk of invasions from the sprouts was low. The sprout-establishment hypothesis assumes that ants obtain delayed benefits for their pruning behavior, but this delay may be relatively short. The colony could receive the benefits of favoring acacia sprouts in the clearing very rapidly nectaries and food bodies are produced by the young acacias after 4 or 5 weeks (on their eighth or ninth leaf, Amador-Vargas unpublished data), and more rapidly from clones that develop faster because of the resources they receive from the host plant [3].

More studies that consider the reaction of resident ants to intruder plants of the host's species are necessary to understand the generality of the results found in this research (but see [14]). We still need to understand in the long-term whether clones are more successful than seedlings inside the clearings (because they are growing faster), and how the ants are able to identify and defend seedlings of the host species when they are not offering rewards.

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Research Article

Nest Digging by Leaf-Cutting Ants: Effect of Group Size and Functional Structures

Roberto da Silva Camargo, Ricardo Toshio Fujihara, and Luiz Carlos Forti

Laboratório de Insetos Sociais-Praga, Departamento de Produção Vegetal, Faculdade de Ciências Agronômicas, UNESP, P.O. Box 237, 18603-970 Botucatu, SP, Brazil

Correspondence should be addressed to Roberto da Silva Camargo, camargobotucatu@yahoo.com.br

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Leaf-cutting ant workers dig underground chambers, for housing their symbiotic fungus, interconnected by a vast quantity of tunnels whose function is to permit the entrance of food (leaves), gaseous exchanges, and movement of workers, offspring, and the queen. Digging is a task executed by a group of workers, but little is known about the group effect and group-constructed functional structures. Thus, we analyzed the structures formed by worker groups (5, 10, 20, and 40 individuals) of the leaf-cutting ant, *Atta sexdens rubropilosa*, for 2 days of excavation. The digging arena was the same for the 4 groups, with each group corresponding to a different density. Our results verified a pattern of tunneling by the workers, but no chamber was constructed. The group effect is well known, since the 40-worker group dug significantly more than the groups of 5, 10, and 20. These groups did not differ statistically from each other. Analysis of load/worker verified that workers of the smallest group carried the greatest load. Our paper demonstrates the group effect on the digging of nests, namely, that excavation is proportional to group size, but without emergence of a functional structure such as a chamber.

1. Introduction

Medium-sized leaf-cutting ant workers are responsible for digging new functional structures within the nest including chambers and tunnels [1]. These workers respond well to the stimulus to dig even when isolated or in small groups. During digging the workers must be involved in coordinated activity and respond actively to regions excavated by other ants. After the initial construction process is established, nestmates must be recruited to the location of the activity. This recruitment of nest companions is governed by a positive feedback mechanism by means of communication between the initial excavators and the recruits. The signal utilized for the recruitment is probably stridulatorial communication [2], which runs through the substrate (soil) to recruit nestmates to the point of activity. When the density increases, this signal ceases, as does its function.

Concomitant with the growth of the structure, we would find a negative feedback coordinating the activity, in other words, conducting the workers to finalize the task of digging. The workers may perceive this via chemical cues (pheromones), metabolic products of the workers (CO_2) [3, 4], or by the encounter rate among them [5]. The touches among these workers are a product of momentary density in the area of activity, that is, a monitoring of the task [5].

This hypothesis is based on the allocation of tasks without centralized control, in which the individuals respond to simple local cues (signals). This rate of signals depends on group characteristics such as their size and density [5]. Thus, the functional structures emerge from the activity of the ants, ceasing with the enlargement of the required structures, whether they be tunnels or chambers.

It is known that among leaf-cutting ants, when small groups of workers are formed (2 to 8) for digging, they do not increase the number of tunnels and instead deepen those already excavated, demonstrating social organization and coordination of work [1]. These indications constitute strong evidence of a nest construction system, although this has not yet been demonstrated experimentally.

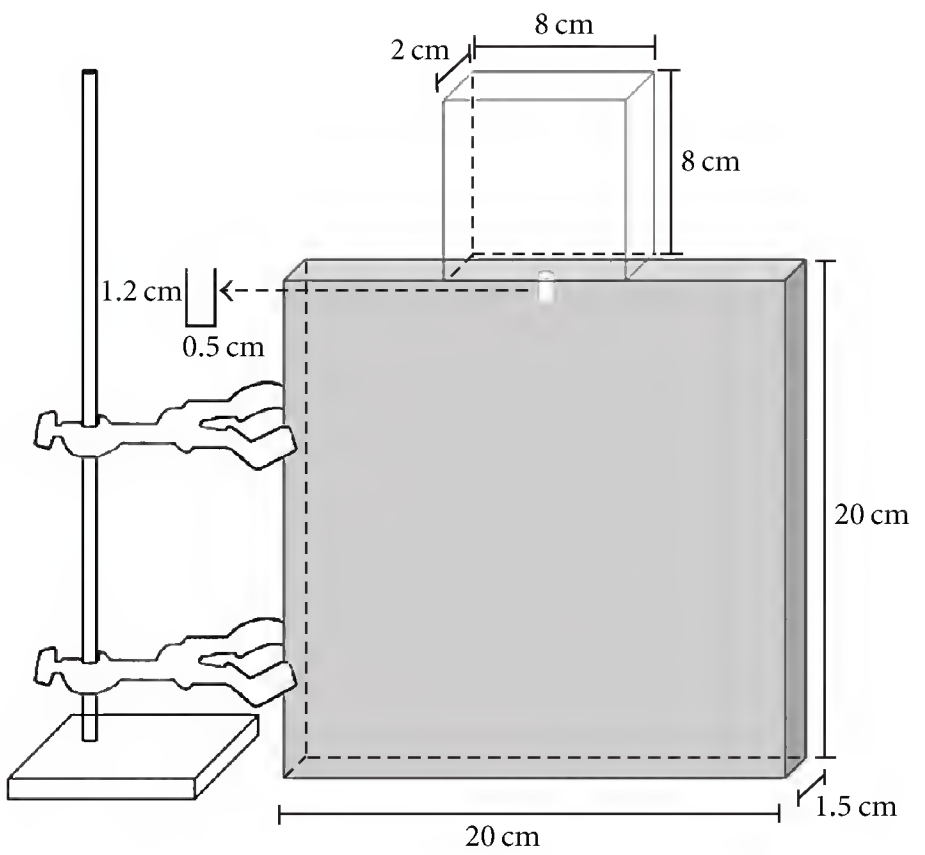

Figure 1: Experimental setup: the workers were allocated into a box with a *pregiven* tunnel to incite digging. The workers continued to dig for 48 hours.

In the present work we analyzed the structures formed by groups of workers (5, 10, 20 and 40 individuals) of the leaf-cutting ant, *Atta sexdens rubropilosa*, for 2 days of digging. The size of the excavation arena was the same for the 4 groups, with each group corresponding to a different density. The excavation arena was disposed vertically, permitting visualization of the digging in a uniform environment. Thus, it was possible to study the group effect on the nest digging by small groups of workers.

2. Material and Methods

2.1. Selection of Workers. Twenty-five workers from the field were collected in adult nests during nest digging. These were isolated, labeled, and transported to the laboratory. The following aspects were measured: head width, body mass, and load mass (soil pellet). This enabled patterning of the size of the ants that effectively do the digging in a natural situation.

In the laboratory, workers were selected according to their size class (head width from 1.2 to 1.6 mm) in midsized colonies (3 years) maintained in the Laboratory of Insect Pests—FCA/UNESP—Botucatu, São Paulo State, Brazil.

2.2. Excavation Boxes. The fifteen glass boxes utilized had dimensions of 20 cm in length and height, and 1.5 cm in thickness. These were filled with soil collected at a depth of 60 cm and sieved (soil density = 1.6 g/cm^3 [6]; water content: 5.4%). A small tunnel, constructed manually (artificially) in this apparatus, was 1.2 cm in length and 0.5 cm in width.

Above this apparatus was placed a small glass box (8 × 8 × 2 cm) into which was transferred a colony for conducting the experiment (Figure 1).

2.3. Rate of Digging and Structures Formed. We analyzed the structures formed by groups of generalist workers (5, 10, 20, and 40 individuals) of the leaf-cutting ant, *Atta sexdens rubropilosa*, for 2 days of digging.

The workers stayed in the digging box for 48 hours, with the excavated soil being removed every 12 hours. The soil volume dug out by the workers in the 12-hour period was collected and dried in an oven for 24 hours at 80°C.

After the 48-hour digging period, liquid plaster was added to obtain the exact dimensions of the architecture of these constructions. After the plaster had dried, these structures and the soil were removed and carefully analyzed.

The structures were analyzed by measuring the area excavated by the workers on two planes; the structures were photographed and their area calculated in mm^2 via the program Image J from the National Institutes of Health, USA., http://rsb.info.nih.gov/ij/. These measures were correlated with the number of workers involved in the excavation, thereby providing a ratio between the number of structures formed and the number of workers.

The volume of these structures was calculated by their weight, with the volume in g, converted to mL.

2.4. Statistical Analyses. A linear regression was applied to correlate the mass of workers with their load in the field, to enable selection of the size class appropriate for digging. The head widths of field workers were compared with those of laboratory workers by the test of Mann-Whitney ($\alpha = 0.05$). The data for the area and volume of excavated soil were submitted to analysis of variance (ANOVA; $\alpha = 0.05$), with subsequent paired comparison by the methods of Student-Newman-Keuls and Dunn.

3. Results

3.1. Selection of Workers. The workers from the field presented a linear relationship between their body mass (0.004 ± 0.002 g) and the mass of soil pellets (0.017 ± 0.01 g) deposited outside the nest ("best-fit" linear equation, $x = 0.00125 + (0.173 * y)$, $R = 0.849$, ($t = 7.702$, $P < 0.001$)) (Figure 2). In their pellets these workers carry on average 4-times their body mass (ratio = soil pellet mass/worker mass, mean = 4.05; standard deviation = 1.66, $N = 25$). These pellets are transported frontally to the exterior of the nest by the mandibles, without projecting all the load mass behind the body, as they do when collecting vegetal matter. This must be the manner by which they fabricate such pellets. The pellets are formed by the behavioral act of "biting" the soil matrix with the mandibles; in other words, the workers remove the soil fragments and aggregate them with the aid of their metathoracic legs. After the soil pellet is formed, it is taken to the exterior of the nest, but is carried frontally, between the mandibles. In unpublished data on leaf-cutting ant queens, we observed that when one of the tibiae of the metathoracic legs is mutilated or cut, these queens become slower and dig more slowly than normal ones. This is due to an inefficiency in aggregating soil to form the soil pellets.

Table 1: Digging rate (g), area excavated (mm^2), and volume of soil excavated (cm^3) by groups of workers (5, 10, 20, and 40), as a function of elapsed time.

Group size	Volume of soil (g)					Load/worker ratio	Trips	Area	Volume
	12	24	36	48	total				
5	3.0	1.5	0.0	0.0	4.5	0.90	53	828.5 ± 436	7.36 ± 4.1
10	4.5	1.1	0.0	0.0	5.6	0.56	33	1066.0 ± 464.64	7.37 ± 3.5
20	9.8	0.3	0.0	0.0	10.1	0.50	30	1176.5 ± 461.01	11.40 ± 4.5
40	21.6	1.7	1.1	2.4	26.9	0.67	40	4530.4 ± 1863.67	36.33 ± 12.7

* Load/worker ratio = total soil volume/number of workers in the group; **trips = (load/worker ratio)/0.017 (average mass of soil pellets).

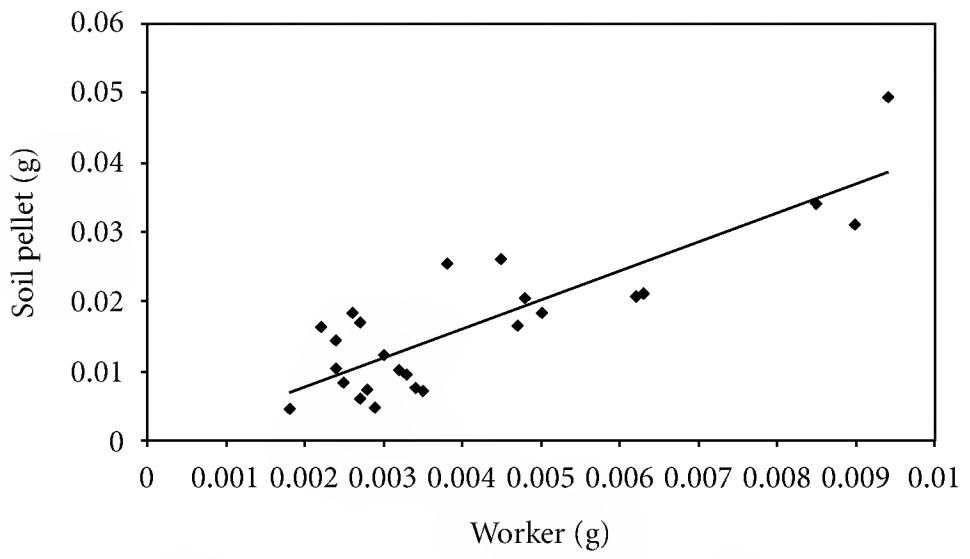

Figure 2: Ratio between mass of workers and their soil load in a natural situation. Linear regression was applied with the "best-fit" linear equation ($x = 0.00125 + (0.173 * y)$, $R = 0.849$, ($t = 7.702$, d.f. = 24, $P < 0.001$)).

In addition to mass, the head width was measured in both field and laboratory workers. The field workers presented a head width of 1.5 ± 0.3 mm while those of the laboratory did not differ significantly at 1.4 ± 0.5 mm ($U = 568.500$, $P = 0.174$). Thus, it may be stated that workers from the field and laboratory were similar, belonging to the same class size, which we can denominate the generalists ([7], on head width from 1.2 to 1.6 mm).

3.2. Digging Rate and Structures Formed. The highest digging rate occurred in the first 12 hours after the release of the workers, and decreased over time (Table 1). There was a significant difference among the times (Kruskal-Wallis test, $H = 10.727$, d.f. = 3, $P < 0.05$) while the posttest (Student-Newman-Keuls) determined that the volume of soil excavated in 12 hours differed significantly from the other evaluation periods.

We calculated that each worker transported from 0.50 to 0.90 g of soil throughout the experiment, within each group. The trips were calculated according to the average volume of soil that each worker transported per trip (0.017 g), resulting in 30 to 53 trips during the experiment (Table 1).

In relation to the structures formed by groups, we verified a specific tunneling pattern by the workers (Figure 3). The area excavated by the groups presented a significant difference (Kruskal-Wallis test, $H = 34.697$, d.f. = 3, $P < 0.001$) while the posttest (Student-Newman-Keuls) determined that the 40-worker group dug significantly more than the groups of 5, 10, and 20. These groups did not differ significantly from each other.

The volume of structures created by the workers, determined by the plaster molds, differed significantly among the groups (Kruskal-Wallis test, $H = 36.359$, d.f. = 3, $P < 0.001$), while the posttest (Dunn) showed that the 40-worker group dug significantly more than the 5-, 10-, and 20-worker groups. There was no significant difference among these groups.

4. Discussion

The results obtained by the present study verified that the workers dug tunnels, but no chamber to house them (Figure 3). The emergence of a functional structure, such as a chamber, is only possible in leaf-cutting ants when the worker group is larger and symbiotic fungus is present as a stimulus for its construction. Fröhle and Roces [8] verified the emergence of functional structures, including chambers, in larger groups (750, 1500, 2500 workers), with diverse volumes of fungus. These workers were apt to enlarge a fungus chamber to house a greater fungus volume, independent of the number of ants.

A higher digging rate was observed at the beginning of the experiment, followed by a reduction as time elapsed (Table 1), as already observed in other species [4, 8, 9]. According to Buhl et al. [9], this decrease in activity at the end of the digging dynamic can be explained by two possible mechanisms: the first corresponds to a reduction in activity by means of a perception of specific signals from individuals such as the concentration of CO_2 [10] or the rate of encounters among ants [11]. The second relies on the propagation properties of the recruitment, leading to a stopping activity according to the determinate density of ants [12].

The volume of excavated soil varies directly with the number of ants in the group (Table 1); however, almost all of the ants were observed to dig. Nevertheless, it is known that in small groups the workers dig more than those in larger groups, as explained by Fröhle and Roces [8]. This is due to the population density; in other words, the smaller the group, the more possibilities the workers will have to dig and all are recruited with opportunities to execute their task.

In summary, our study demonstrates the group effect on the digging of nests, namely, that digging is proportional to group size, but without emergence of a functional structure such as a chamber.

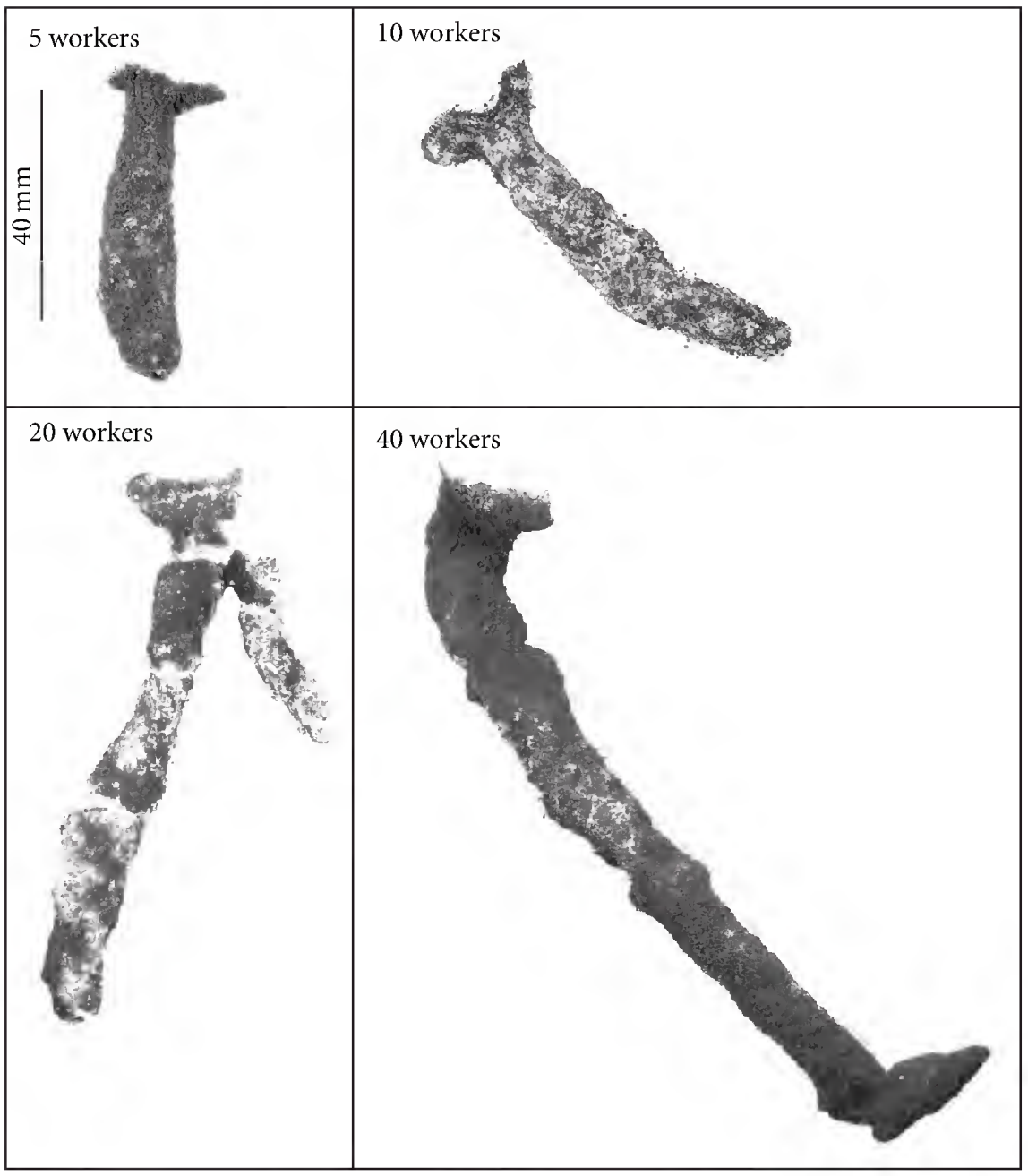

FIGURE 3: Structures produced by groups of *Atta sexdens rubropilosa* workers, in groups of 5, 10, 20, and 40, throughout 48 hours.

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Research Article

A Predator's Perspective of the Accuracy of Ant Mimicry in Spiders

Ximena J. Nelson

School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8041, New Zealand

Correspondence should be addressed to Ximena J. Nelson, ximena.nelson@canterbury.ac.nz

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Among spiders, resemblance of ants (myrmecomorphy) usually involves the Batesian mimicry, in which the spider coopts the morphological and behavioural characteristics of ants to deceive ant-averse predators. Nevertheless, the degree of resemblance between mimics and ants varies considerably. I used *Portia fimbriata,* a jumping spider (Salticidae) with exceptional eyesight that specialises on preying on salticids, to test predator perception of the accuracy of ant mimicry. *Portia fimbriata*'s response to ants (*Oecophylla smaragdina*), accurate ant-like salticids (*Synageles occidentalis*), and inaccurate ant-like salticids (females of *Myrmarachne bakeri* and sexually dimorphic males of *M. bakeri*, which have enlarged chelicerae) was assessed. *Portia fimbriata* exhibited graded aversion in accordance with the accuracy of resemblance to ants (*O. smaragdina* > *S. occidentalis* > female *M. bakeri* > male *M. bakeri*). These results support the hypothesis that ant resemblance confers protection from visual predators, but to varying degrees depending on signal accuracy.

1. Introduction

Predator avoidance of dangerous prey is often exploited by deceptive prey species; the Batesian mimics are those that deceitfully advertise to potential predators that they also can induce the negative repercussions associated with this prey [1, 2], which often use warning (aposematic) signals to indicate their defences to would-be predators. The Batesian mimicry works solely to the advantage of the sender of the counterfeit signal, as both the receiver and the model are exploited. The receiver is cheated out of a source of food, and the model is less likely to benefit from its cues. The negative effect on models is due to frequency-dependent selection: if mimics exist in large numbers, the predators may take longer to learn an aversion or the potential for evolving innate fear of dangerous prey is lessened. Although studies of the Batesian mimicry have usually emphasised learning as a mechanism for the evolution of mimicry (e.g., [3]), both innate and learned fear of dangerous or distasteful prey can favour the evolution of the Batesian mimicry, as is clear from studies using naïve jumping spiders (Salticidae) as potential predators (e.g., [4]).

While we traditionally think of dangerous prey as one using bright, contrasting colours as aposematic signals, as in the case of poison dart frogs [3], not all dangerous species that are mimicked use aposematic signals. Correspondingly, deceitful use of aposematic signals appears to be an evolutionary strategy used by some Batesian mimics, but not others. Many spiders are the Batesian mimics of ants [5], animals which do not intuitively fit into the category of aposematic. Having a slender body, narrow waist, and an erratic style of locomotion, ants have a distinctive appearance, but this is unlikely to have evolved as an antipredator defence signal. Ants are, nevertheless, potentially harmful to predators through their ability to bite, sting, or spray formic acid. Being social, ants are all the more dangerous because they can mount communal attacks on potential predators [6]. Predators often respond to ant-like appearance as a cue for avoidance [4], and to disqualify ant mimicry as examples of the Batesian mimicry on the basis of hypotheses about the evolutionary origin of the ant's appearance places undue emphasis on a distinction that is irrelevant to the predator. In fact, ants appear to be particularly suitable as models for mimicry, especially among spiders. Illustrating how

predation plays an important role in evolutionary diversification, ant mimicry (myrmecomorphy) has evolved in at least 43 spider genera within 13 families [5].

The 300 or so species of described myrmecomorphic spiders are typically characterised by a thin, elongated body, the creation of an antennal "illusion" by waving the forelegs, and an erratic style of locomotion [5, 7–9]. The vast majority of these species are Batesian mimics that are avoided by ant-averse arthropod predators [9–14], although the response of vertebrates is largely unknown. A few rough numbers may best express the efficacy of this deceptive signal. With over 5,300 described species, the Salticidae is the largest family of spiders [15]. The most speciose genus within the Salticidae, *Myrmarachne,* has over 200 described species—all of them ant mimics.

Theoretically the Batesian mimics are under selective pressure to closely resemble their models while the models are under pressure to distance themselves from the deceitful signalling of the mimics, so there should be an arms race in which mimics are expected to converge upon their models (e.g., [16]). Yet polymorphism can also be maintained in populations of the Batesian mimics [17], particularly when more than one model species is available [3]. It is especially noticeable that several species of ant mimics are polymorphic [18, 19]. As judged by humans, there is also considerable range in the accuracy of ant mimicry, with some being imprecise mimics, while others are remarkably similar in appearance to their model. Additionally, species in the large salticid genus *Myrmarachne* are sexually dimorphic as adults [20], with males seeming to be rather poor mimics due to their greatly enlarged chelicerae. Nevertheless, previous findings have suggested that males actually resemble ants carrying something in their mandibles [21]. In other words, they appear to be the Batesian mimics of a compound model (an ant plus the object it is carrying).

The exceptionally acute visual ability of salticids [22] enables them to identify motionless lures made from dead prey [23] and also enables them to escape some interactions with predators [11], such as ants. Although *Myrmarachne* can distinguish conspecifics and other mimics from ants [24–26], current evidence suggests that non-ant-like salticids are unable to make this distinction [4, 21]. The question of interest in this study is whether accuracy of ant mimicry, as judged by humans, is reflected in predator behaviour. The answer is of significance because most salticids will readily prey on each other [27], yet most salticids also appear to avoid ants [4], encounters with which are often lethal to salticids, including *Myrmarachne* [28, 29]. Clearly, it is also pertinent to determine how nonhuman animals classify objects and to determine the differences (or not) that may be found according to very different visual systems.

Here I tested *Portia fimbriata,* an Australian spider-eating (araneophagic) salticid that specialises on capturing other salticids as prey [30], with Asian weaver ants (*Oecophylla smaragdina*). I then compared whether their response toward ant-like salticids was similar to that elicited by *O. smaragdina* by testing *P. fimbriata* with males and females of *Myrmarachne bakeri* from the Philippines. This species is an imprecise ant mimic [19], and males are expected to be less precise than females due to their enlarged chelicerae. Finally, I tested *P. fimbriata* with an unrelated, but accurate, ant-like salticid from North America, *Synageles occidentalis.* In this study I address two specific questions: (1) does the non-ant-like salticid *P. fimbriata* avoid ants? (2) does *P. fimbriata* avoid or stalk ant-like salticids, and does this predators' behaviour differ depending on the accuracy of the mimic?

2. Materials and Methods

I collected *Myrmarachne bakeri* and *Oecophylla smaragdina* in the Philippines and conducted laboratory work at the University of Canterbury (Christchurch, New Zealand), where cultures of Australian *Portia fimbriata* and North American *Synageles occidentalis* were available. Sexually mature female *Portia fimbriata* (body length 8–10 mm) were tested with one of each of a variety of lures of four different types ($N = 15$ for each type), and the distance to which *P. fimbriata* approached lures was measured. Lures were made from dead ants (major workers of *O. smaragdina,* 8 mm in body length) and ant mimics (male and female *M. bakeri,* 8 and 6 mm in body length, respectively, and female *S. occidentalis,* 3.5 mm in body length). While *M. assimilis* is the accurate mimic of *O. smaragdina* [4], there were no longer any individuals of this species in the laboratory in New Zealand when this study was done. As we were unable to procure any more, tests were carried out using another excellent mimic, *S. occidentalis,* instead. No test spiders had any previous experience with ants or with ant mimics.

Spiders were maintained in individual plastic cages, cleaned weekly, with a cotton roll through the bottom that dangled in a small cup of water to provide humidity. Spiders were fed twice a week with house flies (*Musca domestica*). Testing was done between 0800 h and 1700 h (laboratory photoperiod 12L : 12D, lights on at 0800 h). A 200 W incandescent lamp, positioned *ca.* 600 mm overhead, lit the apparatus; fluorescent lamps provided additional, ambient lighting. Using standard protocol for experiments on predatory behaviour, spiders were fasted between 4 to 7 days prior to testing. No individual spider was tested more than once with a given type of lure.

The testing apparatus was a wooden ramp (see Figure 1 for dimensions) raised at a 20° angle, which was supported by a wooden pole, glued to a wooden base. The entire apparatus was painted with two coats of polyurethane and was wiped with 80% ethanol and allowed to dry for 30 min between each test to eliminate possible chemical traces from salticids in previous tests. The ramp was marked in a 5 mm grid to allow accurate distance measurements to be obtained. A thin piece of wood glued to the top end of the ramp served as a background against which the salticid saw the lure. The lure was placed 40 mm from the top end of the ramp, equidistant from both edges, and placed such that it was faced 45° away from the pit, enabling test spiders to view cues from both the body and the head or cephalothorax of the lure. Lures were made by immobilizing an arthropod with CO_2 and placing it in 80% ethanol. One day later, I mounted the arthropod in a life-like posture on the centre

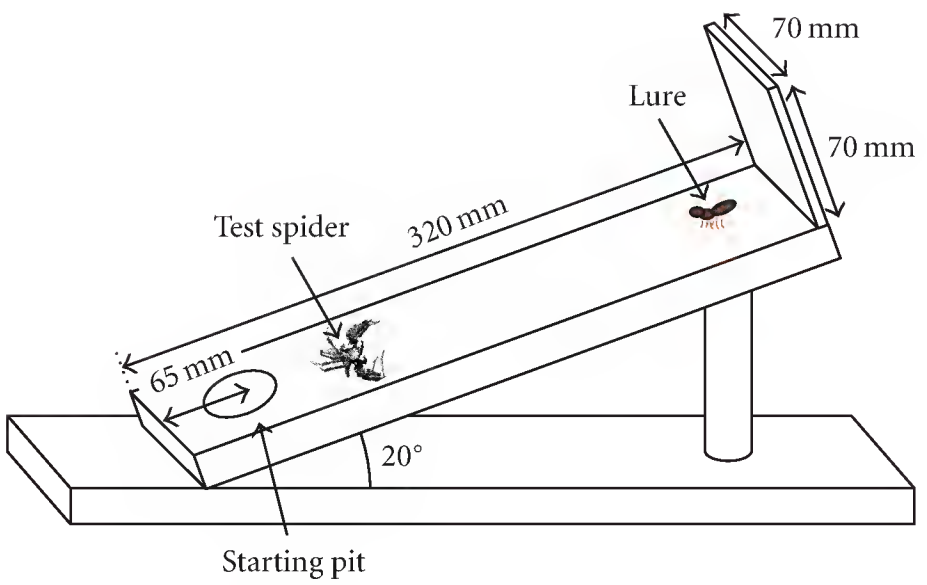

Figure 1: Ramp used for testing *Portia fimbriata* with lures of ants and ant mimics.

of one side of a disc-shaped piece of cork (diameter *c.* 1.25 × the body length of the arthropod; thickness *ca.* 2 mm) using forceps to position the arthropod. Lures were then sprayed with a transparent aerosol plastic adhesive for preservation.

Before each test, *P. fimbriata* was placed in a 32 mm diameter "starting pit" drilled halfway through the thickness of the ramp 200 mm from the lure. The salticid was left in the pit to acclimate for 60 s before a piece of cardboard, which was placed over the pit, was removed, allowing the salticid to exit from the pit. A white paper screen running along three sides surrounded the apparatus, leaving one side open for observations. The ramp was positioned so that the salticid moved away from the observer during tests. Tests began when *P. fimbriata* walked out of the pit and on to the ramp and ended when *P. fimbriata* either attacked the lure or walked off the top end of the ramp. If the salticid jumped off the ramp at a point below the lure or if it stayed in the pit for more than 30 min (no spiders walked under the ramp), tests were aborted. After testing for normality (D'Agostino and Pearson omnibus test), data were analysed using ANOVA in Prism v.5.

3. Results

There was a significant overall effect of lure type on the distance to which *P. fimbriata* approached the lure ($F_3 = 2.794$, $P < 0.05$), although in general *P. fimbriata* showed an aversion to both ants and ant mimics. *P. fimbriata* avoided contact with lures by circling around the lure and then continuing up the ramp. Tukey's post hoc comparisons revealed no differences between responses to *O. smaragdina* and *S. occidentalis* or female *M. bakeri,* but male *M. bakeri* were approached significantly closer than *O. smaragdina* ($P < 0.05$). Overall *P. fimbriata* was kept furthest away from the ant (*O. smaragdina*), followed by *S. occidentalis*, then female *M. bakeri*, and lastly male *M. bakeri* (Figure 2). There were three instances of attacks towards lures, and all of these were aimed at lures of male *M. bakeri*.

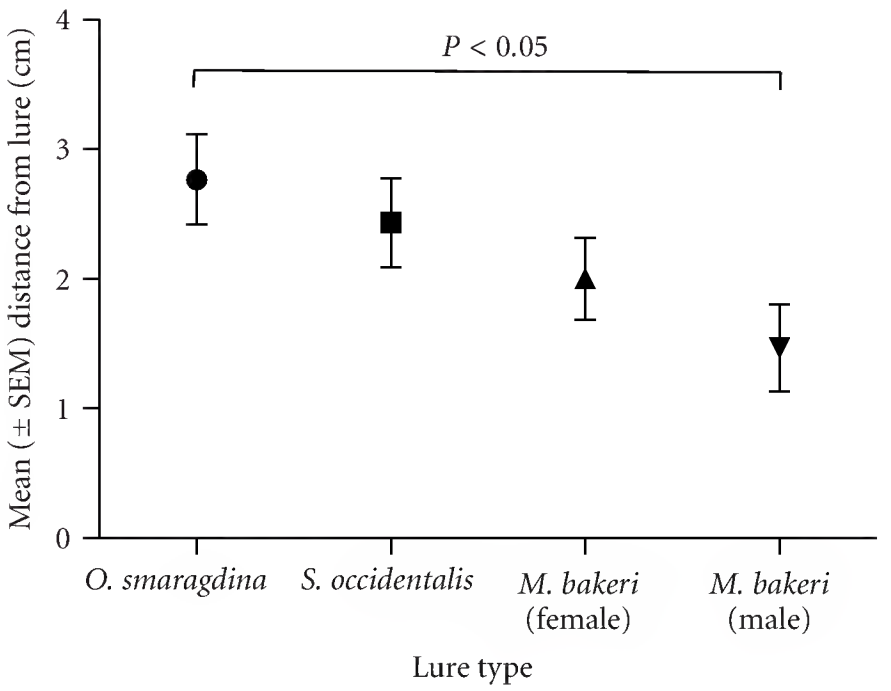

Figure 2: Mean (±SEM) approach distance by the spider-eating salticid *Portia fimbriata* to lures of ants (*Oecophylla smaragdina*) and ant mimics of varying degrees of accuracy of mimicry (*Synageles occidentalis* and male and female *Myrmarachne bakeri*).

4. Discussion

Portia fimbriata was unable to correctly classify the mimics as its preferred prey, salticids [30], and instead generally responded toward the mimics as it did toward ants. These results provide additional evidence that ant mimicry in spiders functions as Batesian mimicry, even with naïve predators. However, it appears that the degree of resemblance to ants may have repercussions when faced with predators with acute eyesight, such as salticids. *Synageles occidentalis* is thought to mimic *Lasius alienus* or *Myrmica americana*, with which it is associated [10]. The salticids we had in the laboratory bore an extremely accurate resemblance to the former ant species. Although *Myrmarachne bakeri* resemble ants, they do not have a specific model to which they render a faithful portrait [19]. *Portia fimbriata* apparently also classified the potential prey with which it was faced in a similar manner to the way in which humans classify these animals, which is by no means a given. Males of *M. bakeri* were significantly less effective at deterring *P. fimbriata* than ants and slightly less aversive than *M. bakeri* females and *S. occidentalis*. Nevertheless, it should be noted that in these experiments prey behaviour was not taken into account. It is known, for example, that some myrmecomorphs will actively display to ant-eating salticid predators, deterring potential attack through mistaken identity [31]. While there is currently no evidence supporting the idea that accurate ant-like spiders behave more like ants than poor mimics, it is conceivable that this might have exacerbated the results of the current study.

The only striking visible difference between the male and the other stimulus animals was the male's large chelicerae. The chelicerae of sexually mature *Myrmarachne* males, which can increase their body size by 30–50% [27], is believed to have evolved as a sexually selected trait [32]. To our eyes, *Myrmarachne* males resemble ants considerably less convincingly than *Myrmarachne* females and juveniles, suggesting

that, along with impaired feeding mechanics [32], impaired predator deterrence through inaccurate mimicry has been a cost of sexual dimorphism for male *Myrmarachne*. Contrary to the other potential prey, lures of male *M. bakeri* were occasionally attacked. Nevertheless, *P. fimbriata* generally avoided lures of male *M. bakeri*, suggesting that mimicry among males, despite possessing some cost in terms of diminished efficacy of mimicry due to their enlarged chelicerae, is still effective at deterring visually based predators. This supports the idea that the shape of the chelicerae of male *Myrmarachne* is in keeping with its mimicry because it looks like an ant worker carrying something in its mandibles [21], as is commonly observed in worker ants [6].

In a study using hoverfly mimics of wasps as prey and pigeons as predators, Dittrich et al. [33] found that despite some species being poor mimics, they were still protected by their mimicry, perhaps due to some constraint in the birds' visual or learning systems. Here it is apparent that imprecise mimics, although not avoided to the same degree as accurate mimics, were nevertheless aversive to naïve predators, suggesting that learning is not essential for the same effects to be seen. A mutually compatible alternative explanation is simply that very numerous and very dangerous models may produce a wider "cone of protection," thus allowing for imprecise mimicry [34] because the payoff to a predator for attacking prey with a given resemblance to a numerous and highly noxious model is limited [35]. Furthermore, polymorphic mimics that do not resemble any particular ant species especially closely may gain other advantages. For example, imprecise ant mimics may not be restricted to the geographical area or microhabitat (e.g., arboreal ants) in which a specific model species is found. Ants are notorious for both their abundance and their formidable defences [6], and it may not be surprising to find that among ant mimics there is considerable variation in form, ranging from accurate to imprecise mimicry. What is unusual is that here we have an example of a mimic resembling one of its own predators [28, 29].

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Research Article

Geographic Spread of *Gnamptogenys triangularis* (Hymenoptera: Formicidae: Ectatomminae)

Joseph A. MacGown[1] and James K. Wetterer[2]

[1] *Department of Entomology and Plant Pathology, Mississippi State University, MS 39762, USA*
[2] *Wilkes Honors College, Florida Atlantic University, 5353 Parkside Drive, Jupiter, FL 33458, USA*

Correspondence should be addressed to Joseph A. MacGown, jmacgown@entomology.msstate.edu

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Gnamptogenys triangularis (Mayr), native to the forests of South and Central America, is a predatory ant that feeds on millipedes. In its native range, this species is known from Buenos Aires, Argentina (38.1°S) in the south to Costa Rica (10.4°N) in the north, with records from eight countries in South America (all except Chile, French Guiana, and Paraguay), and the two southernmost countries of Central America (Panama and Costa Rica). The first records of *G. triangularis* outside its native range came from Florida beginning in 1985 (six sites: 25.5°–30.4°N) and Alabama in 1996 (one site: 30.4°N). Here we present the first records of *G. triangularis* from Mississippi, dating from 2002–2010 (five sites: 30.5°–31.2°N). Based on its South American range, it appears that *G. triangularis* has the potential to spread to forests throughout much of the southeastern USA. There are no documented impacts of *G. triangularis,* and it seems unlikely that this species will ever become a major pest.

1. Introduction

Gnamptogenys triangularis (Mayr), native to the forests of South and Central America, is a predatory ant that feeds on millipedes [1, 2]. Kusnezov [3] wrote that in Tucuman, Argentina, *G. triangularis* occurred by streams in the subtropical cloud forest areas. In Surinam, Kempf [4] recorded *G. triangularis* from a primary forest and a "marshy wood." Lattke et al. [5] wrote that *G. triangularis* nests in trees and broken branches lying on the forest floor in wet primary and secondary forests, from sea level to elevations >1,000 m. L. R. Davis collected specimens in Harrison County, Mississippi, nesting in a rotten pine limb in leaf litter beneath grape vines (from Mississippi Entomological Museum specimen label data). Other collections of this species in Mississippi were in deciduous forests. Lattke [1] reported that *G. triangularis* colonies typically have only 80–120 workers, up to a probable maximum of 150 workers.

The first records of *G. triangularis* outside its native range came from Miami-Dade County, Florida, beginning in 1985 [6, 7]. Deyrup [8] reported *G. triangularis* from two additional counties in Florida (Broward and Escambia). MacGown and Forster [9] were the first to report this ant from Alabama (Mobile County). Here, we report the first records of *G. triangularis* from Mississippi and evaluate its potential spread based on its known native range.

2. Taxonomy and Identification

Ectatomma triangulare (*G. triangularis*) was originally described in Uruguay in 1887 [10]. Junior synonyms include *Ectatomma triangulare richteri* Forel, described in 1913 in Argentina [11] and *Ectatomma aculeaticoxae* Santschi, described from "Haute Carsevenne, French Guiana" in 1921 [12] (now Alto Rio Calçoene, Brazil) [2].

Gnamptogenys triangularis and *Gnamptogenys hartmani* (Wheeler) are the only two members of the subfamily Ectatomminae known to occur in the southeastern USA. These very distinctive ants can be recognized immediately by the deep horizontal grooves covering the entire head and body. *Gnamptogenys triangularis* workers are ~5.0 mm in length and dark brown (Figure 1(a)). Females are similar in appearance to workers, but are slightly larger (~5.5 mm in length) and have brownish gray colored wings (Figure 1(b)).

Figure 1: Profile views of *Gnamptogenys triangularis:* (a) worker, (b) alate female, and (c) alate male. Scale bar equals 1.0 mm.

Males (Figure 1(c)) are approximately the same length as females (~5.5 mm), but are wasp-like in appearance and differ considerably from workers and females. The deep horizontal grooves characteristic of the genus are mostly absent except on the face and first gastral tergite. Sides of head, pronotum, much of the mesonotum, and gaster have reduced sculpture and are shiny, and the remainder of mesosoma and petiole are rugoreticulate. Males are bicolored with the head and mesosoma dark reddish black to black, petiole dark reddish brown, and gaster reddish orange. The legs, antennal scape, and first segment of funiculus are orangish red, the remainder of funiculus is gray, and the wings are dusky gray. In comparison, *Gnamptogenys hartmani* workers are 3.5–4.0 mm and pale reddish brown. Females are ~5 mm and similar in appearance to workers. Males are approximately the same size as females, but horizontal grooves are greatly reduced, and overall they are shinier in appearance. The entire body is reddish brown, legs and scapes are yellowish brown, and the funiculus is dark colored.

3. Methods

Using published and unpublished records, we documented the native and exotic range of *G. triangularis.* We obtained unpublished site records from museum specimens in the collections of the Museum of Comparative Zoology (MCZ, identified by S. Cover) and the Smithsonian Institution (SI, identified by M. Smith). In addition, we used online databases with collection information on specimens by Antweb (http://www.antweb.org/) and the Global Biodiversity Information Facility (http://www.gbif.org/).

4. Results

In South and Central America, *G. triangularis* has been recorded from 12 countries (Table 1) from central Argentina to Costa Rica (Figure 2). References to this species occurring in French Guiana appear to be the type locale of *G. aculeaticoxae* (*G. triangularis*), nowin Brazil (see Section 1).

Table 1: Earliest known records for *Gnamptogenys triangularis* from South and Central America. MCZ: Museum of Comparative Zoology.

	Earliest record
Uruguay	≤1887 [10]
Bolivia	≤1893 [13]
Argentina	1898 [14]
Brazil	1898 (as *E. aculeaticoxae*) [12]
Guyana	1922 (as *G. aculeaticoxae*) [15]
Panama	1941 (as *G. aculeaticoxae*) [15]
Costa Rica	1949 (L. Garling, MCZ): La Selva
Surinam	1959 (as *G. aculeaticoxae*) [4]
Peru	1967 (W. L. Brown and W. Sherbrooke, MCZ): Tingo Maria
Colombia	1972 (M. Corn, MCZ): near Puerto Asis
Ecuador	1975 [16]
Venezuela	1982 (as *G. aculeaticoxae*) [1]

Published records of *G. triangularis* outside its native range come from Florida beginning in 1985 (four sites: 25.5°–30.4° N) and Alabama in 1996 (one site: 30.4° N).

Based on specimens in the Mississippi Entomological Museum (identified by JAM), we report records of *G. triangularis* from five sites in four Mississippi counties: Forrest Co., 10 mi S Hattiesburg at diner near Camp Shelby (one male; 31.188° N, 89.251° W; 12 Nov 2010, leg. D. C. Cross), Stone Co., DeSoto National Forest (one gyne; 30.869° N, 89.001° W; 7–13 June 2002, Malaise sample, leg. T. L. Schiefer and J. Schonewitz), Pearl River Co., White Sand (16 gynes and 32 males; 30.794° N, 89.659° W; 24 May–13 July 2002: five different weekly Malaise samples, leg. T. L. Schiefer and L. Thomas), Harrison Co., 1 mi. NE Wortham (one worker; 30.570° N, 89.129° W; 21 July–7 Aug 2006, Lindgren funnel), and Harrison Co., Wool Market (nine workers; 30.484° N, 88.963° W; 27 June 2004, leg. L. R. Davis).

A collection by L. R. Davis from Alabama represents the northernmost record in the United States: Monroe Co., Frisco City, along rt. 21; 2 miles South of jct. rt. 84, (31.453° N, 87.374 W; 4 May 2005; L. R. Davis, Jr., pers. comm.). In addition, two unpublished records from Florida on Antweb represent a new county record: Marion Co., Ocala; 5454 SW 84th St. (29.101° N, 82.210° W; 7 May 2004; leg. M. Jones, Archbold Biological Station) and Marion Co., Ocala, 6455 SW Hwy 200 (29.111° N, 82.227° W; 21 Jul 2004, leg. J. Mangold, California Academy of Sciences).

5. Discussion

Records of *G. triangularis* come from eight countries in South America (all except Chile, French Guiana, and Paraguay), and the two southernmost countries of Central America (Panama and Costa Rica) (Figure 2, Table 1). Because there are no discernable geographic barriers, it seems very likely that the native range of *G. triangularis* also extends into forest habitats in French Guiana, Paraguay, and farther

Figure 2: Map showing the known records of *Gnamptogenys triangularis*.

Table 2: Earliest known records for *Gnamptogenys triangularis* from the USA. +: no previously published records. LRD: L. R. Davis collection. MEM: Mississippi Entomological Museum.

	Earliest record
Florida	1985 [7]
Alabama	1996 (L. R. Davis, LRD): Mobile [9]
+Mississippi	2002 (T. L. Schiefer and L. Thomas, MEM): White Sand

north in Central America, but has not yet been recorded in these areas due to its rarity.

In its exotic range, *G. triangularis* is now known from four counties in Florida (six sites: 25.5°–30.4°N), two in Alabama, and four in Mississippi (five sites: 30.5°–31.2°N) (Figure 2, Table 2). Based on its South American range, extending to 38.1°S, it is possible that *G. triangularis* could spread to forests throughout much of the southeastern USA, perhaps as far north as Richmond, Virginia (37.5°N) and Lexington, Kentucky (38.0°N).

Many of the Mississippi records are based on specimens collected in Malaise traps. These traps and other flight interception traps may be useful methods for monitoring the movements of exotic species, especially alate males and females.

In both its native and exotic ranges, *G. triangularis* inhabits forests and preys on millipedes [2, 3, 8]. There are no known impacts of *G. triangularis*, even though if this species was having a localized impact on millipede populations, it seems unlikely that this would have been detected. Nonetheless, it seems unlikely that this species will ever become a major pest.

Acknowledgments

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Research Article

Effects of the Trophobiont Herbivore *Calloconophora pugionata* (Hemiptera) on Ant Fauna Associated with *Myrcia obovata* (Myrtaceae) in a Montane Tropical Forest

Roberth Fagundes,[1,2] Kleber Del-Claro,[1] and Sérvio Pontes Ribeiro[2]

[1] *Post-Graduate Program in Ecology and Conservation of Natural Resources, Laboratory of Behavior Ecology and Interactions, Federal University of Uberlandia, 38400-902 Uberlandia, MG, Brazil*
[2] *Post-Graduate Program in Ecology of Tropical Biomes, Laboratory of Evolutionary Ecology of Canopy Insects and Natural Succession, Department of Biodiversity, Evolution and Environment, Federal University of Ouro Preto, 35400-000 Ouro Preto, MG, Brazil*

Correspondence should be addressed to Roberth Fagundes, roberthbio@yahoo.com.br

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Many studies have investigated the mechanisms behind the structure of arboreal ant assemblages. In this study, the objective was to evaluate the effect of availability of honeydew-producing colonies of *Calloconophora pugionata* (Membracidae) on the structure of ant assemblages associated with the host plant *Myrcia obovata* (Myrtaceae) in an Atlantic forest of Minas Gerais (Brazil). Our experiment consisted in a gradual exclusion of hemipteran colonies out of the host plant crown and further record of the ant assemblage response (species richness, composition, and occurrence) to the presence and density of treehopper colonies. The hypothesis was that an increase in the number of trophobiont herbivores results in an increase in tending ant occurrence but a reduction in ant species diversity. Results corroborated our main hypothesis: membracids had a positive effect on the occurrence of ants but negative on species richness. Overall insect occurrence was also reduced with increasing in *C. pugionata* colonies, probably due to strengthening dominant ant species territory sizes and intensification of patrolling.

1. Introduction

The outstanding occurrences of large arboreal ant colonies result in the most remarkable animal biomass found in any tropical canopy [1]. Territory patrolling behaviour causes species dominance which reflects in nest sizes and intense search for food [2, 3]. The most common and rich food resource for ants is extrafloral nectaries secretion and exudates of sap-sucking herbivores, the honeydew [4–6]. These resources are predictable, highly energetic, and nutritive, leading to an increase of ant activity on the foliage [7] and ant fitness [8].

The trophobiosis is a relationship between ants and honeydew-producer insects [9]. It is a mutualistic interaction that involves consumption of honeydew by ants in return for protection of the hemipterans against natural enemies [10]. This kind of resource characterizes its consumers as indirect herbivores and provides energy necessary to achieve higher population sizes [3, 10]. However, the honeydew is a spatially limited resource, despite its importance, and consequently it may trigger interspecific competition [11].

Resource competition is one of the most important forces driving ant assemblage structure [2, 12]. In the canopy habitat, there are a small variety of resources for numerous ant species that share the same kind of preferred food, like honeydew or nectar, and canopy may be indeed one of the most competitive tropical environments [11, 13]. Studies with stable isotopes showed that many ant species frequently use honeydew and nectar as main food item, and thus these resources do shape arboreal ant community [3, 11, 14, 15].

The bottom-up effect caused by the sugar resources consumed in such interactions may lead to a cascade effect at multitrophic scales [15–17]. The resource monopolization by the dominant species may increase the interespecific

competition and the encounter frequencies between competitors, reducing the forage of nondominant ant species [18, 19]. In addition, when colony nutritional demands increase beyond the offer of honeydew, predatory behaviour became more frequent, along with the protective behaviour of removing any arthropod from the foliages close to the sugar resources, thus reducing the arthropods in the host plant [20–22]. The last case may generate an indirect benefit to the host plant by reducing herbivore occurrence and thus leaf chewing damage [20].

Trophobiosis has been largely studied in lowland, close tropical rainforests; and in the Brazilian Cerrado, among a few other ecosystems [17, 18, 21, 23, 24]. Although it is a well-described mutualistic system, likely to result in similar ecological output wherever it happens, it is necessary to investigate such interactions in other habitats [23]. For instance, climatically challenging conditions, or biogeographically isolated and intensely disturbed ecosystems, would result in harsh ecological conditions, capable for altering the expected output of increasing territories and competition around honeydew producers. Namely, how resilient a large colony would be to hemipteran density variation in space or time, under extreme environments?

In the present study we evaluated the effect of variation in the presence and density of hemipterans exudates producers among tree crowns on the arboreal ant species richness and occurrence, in a secondary montane forest ecosystem. We tested the hypothesis that the availability of the *Calloconophora pugionata* Driecht colonies positively affects the feeding behaviour and diversity of ants associated with the host plant *Myrcia obovata* (O. Berg) Nied (Myrsinaceae). Further, we expected that a high density of ants around the hemipteran colonies would affect other arthropods, from plant natural enemies, such as herbivore beetles, to membracid predators, such as spiders.

2. Material and Methods

2.1. Study Sites. The field work was conducted in Itacolomi State Park (20°26′26″S, 43°30′52″W) located between the cities of Ouro Preto and Mariana, Minas Gerais State, Brazil. This ecological reserve has an area of 7,000 ha covered with a mosaic of Atlantic montane forests and Cerrado. The rainfall regime varies between 1,500 to 2,000 mm per year and temperature between 19°C and 22°C. The rainy season extends from September to February and the dry season from March to August.

2.2. Model System. The tritrophic interaction is composed by *Myrcia obovata* (O. Berg) Nied (Myrtaceae), a common Atlantic forest tree, *Calloconophora pugionata* Driecht (Membracidae), a sap-sucking insect and honeydew producer, and the associated ant species, mostly *Camponotus crassus* Mayr, 1862 (Formicidae) and *Camponotus rufipes* Fabricius, 1775 (Formicidae), two of the numerical dominant ants in these forests [25]. This system occurs mostly in tropical montane forests established in the "canga", an ironstone outcrop areas.

The field work was carried out in October and November of 2009 (wet season). Thirty-six individuals of *M. obovata* with similar structure (number of stems, height, crown circumference) were tagged in this site. From this subsample, 20 trees were randomly marked and divided in two groups of 10 plants named according to the presence of membracid as treatment (with membracid colonies) or control (without colonies). The colonies of *C. pugionata* hosted in each experimental plant had 25.4 ± 5.3 membracids (mean ± s.d.; $n = 30$), between adults and nymphs. Trees of *M. obovata* may occur isolated or in the same patch, but with a minimal distance of five meters between each one. Control and treatment plants were randomly selected from the original population, so their crowns are not grouped but mixed in the same canopy.

2.3. Experimental Design. The experiment was set by gradual removal of the membracid colonies (one colony per tree each time), and then we evaluate how the ant assemblage responds to that (Table 1). The procedure was carried out through four periods separated by three-day interval, which simulated a reducing resource gradient. At the end of each period all individuals of one colony of *C. pugionata* were removed from each treatment plant which were then monitored again straight way for the next days. In the last period, treatment plants had all colonies removed. The control plants were not manipulated and had no trophobioses in all periods. Hence, all ten plants of each group (independent variable) were successively observed (six events per period) along the resource gradient (period then used as repeated measures). These observations were made every two days on both control and treatment trees during two weeks for each period and last for 20 minutes per plant individual, and all visiting ants were recorded. Additionally, the other arthropods specimens observed were also quantified. Every species was classified using behaviour and morphological characteristics, as trophobiont ants, nontrophobiont ants, potential *C. pugionata* predator arthropods, potential *M. obovata* herbivore arthropods. Vouchers specimens of ant species were deposited at the insect collection of the Laboratory of Evolutionary Ecology of Canopy Insects and Natural Succession at Federal University of Ouro Preto, Ouro Preto, Brazil.

2.4. Data Analysis. The mean ant species richness (number of species per tree at each observation) and the mean occurrence of ants (number of ants per species observed in each tree) were analyzed using repeated measures ANOVA models, with the experimental groups as group factor (treatment and control) and the four periods as repeated measures (resource gradient). Repeated measure ANOVA was also used to compare the relative proportion of trophobiont ant species between the treatment groups for each period. A Fisher's LSD post hoc test was used, performed as paired test.

The total ant species richness (total number of ant species observed at each period for both experimental groups) for every case was estimated using Jackniffe I with 500 repetitions. After that, the values of total estimated

Table 1: Scheme represented the sampling design of the experiment.

Groups[1]	Experiment (two months)			
	Period 1[2] (Two weeks)	Period 2[2] (Two weeks)	Period 3[2] (Two weeks)	Period 4[2] (Two weeks)
Treatment (10 plants)	3 colonies[3]	2 colonies[3]	1 colony[3]	No colonies[3]
Control (10 plants)	No colonies[3]	No colonies[3]	No colonies[3]	No colonies[3]

[1]The same plants were monitored in all periods.
[2]Six days of observations per period.
[3]Colonies of *Calloconophora pugionata* per tree.

species richness were interpreted for the treatments with visual analyses on the overlap of the confidence intervals (95%) as indicator of difference. Additionally, the ant species composition was compared by discriminant analysis using the periods as grouping factor.

The mean occurrence of herbivores, predators, and all nonant arthropods grouped (including dipterans, cockroaches, etc.) was also analyzed using repeated measures ANOVA models as performed to ants data. Fisher's LSD test was used after ANOVA as paired test. All analyses were performed with data transformed ($X' = \log_{10} X$), but graph was plotted with original data.

3. Results

We recorded a total of 1,897 ants visiting *M. obovata*, belonging to 10 species from four subfamilies, with 81.5% only at the treatment group (Tables 2 and 3). The subfamily Formicinae was the most representative group with *Camponotus* species as the numerically dominant group (89.4%). *M. obovata* was also visited by 1,125 nonant arthropods, including 62.5% in the control plants and 37.5% in the treatment plants (Table 3).

The presence and amount of *C. pugionata* colonies positively affected ant forage in the trees. In the treatment plants we observed an decrease in the ant number responding to reduced availability of honeydew sources, while nothing changed in the control (Repeated measures ANOVA: Group∗Period: $F(3;354) = 4.4$; $P < 0.01$; Figure 1). Besides, the ant foraging was higher in the treatment plants except for trophobiose-lack period, when the ant activity was similar for both groups (LSD: $P < 0.05$). In fact, treatment plants were mostly occupied by honeydew-feeding ants, but again only at the periods when hemipterans colonies occurred (Repeated measures ANOVA: $F(3;354) = 3.6$; $P < 0.02$; Figure 2).

The mean ant species richness per plant, that is, the number of ant species foraging at the same time on each crown, was higher in the presence of mutualistic hemipteran colonies, although the result did not change in response to colony removal (Repeated measures ANOVA by Group: $F(1;118) = 10.47$; $P < 0.01$; Figure 3). Still, mean richness was always lower in the control than in treatment plants (LSD: $P < 0.05$). In opposite, the estimated total number of ant species was higher in the control plants than in the treatment

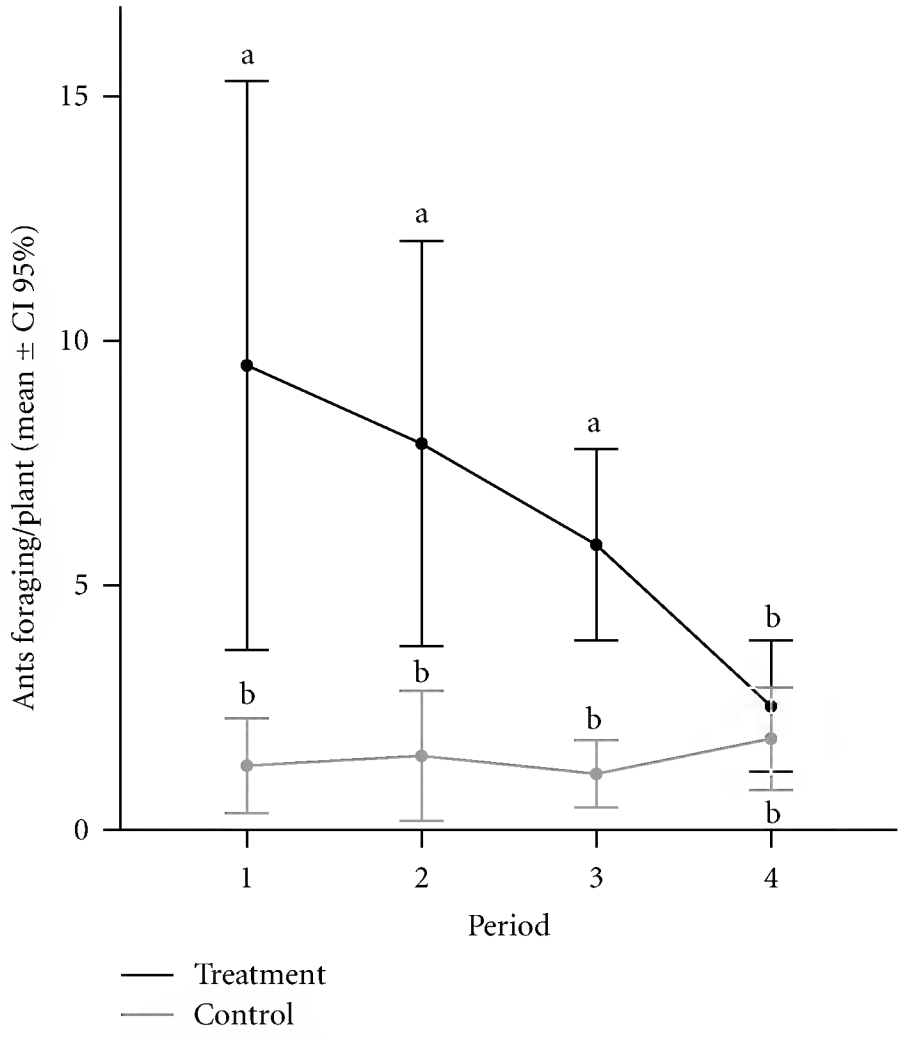


Figure 1: Positive relationship between the availability of hemipterans colonies and the amount of ants foraging on each plant (Repeated measures ANOVA: Group∗Period: $F(3;354) = 4.4$; $P < 0.01$). The largest amount of ants was recorded in period 1 and gradually decreases until period 4 when it became identical to values found in all phases of the control group. Different letters indicate significant differences according to post hoc test (LSD: $P < 0.05$).

but became similar to the control plants in the last period (Figure 4).

The ant species composition also changed in response to trophobiont hemipteran occurrence (Discriminant analysis: Wilk's $\lambda = 0.206$; $\chi^2 = 110.47$; d.f. = 70; $P < 0.01$). Accordingly, with 75.9% of the variance explained by the first function and 8.6% by the second, the ant assemblage associated with plants hosting ant-hemipteran mutualism was significantly different from plants where hemipterans were totally removed or never existed (Figure 5). This difference was mainly associated with the massive presence of the two trophobiont ant species, *Camponotus crassus* and *C. rufipes*, when plants had colonies of *C. pugionata* (*C. crassus*: $F(7;72) = 5.46$; $P < 0.01$; *C. rufipes*: $F(7;72) = 3.31$; $P < 0.01$; other species did not contribute significantly for the model).

Table 2: List of ant species observed associated with *Myrcia obovata* and respective total number of occurrence.

	Total number of ants	
	Treatment	Control
Family Formicidae		
Subfamily Formicinae		
Camponotus rufipes Fabricius 1775	635	23
Camponotus crassus Mayr 1862	1315	132
Camponotus novogranadensis Mayr 1870	122	113
Camponotus fastigatus Roger 1863	22	29
Subfamily Myrmicinae		
Crematogaster sp1	0	118
Cephalotes pusillus Klug 1824	16	13
Pheidole sp1	23	19
Subfamily Pseudomyrmicinae		
Pseudomyrmex gracilis Fabricius 1804	41	35
Pseudomyrmex pallidus Smith 1855	7	10
Subfamily Ponerinae		
Gnamptogenys striatula Mayr 1884	2	0

Table 3: Total number of occurrences registered for each experimental group throughout the experiment.

	Group		
	Treatment	Control	Total
Ants	1546	351	**1897**
Herbivores	276	384	**660**
Predators	102	253	**355**
Others arthropods	44	66	**110**
Total	**1968**	**1054**	**3022**

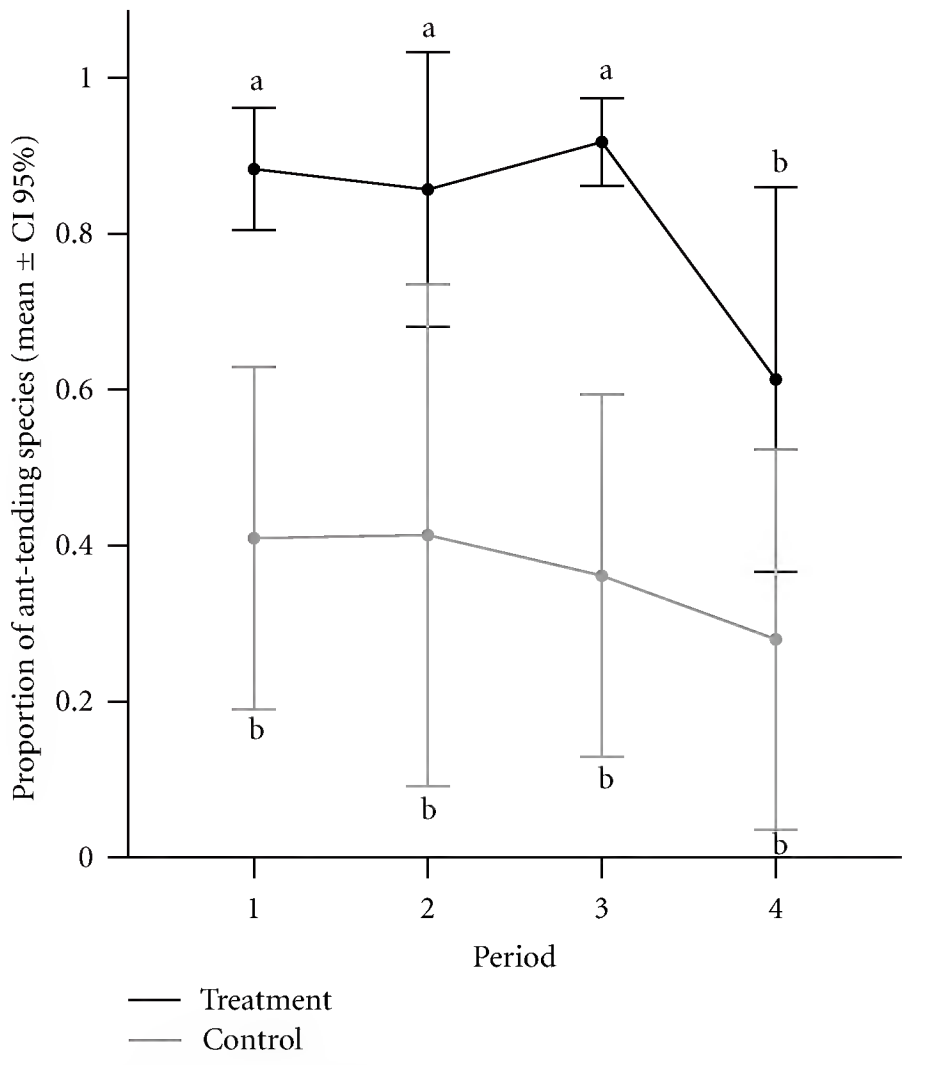


Figure 2: The ant foraging fauna on plants with trophobioses is formed mostly by exudates-feeding species (Repeated ANOVA: $F(3;354) = 3.6$; $P = 0.02$). Lines show the proportion of the occurrence of trophobiont ant species from total ants. Different letters indicate significant differences according to post hoc test (LSD: $P < 0.05$).

Arthropods other than ants visiting *M. obovata* showed a positive response to decrease honeydew source gradient (Repeated measures ANOVA group∗periods: Non-ant arthropods: $F(3;354) = 3.62$; $P < 0.05$; Figure 6(a). The control group did not change through the periods despite low fluctuations (LSD: $P > 0.05$). A similar pattern was observed when herbivores and predators were analyzed separately (Repeated measures ANOVA group∗periods: Herbivores: $F(3;354) = 3.21$; $P < 0.05$; Predators: $F(3;354) = 4.66$; $P < 0.01$; Figures 6(b) and 6(c). The occurrence of every other arthropod guilds in the treatment plants was lower than that in the control plants, except at the last period when both plant groups presented the same number of foreign visitors, as expected (LSD: $P < 0.05$).

4. Discussion

The bottom-up effect caused by *C. pugionata* was noticeable for both tending ants as well as for the rest of the associated fauna in *M. obovata*. The availability of exudate-producing herbivore colonies positively affected the occurrence of foraging ants on the plants. There was an increase in the numerical dominance of species collecting honeydew, along with a decreasing in species richness. Still, the number of species foraging at the same time was greater in the presence of membracid, probably due to the high frequency of trophobiont species. Furthermore, we found a low occurrence of potential host plant herbivores and likely predators when colonies of mutualistic hemipterans were present. Such findings suggest the presence of mutual benefit among the

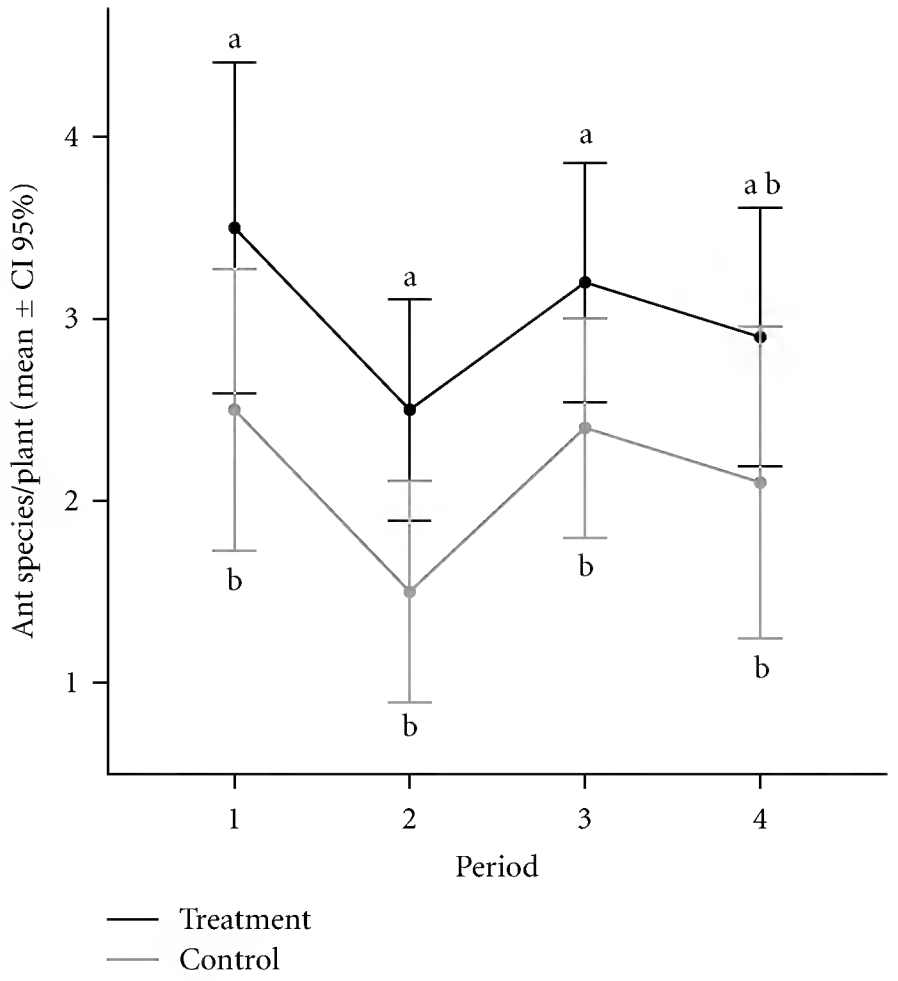


Figure 3: Plants of the treatment group presented higher mean ant richness than control group in all periods (Repeated measures ANOVA: Group: $F(1;118) = 10.47$; $P < 0.01$). Different letters indicate significant differences according to post hoc test (LSD: $P < 0.05$).

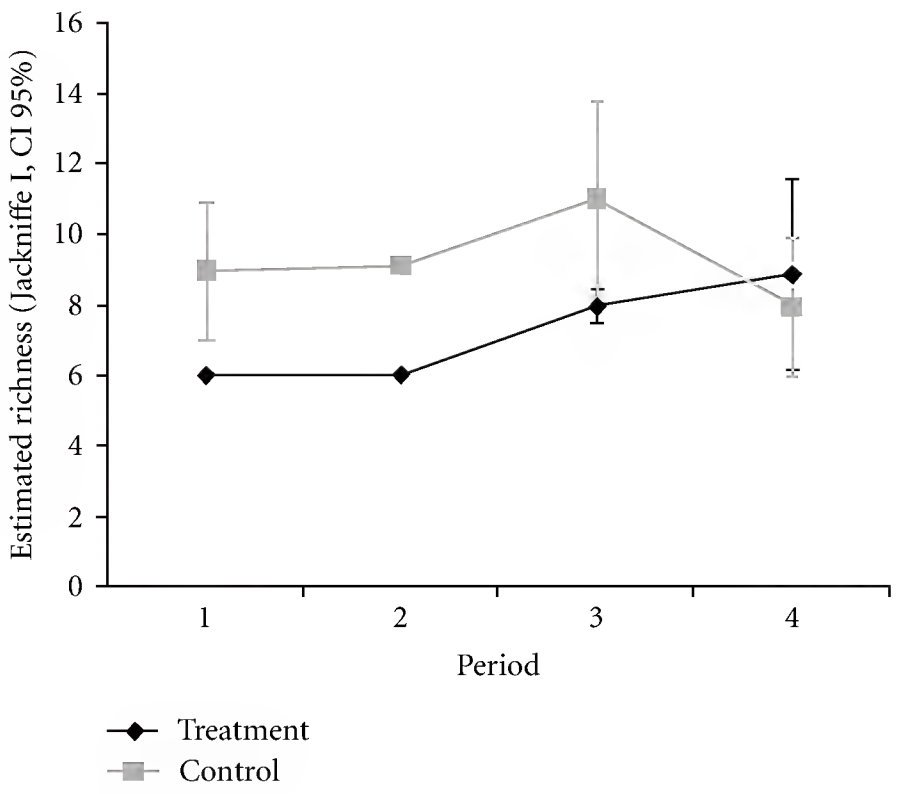


Figure 4: Estimated total richness calculated for the four periods in both groups. Treatment group shows low richness compared to control group in the three first periods.

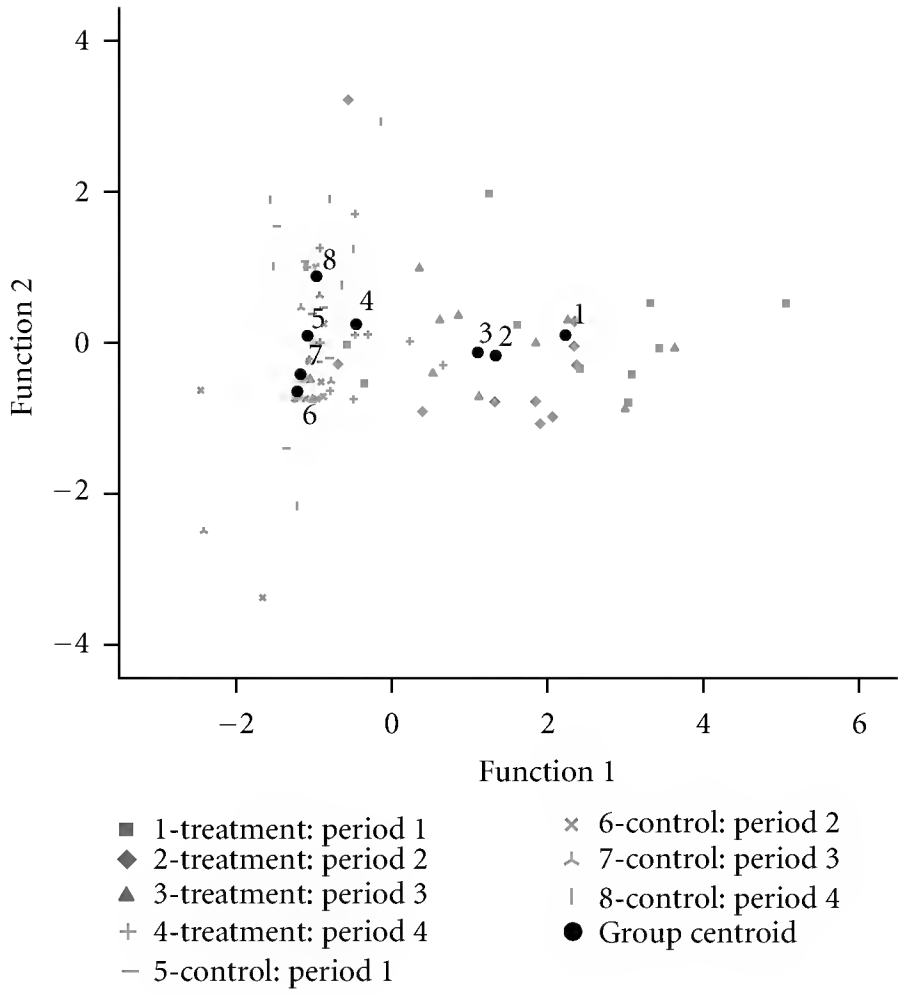


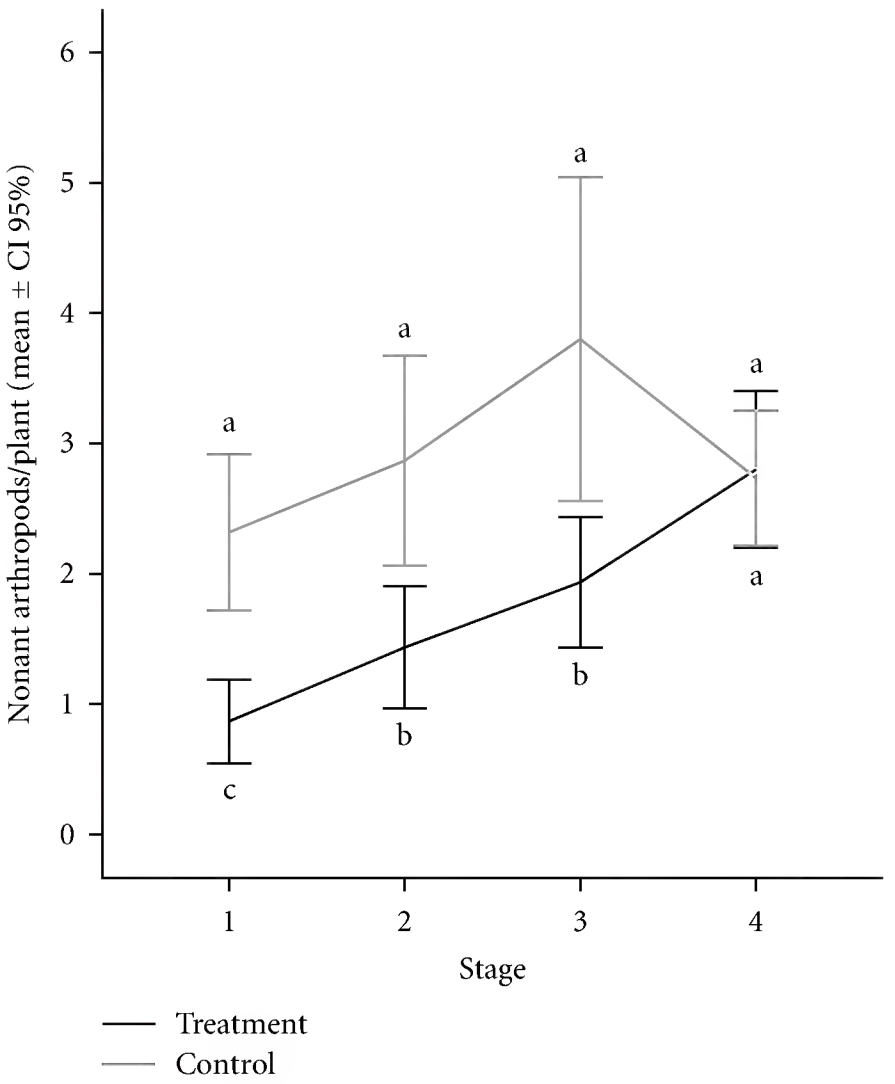
Figure 5: Discriminant analysis comparing the experimental groups (for each period of experiment) using ant species composition and relative occurrence. With 75.9% of the variance explained by the first and 8.6% by the second function, periods were discriminated in cases with trophobioses and without trophobioses by the high occurrence of the trophobiont ant species *Camponotus rufipes* and *C. crassus* in the first group (Wilk's $\lambda = 0.206$; $P < 0.01$; *C. crassus*: $F(7;72) = 5.46$; $P < 0.01$; *C. rufipes*: $F(7;72) = 3.31$; $P < 0.01$; other species did not contribute significantly for the model).

mutualism actors (e.g. [17]), or at least some compensation for the sap-sucking damage on the host plant.

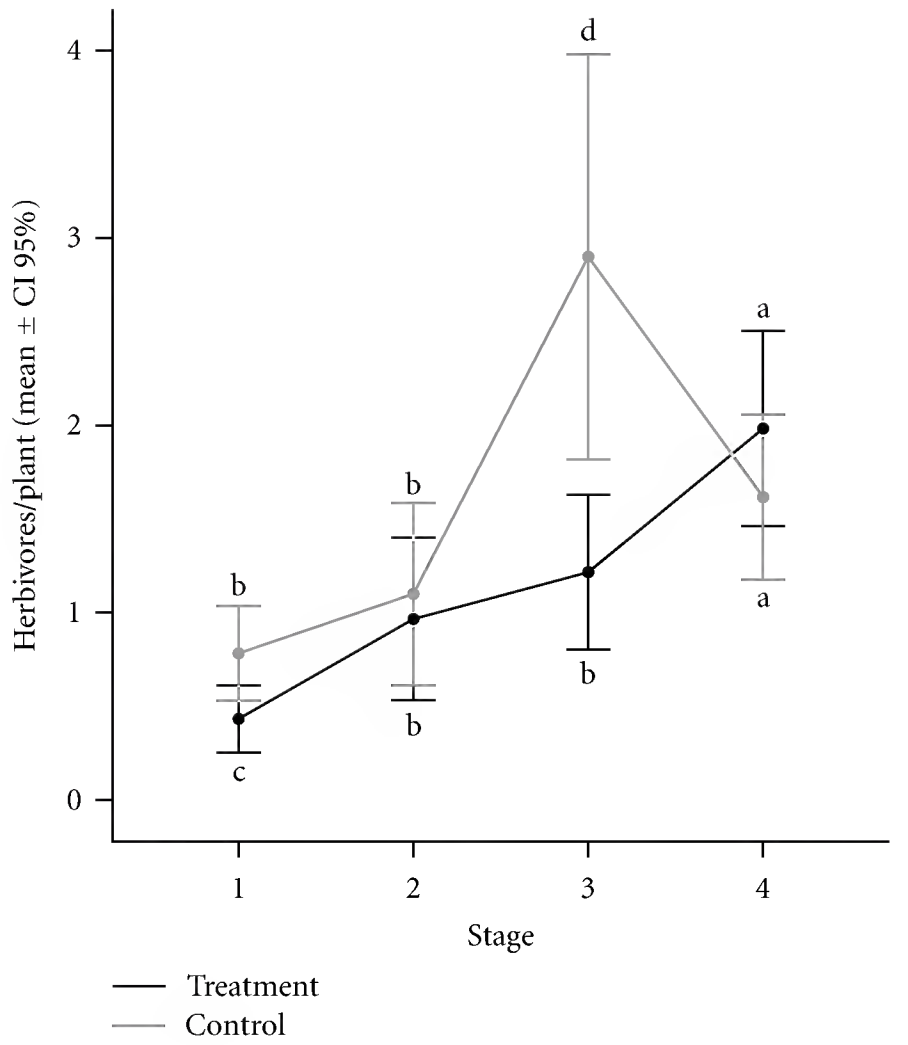
The existence of an ant dominance and patrolled territory in association with hemipteran colonies or extrafloral nectaries is a pattern extensively described for various tropical habitats, although it is relatively unknown for montane forests [3, 11, 26–28]. Clearly, ecology of arboreal ants is strongly related to plant hemipteran sweet exudates, and the dominant ants are the principal consumer of this sort of trophic resource [29]. Such phenomena may even explain the formation of large mosaic type of territories in the canopies of tropical native and cultivated forests [11, 26, 27].

It is common knowledge that the number of ants foraging in the canopy is proportional to the amount of resources, as sources of nectar or exudates [11, 15, 21]. When they engage in trophobiosis, they assume a primary consumers status, and their occurrence is equal or larger than the other herbivores in its community. In the absence of sugary secretions, predation becomes the primary feeding behaviour; then the situation is reversed, and the ants assume low occurrence [6, 10, 29]. Other studies also showed that the increased aggressiveness and occurrence of ants are related to the availability of honeydew sources [4, 5, 11, 21, 30].

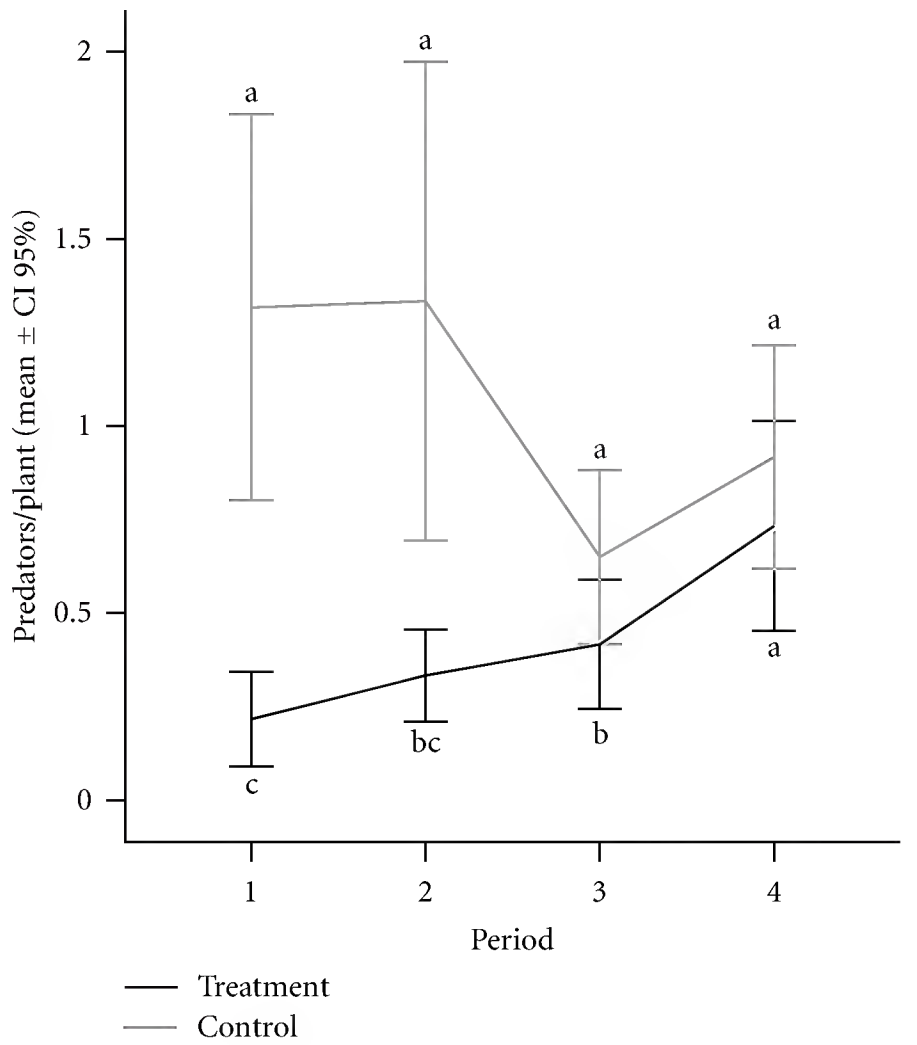
Blüthgen et al. [15] showed that the ant species richness in host plants with trophobiosis was lower compared with plants carrying extrafloral nectaries or with non-myrmecophilous plants. Dominant ants are considered the most aggressive species, and they are also numerous in other food sources such an extrafloral nectaries [15]. This behaviour, along with high territoriality, affects negatively the ant species richness due to the exclusion of competing species, reduction of co-occurrences, and avoiding species replacement [11, 15]. Thus, the same pattern we observed in the system *M. obovata*-*C. pugionata*-ants was similar to



(a)



(b)



(c)

Figure 6: Mean occurrence of the whole nonants arthropods (a) and for herbivores (b) and predators (c) separately, registered in the experimental groups (lines) at each period (x axis). These arthropods respond positively to decrease occurrence of ants and consequently the absence of *C. pugionata* (ANOVA: nonants arthropods: $F(3;354) = 3.62$; $P < 0.01$; Herbivores: $F(3;354) = 3.21$; $P < 0.01$; Predators: $F(3;354) = 4.66$; $P < 0.01$). Different letters indicate significant differences according to post hoc test (LSD: $P < 0.05$).

different ecosystems with other types of myrmecophily [11, 28, 31, 32].

The interaction between trophobiont herbivores and dominant ant species may alter the entire food web [9, 33–35]. This could generate benefits for the host plants and mutualistic treehoppers by killing or repelling their natural enemies [29, 30, 35]. Such protection has consequences as reducing of herbivory [17, 36] and increasing plant fitness [24, 37]. Rosumek et al. [22] and Schoereder et al. [28] reviewed the ant protective role and the importance of nectaries and trophobiont hemipterans, showing that most studies presented a negative correlation between ants and herbivores mainly when myrmecophilous resources are involved. In this interaction system, the host plant is the one taken some costs, due to the constant sap-sucking activity from the hemipterans, plus the damage caused by opportunistic phytopathologies [38]. However, such cost may be compensated by the protective action from the ants against the chewing herbivores [8, 24]. Further studies are needed to evaluate the actual damage caused by *C. pugionata* and its effects on *M. obovata* fitness.

The establishment of trophobioses can also result in an ecological cascade with further evolutionary consequences for the entire community [28]. For example, one quarter of the species and one third of the plant individuals in the Brazilian Cerrado have extrafloral nectaries and/or trophobiont hemipterans [39]. In addition, a third of the ant species that feed in the Cerrado vegetation are typically sugary exudates consumer [28, 40–42]. In one hand, the low, accessible tree crowns in the cerrado, montane forests, and rupestrian field's vegetation allow such type of investigation and a precise quantitative analysis of the actual availability of such resources. Therefore, the investigation of how relevant trophobiosis would be for other tropical ecosystems is constrained mostly by restricted canopy access to a large enough area in order to produce comparative results. The present work was developed in relatively low and also accessible canopies of a secondary montane forest. This unique situation allowed us to explore the very mechanisms of the interactions plant-hemipteran-ants in this distinct forest habitat, where its relevance and ecological strength seem similar to that previously observed in the Cerrado.

Our study provides empirical evidence of bottom-up control exerted by the sap-sucking hemiptera *C. pugionata* in their community, an effect clearly perceptible in the ant assemblage. By means of the trophobioses, the mutualistic membracids are capable of altering not only the ant species richness and occurrence but also the structure of the entire arthropod community associated with its host plant. In the tritrophic system formed by *M. obovata*, *C. pugionata,* and ants, any species seem to benefit from the interaction [17]. In conclusion, myrmecophillous resources produced by several animals and plant species can be key elements for the configuration of hierarchically structured ant assemblage, as observed coherently among different ecosystems, and then affect all the arboreal community associated [3, 11, 14, 15, 28, 40].

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Review Article

Chemical Recruitment for Foraging in Ants (Formicidae) and Termites (Isoptera): A Revealing Comparison

Klaus Jaffe, Solange Issa, and Cristina Sainz-Borgo

Departamento de Biología de Organismos, Universidad Simón Bolívar, Caracas 1080, Venezuela

Correspondence should be addressed to Klaus Jaffe, kjaffe@usb.ve

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All termites secrete trail pheromones from their sternal gland, whereas ants use a variety of glands for this purpose. This and the diversity of chemical compounds that serve as trail pheromones among ants, and the uniformity of chemicals among termite trails, suggest a different evolutionary historical dynamics for the development of chemical mass recruitment in both taxa. Termites in addition show pheromonal parsimony. This suggest a single evolutionary origin of pheromone trails in Isoptera, whereas chemical mass recruitment among Formicidae seems to have evolved many times and in different ways. Despite these very different evolutionary histories, both taxa evolved chemical recruitment systems involving attractants and orientation signals, and at least two divergent decision making system for recruitment. This evolutionary analogy suggests that chemical mass recruitment is constraint by fundamental physical dynamic laws. Artificial intelligence including "mass intelligence" and "ant intelligence", emulates mass recruitment in interacting virtual agents in search of optimal solutions. This approach, however, has copied only the "Democratic" recruitment dynamics with a single compound pheromone. Ant and termite evolution shows more sophisticated recruitment dynamics which, if understood properly, will improve our understanding of nature and applications of artificial "swarm intelligence".

1. Introduction

One of the great advantages of society is the use of large numbers of individuals to perform tasks that a lonely individual is unable to perform [2, 3]. One of the most studied group task in social insects is recruitment for food retrieval, after an individual discovers a food source that is much larger than what it can handle on its own. Some of the communication signals modulating this recruitment are based on auditory or visual signals, but the most important communication signal used in recruitment, in the great majority of ant and termite species, is chemical. In recruitment to food, these signals are at least of two different kinds as first detailed for ants [4] : one used to orient workers to the food source, that is trail pheromones; another to attract workers to the trail and thus to the food source, that is attractants for food recruitment. Some species use chemicals for only one of these signals and signal the other function by means of tactile or acoustic signals. An illustrative intermediate recruitment system is called "Tandem Running" [5], where the scout physically carries a nestmate to the food source. In tandem calling [6], the recruiting workers lead nestmates to the newly discovered food source by physically guiding them to the source, sometimes using chemical trails to help orientate to the food. Other species lay chemical trails that fulfill both functions, requiring different chemicals for attracting and orienting ants [7, 8]. These intermediate stages in the evolution of chemical mass recruitment, starting from individual foraging, allow us to suggest phylogenies for recruitment systems illuminating the possible evolutionary history of chemical mass recruitment. Such comparisons suggest that the evolution of chemical recruitment seems to have happened several times, at least in ants [9].

Termites seem also to use both type of chemicals, attractants and orientation signals, in their foraging trails [10, 11], although the details of the chemical communication system used by termites are less well known than in ants.

As both, ants and termites, are terrestrial and arboreal, and that both use chemical mass recruitment, we can compare the different chemical recruitment systems known

among ants and among termite in order to extract some general rules.

2. Methods

We explored the existing literature collected by Pherobase [1] for publications on trail pheromones for ants and Isoptera and by Bordereau and Pasteels [12] for additional data on Isoptera. Pherobase, among many other things, reports for Isoptera and for Formicidae all publications mentioning chemicals that had been related to trail pheromones by the author of the publication. Pherobase provides, if available, the exact molecular structure of the chemical and the link to the reference where the trail pheromone was published, grouped by taxonomic or by chemical criteria. Thus, for more details of all references indicated in the Tables, the reader should consult Pherobase for ants and Isoptera, and Bordereau and Pasteels for Isoptera.

3. Results

3.1. Ants. Reports of the chemical nature of the communication signal used for recruitment in ants revealed an interesting pattern of chemical compounds. The summary of available data for ants is presented in Table 1. This table shows that, in many cases, the various compounds produced by a single species are very similar as they constitute small variants of a common chemical skeleton, as is the case for *Monomorium pharaonis*. We suggest that this might be due to the fact that in the biochemical process leading to the synthesis of one or a few active compounds, other chemicals are produced in the process. Indirect evidence for this suggestion comes from other insects where it was shown that synthesizing pure chemicals in pheromone secreting glands is very difficult, if not impossible [13]. In other cases, an adaptive purposeful chemical diversity seems to be present, as chemicals from completely different biochemical pathways are produced as a substrate for the chemical recruitment signal. This is the case for the *Atta* and *Acromyrmex* species and *Daceton armigerum*. In these cases, as shown in Table 1, some compounds have high carbon numbers and low volatility, and others have high volatility, appropriate for the fulfillment of different communication functions such as orientation and attraction.

The chemical survey presented in Table 1 reveals that the pattern of chemical compounds related to trail pheromones in ants correlates with what we know about the decision making behavior used during chemical mass recruitment to food [14]. We know that ants use either one of two decision making systems regulating chemical mass recruitment. The "Democratic" mass recruitment was described in detail for *Solenopsis invicta* [15] and the "Autocratic" system first described for *Atta cephalotes* [16]. The main difference is that in the Democratic system, all workers eventually perform all tasks as in *Solenopsis*; in the Autocratic system, workers specialize either in scouting or in food retrieval [17], as in *Atta*. The Democratic recruitment system is adapted for fast recruitment towards ephemeral food sources. Here all workers participating in the recruitment process have the same responsibility and add a fixed amount of recruitment pheromone to the trail. The more trail pheromone, the stronger the signal, the more workers are recruited. This leads to an increase in the workforce allowing engaging the maximum worker strength in the shortest possible time, so as to collect a scarce recourse (a recently discovered dead cockroach for example) before a competitor does.

The Autocratic recruitment system is adapted for the simultaneous exploitation of a diversity of durable food sources. Here workers specialize in chemical communication or in food retrieval. Communication specialists then visit different food sources and signal the palatability, quality, or quantity of a food source with varying levels of chemical concentrations. Thus, a very good food source will trigger trail laying with plenty of an attractive chemical, whereas food sources of low quality will be signaled with low amounts of this chemical laid on the trail. This system allows for the fine tuning of sophisticated recruitment activity such as described for several *Atta* species, where one group of workers recruit nestmates to the tree canopy where they cut large leaves at their base, so that they fall whole to the ground. There, another group of workers is recruited to each of the leaves that accumulate on the ground, where the workers cut the leave in smaller pieces and transport these pieces to intermediate sites, from where another group of workers transport the leaf fragments to the nest [18].

In both cases, the trail needs to be marked with a chemical that will orient workers towards the food source. If the food source is ephemeral in its existence, an efficient chemical mark does not need to last long. As soon as the food has been collected, the chemical evaporates and the trail disappears. For the simultaneous exploitation of several food sources, however, several longer lasting chemical signals could be very useful, as the source could be revisited fast after spots of inactivity due to rain, heat, cold, or other daily rhythmic patterns. Yet a long lasting chemical signal is not appropriate if it has to work also as an attractant, as any changes in the required workforce will take a long time to achieve if the long lasting chemical need, to evaporate first. Therefore in this later case, highly volatile chemicals, together with some of low volatility, are required to modulate recruitment. Species using chemicals to only attract or orient ants need only one—or a few—chemical compounds to perform this function, whereas species using chemical trails for both, attraction and orientation of nestmates, have to produce a range of chemicals for these two purposes.

As Table 1 shows, most ant species seem to use a few compounds as trail pheromone. Only 14 out of 57 species (25%) seem to use more than 3 chemicals, and only 10% of the species listed use six or more compounds. The use of a few compounds corresponds well to Tandem Calling or even to a Democratic recruitment system. In contrast, species such as the leaf cutting and fungus growing ants *Atta, Trachymyrmex,* and *Acromyrmex* secrete over six different chemicals on their trails. Other species using the Democratic system, such as *Solenopsis*, seem to produce much simpler trail pheromones from the standpoint of chemical diversity of compounds. The trail pheromone composition of

Table 1: Chemical compounds reported in trail pheromones of ants. All data were extracted from Pherobase [1].

Myrmicinae
Acromyrmex octospinosus
Cross JH 1982. J. Chem. Ecol. 8 : 1119
me-4me-pyrrole-2-carboxylate
2me5me-3-ethylpyrazine
3me5me-2-ethylpyrazine
Acromyrmex subterraneus subterraneus
Do Nascimento RR 1994. J. Chem. Ecol. 20 : 1719
me-4me-pyrrole-2-carboxylate
Aphaenogaster albisetosus
Hölldobler B 1995 J. Insect Physiol. 41 : 739
4Sme-7-3Kt
4Rme-7-3Kt
Aphaenogaster cockerelli
Hölldobler B 1995. J. Insect Physiol. 41 : 739
1R-phenylethanol
4Sme-7-3Kt
Aphaenogaster rudis
Attygalle AB 1998b Naturwissenschaften 85 : 38
anabasine
anabaseine
2,3-bipyridyl
isopentyl-2-phenylethylamine
Atta bisphaerica
De Oliveira JS 1990 An. Soc. Entomol. Brasil 19 : 145
me-4me-pyrrole-2-carboxylate
2me5me-3-ethylpyrazine
2-phenylacetic acid
bornylene
8OH
Atta cephalotes
Evershed RP 1983 Insect Biochem. 13 : 469
me-4me-pyrrole-2-carboxylate
2me5me-3-ethylpyrazine
2Ald
Riley RG 1974b J. Insect Physiol. 20 : 651
me-4me-pyrrole-2-carboxylate
Atta laevigata
De Oliveira JS 1990 An. Soc. Entomol. Brasil 19 : 145
me-4me-pyrrole-2-carboxylate
2-phenylacetic acid
bornylene
8OH
Atta sexdens
Robinson SW 1978 Bull. Entomol. Res. 68 : 159
me-4me-pyrrole-2-carboxylate
Atta sexdens rubropilosa
Evershed RP 1983 Insect Biochem. 13 : 469
me-4me-pyrrole-2-carboxylate

Table 1: Continued.

Myrmicinae
2me5me-3-ethylpyrazine
2Ald
Cross JH 1979 J. Chem. Ecol. 5 : 187
2me5me-3-ethylpyrazine
methyl phenylacetate
ethyl phenylacetate
me-4me-pyrrole-2-carboxylate
Atta sexdens sexdens
Billen J 1992 Ethol. Ecol. Evol. 4 : 197
2me5me-3-ethylpyrazine
me-4me-pyrrole-2-carboxylate
Evershed RP 1983 Insect Biochem. 13 : 469
me-4me-pyrrole-2-carboxylate
2me5me-3-ethylpyrazine
2Ald
Atta texana
Tumlinson JH 1972b J. Insect Physiol. 18 : 809
me-4me-pyrrole-2-carboxylate
Sonnet PE 1972 J. Agric. Food Chem. 20 : 1191
me-4me-pyrrole-2-carboxylate
Crematogaster castanea
Morgan ED 2004 Chemoecology 14 : 119
R-dodecan-2-ol
Daceton armigerum
Morgan ED 1992 J. Chem. Ecol. 18 : 2161
2me5me-pyrazine
2me3me5me-pyrazine
2me5me-3-ethylpyrazine
delta9-23Hy
23Hy
delta9-25Hy
Eutetramorium mocquerysi
Tentschert J 2000 Naturwissenschaften 87 : 377
2me3me-5-2-methylpropylpyrazine
Manica rubida
Attygalle AB 1986a Physiol. Entomol. 11 : 125
2me5me-3-ethylpyrazine
Mayriella overbecki
Kohl E 2000 Naturwissenschaften 87 : 320
me-2-hydroxy-6me-benzoate
Messor bouvieri
Jackson BD 1989a Experientia 45 : 487
anabasine
2me5me-3-ethylpyrazine
Messor capensis
Brand JM 1993 J. Chem. Ecol. 19 : 1315
anabasine
anabaseine

Table 1: Continued.

Myrmicinae
Messor ebeninus
Coll M 1987 Z. Naturforsch. C 42 : 1027
anabasine
Metapone madagascarica
Hölldobler B 2002 Chemoecology 12 : 147
me-pyrrole-2-carboxylate
Metapone madagascarica
Hölldobler B 2002 Chemoecology 12 : 147
me-pyrrole-2-carboxylate
Monomorium pharaonis Linnaeus
Edwards JP 1978 Ann. Appl. Biol. 89 : 395
3-butyl-5me-octahydroindolizine
Ritter FJ 1977b Crop Prot. Agents : 195
monomorine I
Ritter FJ 1977a Tetrahedron Lett. 30 : 2617
faranal
Ritter FJ 1975b Uni. Dijon : 99
monomorine I
monomorine II
monomorine III
monomorine IV
monomorine V
Pheidole pallidula
Ali MF 1988c Physiol. Entomol. 13 : 257
2me5me-3-ethylpyrazine
Pogonomyrmex barbatus
Liu Y 2002 Fenxi Huaxue 47 : 369
2me3me5me-pyrazine
2me5me-3-ethylpyrazine
2me5me-pyrazine
Hölldobler B 2001 J. Insect Physiol. 47 : 369
2me5me-pyrazine
2me3me5me-pyrazine
2me5me-3-ethylpyrazine
Pogonomyrmex maricopa
Hölldobler B 2001 J. Insect Physiol. 47 : 369
2me5me-pyrazine
2me3me5me-pyrazine
2me5me-3-ethylpyrazine
Pogonomyrmex occidentalis
Hölldobler B 2001 J. Insect Physiol. 47 : 369
2me5me-pyrazine
2me3me5me-pyrazine
Pogonomyrmex rugosus
Hölldobler B 2001 J. Insect Physiol. 47 : 369
2me5me-pyrazine
2me3me5me-pyrazine
2me5me-3-ethylpyrazine
Solenopsis invicta
Van der Meer RK 1983 Fla. Entomol. 66 : 39

Table 1: Continued.

Myrmicinae
Z,E-alpha-farnesene
E,E-alpha-farnesene
Z,E-alpha-homofarnesene
Z,Z-alpha-homofarnesene
17Hy
Z,Z,Z-allofarnesene
Williams HJ 1981b Experientia 37 : 1159
Z,Z,Z-allofarnesene
Van der Meer RK 1981 Tetrahedron Lett. 22 : 1651
Z,E-alpha-farnesene
E,E-alpha-farnesene
Z,E-alpha-homofarnesene
Z,Z-alpha-homofarnesene
Tetramorium caespitum
Attygalle AB 1984J. Chem. Ecol. 10 : 1453
2me5me-pyrazine
2me5me-3-ethylpyrazine
Attygalle AB 1983b Naturwissenschaften 70 : 364
2me5me-pyrazine
2me5me-3-ethylpyrazine
Tetramorium impurum
Morgan ED 1990 J. Chem. Ecol. 16 : 349
me-2-hydroxy-6me-benzoate
Morgan ED 1987 Naturwissenschaften 74 : 596
me-2-hydroxy-6me-benzoate
Tetramorium meridionale Emery
Jackson BD 1990c Naturwissenschaften 77 : 294
2me-pyrazine
2me5me-pyrazine
2me3me5me-pyrazine
2me5me-3-ethylpyrazine
Formicinae
Camponotus atriceps
Haak U 1996 Chemoecology 7 : 85
6-butyl-tetrahydro-3me5me-pyran-2Kt
nerolic acid
Camponotus balzani
Kohl E 2003 Chemoecology 13 : 113
8-hydroxy-3me5me7me-isochromanone
Camponotus castaneus
Kohl E 2003 Chemoecology 13 : 113
6-butyl-tetrahydro-3me5me-pyran-2Kt
Camponotus floridanus
Haak U 1996 Chemoecology 7 : 85
6-butyl-tetrahydro-3me5me-pyran-2Kt
nerolic acid
Camponotus herculeanus
Bestmann HJ 1999 Chem. Eur. J. 5 : 2984
2Sme4Rme5S-5-hexanolide
Payne TL 1975 Ann. Entomol. Soc. Am. 68 : 385

Table 1: Continued.

Formicinae
me-2-hydroxy-6me-benzoate
mellein
Hölldobler B 1965 Z. vergl. Physiol. 50 : 551
me-2-hydroxy-6me-benzoate
mellein
10me-12Acid
Camponotus inaequalis
Bestmann HJ 1997 Angew. Chem. 36 : 395
3,4-dihydro-8-hydroxy-3me5me7me-isocoumarin
Camponotus ligniperda
Bestmann HJ 1999 Chem. Eur. J. 5 : 2984
2Sme4Rme5S-5-hexanolide
Bestmann HJ 1999 Chem. Eur. J. 5 : 2984
2Sme4Rme5S-5-hexanolide
Camponotus rufipes
Uebler E 1995 Naturwissenschaften 82 : 523
mellein
Camponotus sericeiventris
Kohl E 2003 Chemoecology 13 : 113
8-hydroxy-3me5me7me-isochromanone
Camponotus silvicola
Uebler E 1995 Naturwissenschaften 82 : 523
8-hydroxy-3me5me7me-isochromanone
Camponotus socius
Kohl E 2001 Chemoecology 11 : 67
2Sme4Rme5S-5-hexanolide
2,3-dihydro-3,5-dihydroxy-6me-pyran-4Kt
Bestmann HJ 1999 Chem. Eur. J. 5 : 2984
2Sme4Rme5S-5-hexanolide
Formica rufa
Bestmann HJ 1992 Angew. Chem. 31 : 795
R-mellein
Lasius fuliginosus
Kern F 1997 J. Chem. Ecol.23 : 779
mellein
2,3-dihydro-3,5-dihydroxy-6me-pyran-4Kt
Akino T 1996 Jap. J. Appl. Entomol. Zool.40 : 233
caproic acid
enanthic acid
caprylic acid
pelargonic acid
caprinic acid
lauric acid
Huwyler S 1975 J. Insect Physiol. 21 : 779
caproic acid
enanthic acid
caprylic acid
pelargonic acid
caprinic acid
lauric acid

Table 1: Continued.

Formicinae
Lasius niger
Bestmann HJ 1992 Angew. Chem.31 : 795
3,4-dihydro-8-hydroxy-3me5me7me-isocoumarin
Linepithema humile
Greenberg L 2000 J. Econ. Entomol. 93 : 119
Cordova YL 1998 Eur. J. Entomol.95 : 501
sulcatone
sulcatol
13-2Kt
Z9-16Ald
Van Vorhis Key SE 1982 J. Chem. Ecol. 8 : 3
Z9-16Ald
Cavill GWK 1979 Experientia 35 : 989
Z9-16Ald
Dolichoderinae
Dolichoderus thoracicus
Attygalle AB 1998a Naturwissenschaften 85 : 275
Z9-18Ald
Z9-16Ald
Tapinoma simrothi
Simon T 1991 Insectes Soc. 38 : 17
iridodial
iridomyrmecin
Ectatomminae
Ectatomma ruidum
Bestmann HJ 1995 Naturwissenschaften 82 : 334
geranylgeraniol acetate
geranylgeraniol
Gnamptogenys striatula
Blatrix R 2002 J. Chem. Ecol. 28 : 2557
4-methylgeraniol
bishomogeraniol
E2,4S6-3me4me7me-octadienyl decanoate
E2,4S6-3me4me7me-octadienyl dodecanoate
Ponerinae
Leptogenys diminuta
Kern F 1993 Naturwissenschaften 80 : 424
3R4Sme-heptan-3-ol
Attygalle AB 1991b Naturwissenschaften 78 : 90
isogeraniol
Attygalle AB 1988c Naturwissenschaften 75 : 315
3R4Sme-heptan-3-ol
isogeraniol
Leptogenys peuqueti
Janssen E 1997b Naturwissenschaften 84 : 122
1-ethyl-4me-heptyl acetate
1-isopropyl-4me-heptyl acetate
1-propyl-4me-heptyl acetate
4me-dodecan-7-ol
3me9me-dodecan-6-ol

Table 1: Continued.

Ponerinae
1-pentyl-4me-heptyl acetate
4me-tridecan-7-ol
4me10me-tridecan-7-ol
4me-tetradecan-7-ol
3me-hexyl-4me-heptyl acetate
3me-hexyl-octyl acetate
heptyloctyl acetate
4me-hexadecan-7-ol
3me-hexyl-decyl acetate
Megaponera foetens
Janssen E 1995 J. Chem. Ecol. 21 : 1947
dimethyluracil
actinidine
Longhurst C 1979 J. Chem. Ecol. 5 : 703
1me2me-disulfane
1me3me-trisulfane
benzyl methyl sulfane
Longhurst C 1979 J. Chem. Ecol. 5 : 703
11Hy
13Hy
Pachycondyla tarsata
Janssen E 1999 Chemoecology 9 : 9
17-9Kt
Rhytidoponera metallica
Meinwald J 1983Naturwissenschaften 70 : 46
isogeraniol
3-hydroxybenzaldehyde
Aenictinae (dorylinae)
Aenictus sp
Oldham NJ 1994, Experientia 50 : 763
methyl anthranilate
methyl nicotinate

Solenopsis invicta recalls the case of *Monomorium pharaonis* discussed above. Although over 5 different chemicals can be recognized in Table 1, all these chemicals have the same chemical skeleton. Thus, the Autocratic chemical recruitment system could be associated to a more advanced chemical signaling. The case of the hunting and recruiting foragers of *Daceton armigerum* [19] that use a multitude of recruitment strategies is interesting. Table 1 shows that its trail pheromone has many chemical compounds, hinting to a sophisticated diverse chemical communication system.

Many ant species in the subfamily Myrmicinae with large colonies and a sophisticated social structure, use carboxylates and pyrazines to lay their pheromone trail. These are semivolatile compounds. The Myrmicinae, *Atta,* and *Acromyrmex*, for example, need to constantly recruit many workers to supply big colonies with a great quantity of leaves which they use as a substrate to grow their fungus. In contrast, ants with less developed societies living in smaller colonies, such as species of the subfamily Ponerinae, use alcohols and acetate, which are more volatile and thus might serve as chemical attractants to trigger foraging to collect ephemeral food sources. Ponerinae individuals feed opportunistically on dispersed food items. This requires quick recruitment of workers, and, as a consequence, the compounds of the pheromone trail are more volatile and less permanent in time, compared to the carboxylates of the leaf cutter ants. In some species of Ponerinae, chemical trails also regulate nest moving [20].

The Formicinae ants are mostly predators but differ from Ponerinae by their greater social complexity, larger colonies, and more diverse worker castes or polymorphism. The trail pheromones of Formicinae species use a mix of compounds that are more complex than that of Ponerinae, probably due to a more elaborate recruitment system. Table 1 reflects this showing among Formicinae, compounds with elevated molecular weights, such as mullein, in addition to compound of low molecular weight and probably low volatility. Formicinae trail pheromone chemistry seems to be closer to the Myrmicinae than the Ponerinae. This suggests trails with both short-term attractant and long-term orientation function. In the case of Dolichoderinae species, the information is scarcer. In the Argentine ant, *Linepithema humile*, a tramp species with supercolonies of hundreds of thousands of workers, the trail pheromone has short-chain volatile aldehydes, suggesting a foraging strategy with fast short term bouts of recruitment. The continuous reinforcement of a trail made with short lasting volatiles can last long if it is reinforced by hundreds of workers.

3.2. Termites. Termite species also show diverse ecological life types. We know species that live and feed in the same piece of wood, and species that have their nest separated from their food source [21]. But even the "one-piece" life type species possess trail pheromones which they use to recruit workers for defense or nest moving. Termites of "one-piece" life type do not require orientation systems a priori. Secretions of their sternal gland are considered to function in the recruitment of nestmates to source disturbance within the nest. These termites might also use trail following pheromones to colonize new food sources to where they move their nest [22, 23]. Most termites forage on relatively durable food sources containing cellulose. In addition, most termite species forage on several food sources simultaneously, suggesting a recruitment system closer to the above described Autocratic chemical recruitment system, which seem to be the case in the only termite species where this has been explored so far [24]. Table 2 presents what we know about the chemicals used in trail pheromones by termites. The available data shows that pheromone trails among each termite species are constructed with one or a few compounds among a total of 8 chemicals. For the families where chemical trail pheromones have been reported, the Rhinotermitidae, Termitidae, and Kalotermitidae seem to use mainly neocembrene and a dodecatrienol; *Nasutitermes corniger* uses in addition to these two compounds trinervitatriene; whereas Mastotermitidae and Termopsidae use a trimethylundecadienol for trail following. That is, all trail pheromones in Isoptera are synthesized from a much

Table 2: Chemical compounds reported from trail pheromones of termites. All data were extracted from Pherobase [1], and from Bordereau and Pasteels [12].

Mastotermitidae
Mastotermes darwiniensis
Sillam-Dussès, D. et al. 2007. J Chem. Ecol 33 : 1960–1977
(*E*)-2,6,10-trimethyl-5,9-undecadien-1-ol
Termopsidae
Porotermitinae
Porotermes adamsoni
Sillam-Dussès, D. et al. 2007. J Chem. Ecol 33 : 1960–1977
(*E*)-2,6,10-trimethyl-5,9-undecadien-1-ol
Stolotermitinae
Stolotermes victoriensis
Sillam-Dussès, D. et al. 2007. J Chem. Ecol 33 : 1960–1977
(*E*)-2,6,10-trimethyl-5,9-undecadien-1-ol
Termopsinae
Zootermopsis angusticollis
Greenberg SL 1986 Int. J. Insect Morphol. Embryol. 15 : 283
Heneicosano
Tricosane
Bordereau C. et al. 2010 Biol J Linn Soc 100 : 519–530
4,6-dimethyldodecanal
Zootermopsis nevadensis
Karlson P. et al. 1968 J. Insect Physiol. 14 : 1763
n-Hexanoic acid
Caproic acid
Bordereau, C. et al. 2010 Biol J Linn Soc 100 : 519–530
4,6-Dimethyldodecanal
Kalotermitidae
Cryptotermes brevis
Sillam-Dussès D. et al. 2009 Chemoecology19 : 103–108
(*Z*)-dodec-3-en-1-ol
Cryptotermes darlingtonae
Sillam-Dussès D. et al. 2009 Chemoecology 19 : 103–108
(*Z*)-dodec-3-en-1-ol
Cryptotermes pallidus
Sillam-Dussès D. et al. 2009 Chemoecology 19 : 103–108
(*Z*)-dodec-3-en-1-ol
Incisitermes tabogae
Sillam-Dussès D. et al. 2009 Chemoecology 19 : 103–108
(*Z*)-dodec-3-en-1-ol
Kalotermes flavicollis
Klochkov and Zhuzhikov 1990. Advances in life science. Birkhäuser, Basel, pp 41–43
Nonanol
Decanol
Undecanol
dodecanol

Table 2: Continued.

Kalotermitidae
Sillam-Dussès D. et al. 2009 Chemoecology 19 : 103–108
(*Z*)-dodec-3-en-1-ol
Neotermes holmgreni
Sillam-Dussès D. et al. 2009 Chemoecology 19 : 103–108
(*Z*)-dodec-3-en-1-ol
Postelectrotermes howa
Sillam-Dussès et al. 2009 Chemoecology 19 : 103–108
(Z)-dodec-3-en-1-ol
Procryptotermes falcifer
Sillam-Dussès D. et al. 2009 Chemoecology 19 : 103–108
(*Z*)-dodec-3-en-1-ol
Procryptotermes leewardensis
Sillam-Dussès D. et al. 2009 Chemoecology 19 : 103–108
(*Z*)-dodec-3-en-1-ol
Rhinotermitidae
Prorhinotermitinae
Prorhinotermes canalifrons
Sillam-Dussès D. et al. 2005 Chemoecology 15 : 1–6
Neocembrene A
Prorhinotermes simplex
Sillam-Dussès D. et al. 2005 Chemoecology 15 : 1–6
Neocembrene
Sillam-Dussès D. et al. 2009 J. Insect Physiol 55 : 751-757
Neocembrene A
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Coptotermitinae
Coptotermes formosanus
Tokoro M. et al. 1994 J. Chem. Ecol. 20 : 199
(*Z*,*E*,*E*)-dodeca-3,6,8-trien-1-ol
Coptotermes gestroi
Arab A. et al. 2004 Sociobiology 43 : 377
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Sillam-Dussès D. et al. 2006 Proceedings XV IUSSI. Washington 100-101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Heterotermitinae
Heterotermes tenuis
Arab A. et al. 2004 Sociobiology 43 : 377
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Sillam-Dussès D. et al. 2006 Proceedings XV IUSSI. Washington 100-101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Reticulitermes flavipes
Howard R. et al. 1976 J. Chem. Ecol. 2 : 147
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Matsumura F. et al. 1968 Nature 219 : 963
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol

Table 2: Continued.

Rhinotermitidae
Reticulitermes hesperus
Zhong CM 1979 Sci. Silvae Sin. 15 : 15
Z3-4-phenyl-4OH
Reticulitermes lucifugus grassei
Wobst B. et al. 1999 J. Chem. Ecol. 25 : 1305
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Reticulitermes santonensis
Wobst B. et al. 1999 J. Chem. Ecol. 25 : 1305
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Laduguie N. et al. 1994 J. Insect Physiol. 40 : 781
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Reticulitermes speratus
Tokoro M. et al. 1990 J. Chem. Ecol. 16 : 2549
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Yamaoka R. et al. 1987 J. Chromatogr. 399 : 259
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Reticulitermes tibialis
Bernklau EJ 2005 J. Econ. Entomol. 98 : 476
CO2
Zhong CM 1979 Sci. Silvae Sin. 15 : 15
Z3-4-phenyl-4OH
Howard R. et al. 1976 J. Chem. Ecol. 2 : 147
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Reticulitermes virginicus
Howard R. et al. 1976 J. Chem. Ecol. 2 : 147
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Tai A. et al. 1969 J. Org. Chem. 34 : 2180
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Matsumura F. et al. 1968 Nature 219 : 963
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Rhinotermitinae
Rhinotermes marginalis
Sillam-Dussès D. et al. 2006 Proc. XV Congress IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Schedorhinotermes lamanianus
Sillam-Dussès D. et al. 2006 Proc. XV Congress IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Termitidae
Macrotermitinae
Ancistrotermes pakistanicus
Robert A. et al. 2004 Naturwissenschaften 91 : 34–39
(*Z*,*Z*)-dodeca-3,6-dien-1-ol
Macrotermes annandalei
Peppuy A. et al. 2001 Insectes Soc. 48 : 245
(*Z*)-dodec-3-en-l-ol
Peppuy A. et al. 2001 J. Insect Physiol. 47 : 445

Table 2: Continued.

Termitidae
(*Z*)-dodec-3-en-l-ol
Macrotermes barneyi
Peppuy A. et al. 2001 Insectes Soc. 48 : 245
(*Z*)-dodec-3-en-l-ol
Macrotermes bellicosus
Peppuy A. et al. 2001 Insectes Soc. 48 : 245
(*Z*)-dodec-3-en-l-ol
Macrotermes subhyalinus
Peppuy A. et al. 2001 Insectes Soc. 48 : 245
(*Z*)-dodec-3-en-1-ol
Odontotermes formosanus
Deng XJ. et al. 2002 Acta Entomol. Sin. 45 : 739
(*Z*,*Z*)-dodeca-3-6-dien-1-ol
Du TY 1982 Acta Entomol. Sin. 25 : 172
(*Z*,*Z*)-dodeca-3-6-dien-1-ol
Odontotermes hainanensis
Peppuy A. et al. 2001 Insectes Soc. 48 : 245
(*Z*)-dodec-3-en-1-ol
Odontotermes maesodensis
Peppuy A. et al. 2001 Insectes Soc. 48 : 245
(*Z*)-dodec-3-en-1-ol
Pseudacanthotermes militaris
Bordereau C. et al. 1993 Actes Coll. Insectes Soc. 17 : 2177
(*Z*,*Z*,*E*)-dodeca-3,6,8-trienol-1-ol
Pseudacanthotermes spiniger
Bordereau C. et al. 1991 J. Chem. Ecol. 17 : 2177
(*Z*,*Z*,*E*)-dodeca-3,6,8-trienol-1-ol
Termitinae
Cubitermes sp.
Sillam-Dussès D. et al. 2006 Proc. IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Drepanotermes perniger
Sillam-Dussès D. et al. 2006 Proc. IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Termes hispaniolae
Sillam-Dussès D. et al. 2006 Proc. IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Amitermes evuncifer
Kotoklo E. et al. 2010 Sociobiology 55 : 1-10
Dodecatrienol
Neocembrene A
Syntermitinae
Cornitermes bequaerti
Sillam-Dussès D. et al. 2006 Proc. IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol

Table 2: Continued.

Termitidae
Cornitermes cumulans
Sillam-Dussès D. et al. 2006 Proc. IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Cornitermes snyderi
Sillam-Dussès D. et al. 2006 Proc. IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Syntermes grandis
Sillam-Dussès D. et al. 2006 Proc. IUSSI, Washington, DC, 100–101
(*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol
Nasutitermitinae
Constrictotermes cyphergaster
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Nasutitermes corniger
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Trinervitatriene
Nasutitermes diabolus
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Nasutitermes ephratae
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Nasutitermes exitiosus
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Birch AJ 1972 J. Chem. Soc. 1 : 2653
Neocembrene-A
Nasutitermes graveolus
Moore P 1966 Nature 211 : 746–747
Neocembrene-A
Birch A. et al. 1972 J Chem Soc Perkin Trans 1 : 2653–2658
Neocembrene-A
Nasutitermes guayanae
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Nasutitermes kemneri
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol

Table 2: Continued.

Termitidae
Neocembrene-A
Nasutitermes lujae
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Nasutitermes walkeri
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Neocembrene-A
Nasutitermes voeltzkowi
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Trinervitermes bettonianus
McDowell PG and Oloo G. 1984 J. Chem. Ecol. 10 : 835
Neocembrene-A
Trinervitermes geminatus
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A
Trinervitermes trinervoides
Sillam-Dussès D. et al. 2010 Biol. J. Linnean Soc. 99 : 20
Dodecatrienol
Neocembrene-A

conserved metabolic route eventually leading to a compound with the same carbon skeleton as that of a dodecatrienol, where two subfamilies have diverged somewhat from the rest in that they synthesize trimethylundecadienol instead of dodecatrienol as the rest of termite species does.

In the case of termites, volatile chemicals for modulating the recruitment of workers are most likely to be used outside the nest or outside the covered galleries. This seems natural if we take into account that most termites forage in galleries which orient workers to their food sources. Very volatile chemicals are of little use in closed environments where they cannot disperse. Long-lasting, low-volatility chemicals may be useful for trail orientation outside the nest and might form the substrate around which galleries are built [25]. Thus, other signals seem to be more appropriate here in modulating communication. Many termite species add feces, saliva, and other secretions to the trail. This explains foraging trails that are reused after several years. A different situation may occur among termites foraging on grasses or leaves in open habitats and foraging at the end of their galleries where they can display very sophisticated foraging and recruitment strategies [26]. When recruitment behavior was explored in an open setting in a Nasutitermitinae [24], the decision making systems used to modulate the recruitment dynamics conformed to the Autocratic kind described for *Atta*.

4. Discussion

This paper is based only on published reports, and many more compounds used as trail pheromones are surely to be discovered in the future. For example, it is very likely that *Atta texana* uses a larger pool of compounds as trail pheromones as that reported in Table 1, as it is unlikely to differ very much from other *Atta* species in this regard. Thus, results in the Tables are biased towards species that have drawn more attention from researchers. Another cautionary remark regards the assessment of volatility based on chemical structure alone. In general, compounds of the same kind of lower molecular weight are more volatile than the ones of higher molecular weight or longer carbon chains. Biologically relevant volatility, however, depends not only on the compound but also on the substrate on which the chemical is secreted, on its concentrations on the substrate, and on the humidity and temperature of the surrounding air. Thus, simple direct correlations between molecular weight, assumed volatility, and behavioral function of a compound should be avoided.

The work behind the literature used for this study, evidently, was not performed with our objectives in mind, but it is unlikely that methodological limitations explain the lack of more chemical compound associated with trail pheromones among termites than among ants. Despite many possible limitations of this study, the large extend of the research effort explored and the large number of species covered guarantee a minimum of robustness that makes drawing conclusion from these data reasonable.

Despite these and other limitations of this paper, we might suggest two basic trends: (1) evolutionary history of the evolution of ant and termite trails is very different, and (2) the dynamics of interacting individuals achieving a recruitment process mediated by chemicals follow basic rules.

4.1. Different Evolutionary Histories between Ants and Termites. The diversity of chemical structures among ant trail pheromones and the uniformity of chemical compounds among termite trails suggest a different evolutionary history for the development of chemical mass recruitment in both taxa. In termites, often trail pheromone compounds are synthesized also by other exocrine glands and are used as sex pheromones. This pheromonal parsimony seems to be characteristic of termites [12] and is not common among ants.

Chemical mass recruitment among ants seems to have evolved at least 8 times [9], whereas chemical mass recruitment among termites seems to be a more conservative phenomenon where all species seem to share a common ancestor that had already developed chemical recruitment. This explains also the large difference between ants and termites in the glands responsible for the secretion of the trail pheromones. Many different glands are used by different species among ants [27], whereas only the sternal gland is used by termites [12]. Another factor explaining this difference is the ecological diversity of ant species, each exploring different food source. Termites in contrast exploit more uniform ecological niches in their search for cellulose.

4.2. Basic Rules Govern the Recruitment Dynamics. The main conclusion from this study is that despite the fact that the evolutionary history of the chemical mass recruitment of ants and termites is different, a similar recruitment dynamics has evolved in both groups. This evolutionary analogy suggests that chemical mass recruitment is constraint by basic physical-dynamic laws. This would explain the convergence to chemical mass recruitment in the two evolutionary processes studied. A third convergence towards similar solution for the modulation of mass recruitment dynamics is nowadays repeated in the development of artificial intelligence, where the "mass intelligence" of ants copied in the interaction of simple virtual computer agents is in search of optimal solutions. Artificial intelligence, however, has copied only the simple recruitment dynamics named here as the Democratic system with a single compound pheromone. More sophisticated modeling could bear fruits to artificial intelligence that might echo the fruits chemical mass recruitment that has brought to social insect species evolving them.

References

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Research Article

Leafcutter Ant Nests Inhibit Low-Intensity Fire Spread in the Understory of Transitional Forests at the Amazon's Forest-Savanna Boundary

Karine S. Carvalho,[1] Ane Alencar,[2] Jennifer Balch,[3,4,5] and Paulo Moutinho[2]

[1] *Departamento de Ciências Biológicas, Universidade Estadual do Sudoeste da Bahia, Avenida José Moreira Sobrinho, 45206-190 Jequié, BA, Brazil*
[2] *Instituto de Pesquisa Ambiental da Amazônia, Avenida Nazaré 669, 66035-170 Belém, PA, Brazil*
[3] *Woods Hole Research Center, 149 Woods Hole Road, Falmouth, MA 02450, USA*
[4] *National Center for Ecological Analysis and Synthesis, 735 State Street, Santa Barbara, CA 93101, USA*
[5] *Department of Geography, The Pennsylvania State University, 302 Walker Building, University Park, PA 16802, USA*

Correspondence should be addressed to Karine S. Carvalho, ksczool@yahoo.com.br

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Leaf-cutter ants (*Atta* spp.) remove leaf litter and woody debris—potential fuels—in and around their nests and foraging trails. We conducted single and three annual experimental fires to determine the effects of this leaf-cutter ant activity on the behavior of low-intensity, slow-moving fires. In a transitional forest, where the southern Amazon forest meets the Brazilian savanna, we tested whether leaf-cutter ant nests and trails (i) inhibit fire spread due to a lack of fuels, and (ii), thereby, reduce the total burned area during these experimental low-intensity fires, particularly at forest edges where leaf-cutter ant abundance was higher. Fine-medium fuel mass increased with an increase in distance from ant nest, and the mean area of bare soil was greater on nests than on the forest floor. Between 60 to 90 percent of the unburned area was within 30 m of ant nests, and burned area significantly increased with increasing distance to ant nests. In addition, the number of ant nests declined with increasing distance from the forest edge, and, with exception of the first experimental fire, burned area also increased with increasing distance from the edge. The present study provides new insight to fire ecology in Amazon environments.

1. Introduction

Leaf-cutter ants (*Atta* spp.) are considered conspicuous herbivores in the neotropics [1, 2]. Their role in the ecosystem, however, goes well beyond their herbivory because their construction and maintenance of nests causes diverse impacts to soil [3, 4] with consequences for recruitment dynamics, [5–9] nutrient access [10, 11], and growth of nearby vegetation [12].

Impacts caused by leaf-cutter ants, or bioperturbation, are associated with their behavior of cultivating symbiotic fungus in subterranean chambers linked through a network of tunnels [13–15]. In order to build these chambers, worker ants remove soil to depths up to 7 m [16] and deposit it on the soil surface, forming a mound, a characteristic heap of soil [15, 17, 18].

During ant nest excavation and expansion, the leaf litter and nearby seedlings are buried or removed, which effectively leaves the mound completely bare. Moreover, the worker ants remove debris near the nest (and along foraging trails) as part of their maintenance activities and thereby leave the nest area free of small plants and debris [7, 19, 20].

Therefore, leaf-cutter ants are considered resilient to fire because they (1) consume leaf biomass, often 12–17% of annual production of a tropical forest [1, 21–23], which is potential fuel; (2) construct subterranean nests out of non-flammable materials, for example, soil; (3) clear trails to bare mineral soil, which are effectively firebreaks for low-intensity fires. Moreover, leaf-cutting ants regulate the temperature of the fungus garden by opening or closing entrances to the nest or by modifying the culture's location inside the nest during different times of the year [2, 24]. These combined effects

may allow leaf-cutter ants to avoid immediate fire damage. However, the longer-term consequences of fire, for example, habitat modification, food availability, and so forth, are not tested or discussed in this study.

Leaf cutter ants are more abundant in disturbed habitats [25–28] with degraded edges [22, 29–31]. These habitats are dominated by pioneers which have less defenses against herbivory [25, 26, 32, 33]. Based on that assumption, we propose to address the following questions: can leaf-cutter ants inhibit fire and effectively protect nearby vegetation in a forest that has experienced this type of disturbance? And if the answer is yes, is this protection more effective at the forest edge? In order to respond to these questions, we hypothesize that leaf-cutter ant trails and nest building (i) blocks fire spread by removing potential fuels and therefore (ii) reduces the total burned area, especially at the forest edge, where they are more abundant.

2. Methods

2.1. Study Site. The study was conducted in seasonally dry forests of the southern Amazon basin on Tanguro Ranch, Mato Grosso, Brazil (13°04′35.39″S, 52°23′08.85″W). The forest biome is at the Cerrado-Amazônia ecotone, and is described as the dry forests of Mato Grosso [34]. In this region, a severe dry season occurs between May and September, while the rainy season occurs between October and April. Annual mean temperature is 23.5°C with annual precipitation between 1800 to 2000 mm [35].

This study is part of the "Savannization" project created in 2004 by the Amazon Environmental Research Institute (IPAM) and the Woods Hole Research Center (WHRC), with the objective of evaluating the effects of repeated understory wildfires on the susceptibility of forests to future fires. In the context of this greater project, we worked within a 150 ha experimental block divided into three 50 ha treatments, defined as: plot (a) unburned control; plot (b) once-burned; and plot (c) thrice-burned. The scale at which wildfires occur in the Amazon required a large-scale ecosystem approach, which makes adequate experimental replication challenging [36]. A necessary limitation of this experiment is that we treat sampling within the 50 ha treatment plots as independent, which we acknowledge as a form of pseudoreplication that is often associated with experimental fires [37]. Moreover, conducting the experimental burns required that the burned plots be adjacent, and therefore treatment was not randomly assigned to each 50 ha block.

Three annual experimental burns were conducted in August or September (2004–6), near the end of the dry season, when many escaped wildfires typically occur (see [35] for a complete description of the site, experimental design, and fire behavior). During all burns, mean daily temperature ranged between 24 to 29°C, and relative humidity ranged between 51 to 57% (measured at the meteorological station). Wind speed was low in the understory (<0.5 m/s) and had little noticeable effect on fire behavior during all years. Fires were set with kerosene drip torches; a total of 10 km of fire lines were set per plot during three to four consecutive days between 9 : 00 h and 16 : 00 h. During all years, fires were extinguished at night and were relit on subsequent days. Combining both burn plots, initial mean flame height and fire spread rate (FSR; ±SE) were 31 (±1) cm and 0.21 (±0.01) m/min, demonstrating that these experimental fires were low-intensity and slow-moving. It is worth noting that fire intensity and spread significantly declined during the second and third burn [35]. Compared with the first burn in 2004, mean flame heights declined by ~10 cm in subsequent burns, and the burned area declined by half in the third fire [35].

2.2. Measurement of Fire Inhibition of Ant Nests and Trails. In order to test the effect of ant nests on fire spread, two measurements were taken: (i) quantification of the amount of fine and small-medium woody fuels (defined here as leaves and twigs with diameter ≤5 cm) which dry faster than large woody debris on the forest floor on and near ant nest mounds and soil, and (ii) calculation of the total area of bare soil created by nests and trails.

Measurement of fuels was conducted between August and September 2005, within several weeks before the experimental fires of that year. For this part of the study, only the experimental burn plots were used (plots (b) and (c)), and all mature, inventoried *Atta* ant nests within the limits of these two plots were utilized (plot (b) = 11 nests and plot (c) = 4 nests).

Maximum height of small twigs was measured within a 40 cm diameter metal ring with increasing distance from each ant nest. Six rings were distributed along a 15 m transect, starting from the nest center (0 m) and extending 3, 6, 9, 12, and 15 m from the nest. After measuring fuel height, all the leaf litter fuels within the 40 cm diameter ring were collected and dried in an oven at 50°C for 48 hours.

To quantify the amount of bare soil associated with ant nests, a wooden frame (100 × 20 cm) was thrown in the nest center and in the nest extremes point. The area within the frame of covered and bare soil was noted. In addition, the length and width of the foraging trails of six nests were measured to calculate the average total area of bare soil associated with a single nest and thereby infer the total forest floor area that was inflammable due to an absence of fuels.

2.3. Relationship between Nest Abundance and Unburned Areas. In order to determine whether ant nests reduce the forest burned area, the annual burn plot (plot (c)) was selected because it was the only one that permitted comparisons between years (2004, 2005, and 2006) and was appropriate for the time period of the present study.

The location of the existing ant nests in the experimental area was registered with an inventory conducted in February of 2005. This inventory used the existing 31 transects (N-S trails which were cut every 50 m in July 2004) in the 150 ha area (each transect was 1 km in length and 40 m in width, totaling a "scanned" area of 116 ha). All of the present ant nests of *Atta* species that were seen within these transects were registered, mapped, and classified.

Nests were classified as active (when ants responded to the stimulus provided by a stick introduced into a nest

opening) or inactive (when there was no response to this stimulus or no observed signs of ant activity). Only nests with active colonies were used because of the cleaning and maintenance activities by worker ants for the upkeep of trails and nest mounds.

Nests were also classified as mature (nest mound $\geq 15\,m^2$) or immature (nest without one big mound, with dispersed small mounds). The species of *Atta* that were registered were: *A. cephalotes*, *A. laevigata* and *A. sexdens*, with this last species being the most common (80% of active colonies). The average area covered by mature nests was $40\,m^2$ (± 15.7), with an estimated volume of removed soil of $6.9\,m^3$ (± 3.2).

2.4. Statistical Analyses. The effect of ant nests on the quantity of combustible material was evaluated using regressions with distance from nest as the independent variable and the fuel parameters (height of small woody debris and weight of leaf litter) as dependent variables. The distance from nest was defined as the distance to the edge of the mound.

In order to test the relationship between nest presence and unburned vegetation, the cumulative percentage of unburned area was calculated at 5 m intervals from each active ant nest. It was then possible to conduct linear regressions using distance from nest as an independent variable and unburned area (log-transformed, base 10) as a dependent variable. Linear regressions were used to test the effects of distance from forest edge on nest number and unburned area.

3. Results

3.1. Nests as Inhibitors of Fire Spread. The average height of small woody debris on nests was 4.1 cm (± 2.1). However, on the forest floor, the values were highly variable. In general, fuel height increased with increasing distance from the nest (Figure 1).

Leaf litter mass also increases with distance from nest (Figure 1). The least amount of leaf litter was documented on top of nest mounds (33.8 ± 136.1 g), and the greatest amount on the forest floor 15 m from ant nests (55.1 ± 12.1 g).

The average area of uncovered soil on top of ant nests was 1.58 (± 0.2) m^2, which was significantly greater when compared to that near or around ant nests ($0.41 \pm 0.2\,m^2$; $t = -9,116$; $P = 0,000$; $N = 15$).

The area of uncovered soil on top of mounds and foraging trails averaged $19\,m^2$ per nest. Considering the number of nests with active colonies (269) inventoried in the 150 ha block, it can be inferred that 0.53 ha (or 0.35%) would be under the protection of ants nests, if in fact all the colonies had reached maturity.

3.2. Relationship between Nest Abundance and Unburned Areas. Between 60 to 90% of the area that was unburned during the experimental understory fires occurred within approximately 30 m of leaf-cutter ant nests and declined with increasing distance from nests (Figure 2).

The number of nests diminished with increasing distance from the edge (Figure 3). Also it was noted that, with the

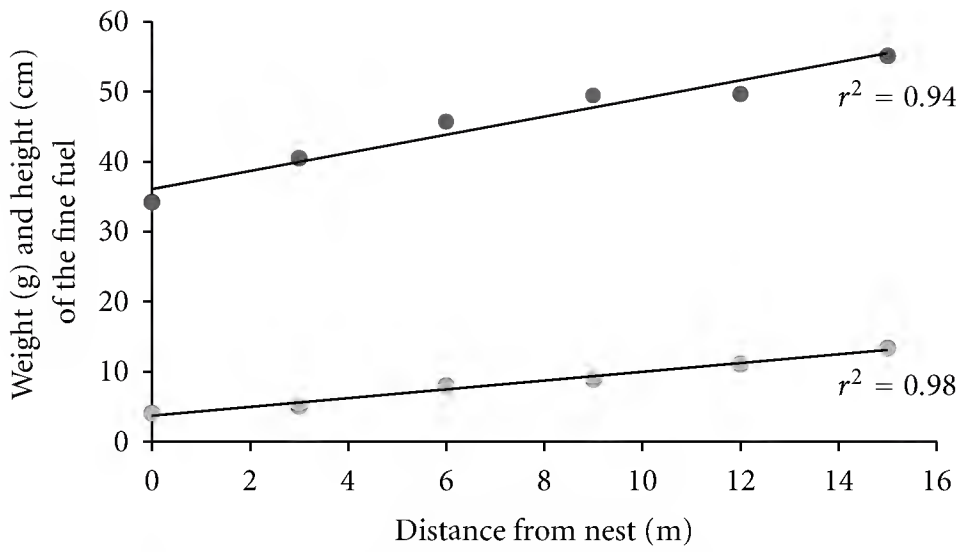
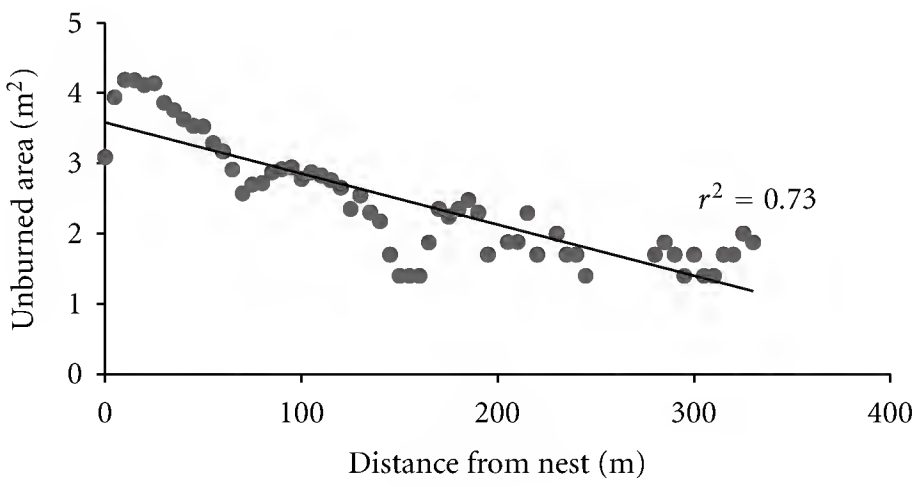
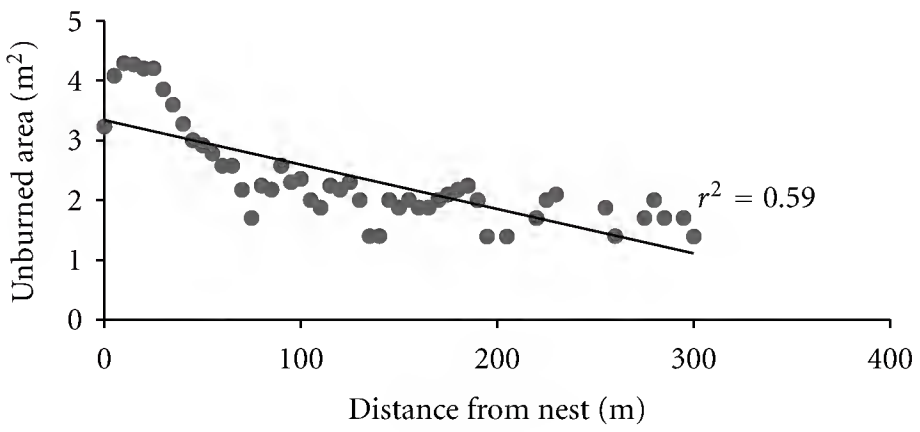


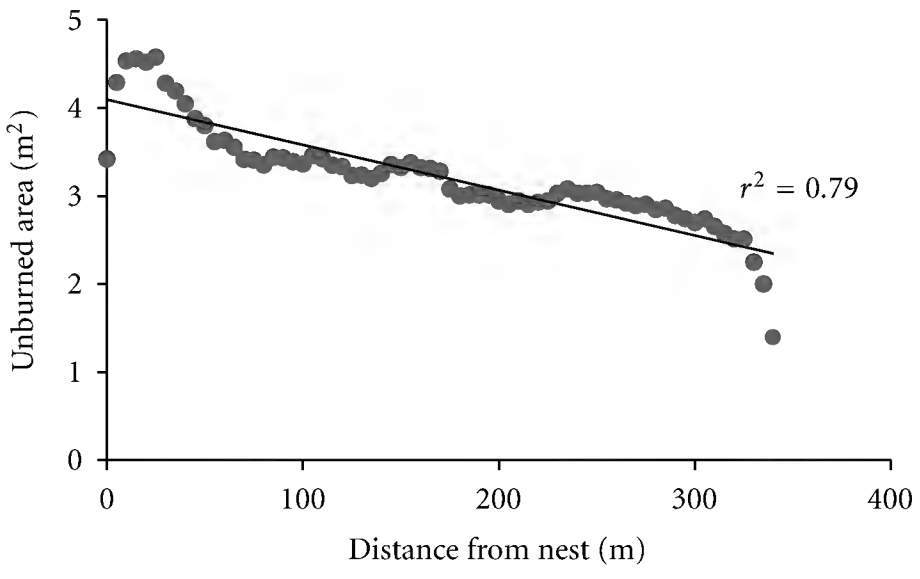
FIGURE 1: Litter mass (g) and height (cm) of small woody debris (≤ 5 cm) as a function of distance from leaf-cutter ant nests in a forest at the Amazon-Cerrado transition. Black circles: litter mass; gray circles: height of small woody debris, $N = 6$.



(a) 2004



(b) 2005



(c) 2006

FIGURE 2: Relationship between unburned area and distance to nests in the 50 ha thrice-burned plot in a transitional forest near the Amazon-Cerrado boundary, $N = 200$.

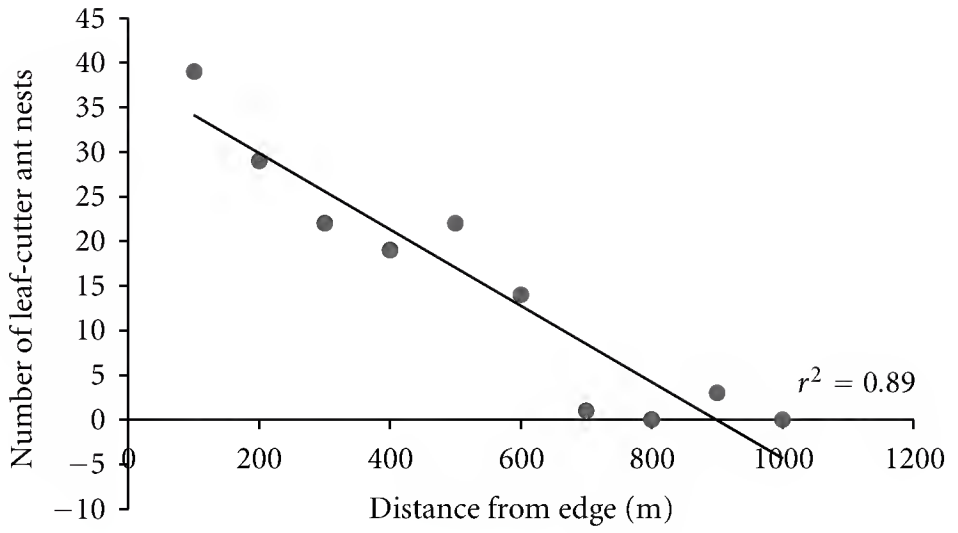


FIGURE 3: Number of leaf-cutter ant nests in relation to distance from the edge of a transitional forest at the Amazon-Cerrado boundary, $N = 10$.

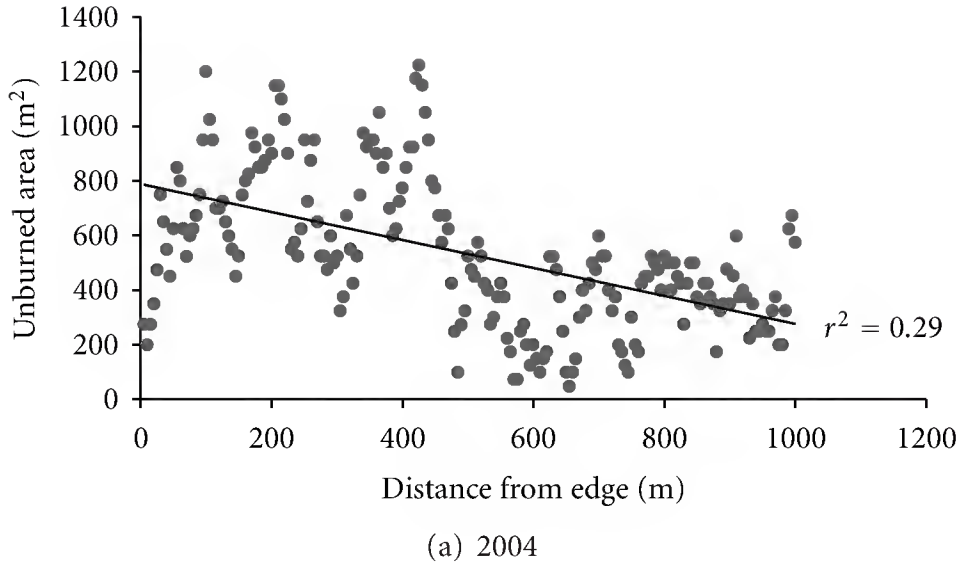
exception of the first experimental fire in 2004, there was more unburned area at the edge that in the forest interior (Figure 4).

4. Discussion

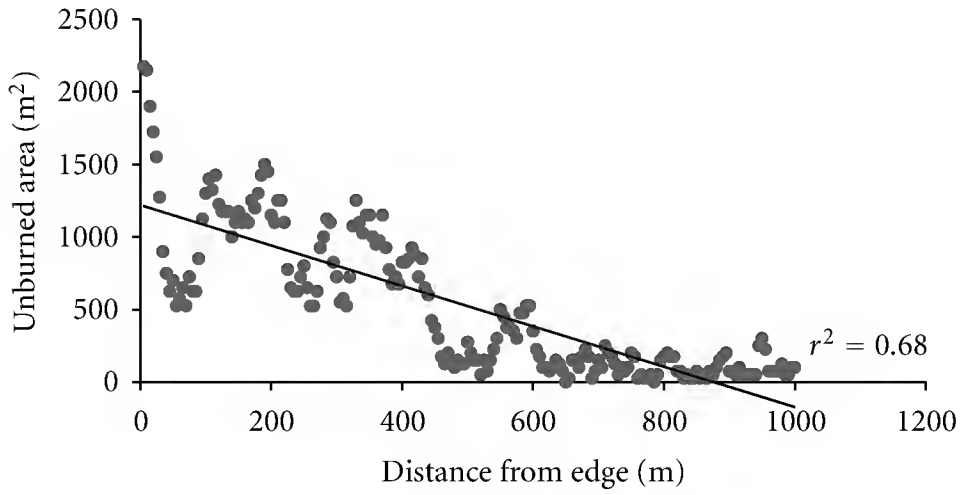
The reduction in spread of low-intensity fires (small flame heights ~30 cm) and thereby burned area, is associated with the presence of ant nests. Although this study primarily demonstrates a correlation, the shortage of leaf litter and small-medium woody debris (combustible material), provoked by leaf-cutter ant bioperturbation, likely provides a mechanism whereby fire spread of low-intensity fires is diminished. In this transitional forest, fuel quantity can be more important in determining fire intensity and spread than relative humidity and other microclimate variables, which control fire behavior during the dry season of a typical humid Amazon forest [38–41]. In fact, fuel mass determined fire behavior in this transitional forest at the Amazon-Cerrado boundary, where a slight decline in fuels after two annual burns limited fire intensity and spread rates [35].

The capacity of leaf-cutter ants to diminish available surface fuels at a fine scale can be extended to the landscape scale where ant nest density is high, as is the case at forest edges [22, 29–31]. The most important result of this study is the documentation that ant nests and trails can function as effective firebreaks at forest edges, which have been traditionally known to be vulnerable to fire entry and spread [42–44]. Edge formation causes alterations in microclimate—such as a decline in humidity and increase in temperature and wind speed—all of which promote fuel drying and fire spread [45]. Further, edge formation dries out adjacent forest fragments and increases available surface fuels, as much as from leaf and branch fall due to plants that are subjected to increased wind exposure [46, 47], as from the forest damage caused by timber removal in these regions [44, 48].

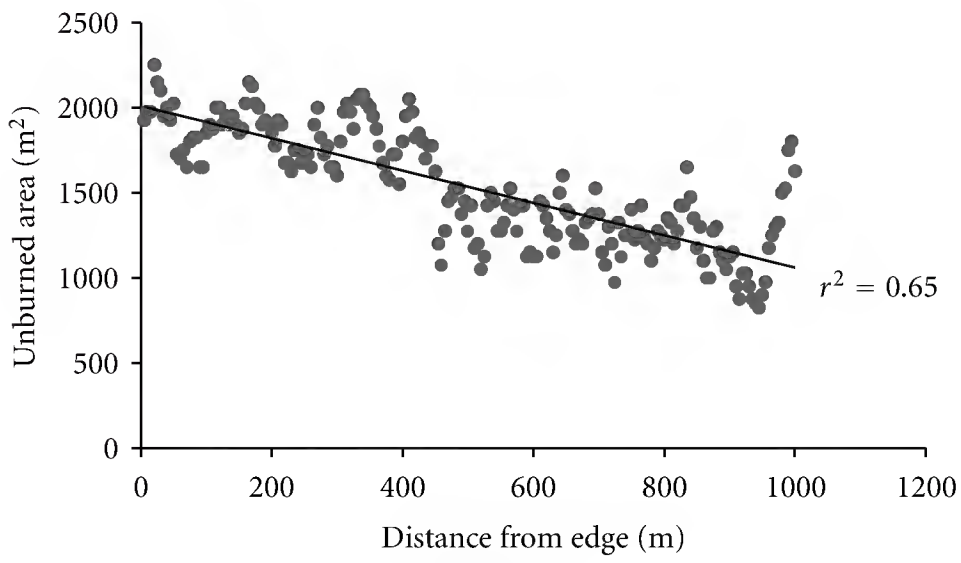
Given this context, populations of leaf-cutter ants may increase, as invertebrate herbivores are repeatedly observed more in edges than forest interiors [22, 29, 30]. Ant nest abundance in these edge areas must be related, in part, to the founding queens' choice [49], which could be driven by their attraction to sunny areas when in nuptial flight.



(a) 2004



(b) 2005



(c) 2006

FIGURE 4: Relationship between unburned area and edge distance in the 50 ha annual burn plot in a transitional forest at the Amazon-Cerrado boundary, $N = 4$.

Beyond the founding queen's dispersal choice, the majority of ant nests in border areas also could be explained by greater colony survival rates in these locations. Leaf-cutter ants require a certain quantity of solar radiation to reach their mounds, and, because of this, they may establish better in locations without shade [14, 32].

Leaf-cutter ant nests occupy large areas, and their populations have a high turnover rate [21, 50]. This means that, beyond the area free of debris that is potentially important in protecting against fire, these deep nests represent frequent and intense disturbance covering large areas in the forest, beyond even what is visible. Fire-induced mortality rate of plants from these mimicked understory fires, calculated for this transitional forest, was the lowest documented for Amazon forests [35]. A possible explanation for this low

mortality was the lowered flammability related to a lack of fuel mass during a third annual experimental burn [35], which limited ignition of larger woody debris or standing dead wood. The degree of flammability depends on the rate of accumulation of fuels and production of litterfall [51] which, in the study area, was substantially lower than in other Amazon forests [35].

The reduction in fuels close to leaf-cutter ant nests could also be influenced by other factors, such as the selectivity of these ants to nest in areas with low leaf litter and woody debris. However, even if other factors contribute to lowered forest flammability and attenuate the damaging effects of fire, the contribution of ant nests in protecting nearby vegetation from low-intensity fires cannot be ignored. This study demonstrates that the behavior of these leaf-cutter ants diminishes the volume of fuels in the environment, by creating, establishing, and maintaining their conspicuous nests in the surface soil of a transitional forest at the Amazon-Cerrado boundary.

This study provides new insights into fire ecology from Amazon studies because the nest effects are off-setting, so that edge areas with lots of nests may not be more susceptible to low-intensity fire than interior plots, as had previous been thought.

Further study should investigate how effective ant nests and trails are at inhibiting more intense or faster fires, such as those observed during more severe droughts. *Atta* is a neotropical genus, and does not have an equivalent organism in well-studied fire-prone ecosystems in Old World systems, as South Africa or Australia; future study may also reveal if there are analog behaviors in Old World invertebrate species. It can be hypothesized from this work that increasing fire frequency, associated with an expanding agricultural frontier, will select for *Atta* species over wood-building taxa, due to their fire-proof construction materials and firebreak trails. This selection may leave lasting effects on arthropod community structure and composition. Given the inherent fire-protection that *Atta* provides, this provides an incentive for farmers and ranchers to avoid using insecticides at agriculture-forest edges. Further study should document the abundance and distribution of *Atta* colonies in burned-over forests through time and their influence on fire behavior of more intense, repeated fires.

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Research Article

Evaluation of the Toxicity of *Virola sebifera* Crude Extracts, Fractions and Isolated Compounds on the Nest of Leaf-Cutting Ants

Keylla Utherdyany Bicalho,[1] Ana Paula Terezan,[1] Denise Cristina Martins,[1] Taís Garcia Freitas,[2] João Batista Fernandes,[1] Maria Fátima das Graças Fernandes da Silva,[1] Paulo Cezar Vieira,[1] Fernando Carlos Pagnocca,[2] and Odair Côrrea Bueno[2]

[1] *Departamento de Química, Universidade Federal de São Carlos, CP 13565-905 São Carlos, SP, Brazil*
[2] *Centro de Estudos de Insetos Sociais, Universidade Estadual Paulista, Campus Rio Claro, CP 199, 13506-900 Rio Claro, SP, Brazil*

Correspondence should be addressed to João Batista Fernandes, djbf@power.ufscar.br

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The phytochemical study of *Virola sebifera* leaves led to the isolation of three lignans: (+)-sesamin, (−)-hinokinin, and (−)-kusunokinin and three flavonoids: quercetin-3-*O*-α-L-rhamnoside, quercetin-3-*O*-β-D-glucoside, and quercetin-3-methoxy-7-*O*-β-D-glucoside by using techniques as high-speed counter-current chromatography and high-performance liquid chromatography. The crude extracts, fractions, and isolated compounds were evaluated for their insecticidal and fungicidal potential against *Atta sexdens rubropilosa* and its symbiotic fungus *Leucoagaricus gongylophorus*. The bioassay results showed a high insecticidal activity for the methanol crude extract of the leaves of *V. sebifera* and its *n*-hexane, dichloromethane and ethyl acetate fractions. The fungicidal bioassay revealed high toxicity of the lignans against *L. gongylophorus*.

1. Introduction

Virola sebifera is one of the most widely spread Myristicaceae species through Brazil [1, 2]. Although it has been mainly known by its hallucinogenic effects [3, 4], this species is also employed, in folk medicine, as antiulcer and in treatment of rheumatism [5, 6]. Previous phytochemical investigations revealed a high diversity of secondary metabolites, where were found 12 lignans [5, 7–10], 23 neolignans [2, 6, 8, 11, 12], one dimeric neolignan [8], 5 polyketides [6, 13, 14], and two alkaloids [3].

Leaf-cutting ants (Hymenoptera), dominant herbivores in the tropics, are considered a serious pest for agriculture, especially when they attack cultivated plants [15]. Control of this pest is still challenging and mostly made by using synthetic insecticides that have often temporary effects and affect nontarget species [16].

They cut high amounts of vegetal matter to feed its symbiotic fungus, *Leucoagaricus gongylophorus* that produce enzymes which are necessary to metabolize polysaccharides to mono and disaccharides, which supplies the major part of the energy needs of adult workers [17]. This relationship between the leaf-cutting ants and its symbiotic fungus is essential to their survival.

Due to the difficulty in controlling this pest, an intense search for alternative methods have been made and may involve both the search for insecticides compounds, or chemical fungicides that inhibit the symbiotic fungus growth. In the present work was evaluated the toxicity of crude extract, fractions, and isolated compounds against *Atta sexdens rubropilosa* and its symbiotic fungus *L. gongylophorus*.

2. Experimental

2.1. General Procedures. The high-speed counter-current chromatography (HSCCC) system employed in the present work was a P. C. Inc. (Potomac, MD, USA) instrument equipped with a quadruple multilayer coil of 1.68 mm

Table 1: *A. sexdens rubropilosa* workers mortality (%) and survival median (Md) when fed with diet containing crude extracts (2000 $\mu g \cdot mL^{-1}$) from *V. sebifera*.

Crude extracts	Days										Md
	1	2	3	6	8	10	14	17	21	25	
Control	2	8	14	22	36	36	42	52	62	72	17[a]
Dichloromethane leaves (**I**)	0	10	18	40	64	92	98	100	100	100	7.5[b]
Methanol leaves (**II**)	0	2	4	20	38	60	70	70	76	78	10[a]
Ethanol branches (**III**)	2	10	14	32	42	48	58	64	64	66	11[a]

[a] Control or no significant and [b]significant difference according to long-rank test ($P < 0.05$).

Table 2: *A. sexdens rubropilosa* workers mortality (%) and survival median (Md) when fed with diet containing the fractions (1000 $\mu g \cdot mL^{-1}$) of the methanol crude extract of leaves of *V. sebifera*.

Fractions	Days										Md
	1	2	3	6	8	10	14	17	21	25	
Control	0	0	2	6	8	14	18	30	42	62	23[a]
n-Hexane (**IV**)	0	8	18	36	54	72	82	88	92	94	8[b]
Dichloromethane (**V**)	2	12	20	60	82	92	94	94	94	94	5[b]
Ethyl acetate (**VI**)	0	0	4	14	36	40	60	64	82	92	12[b]
Hydroalcoholic (**VII**)	2	3	10	14	26	32	46	50	62	76	17[b]

[a] Control or no significant and [b]significant difference according to long-rank test ($P < 0.05$).

I.D. polytetrafluoroethylene (PTFE) tubing and had a total capacity of 443 mL. The β value varied from 0.50 at the internal terminal to 0.85 at the external terminal, and the revolution radius was 10 cm ($\beta = r/R$, where r is the distance from the coil to the holder and R the revolution radius or the distance between the holder axis and the central shaft). The loop volume of the sample injection was 5 mL, and the revolution speed of the apparatus was regulated with a speed controller in the range between 0 and 1000 rpm. The flow rate was controlled with an FMI-50 QD SSY, BS/BS (Fluid Metering, New York, USA) constant flow pump. The fractions were obtained by an automated fraction collector DC-1200 (Eyela, Sunnyvale, CA, USA).

High-performance liquid chromatography (HPLC) analyses were performed using Shimadzu pump LC-6AV and a SPD 6AV UV detector set at 254 nm. HPLC grade solvents were obtained from Tedia, and H_2O was purified in a Milli-Q system.

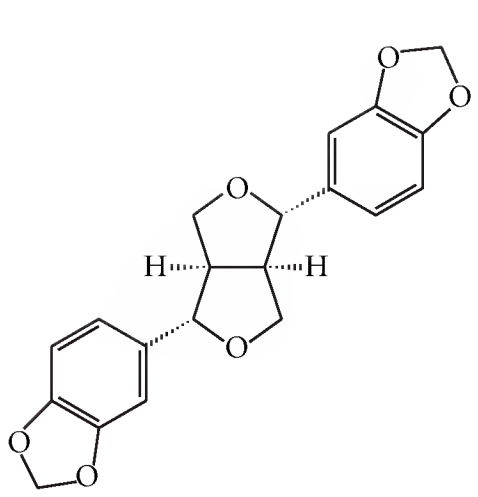
LC-UV-electrospray ionization (ESI) MS/MS data were obtained using an Alliance 2695 liquid chromatography equipped with a Waters (Milliford, MA, USA) PDA2996 photodiode array detection (DAD) system; mass spectral data were acquired in negative ion mode on a triple quadrupole Micromass Quattro LC spectrometer (Manchester, U.K.), equipped with a Z-Spray API ion source and a megaflow electrospray probe.

The ^1H NMR, ^{13}C NMR, and 2D correlations spectra were obtained using Bruker DRX-400 spectrometer, with $CDCl_3$ and DMSO-*d6*.

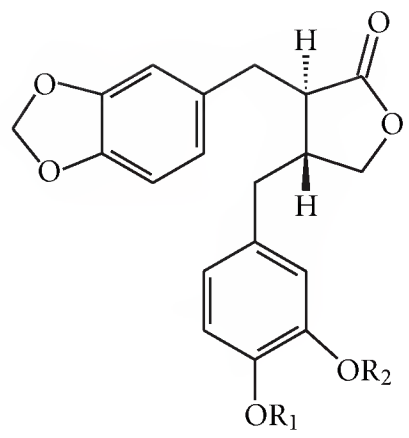
2.2. Plant Material. *V. sebifera* was collected at the cerrado reserve of Canchim Farm, São Carlos, São Paulo state, Brazil and identified by Dra. Maria Helena Antunes de Oliveira e Souza from the Botanic Department of Federal University of São Carlos, where can be found the voucher specimens.

2.3. Extraction and Isolation of Compounds. The extracts were prepared from leaves and branch. The leaves air-dried powered (362 g) of *V. sebifera* were subsequently extracted with dichloromethane (**I**) and methanol (**II**), and the branches air-dried powered (407 g) were extracted with ethanol (**III**). The crude methanol extract (**II**) was submitted to a liquid-liquid partition with *n*-hexane (**IV**), dichloromethane (**V**), ethyl acetate (**VI**), remaining, the hydroalcoholic phase (**VII**). The dichloromethane fraction was purified through high-speed counter-current chromatography (HSCCC) obtaining three lignans (**1–3**), and the ethyl acetate fraction was purified through exclusion chromatography using Sephadex LH-20 as stationary phase and high-performance liquid chromatography (HPLC) obtaining three flavonoids (**4–6**). The compounds were identified using NMR and MS techniques.

2.4. Fungicidal Bioassay. The experiments with the symbiotic fungus *L. gongylophorus* were conducted in Bioassays Natural Products Laboratory, Federal University of São Carlos (UFSCar). The fungus *L. gongylophorus* (Singer) Möller (syn *Rozites gongylophorus*) was isolated from an *A. sexdens rubropilosa* laboratory nest and maintained in laboratory in a culture medium composed of malt extract (20 $g \cdot L^{-1}$), bacto-peptone (5 $g \cdot L^{-1}$), yeast extract (2 $g \cdot L^{-1}$), and agar (20 $g \cdot L^{-1}$) [19]. The samples submitted for assay with the symbiotic fungus were incorporated into the culture medium and followed by the addition of distilled water. Then, in each tube were added 10 mL of culture medium with sample or only culture medium. All the material was autoclaved under the conditions 120°C and 1.0 atm. for 20 minutes. After the sterilization of the material, culture media were poured in Petri plates (80 × 15 mm) inside the laminar flow

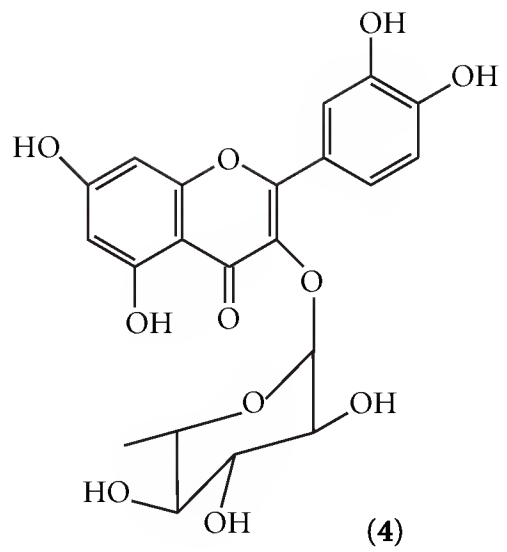


(**1**)



(**2**) R_1–R_2 = CH_2

(**3**) R_1 = R_2 = CH_3



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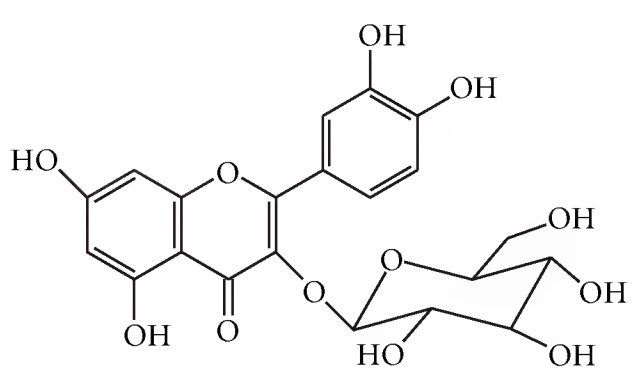

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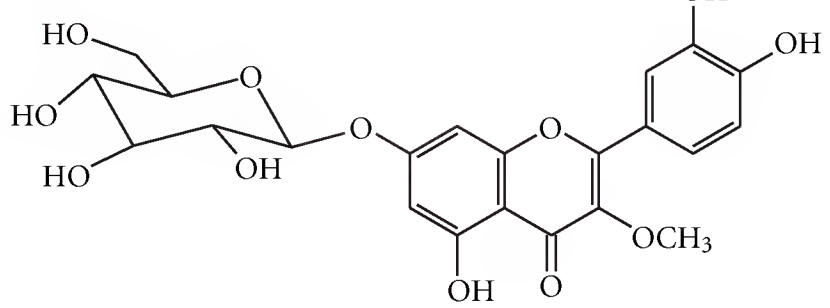

(**6**)

Figure 1: Chemical structures of the isolated compounds from *V. sebifera*.

cabinet, previously sterilized for 30 minutes by ultraviolet light. After solidification of the culture medium, each Petri plate was inoculated with a disc of agar (8 mm) at the center position, previously colonized by the symbiotic fungus *L. gongylophorus*. After the incubation period of 30 days at 25°C, calculations were performed in the areas of mycelial growth of the symbiotic fungus in each sample.

2.5. Leaf-Cutting Ant Insecticide Bioassay. The *A. sexdens rubropilosa* workers used in the assays were randomly removed from laboratory nests. They had a body mass of 20–25 mg. Before the assays, the nests were supplied daily with leaves of *Eucalyptus* sp., oat seed and occasionally with leaves of other plants such as *Hibiscus* sp., *Ligustrum* sp., or rose petals. Fifty ants were removed from the nests and put into five Petri dishes (ten ants each) for each treatment. During the assay, the ants were maintained on an artificial diet prepared with glucose ($50.0\,g\cdot L^{-1}$), bacto peptone ($10.0\,g\cdot L^{-1}$), yeast extract ($1.0\,g\cdot L^{-1}$), and agar ($15.0\,g\cdot L^{-1}$) in distilled water (100 mL) [20]. The diets (0.4-0.5 g per dish) with the addition of compounds (experiment) or without it (control) were offered daily in a small plastic cap. The control was prepared with the diet and the solvent. To ensure that undetectable remaining amounts of the solvent did not affect the ants, a comparison was made with another set of dishes in which water was used instead of solvent. As expected, the same survival rates were obtained with both systems (data not shown). The compounds were poured into the hot diet immediately after it was autoclaved. The final concentration of the extracts added to the diet was $2000\,\mu g\cdot mL^{-1}$, of the fractions were $1000\,\mu g\cdot mL^{-1}$, and of the compounds were 200 or $400\,\mu g\cdot mL^{-1}$. During the assays, the material was maintained in an incubator at the temperature 25 (±1)°C and relative humidity ranging between 70 and 80%. The maximum length of observation was 25 days, and the number of dead ants was registered daily.

Percentage survival was plotted as a function of time in a survival curve which was then used to calculate the median survival time (*S*50, the time at which 50% of the ants in each experiment remained alive). The *S*50 was calculated and survival curves were compared using the nonparametric log-rank test at the 95% significant level [21].

3. Results and Discussion

The compounds isolated from the dichloromethane fraction of the methanol crude extract of the leaves of *V. sebifera* were the lignans (+)-sesamin (**1**), (−)-hinokinin (**2**), and (−)-kusunokinin (**3**) (Figure 1). These compounds were identified by the comparison of NMR spectral data with those described in the literature [7, 22–24]. The compounds isolated from the ethyl acetate were the flavonoids quercetin-3-*O*-α-L-rhamnoside (**4**, synonyms: quecetrin or quecitrin), quercetin-3-*O*-β-D-glucoside (**5**), and quercetin-3-methoxy-7-*O*-β-D-glucoside (**6**) (Figure 1). These flavonoids were also identified by the comparison of NMR spectral data with those described in the literature [25–27].

The *V. Sebifera* crude extracts (**I**, **II,** and **III**) were evaluated by their insecticidal potential, and only the methanol crude extract of the leaves (**II**) presented insecticidal activity

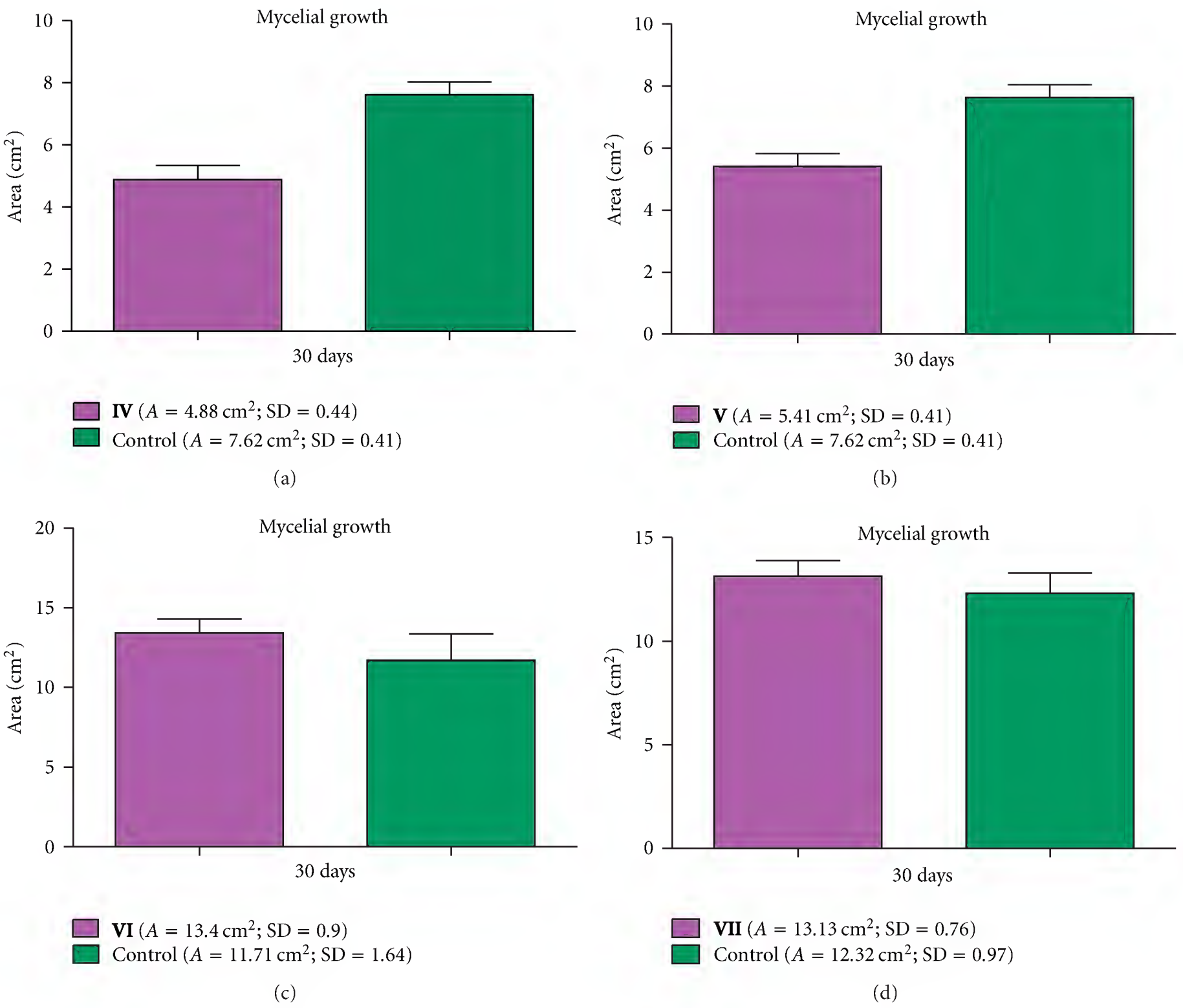

FIGURE 2: Effect of the fractions **IV** (600 μg·mL^{-1}), **V** (600 μg·mL^{-1}), **VI** (600 μg·mL^{-1}), and **VII** (600 μg·mL^{-1}) on the mycelial growth of the symbiotic fungus *L. gongylophorus*.

on *A. sexdens rubropilosa* (Table 1), and it was chosen for the beginning of the fractionation studies. In this treatment, the mortality of ants reached 100% at the 17th day while the control with the pure diet did not exceed 72% of mortality at the end of the 25 days of experiment.

The *n*-hexane (**IV**), dichloromethane (**V**), ethyl acetate (**VI**), and hydroalcoholic (**VII**) fractions from partition of methanol extract **II** were tested in ants (*A. sexdens rubropilosa*) ingestion bioassay (Table 2). All the fractions presented statistic difference in comparison with control; however, *n*-hexane, dichloromethane, and ethyl acetate fractions showed high cumulative mortality, 94%, 94%, and 92% of dead ants on the 25th day of experiment, respectively. The *n*-hexane and dichloromethane fractions showed the highest insecticidal activity in a short time, presenting 82% and 94% of mortality in only 12 days of experiment.

In the *in vivo* fungicidal bioassay of these fractions, the *n*-hexane and dichloromethane fractions showed 36% and 29% of inhibitory activity on symbiotic fungus; respectively, while the ethyl acetate and hydroalcoholic fractions promoted the mycelial growth of the fungus in 14% and 7%, respectively (Figure 2).

All the six compounds **1–6** were evaluated as their insecticidal potential against *A. sexdens rubropilosa* workers by an ingestion bioassay (Table 3). Although the compounds **2**, **3**, **5**, and **6** presented statistic difference in comparison with control, only the lignan **3** resulted in higher cumulative mortality, 90%, than the control with pure diet. These results indicate that none of the others substances were toxic to the ants workers, and only the lignan (−)-kusunokinin (**3**) was considered biologically active as an insecticide.

In contrast with the weak activity presented by most of the compounds tested, the three lignans showed a high fungicidal potential against the symbiotic fungus *L. gongylophorus*. The lignans (+)-sesamin (**1**), (−)-hinokinin (**2**), and (−)-kusunokinin (**3**) inhibited the mycelial growth in 74%, 72%, and 100%, respectively (Figures 3 and 4). In previous studies [18] were already evaluated the fungicidal

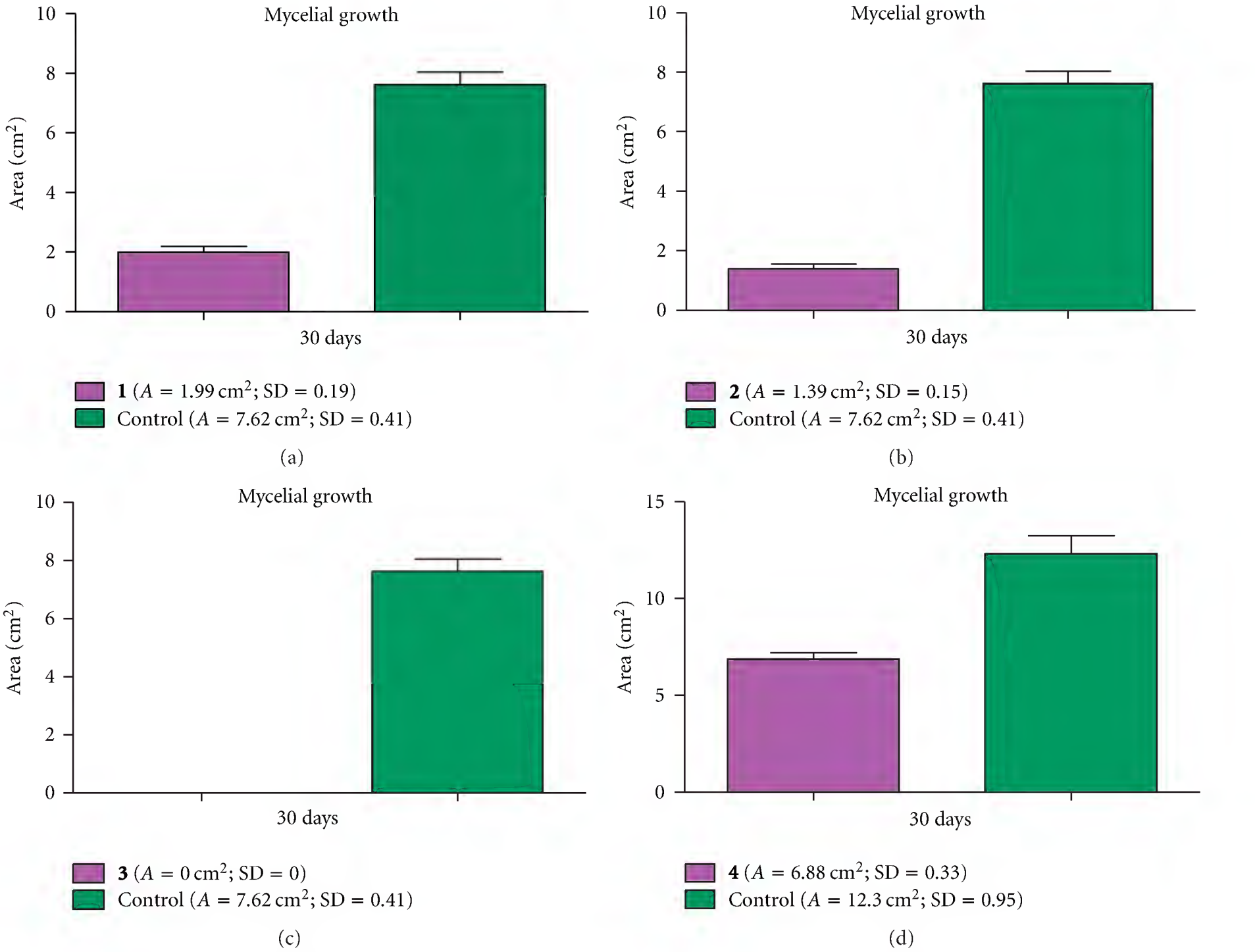

FIGURE 3: Effect of the compounds (+)-sesamin (**1**) (100 $\mu g \cdot mL^{-1}$), (−)-hinokinin (**2**) (100 $\mu g \cdot mL^{-1}$), (−)-kusunokinin (**3**) (120 $\mu g \cdot mL^{-1}$), and quercetin-3-*O*-α-L-rhamnoside (**4**) (100 $\mu g \cdot mL^{-1}$) [18] on the mycelial growth of the symbiotic fungus *L. gongylophorus*.

TABLE 3: *A. sexdens rubropilosa* workers mortality (%) and survival median (Md) when fed with diet containing the compounds **1**–**4** (400 $\mu g \cdot mL^{-1}$) and **5**, **6** (200 $\mu g \cdot mL^{-1}$).

Fractions	Days										Md
	1	2	3	6	8	10	14	17	21	25	
Control	0	0	0	6	10	10	24	44	78	84	18.5[a]
(+)-sesamin (**1**)	0	2	2	4	4	6	16	44	56	62	19[a]
(−)-hinokinin (**2**)	0	2	4	4	6	10	18	22	42	64	23[b]
(−)-kusunokinin (**3**)	2	6	6	18	22	24	34	70	82	90	16[b]
Quercetin-3-*O*-α-L-rhamnoside (**4**)	0	2	4	10	12	20	34	42	58	74	19[a]
Quercetin-3-*O*-β-D-glucoside (**5**)	0	0	2	2	4	14	26	36	40	60	25[b]
Quercetin-3-methoxy-7-*O*-β-D-glucoside (**6**)	0	2	2	6	8	14	24	30	48	54	23[b]

[a] Control or no significant and [b]significant difference according to long-rank test ($P < 0.05$).

potentials for furofuran and dibenzylbutyrolactone lignans such as (+)-sesamin and (−)-kusunokinin, respectively. For (+)-sesamin was confirmed its high toxicity against the fungus *L. gongylophorus,* but the results were quite different for (−)-kusunokinin. The actual bioassay uses a different methodology than that used initially, and the fact that the lignan (−)-hinokinin, another dibenzylbutyrolactone lignan, also showed toxicity against the fungus validates the results of this bioassay.

The flavonoids quercetin-3-*O*-β-D-glucoside (**5**) and quercetin-3-methoxy-7-*O*-β-D-glucoside (**6**) were not tested in this bioassay, and the flavonoid quercetin-3-*O*-α-L-rhamnoside (**4**) showed weak inhibition of fungal growth, only 44% [28].

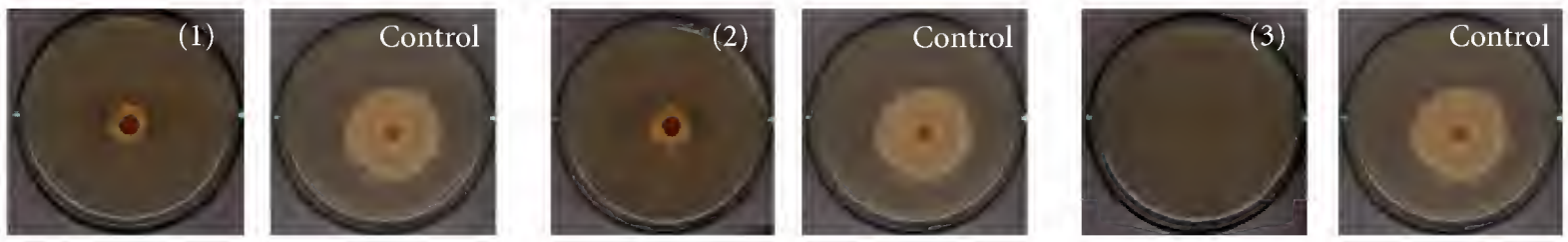

FIGURE 4: Images of the symbiotic fungus in the presence of the lignans (+)-sesamin (**1**), (−)-hinokinin (**2**), and (−)-kusunokinin (**3**) after 30 days of inoculations and the respective controls.

4. Conclusions

The results observed in the ingestion bioassay with ants workers for the methanol crude extract of the leaves of *V. sebifera* and the *n*-hexane, dichloromethane, and ethyl acetate fractions suggest the use of this plant to control nests of *A. sexdens rubropilosa*. Besides, the high fungicidal potentials of the lignans reveal a rich source for new fungicides.

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Research Article

Division of Labor in *Pachycondyla striata* Fr. Smith, 1858 (Hymenoptera: Formicidae: Ponerinae)

Adolfo da Silva-Melo and Edilberto Giannotti

Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Campus Rio Claro SP, Caixa Postal 199, 13506-900, Rio Claro, SP, Brazil

Correspondence should be addressed to Adolfo da Silva-Melo, adolfoants@yahoo.com.br

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Four colonies of the ant *Pachycondyla striata* were used to analyze the specie behavioral repertoire. Forty-six behavioral acts were recorded in laboratory. Here, we present the record the division of labor between the castes and the temporal polyethism of monomorphic workers. The queens carried out many of the behavioral traits recorded in this work however; they performed them less frequently compared to the worker. The workers activity involved chasing and feeding on fresh insects and usingthem to nourish larvae besides laying eggs in the C-posture, an activity also performed by queens, which is similar to that of wasps of the subfamily *Stenogastrinae*. The young workers were involved in activities of brood care, sexuate care, and nest maintenance, and the older workers were involved in defense, exploration, and foraging.

1. Introduction

The evolution of social behavior may be defined as the combination of care for young individuals by adults, overlapping generations, and division of labor in the reproductive and nonreproductive castes [1–4]. The ants are eusocial, and their behavior differs from that one of other social insects in three respects: (a) they have a varied diet, (b) nest building retains characteristics unique to this group, parental care in galleries, and workers performing tasks according to their age or size, and (c) adults remaining long time with their brood [5].

Among the aspects covered in ethologic studies of ants, division of labor (when individuals within a group perform different roles) or polyethism comprehends a widely explored subject and may present two divisions: (a) physical polyethism, when individuals show distinct morphological characteristics to perform specific tasks and (b) temporal polyethism, when the variation of tasks occurs according to age [1, 2, 4, 6]. Therefore, temporal polyethism may occur both in populations of monomorphic workers and in polymorphic workers [7, 8]. The ants of the genus *Pachycondyla* have a wide pantropical distribution with about 270 species being described [9]. The *Pachycondyla species* are diverse in their morphology and their behavior [10].

Pachycondyla striata Smith 1858 [11], classified into the subfamily Ponerinae [12], presents relatively large individuals (13.2–16.7 mm long). The castes are slightly different. The workers are different from the queens by the absence of ocelli and wing scars. This species is distributed through northern Argentina, Paraguay, Uruguay, and Brazil [13–15].

The aim of this study was to verify whether there is division of labor among castes and age polyethism in *P. striata*. The results will contribute to better understanding and interpretation of its social organization and allow comparison with other species of the family Formicidae.

2. Materials and Methods

Four colonies were collected on the campus of the University UNESP—Universidade Estadual Paulista, Rio Claro (22°32′40″S/47°32′44″W), São Paulo State. The ethological analysis began two days after the collection. Observations were done in the foraging area and plaster nest.

Table 1: Composition of the colonies of *Pachycondyla striata*.

Colony	Number of individuals							Date of collection
	eggs	larvae	pupae	workers	winged females	males	queens	
N. 2	—	—	—	20	5	—	—	04/13/2006
N. 3	—	—	37	178	38	33	—	04/14/2006
N. 7	264	65	—	382	7	8	—	08/06/2006
N. 8	30	231	240	384	—	—	1	11/17/2006

The colonies selected in field contained queens and/or winged females. The latter were regarded as queens after wing loss. The colonies were transferred to a laboratory and placed in plastic containers (width: 30.0 cm; length: 48.0 cm; height: 12.0 cm). In each container, there was a plaster nest consisting of three chambers in different sizes, interconnected by tunnels of 1.0 cm in width and 3.0 cm in depth, covered with glass to avoid disturbance and red cellophane paper to prevent the passage of the full spectrum of light.

The diet of the ants consisted of sugar and water in a ratio of 1:1 (offered in test tubes, with cotton wool in the opening), termites, worms, cockroaches, larvae of Coleoptera (*Tenebrio molitor*), flies, and papaya seeds.

Previous observation was performed for 20 hours to obtain behavioral data, with the aim of identifying queens and workers. The ants were differentiated by covering their thorax with quick-drying paint for model airplanes (Revel), allowing the identification of the individuals by age group just after their emergence. Young workers are known for having a paler color in relation to older ones. Later, the scan sampling method described by Altmann [16] was used to qualify the acts.

The quantitative observation of the behavioral acts of the individuals in each colony was performed for five minutes, with one-minute intervals. The observation time was one hour a day, four times a week, during six months, for a total of 94 hours. A comparative ethogram for the individuals was developed. Sample coverage was defined by the formula $\varnothing = 1 - (\mathbf{N1}/\mathbf{i})$, where **N1** = number of behavioral acts observed once and **i** = total number of behavioral acts, the more this value approaches to 1, the more complete the sample [17]. The behavioral catalog was divided into ten categories and used to build histograms and a dendrogram with clustering method (UPGMA) of Euclidean distance [18] (Table 1).

3. Results

3.1. Division of Labor. When introduced in laboratory, the individuals of *P. striata* immediately occupied the artificial nest. The ants carried the immature from the foraging area and accommodated them in the first and minor chamber for 12 hours. Only after this, they carried them to the last and bigger chamber. In the nest seven, the workers distributed randomly the immature to the chambers and tunnels of the nest.

As previously announced for this study, we considered the existence of two castes morphologically and subtly differentiated, containing monomorphical workers. In Table 2 the different categories, are distributed and quantified and behavioral acts of queens, workers, winged females, and males of *P. striata are defined as well.*

The sample coverage value ($\varnothing$) was 0. 981 meeting the expectations of Fagen and Goldman [17]. The dissimilarity dendrogram informs a great ethological difference between the castes. (Figure 1).

The inactivity of the males into the nest suggests their action to be more prevalent in the mating season, but this was not verified in this study (Table 2).

The behavorial acts supposedly regarded as less derived have been identified in the castes, such as feeding larvae and adults on fresh insects, and laying eggs in the C-posture. Furthermore, the queens performed activities that are exclusively carried out by workers in other more derived species, such as brood care, exploring, foraging, and nest maintenance (Table 2).

The dominance behavior involved both individuals for recruiting and reproductive labor. The latter case, the interaction of dominance occurred between queen and worker and among workers. Some workers developed ovaries to lay eggs. However, this data were not quantified.

3.2. Temporal Polyethism. Some activities were preferably carried out by younger workers or older workers. This suggests division of labor by age (Figure 2).

The younger workers (7 to 56 days of age) stayed in the nest for approximately 27.03 ± 12.72 days (7–56, $N = 27$). For this time, took they care the pupae, larvae, eggs, males, and winged females (Figure 2). However, some newly hatched ants did not taken care for the young individuals. This might be related the presence of physiological problems, because they died within two or three days.

The older workers (those at more than 56 days of age) performed several categories, but they pointed in the activities out of the nest, as defense, foraging, and exploring (Figure 2). Furthermore, the dominance is a category that deserves attention. It may be linked to the maintenance of the colony, as a measure of protection from the nest and obtaining food, or reproduction.

The intermediate group (queens, virgin queens, and winged females), which is regarded as a caste, showed clear transition tasks. The quantitative results of the group are

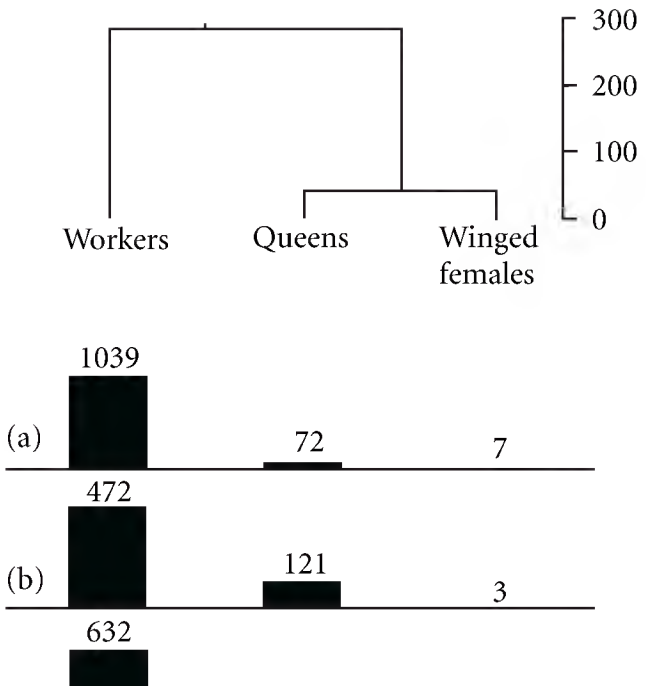

Figure 1: Dissimilarity dendrogram of individuals of *P. striata*. Behavioral categories: (a) feeding, (b) communication, (c) brood care, (d) sexuate care, (e) defense, (f) exploring and foraging, (g) grooming, (h) inactivity, (i) dominance, and (j) nest maintenance.

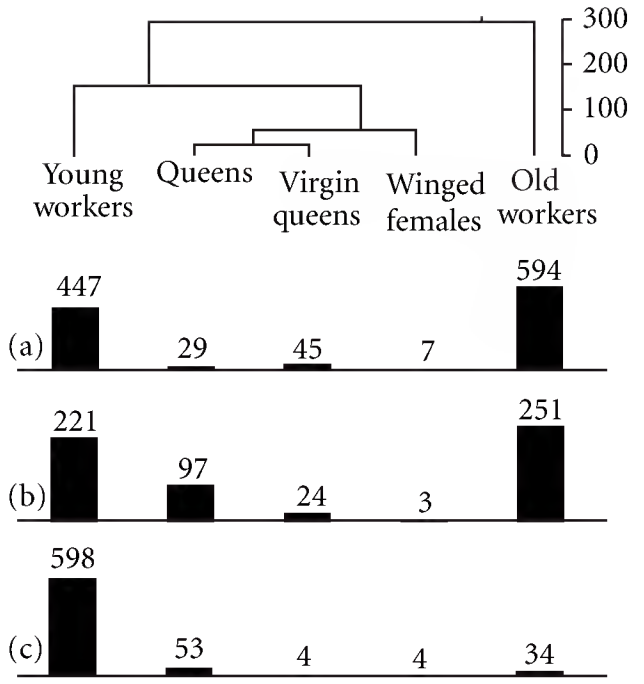

Figure 2: Dissimilarity dendrogram of individuals of *P. striata* showing the division of labor by age. Behavioral categories: (a) feeding, (b) communication, (c) brood care, (d) sexuate care, (e) defense, (f) exploring and foraging, (g) grooming, (h) inactivity, (i) dominance, and (j) nest maintenance.

smaller when compared to workers, and the activties have been concentrated within the nest.

4. Discussion

It is interesting to note that a small portion of behavioral acts is performed by queens within the nest. This type of occurrence is mentioned to the species of *P. (Neoponera) villosa*, *P. (Neoponera) apicalis*, and *P. (Neoponera) obscuricornis* [19]. The queens of *P. striata* presented more care for eggs than to the other immature individuals, while *P. (Neoponera) villosa* spends more energy caring for eggs and pupae, *P. (Neoponera) apicalis* and *P. (Neoponera) obscuricornis* invest more energy in caring for larvae and pupae [19]. The involvement of queens in brood care seems to be a little derived characteristic [20].

Feeding was a behavioral act frequently observed in the queens of *P. striata*, while the queen of *Nothomyrmecia macrops* was seen feeding once [21].

The queens and workers which to perform the laying eggs, retained the position in the form C. *Ectatomma planidens* [22, 23] and *Platythyrea punctata* [24], also acquired the same position.

This is characteristic of wasps of the genera *Listenogaster* [25] and *Eustenogaster* [26]. This condition may be an evidence of an attribute that might have been preserved.

Table 2: Behavioral catalog of *Pachycondyla striata*.

Category and behavioral acts	Queens	Workers	Winged females	Males
(A) Feeding				
01-Feeding on prey	0.0355	0.0700	0.0128	
02-Intake of liquids	0.0264	0.0587	0.0171	0.0116
44-Cannibalism	0.0030	0.0020	0.0059	
(B) Communication				
03-Antennate workers	0.0755	0.0549	0.0128	0.0516
04-Antennate queens	0.0345	0.0052		
(C) Brood care				
05-Antennate egg	0.0218	0.0129		
06-Antennate larvae	0.0010	0.003		
07-Antennate pupae		0.004		
08-Standing on eggs		0.0006		
09-Standing on larvae	0.0010	0.0014		
10-Standing on pupae		0.0009		0.0058
11-Handling eggs	0.0127	0.0045		
12-Handling larvae		0.0063	0.0043	
13-Handling pupae		0.0015		
15-Feeding larvae		0.0058		
16-Cleaning larvae		0.0085	0.0043	
18-Carrying eggs	0.0063	0.0126	0.0086	
19-Carrying pupae		0.0071		
20-Carrying larvae		0.0031		
22-Standing and holding an egg	0.0045	0.0061		
23-Standing and holding a pupa		0.0012		
24-Egg laying	0.0045	0.0004		
(D) Sexuate care				
14-Handling winged females		0.0003		
17-Cleaning males		0.0059		
21-Carrying males		0.0006		
(E) Defense				
25-Guarding the nest entrance	0.0110	0.0310	0.0210	
(F) Exploring and foraging				
26-Capturing prey	0.0118	0.0582		
27-Walking in the foraging arena	0.0427	0.0478	0.1070	0.0580
28-Tanden running	0.0010	0.0047	0.0043	
(G) Grooming				
29-Self-grooming their antennae	0.1164	0.082	0.059	0.0860
30-Self-grooming their 1st pair of legs	0.0582	0.03	0.0043	0.0660
31-Self-grooming their antennae and 1st pair of legs	0.0282	0.0252	0.0259	0.0233
32-Self-grooming their 2nd and 3rd pairs of legs	0.0591	0.0722	0.0212	0.0290
33-Self-grooming their anus	0.0054	0.0200		0.0260
34-Social grooming	0.0363	0.0062	0.0530	
(H) Inactivity				
38-Inactivity in the nest	0.3700	0.0323	0.4369	0.5000
39-Inactivity in the foraging arena	0.0219	0.0163	0.0350	0.0630
(I) Dominance				
35-Antennal boxing	0.0218	0.0405	0.0027	
36-Blocking	0.0054	0.0005		
37-Immobilization	0.0010	0.0081		0.0160

Table 2: Continued.

Category and behavioral acts	Queens	Workers	Winged females	Males
(J) Nest maintenance				
40-Carrying a dead ant	0.0010	0.1512		
41-Handling a dead ant		0.133		
42-Carrying garbage	0.0010	0.0103		
43-Handling garbage	0.0010	0.0085		
45-Exploring the plaster nest	0.0700	0.0160	0.1639	0.0643
46-Digging in the plaster nest		0.0080		
Total frequency	1	1	1	1
Total categories	9	10	9	8
Total behavioral acts	31	46	19	13

The agonistic behavioral acts were almost always related to reproduction or foraging activities. Antennal boxing occurred with winged females, queens, and workers. This behavior may be related to the recruitment of workers, as the measure was implemented in the nest, and a larger number of workers moved to the foraging arena. The same happens to *P. bertholudi* [27].

In nest 8, after the queen's death, one worker started laying eggs. Afterwards, agonistic encounters became frequent, and another worker that started laying eggs was mutilated. This suggests that *P. striata* presents a reproductive dominance, as does *P. crassinoda* [28]. Agonistic encounters were also reported for *P. (Neoponera) obscuricornis* [29, 30] and *P. bertholudi* [27].

Chagas and Vasconcelos [31] described the fighting behavior between workers of *P. striata* and *P. (Neoponera) obscuricornis* in the field. According to these researchers, this event occurred because *P. striata* invaded the foraging and/or life area of *P. obscuricornis*.

The agonistic behavioral acts observed in *P. striata* were also reported for *Dinoponera quadriceps* [32], *P. (Neoponera) apicalis* [33], *P. (Neoponera) obscuricornis* [29], *Rhytidoponera* sp. 12 [34], *P. inversa* [35], and *P. bertholudi* [27].

We checked that the workers ate larvae, pupae, other workers, and males. Some alive males had their abdominal region pulled off by workers. These behaviors may indicate stress or cannibalism. Wilson [1] reported that dead workers might be used as food or were discarded.

The eggs of *P. striata* collected from the natural environment and those laid by queens and workers in laboratory did not develop. They were predated by dominant individuals or by the whole group under stress. Egg predation was reported in *Ectatomma planidens* [22, 23] and *E. vizottoi* [36] although it has been absent or not observed in *Pachycondyla bertholudi* [37]. The eggs laid by workers are usually eaten by queens and larvae, which represents a stereotyped, conspicuous behavior pattern [1].

Oophagy is indispensable to the social Hymenoptera [1]. It is important because workers do not regurgitate food either for larvae or for queens, so they can use their own resources to produce immature oocytes [38]. This event seems restricted to some genera in the subfamily Ponerinae [38].

In the presence of a large number of eggs, the workers gathered them and stood still on them. They standing motionless on eggs, pupae, and larvae. This may suggest warming and protection of the immature individuals. When the number of eggs in the nest was small, the ants of this species kept the eggs clustered between their mandibles.

The behavioral act *tandem running* was carried out to recruit workers into the foraging arena. Medeiros and Oliveira [39] observed this as well. This behavior is common in several species such as *Pachycondyla (Brotoponera) tesserinoda* [40] and *Pachycondyla obscuricornis* [31].

The larvae of *P. striata* display a characteristic behavior to order food. They shake their necks and heads several times towards the ventral region of their body until a worker answers. This behavior is similar to that one of larvae of *Gnamptogenys striatula* [41]. The workers moved the larvae towards the prey. In some cases, the workers held the prey between their mandibles, while the larvae inserted their head into the sectioned part of the mealworm and fed on hemolymph. The workers feed preferentially larvae closer to them. Asking for food was a behavioral act observed more often in larvae in the last instar. The workers touched the buccal apparatus of the larvae with their mandibles open, but it was not possible to see the food transfer or the projection of the glossa of the workers. A similar behavioral act was described for *P. crassinoda* [28].

Small pieces of mealworm were placed in the ventral region of the larvae of ants by the workers. The larvae curved their necks and fed in the same manner as described for *Gnamptogenys horni* [42], *Ponera pennsylvannica* [43], and *Pachycondyla crassinoda* [43]. According to Wilson [1] and Traniello and Jayasuriya [44], feeding larvae on small fragments of prey is a less derived characteristic.

P. striata use their stinger to paralyze their prey. The sting might be stimulated by sudden movements of the prey, similar to way what happens to workers of *P. caffraria* [45]. According to Traniello and Jayasuriya [44], using the stinger to paralyze prey is a less derived characteristic.

The state of inactivity or deep sleep exhibited by *P. striata* is similar to one that described by Cassill et al. [46]. Many workers remained motionless in foraging area. This category may reflect the restricted space of the arena or, as Miguel and Del-Claro [47], the state, containment of spent

energy. The inactivity behavior was observed in *Pachycondyla (Neoponera) villosa*, *P. (Neoponera) apicalis*, *P. (Neoponera) obscuricornis* [19], *P. crassinoda* [48], *Nothomyrmecia macrops* [21], *E. planidens* [22, 23], and *E. opaciventre* [47].

The monomorphic workers of *P. striata* present specialized task division, forming work groups to performing tasks linking to individuals with similar ages. Young individuals provide parental care, whereas older individuals carry out the activities of defense, exploration, and foraging.

Young workers stayed in the nest for 56 days, but some left earlier. They were recruited into the foraging area according to the necessity of food or to substitute the dead workers. In the first 45 days after emergence, *Ectatomma tuberculatum* performs tasks progressively according to the age of the individuals [49]. The same happens to workers of *Platythyrea lamellosa*, which after hatching (0–5 days of age) present association with pupae and later take care of eggs and larvae, performing specific tasks influenced by their age [50]. Unlike *P. striata*, newly hatched individuals of the species *Pachycondyla caffraria* (0–5 days of age) present four types of behavioral acts and are capable of foraging early at this age [51]. Each colony of this species has precise requirements as to carbohydrates and proteins, appropriate for labor division, which happens in relatively fixed proportions between hunting foragers and those which collect water with sugar [45]. Workers of *P. striata* were seen at the carbohydrate source in a very small frequency. This activity was included in the behavioral act of taking water in from the cotton wool. *P. striata* preferred to capture other insects to provide protein intake.

This research shows the profile of social organization of *P. striata*. We see that many behavioral acts are common for species of the subfamily Ponerinae. Although there is a narrow dimorphism in castes of *P. striata*, there is a great difference of division of labour between them. The age is a factor that controls the performance of tasks in workers.

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Research Article

Community Structure of Leaf-Litter Ants in a Neotropical Dry Forest: A Biogeographic Approach to Explain Betadiversity

Rogério Silvestre,[1] Manoel F. Demétrio,[2] and Jacques H. C. Delabie[3]

[1] *Laboratório de Ecologia de Hymenoptera (HECOLAB), Universidade Federal da Grande Dourados, Rodovia Dourados, Itahum, km 12, Cidade Universitária, 79.804-970 Dourados, MS, Brazil*
[2] *Programa de Pós-Graduação em Entomologia e Conservação da Biodiversidade, Universidade Federal da Grande Dourados (UFGD), Cidade Universitária, 79.804-970 Dourados, MS, Brazil*
[3] *Laboratório de Mirmecologia, Centro de Pesquisas do Cacau, Convênio UESC/CEPLAC, 45.651-630 Ilhéus, BA, Brazil*

Correspondence should be addressed to Rogério Silvestre, rogeriosilvestre@ufgd.edu.br

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This paper describes habitat and geographic correlates of ant diversity in Serra da Bodoquena, a poorly surveyed region of central-western Brazil. We discuss leaf-litter ant diversity on a regional scale, with emphasis on the contribution of each of the processes that form the evolutionary basis of contemporary beta diversity. The diversity of leaf-litter ants was assessed from a series of 262 Winkler samples conducted in two microbasins within a deciduous forest domain. A total of 170 litter-dwelling ant species in 45 genera and 11 subfamilies was identified. The data showed that the study areas exhibited different arrangements of ant fauna, with a high turnover in species composition between sites, indicating high beta diversity. Our analysis suggests that the biogeographic history of this tropical dry forest in the centre of South America could explain ant assemblage structure more than competitive dominance. The co-occurrence analysis showed that species co-occur less often than expected by chance in only two of the localities, suggesting that, for most of the species, co-occurrences are random. The assessment of the structure of the diversity of litter-dwelling ants is the first step in understanding the beta diversity patterns in this region of great biogeographic importance.

1. Introduction

The highly diverse ant fauna of the leaf litter in tropical forests has been the focus of many studies investigating the structure of ecological communities, particularly in the last twenty years [1–3]. Approximately 60% of the entire ant species that are currently known live in leaf litter, where the ant fauna is especially diverse, taxonomically, morphologically, and ecologically [4–6]. Studies on the biogeography and diversity of Formicidae, as well as the processes affecting their maintenance, can be of great interest for planning effective conservation of the biota at a regional scale. Such studies can also contribute to producing new ecological and taxonomic data, particularly in areas where no previous records exist for the group [7, 8].

The Serra da Bodoquena, within the Chacoan subregion, borders the provinces Chaco, Cerrado, Pantanal and Parana Forest [9, 10] and is a place with no previous ant records. Prado and Gibbs [11] pointed out that seasonal deciduous forests are remnants of a broader continuous distribution that was present in the past, ranging from north-eastern Brazil to Argentina in the Pleistocene dry period. This currently fragmented structure is the result of the dry, cold climate that caused the retraction of wet forests to riversides and the spread of seasonal forests [12]. Deciduous forests comprise discontinuous patches along fertile valleys and basaltic and calcareous rocks in a matrix of Cerrado on the Brazilian Central Plateau. This matrix, intersected by riparian forests, acts as a connection among dry forests in north-eastern Brazil, east of Minas Gerais and São Paulo States, and forest remnants in Pantanal. The vegetation has some floristic similarities to the Amazon and the Paraguayan Chaco [13, 14].

We investigated whether the leaf-litter ant assemblages at Serra da Bodoquena could be explained by current factors, such as ant dominance and competition, or if the community structure was influenced by its geography, which differs between the northern and southern portions of Park.

Some hypotheses regarding the array of situations found in the region could be tested, assuming that the vegetation properties are also valid for the ant assemblage. Despite the biogeographic relationships of the vegetation, current and evolutionary effects of environmental formations may be reflected in the structure of the ant community. Interspecific competition is usually associated with significant divergence and with the principle of limiting similarity [15]. Although niche differentiation is undoubtedly an important concept, it seems insufficient to wholly determine the high levels of local diversity commonly observed in warm climates [5, 6, 16, 17].

Significant aggregations of assemblages have been associated with the presence of environmental filters [18]. The coexistence of species would be more frequent than expected if randomly organized, because of environmental conditions that act as environment filters, allowing only a narrow spectrum of species to survive. We discuss the possibility that the structure of the leaf-litter ant community in Serra da Bodoquena could be influenced by neighbouring landscapes, as it is situated at the intersection between the Pantanal, Chaco, Cerrado, Brazilian Atlantic Forest, and Amazon Forest biomes. Alternatively, the fauna could be completely different and specific to this Seasonal Deciduous Forest.

The following issues were based on three sets of arguments; namely, (i) if the similarity between the sampling sites is high, the ant fauna of the north and south portions of the dry forest could be derived from the same historic processes and by the same selective ecological pressures and could be driven by a single colonisation process (this argument assumes that all species have an equal probability of colonisation in all sites); (ii) if the north and south portions of the forest have a distinct fauna, this suggests that the geographic basis is important to the formation of the ant assemblages once the different portions attained a distinct physiographic structure. (Therefore, the question is whether the faunistic similarity of ant communities between the northern and southern portions of Serra da Bodoquena is low, blocks are likely to be formed through different colonisation processes;) or (iii) if the samples are dissimilar among sites, a series of distinct ecological, spatial, and temporal situations may have contributed to the formation of leaf-litter ant assemblage in the region, and the surrounding environments influence the faunistic colonisation.

The goal in the present study was thus to identify associating parameters between the community structure of leaf-litter ants and the phytophysionomic matrix within the two distinct land portions in Serra da Bodoquena National Park.

In central-western Brazil, the expansion of agriculture and intensive cattle farming has led to a dramatic loss of forests. Thus, it is likely, this insect diversity has already been affected before it is has been thoroughly evaluated. Therefore, the assessment of the structure of the diversity of leaf-litter ants is the first step in understanding these patterns in this region of great biogeographic importance.

2. Materials and Methods

This study was carried out in a seasonal deciduous forest area in Serra da Bodoquena National Park (core coordinates: 21°07′16″S 56°28′55″W). This is the only fully protected Federal Reserve of Mato Grosso do Sul, Brazil. It harbours significant portions of seasonal deciduous and semideciduous forests, transitional areas between Cerrado and Brazilian Atlantic Forest, Cerrado and Tropical Seasonal Deciduous Forest, marshes, rocky fields, and anthropic lands with cattle farms.

The western region of the Bodoquena mountain range is formed by a mosaic of vegetational types; lowlands, including savannas steppe, arborous and gramineous Chaco, plus xeromorphic and mesoxeromorphic forests. To the east, there are many cattle farms within what used to be Cerrado vegetation, to the south, there are soybean farms and islands of semi-deciduous forest, and to the north, there are the Pantanal plains. The island of preserved dry forest areas in this region is the largest of those in the centre of South America.

The Serra da Bodoquena National Park has an area of 77,200 ha, made up of a steep plateau in the west, and comprising two distinct land portions that together cover a 300 × 50 km area. The area is preserved because it is a watershed that supplies the drainage basins of the Western region of Brazil [19]. The region divides important water catchments. Salobra River, in the Northern land portion, fuels the Miranda River on Pantanal plains, and Perdido River, in the Southern land portion, fuels the Apa River. Both rivers are tributaries of the Paraguay River, although their respective waters only mix after a thousand kilometres (Figure 1).

The locality is sustained by calcareous rocks of the Corumbá group-Neoproterozoic III. It is characterised by a high rocky massif, with altitudes varying between 200 m and 770 m asl. Exposed limestone from the Tamengo formation predominates in this karstic region, where rivers are found within canyons [20, 21].

The annual average temperatures of the area vary between 22°C and 26°C. The minimum temperature can be as low as 0°C. The relative humidity is low and rarely reaches 80%, and rainfall varies between 1300 mm and 1700 mm a year. The hot and rainy season occurs between October and April, and the cold and dry season from May to September [22].

The survey was carried out from September 2005 to February 2008, with samples taken in the dry and wet seasons, at 10 selected sites, in eight collecting expeditions (in two expedictions has two sites) along the Bodoquena ridge (Table 1), covering the microbasin of Salobra River in the Northern land portion, including the Kadiwéu Indian Reserve, and the microbasin of Perdido River, in the Southern land portion (Figure 2).

The leaf-litter sampling ant was carried out according to the ALL protocol [2], with a few adaptations due to

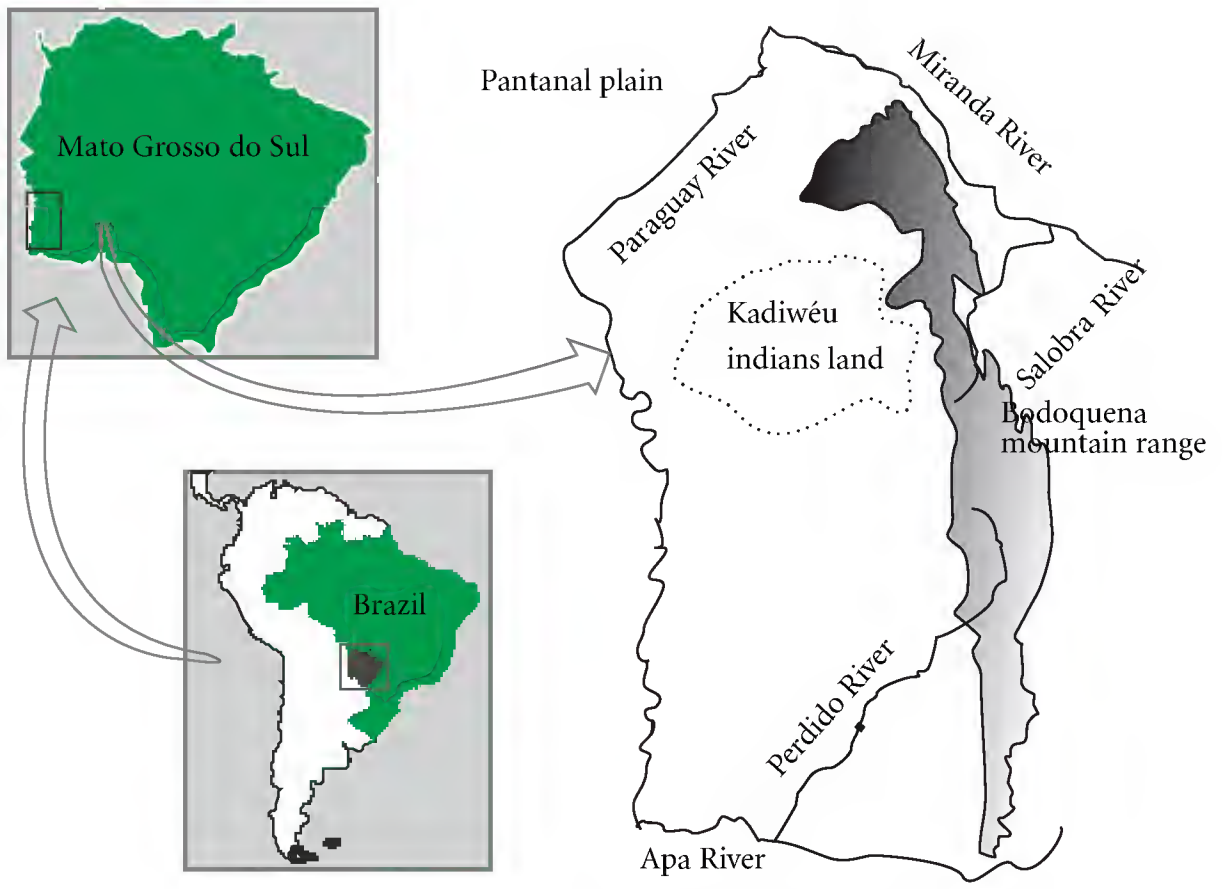


Figure 1: Map of Serra da Bodoquena (Chacoan sub-region), bordering the Pantanal province, State of Mato Grosso do Sul, Brazil.

Sites of capture

Image chart of study area: Serra da Bodoquena National Park

Polyconic projection-South American datum 1969

Cartographic base: landsat mosaic, path/rows 266/74 + 75;

acquisition dates: 2/Sept/07 + 13May/07

1 : 750000

(1) Balneário Perdido River

(2) Sta Maria Farm-Perdido River

(3) Harmonia Farm

(4) Boqueirão Farm

(5) DaMata Farm

(6) Sta Laura Farm and Salobra River-left and right margin

(7) California Farm

(8) Kadiwéu reserve

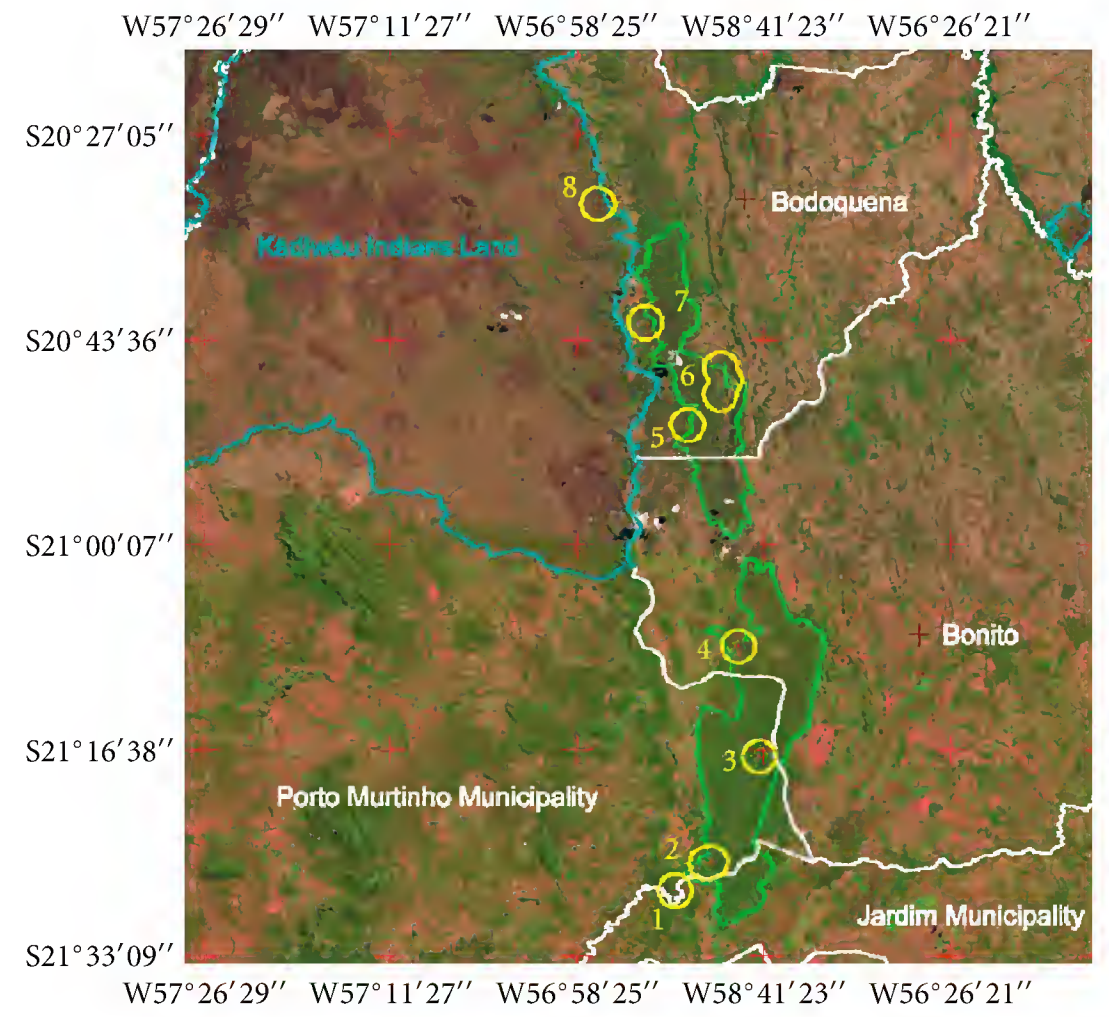


Figure 2: Landsat image indicating eight localities studied in Serra da Bodoquena National Park, Brazil. Obs: in Salobra river (site 6) three sampling points were performed (III, IV, and IX).

the habitat being composed of limestone rock floors which made it impossible to use pitfall traps in most areas. A total of 262 leaf-litter samples of 1 m^2 were extracted using mini-Winkler sacks [23]. A high diversity of microhabitats with a stratified structure was observed in the study areas. Inside the forest, there are calcareous floors and rocks with little-litter accumulation. The sampled points were chosen randomly along each transect of 40 m × 500 m but set at minimum intervals of 20 m. In each transect a minimum of 25 samples were taken. We searched for microhabitats in dry forest with sufficient leaf-litter accumulation so as to obtain approximate 2 kg. The sample exposure time of the material inside the extractor was 24 hours.

The ant identifications follow Bolton [24, 25], Fernández [26], Baroni-Urbani and De Andrade [27], and LaPolla et al. [28]. Voucher specimens were deposited at the Museu de Biodiversidade da Universidade Federal da Grande Dourados (MuBio-UFGD, Mato Grosso do Sul, Brazil) under the

Table 1: Sampling localities, number of samples per site, altitude above sea level, sampling season, and geographic coordinates.

Points/sites	Number of samples	ASL	Portion	Season	Coordinates
(I) Balneário Perdido river	25	357 m	South	Dry	21°27′55.00″S 56°48′34.31″W
(II) Boqueirão farm	32	511 m	South	Wet	21°08′13.94″S 56°43′28.00″W
(III) Salobra river-left margin	25	221 m	North	Dry	20°46′48.87″S 56°44′32.78″W
(IV) Salobra river-right margin	25	248 m	North	Dry	20°47′59. 94″S 56°44′54.05′W
(V) Harmonia farm-Perdido river	25	460 m	South	Wet	21°17′09.8′S 56°41′45.5′W
(VI) Califórnia farm	25	464 m	North	Wet	20°42′11.81″S 56°50′57.56″W
(VII) Kadiweu reserve	25	306 m	North	Wet	20°32′41.48″S 56°54′44.66″W
(VIII) Da Mata farm	25	578 m	North	Dry	20°50′26.16″S 56°47′31.85″W
(IX) Sta Laura farm-Salobra river	30	233 m	North	Wet	20°45′53.6″S 56°44′53.11″W
(X) Sta Maria farm-Perdido river	25	402 m	South	Wet	21°25′39.24′S 56°45′48.90″W

reference numbers Hym00108F to Hym02332F, at the Laboratório de Mirmecologia, Cocoa Research Centre, (CPDC, Ilhéus, Bahia, Brazil), and at the Museu de Zoologia da Universidade de São Paulo (MZ-USP, São Paulo, Brazil).

The data were considered for sites independently and grouped by land portions, South (presumably under Atlantic Forest influence- Paraná subregion), and North (presumably under Pantanal influence- Amazonian subregion).

The data analysis was based on the species occurrence in samples (frequency), as accepted for quantifying social insects [29]. To estimate species richness, the Chao 2 and Jackknife 2nd-order estimators were calculated using-EstimateS 7.5 [30], which are widely used in ant diversity studies [31–33].

Rarefaction curves showing the expected species richness versus species occurrence were used to assess the sampling efficiency for each sample area [34]. From the observed species richness per site, we estimated the number of species remaining to be sampled using the second-order Jackknife estimator (incidence based). The expected number of species was plotted against number of species records on the x-axis (individual-based accumulation curve). This plot provides a measure of species diversity which is robust to sample size effects.

In order to verify if there are differences in betadiversity increasing between northern and southern land portions, the two data sets were compared following a north-south axis and were plotted by increasing ant diversity against the distance between successive sample series in the eight localities.

To analyse site similarity, we used a principal coordinate analysis (PCO) using the Bray-Curtis dissimilarity index [35, 36]. The similarity among the ant assemblages at the different seasons and altitude was assessed using a cluster analysis (Jaccard coefficient of similarity). The resulting similarity matrix was analysed through a sequential, agglomerative, hierarchical, and nested clustering algorithm, described by Sneath and Sokal [37]. The option used was the Unweighted Pair-Group Method, arithmetic average (UPGMA). This analysis was conducted using the MVSP 3.1 software [38].

The diversity and similarity analyses were run using EstimateS 7.5 [30]. Similarity and distance matrices (Euclidian distance) were compared using a Mantel's test [39]. The data set was analysed using R software [40], using the Vegan package [41]. The graphic design was constructed with Statistica for Windows 6 [42]. The Morisita-Horn index was used too to evaluate the similarity among the localities, pairwise, because this index is not affected by the number of samples or the species richness, except for very small sampling niches [43, 44].

We used EcoSim (version 7.72) to compute random matrices of species co-occurrences [45] to determine whether the mean and variance C-score among samples is larger or smaller than expected by chance. Co-occurrences based on averages that were calculated across all possible pairs of species were randomised (5,000 repetitions) within the constraint of fixed marginal totals, which is an appropriate null model for detecting patterns caused by species interactions [46].

3. Results

More than 20,000 ants were captured in the seasonal deciduous forest. We recorded 170 species from 45 genera and

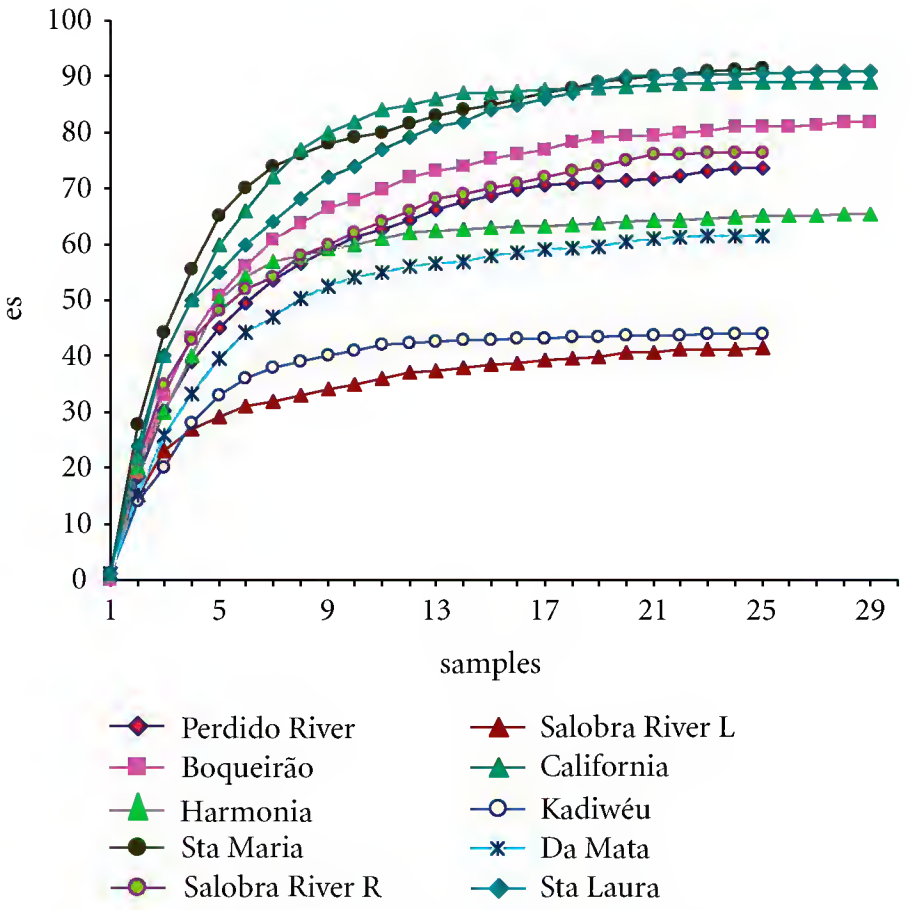


FIGURE 3: Comparison of incidence-based ant species rarefactions curves showing expected species richness (es) versus sampling effort in ten sampling sites (samples) in the Bodoquena mountain range.

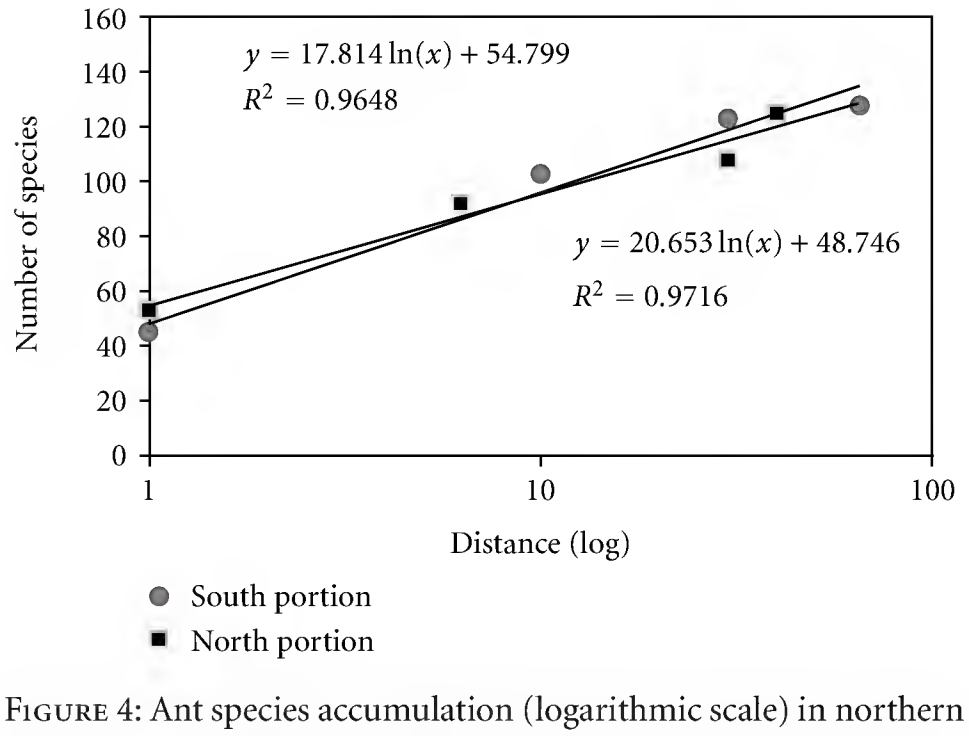


FIGURE 4: Ant species accumulation (logarithmic scale) in northern and southern portions in Serra da Bodoquena, Mato Grosso do Sul, Brazil. Obs: eight localities were considered, as collecting points III, IV, and IX were grouped as a single sample.

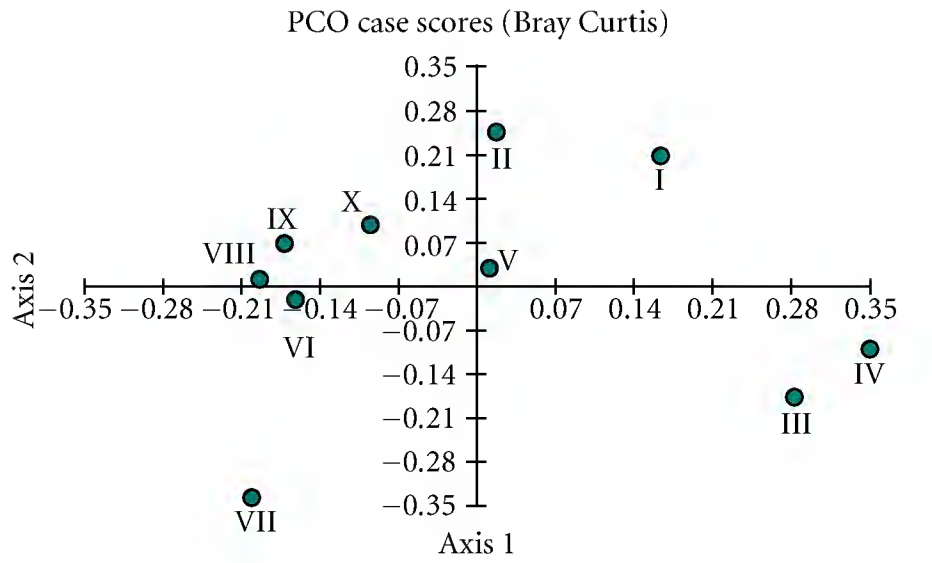


FIGURE 5: PCO analysis of the sites located in the northern and southern portions of Serra da Bodoquena using the Bray-Curtis dissimilarity index (eigenvalues: 0001).

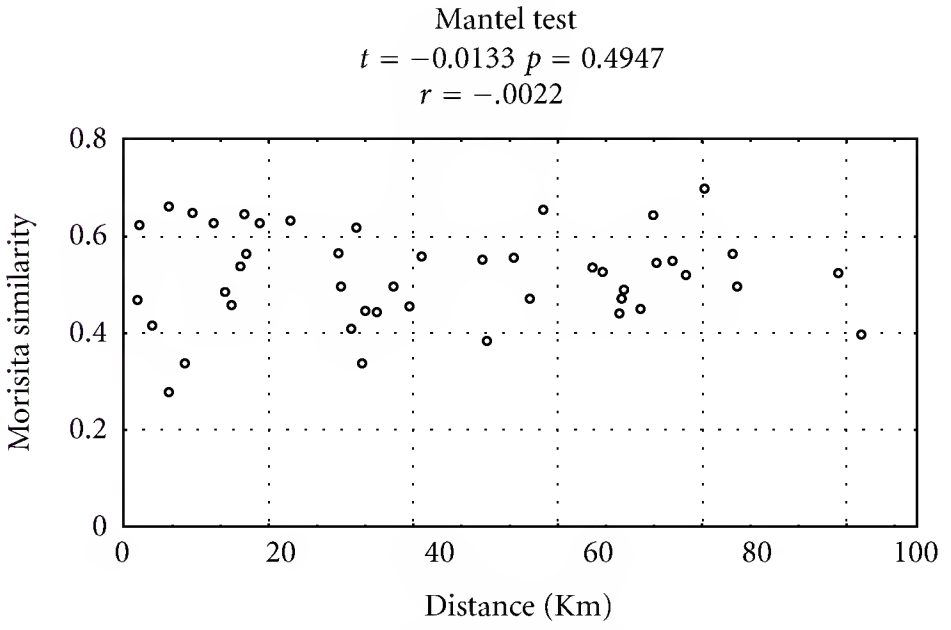


FIGURE 6: Mantel's test analysis between the values obtained using the Morisita-Horn similarity index and the distance between sites (km).

11 subfamilies out of the 15 subfamilies of Formicidae known to occur in the Neotropical Region (Table 2).

The most frequently observed species were *Solenopsis* (*Diplorhoptrum*) sp. 1 (91 occurrences), *Cyphomyrmex* (gr. *rimosus*) sp. 1 (85), *Solenopsis* sp. 2 (81), and *Hypoponera* sp. 7 (77). The most speciose genera were: *Hypoponera* (21 species), *Pheidole* (17), *Cyphomyrmex* (12), *Strumigenys* (13), *Solenopsis* (11), and *Basiceros* (9). We recorded the first observations of the genus *Cryptomyrmex* in the central-west Brazilian region. Three new species were found in deciduous forest: *Asphinctanilloides* sp. new, *Amblyopone* sp. new, and *Probolomyrmex* sp. new.

A total of 37 species were recorded only once (singletons). The total number of singletons represents approximately 20% of all ant species collected in the present study (Table 3).

Rarefaction curves (Figure 3) show the sampling effort in each sample site, for each land portion. Evidence of asymptotes indicates that most leaf litter ant species were sampled with the number of samples used.

The comparison of northern and southern data sets did not reveal any difference in the betadiversity of the ant communities. The two distributions follow more or less the same pattern of species substitution in relation to increasing the distance of sample sites (Figure 4).

The PCO analysis (Figure 5) shows a consistency of groups between northern and southern land portions. The analysis grouped areas of southern (I, II, and V) and also grouped the sites of northern (VI, VIII, and IX); the site X (southern) is close with this group. The samples most similar were taken in the Salobra River (III and IV) in the same season (dry).

There was no correlation between the similarity (Morisita-Horn) among species frequencies in the communities

Table 2: Records of occurrences of the 170 species collected at 10 sampling sites in Serra da Bodoquena National Park, Mato Grosso do Sul, Brazil.

Species	I	II	III	IV	V	VI	VII	VIII	IX	X
Amblyoponinae										
Tribe Amblyoponini										
Amblyopone elongata (Santschi 1912)	—	—	—	—	2	—	—	—	—	—
Amblyopone lurilabes Lattke 1991	—	5	—	—	—	—	1	—	—	—
Amblyopone sp. new	1	—	—	—	—	—	—	—	—	—
Cerapachyinae										
Tribe Cerapachyini										
Cerapachys splendens Borgmeier 1957	—	—	—	—	—	1	—	—	—	1
Dolichoderinae										
Tribe Dolichoderini										
Azteca alfari Emery 1893	4	3	2	1	4	3	—	1	4	—
Dolichoderus sp. 1	1	—	—	—	—	—	—	—	—	—
Dorymyrmex sp. 1	1	—	—	—	—	—	—	—	—	—
Linepithema humile (Mayr 1868)	2	—	1	—	4	—	—	—	—	—
Ecitoninae										
Tribe Ecitonini										
Neivamyrmex sp. 1	—	—	2	—	—	2	—	1	2	2
Neivamyrmex sp. 2	—	1	—	—	—	—	—	—	—	—
Ectatomminae										
Tribe Ectatommini										
Ectatomma brunneum Smith 1858	—	—	—	—	—	—	—	—	—	1
Ectatomma edentatum Roger 1863	—	—	—	—	—	—	—	—	1	—
Gnamptogenys striatula Mayr 1884	—	—	—	—	3	—	—	—	2	2
Gnamptogenys (gr. *striatula*) sp. 1	—	—	1	—	—	—	—	—	—	1
Gnamptogenys sulcata (Smith 1858)	—	—	—	—	—	—	—	—	1	—
Tribe Typhlomyrmecini										
Typhlomyrmex rogenhoferi Mayr 1862	—	—	1	—	—	—	—	—	—	—
Typhlomyrmex sp. 1	—	—	—	1	—	—	—	—	—	—
Formicinae										
Tribe Camponotini										
Camponotus crassus Mayr 1862	—	—	—	—	—	—	—	—	1	—
Camponotus sp. 1	—	—	—	—	—	1	—	—	—	—
Camponotus sp. 2	—	—	—	—	—	—	—	—	—	1
Tribe Plagiolepidini										
Brachymyrmex sp. 1	4	3	1	2	—	3	1	—	—	3
Brachymyrmex sp. 2	7	—	4	—	—	—	—	3	—	—
Brachymyrmex sp. 3	1	1	—	—	—	—	—	—	2	—
Brachymyrmex sp. 4	—	—	—	—	—	—	—	3	—	—
Nylanderia fulva (Mayr 1862)	—	1	—	—	3	2	—	1	—	1
Nylanderia sp. 1	—	—	2	—	1	1	3	1	—	—
Nylanderia sp. 2	1	—	1	—	3	1	—	3	1	1
Nylanderia sp. 3	—	—	—	—	—	—	2	—	—	2
Nylanderia sp. 4	—	—	—	—	—	1	—	—	—	—
Nylanderia sp. 5	—	—	—	—	—	1	—	—	—	—
Paratrechina longicornis (Latreille 1802)	1	3	—	2	3	—	—	2	4	1

Table 2: Continued.

Species	I	II	III	IV	V	VI	VII	VIII	IX	X
Leptanilloidinae										
Asphinctanilloides sp. new	—	—	—	—	—	1	—	—	—	—
Myrmicinae										
Tribe Adelomyrmecini										
Cryptomyrmex boltoni (Fernández 2003)	—	—	—	—	1	—	—	—	—	—
Tribe Attini										
Acromyrmex subterraneus (Forel 1893)	—	3	—	—	—	—	—	—	—	—
Acromyrmex sp. 1	2	—	—	—	—	—	—	—	—	—
Acromyrmex sp. 2	—	4	—	—	—	—	—	—	—	—
Apterostigma manni Weber 1938	—	1	—	—	—	—	—	—	—	—
Apterostigma pilosum Mayr 1865	1	1	—	—	—	—	—	—	—	—
Apterostigma wasmanni Forel 1892	4	1	—	—	—	—	—	—	—	9
Atta sp. 1	2	—	—	—	—	—	—	—	—	—
Cyphomyrmex lectus (Forel 1911)	—	—	5	—	—	—	—	—	—	—
Cyphomyrmex olitor Forel 1893	—	1	—	—	—	—	—	—	—	—
Cyphomyrmex (gr. *rimosus*) sp. 1	11	9	7	4	6	13	8	3	10	14
Cyphomyrmex (gr. *rimosus*) sp. 2	6	8	5	—	8	4	—	6	7	6
Cyphomyrmex (gr. *rimosus*) sp. 3	—	2	—	—	—	6	—	1	4	5
Cyphomyrmex (gr. *rimosus*) sp. 4	—	1	—	—	2	1	—	—	—	—
Cyphomyrmex (gr. *rimosus*) sp. 5	—	2	—	—	—	1	—	—	—	5
Cyphomyrmex (gr. *rimosus*) sp. 6	—	—	—	—	—	2	—	—	—	—
Cyphomyrmex (gr. *rimosus*) sp. 7	—	1	—	—	—	2	—	—	3	—
Cyphomyrmex (gr. *rimosus*) sp. 8	—	2	—	—	—	—	—	2	2	—
Cyphomyrmex (gr. *strigatus*) sp. 1	—	—	—	—	8	—	—	—	—	—
Cyphomyrmex (gr. *strigatus*) sp. 2	—	—	—	—	—	—	—	—	—	3
Mycocepurus goeldii (Forel 1893)	—	1	12	11	3	5	5	2	6	6
Mycocepurus smithii (Forel 1893)	—	1	—	—	—	—	—	—	—	—
Mycocepurus sp. 1	1	—	—	—	—	—	—	—	—	—
Myrmicocrypta sp. 1	—	3	—	1	1	—	—	—	—	—
Sericomyrmex (gr. *amabilis*) sp. 1	—	—	3	2	—	2	—	2	—	—
Sericomyrmex (gr. *amabilis*) sp. 2	—	—	—	—	—	—	—	1	—	—
Sericomyrmex sp. 1	—	4	—	—	—	3	—	—	—	—
Trachymyrmex sp. 1	4	—	—	—	—	—	—	—	—	—
Trachymyrmex sp. 2	1	1	—	—	—	—	—	—	—	—
Tribe Blepharidattini										
Wasmannia auropunctata (Roger 1863)	—	2	3	2	—	9	—	3	—	1
Wasmannia lutzi Forel 1908	1	2	1	—	—	—	—	4	1	4
Wasmannia sp. 1	—	—	—	—	3	4	5	1	—	1
Wasmannia sp. 2	—	—	—	—	—	2	—	3	1	4
Wasmannia sp. 3	—	—	—	—	—	—	—	1	2	—
Tribe Cephalotini										
Cephalotes atratus (Linnaeus 1758)	1	—	—	—	—	—	—	—	—	—
Cephalotes sp. 1	—	—	—	—	—	—	—	—	1	1
Cephalotes sp. 2	—	—	—	—	—	—	—	1	1	—
Procryptocerus alternatus Smith 1876	1	—	—	—	—	—	—	—	—	—
Tribe Crematogastrini										
Crematogaster curvispinosa Mayr 1862	—	—	4	—	—	1	1	—	—	—
Crematogaster sp. 1	—	—	3	—	2	—	—	—	—	—

Table 2: Continued.

Species	I	II	III	IV	V	VI	VII	VIII	IX	X
Crematogaster sp. 2	1	—	—	2	4	—	1	—	—	—
Crematogaster sp. 3	—	2	—	—	—	—	—	—	—	—
Crematogaster sp. 4	—	3	—	—	—	—	—	—	—	—
Tribe Dacetini										
Basiceros disciger (Mayr 1887)	—	—	—	—	1	—	—	—	—	—
B. stenognathum (Brown & Kempf 1960)	4	9	—	13	11	11	4	—	8	7
Basiceros (*Octostruma*) *balzani* (Emery 1894)	7	—	10	14	12	10	—	—	7	3
Basiceros (*Octostruma*) *simoni* (Emery 1887)	—	—	—	—	7	7	—	—	7	4
Basiceros (*Octostruma*) *rugifera* (Mayr 1887)	—	—	—	—	6	6	6	—	3	6
Basiceros (*Octostruma*) sp. 1	—	—	—	—	5	—	—	4	2	4
Basiceros (*Octostruma*) sp. 2	—	—	—	—	2	—	—	—	2	—
Basiceros (*Octostruma*) sp. 3	—	5	—	—	—	—	—	—	—	—
Basiceros (*Octostruma*) sp. 4	4	—	—	—	—	2	—	—	—	—
Strumigenys eggersi Emery 1890	3	3	7	—	12	9	5	3	16	4
Strumigenys (*gr. elongata*) sp. 1	—	—	2	—	—	3	—	—	—	—
Strumigenys xenochelyna (Bolton 2000)	3	4	—	—	14	—	—	—	—	—
Strumigenys sp. 1	—	—	—	7	2	—	—	—	—	2
Strumigenys sp. 2	—	2	—	—	2	4	—	—	2	4
Strumigenys sp. 3	—	—	—	—	—	—	3	—	—	—
Strumigenys sp. 4	1	—	—	—	—	—	—	—	—	—
Strumigenys sp. 5	3	6	—	—	8	—	—	—	—	—
Strumigenys sp. 6	—	5	9	2	5	4	6	1	14	—
Strumigenys sp. 7	—	—	1	—	—	2	—	—	1	—
Strumigenys sp. 8	—	—	4	—	—	—	—	—	2	—
Strumigenys sp. 9	—	—	1	—	2	3	—	—	—	2
Strumigenys sp. 10	—	—	—	—	—	—	1	—	—	—
Tribe Myrmicini										
Hylomyrma balzani (Emery 1894)	—	—	—	—	1	—	—	—	—	—
Hylomyrma sp. 1	—	—	2	—	—	—	—	—	—	—
Tribe Pheidolini										
Pheidole (gr. flavens) sp. 1	—	—	—	—	—	—	—	—	—	1
Pheidole gertrudae Forel 1886	—	—	—	1	—	—	—	—	1	2
Pheidole sp. 1	4	3	2	2	6	7	5	5	8	4
Pheidole sp. 2	2	11	3	2	5	5	7	5	—	3
Pheidole sp. 3	—	4	2	13	8	—	—	4	14	3
Pheidole sp. 4	1	3	1	4	7	—	—	—	4	3
Pheidole sp. 5	2	—	2	—	5	—	—	—	11	2
Pheidole sp. 6	2	—	1	—	—	—	—	—	3	2
Pheidole sp. 7	—	—	2	2	2	—	—	—	—	—
Pheidole sp. 8	—	—	—	—	—	—	—	—	1	—
Pheidole sp. 9	—	—	—	—	1	2	2	—	2	—
Pheidole sp. 10	—	—	2	3	—	—	—	—	1	—
Pheidole sp. 11	—	—	—	—	—	3	3	—	—	—
Pheidole sp. 12	—	—	—	—	—	1	3	—	—	—
Pheidole sp. 13	—	—	—	—	—	4	2	—	—	—
Pheidole sp. 14	—	—	—	—	—	—	—	1	—	—
Pheidole sp. 15	—	—	—	—	—	9	6	—	1	—

Table 2: Continued.

Species	I	II	III	IV	V	VI	VII	VIII	IX	X
Tribe Solenopsidini										
Carebara sp. 1	8	10	6	3	6	9	2	3	9	3
Carebara sp. 2	2	3	1	—	7	4	6	—	8	2
Megalomyrmex silvestrii Wheeler 1909	—	—	—	—	—	3	—	3	—	—
Megalomyrmex wallacei Mann 1916	—	—	—	—	—	—	3	—	—	—
Monomorium sp. 1	—	—	14	2	—	—	—	—	—	—
Oxyepoecus sp. 1	—	—	1	—	—	—	—	—	—	—
Solenopsis (gr. *geminata*) sp. 1	—	—	1	—	—	—	—	—	—	—
Solenopsis (gr. *invicta*) sp. 1	4	2	—	—	—	4	—	1	3	14
Solenopsis (gr. *invicta*) sp. 2	1	—	—	—	—	—	—	—	1	—
Solenopsis (*Diphorhoptrum*) sp. 1	10	8	6	10	10	12	5	8	7	15
Solenopsis sp. 2	5	6	4	2	3	9	—	22	20	10
Solenopsis sp. 3	3	2	5	4	2	4	9	3	2	4
Solenopsis sp. 4	8	6	8	9	—	4	—	3	1	2
Solenopsis sp. 5	1	—	—	—	—	4	—	—	5	4
Solenopsis sp. 6	—	—	—	—	—	9	—	3	3	3
Solenopsis sp. 7	2	6	—	—	—	4	—	4	4	1
Solenopsis sp. 8	—	—	—	—	—	—	—	—	10	—
Tribe Stenammini										
Rogeria alzatei Kugler 1994	—	—	—	—	—	—	1	—	—	—
Rogeria lirata Kugler 1994	—	1	—	—	—	1	7	—	—	—
Rogeria sp. 1	—	—	1	—	—	—	—	—	2	—
Rogeria sp. 2	—	—	1	—	—	—	—	—	2	—
Ponerinae										
Tribe Ponerini										
Anochetus diegensis Forel 1912	2	—	7	4	3	5	6	2	9	10
Hypoponera sp. 1	10	11	8	11	15	6	—	—	—	8
Hypoponera sp. 2	4	13	9	9	7	—	4	—	7	9
Hypoponera sp. 3	—	—	7	6	—	17	6	3	—	4
Hypoponera sp. 4	—	6	—	—	3	8	2	—	7	4
Hypoponera sp. 5	—	1	—	—	4	—	—	—	—	6
Hypoponera sp. 6	2	3	—	—	—	18	7	8	11	11
Hypoponera sp. 7	4	5	—	—	13	6	13	12	17	7
Hypoponera sp. 8	—	9	—	—	2	3	—	—	3	6
Hypoponera sp. 9	5	2	—	—	—	—	—	—	2	4
Hypoponera sp. 10	—	3	—	—	6	1	3	1	9	2
Hypoponera sp. 11	—	—	—	—	—	—	—	—	1	1
Hypoponera sp. 12	—	—	—	—	—	—	—	—	—	1
Hypoponera sp. 13	—	—	—	—	—	—	—	—	—	4
Hypoponera sp. 14	—	—	—	—	—	6	—	—	—	2
Hypoponera sp. 15	—	—	—	—	—	1	—	—	—	1
Hypoponera sp. 16	—	—	—	—	3	—	—	—	—	—
Hypoponera sp. 17	—	—	—	—	—	—	—	—	—	2
Hypoponera sp. 18	—	—	—	—	1	3	—	2	—	—
Hypoponera sp. 19	—	2	—	—	—	2	—	3	—	—
Hypoponera sp. 20	—	1	—	—	—	1	—	—	—	—
Hypoponera sp. 21	—	6	—	—	—	4	—	—	—	—

Table 2: Continued.

Species	I	II	III	IV	V	VI	VII	VIII	IX	X
Leptogenys sp. 1	—	1	1	—	1	—	—	—	—	—
Odontomachus bauri Emery 1892	—	—	2	—	—	2	—	—	—	—
Odontomachus chelifer (Latreille 1802)	—	—	—	—	—	2	—	—	—	—
Odontomachus meinerti Forel 1905	2	—	4	—	2	2	1	—	3	1
Pachycondyla harpax (Fabricius 1804)	—	—	—	—	—	—	—	—	4	—
Pachycondyla lunaris (Emery 1896)	—	—	—	—	—	—	—	—	—	1
Pachycondyla marginata (Roger 1861)	—	—	—	—	—	—	—	—	1	—
Pachycondyla ferruginea (Smith 1858)	—	—	12	2	—	—	—	1	—	—
Proceratiinae										
Tribe Probolomyrmecini										
Probolomyrmex sp. new	—	—	—	4	—	—	—	—	1	7
Probolomyrmex petiolatus Weber 1940	—	—	1	—	—	—	—	—	—	—
Pseudomyrmecinae										
Tribe Pseudomyrmecini										
Pseudomyrmex gracilis (Fabricius 1804)	2	—	—	—	—	—	—	—	—	3

Table 3: Species richness, estimated richness (Chao 2, Jackknife 2), diversity index, and the number of "singletons" and "doubletons".

Sites	Number of observed species	(Chao 2)	Jackknife2[a]. order	Shannon-Wiener index	Singletons	Doubletons
I	52	65.14	74.26	3.67	17	11
II	62	73.64	82.52	3.85	16	11
III	54	67.14	76.26	3.66	17	11
IV	33	33.67	29.94	3.18	4	12
V	57	59.91	62.29	3.79	8	11
VI	72	80.65	88.80	3.98	15	13
VII	36	39.60	42.87	3.39	6	5
VIII	45	61.33	66.03	3.45	14	6
IX	69	79.80	89.68	3.86	18	15
X	70	82.04	91.38	3.96	17	12
Total	170	231.70	250.38	4.40	37	17

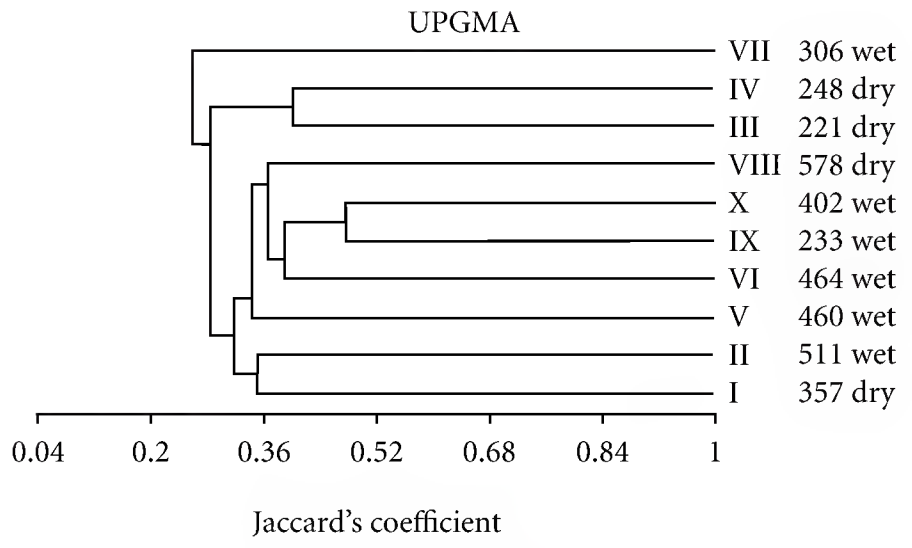


Figure 7: Dendrogram of the similarities (Jaccard coefficient, UPGMA cluster analysis) among 10 sampling sites in the Bodoquena mountain range to leaf-litter ant's fauna. Wet and dry mean seasons, and numbers mean altitude (als).

and the geographical distance (km) between the sites ($r = -0.002$; $P = 0.49$) (Table 4; Figure 6).

The similarity was compared between areas in relation to season and altitude. The samples made during wet season were richer in species, and samples performed in same seasons (VI, IX, and X) appeared grouped. There was no consistent pattern between the assemblages according the altitude ($r = 0.214$; $P = 0.55$) (Figure 7).

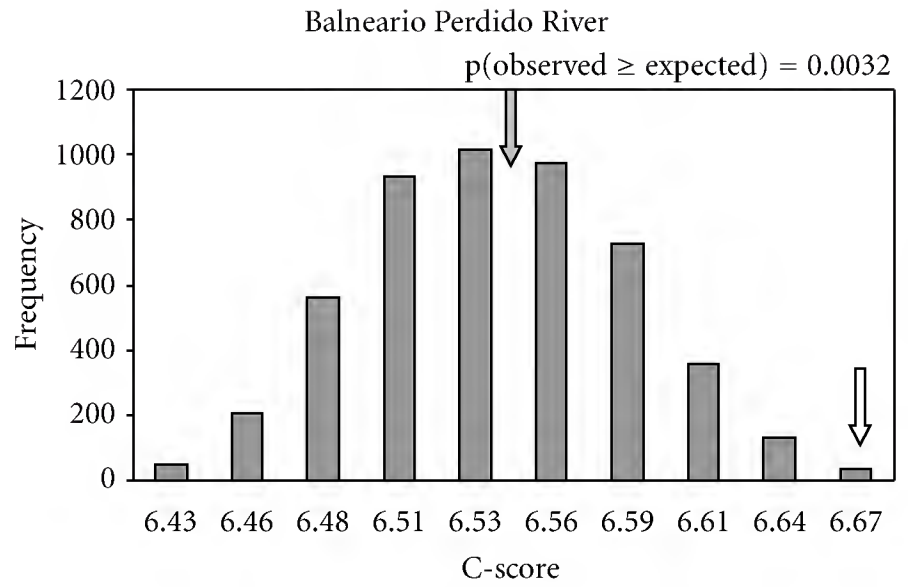
The co-occurrence analysis indicated that species co-occurred less often than expected by chance in only two of the localities sampled P (observed $\geq$ expected) = 0.003, for the Balneário Perdido river, and P (observed $\geq$ expected) = 0.042 for the Santa Maria Perdido River, both in the southern micro-basin, suggesting that species co-occurrences are random (Figure 8).

4. Discussion

Our results suggest that the studied sites exhibit different arrangements of ant fauna with a replacement in abundant species across sampling units, which results in high beta diversity. Gotelli and Ellison [47] suggested that species-energy relationships, in addition to other factors that are

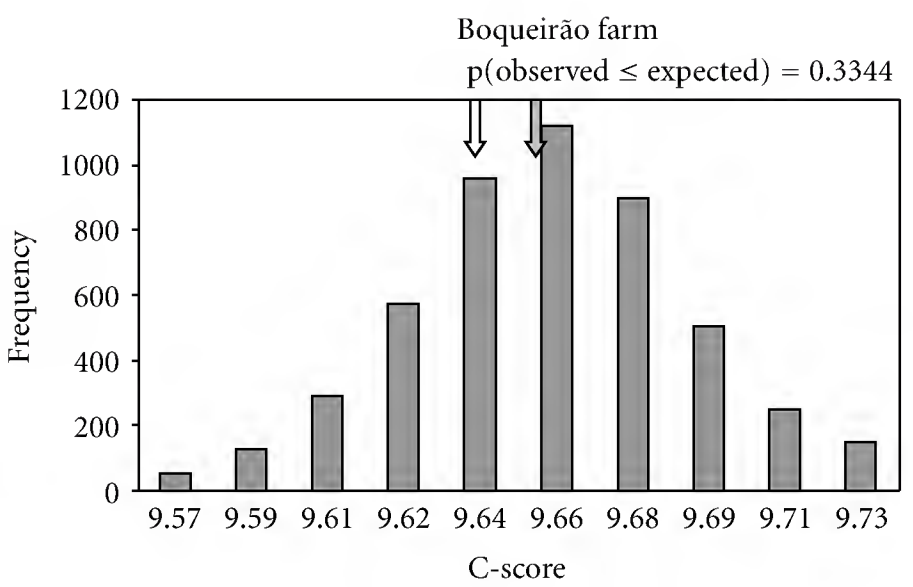
Table 4: Similarity analysis, richness, species shared and distance between sample sites in a pairwise comparison of the leaf-litter ant assemblages from Serra da Bodoquena, Brazil.

Localities		Shared species	Chao shared (estimator)	Morisita-Horn	Distance (Km)
Balneário	Boqueirão	30	36.14	0.67	30.05
	Salobra left margin	25	30.02	0.54	76
	Salobra right margin	18	21.14	0.54	73.77
	Harmonia	26	29.18	0.63	23
	Califórnia	25	25.83	0.56	84.4
	Kadiweu reserve	16	16.59	0.39	102
	Da Mata	20	23.02	0.47	69
	Sta Laura	30	36.44	0.51	77.73
	Sta Maria	32	35.76	0.66	6.2
Boqueirão	Salobra left	22	24.69	0.45	39.5
	Salobra right	20	21.49	0.49	37.3
	Harmonia	31	33.99	0.64	16.7
	Califórnia	36	40.73	0.55	49.7
	Kadiweu reserve	18	19.14	0.44	68.6
	Da Mata	26	30.15	0.44	33.5
	Sta Laura	32	34.93	0.55	41.3
	Sta Maria	35	39.71	0.61	32.2
Salobra left	Salobra right	25	26.48	0.62	2.25
	Harmonia	28	32.67	0.47	56.13
	Califórnia	30	34.48	0.48	13.96
	Kadiweu reserve	17	18.28	0.40	31.46
	Da Mata	24	26.26	0.33	8.43
	Sta Laura	29	38.81	0.46	1.81
	Sta Maria	29	37.35	0.45	71.5
Salobra right	Harmonia	22	24.07	0.55	54
	Califórnia	19	19.22	0.45	14.95
	Kadiweu reserve	14	14.23	0.33	33
	Da Mata	18	20.67	0.27	6.28
	Sta Laura	20	23.23	0.41	3.88
	Sta Maria	23	24.24	0.49	69.3
Harmonia	Califórnia	33	35.42	0.52	66.34
	Kadiweu reserve	22	22.82	0.49	85
	Da Mata	23	28.73	0.38	50.3
	Sta Laura	33	33.54	0.65	58
	Sta Maria	36	37.78	0.56	17
Califórnia	Kadiweu reserve	29	31.70	0.62	18.84
	Da Mata	33	37.57	0.53	16.26
	Sta Laura	37	40.34	0.62	12.5
	Sta Maria	43	48.18	0.69	80.5
Kadiweu	Da Mata	16	18.11	0.44	35
	Sta Laura	20	20.42	0.56	29.7
	Sta Maria	22	22.97	0.52	98.9
Da Mata	Sta Laura	30	39.10	0.64	9.56
	Sta Maria	30	33.74	0.53	64.9
Sta Laura	Sta Maria	45	52.68	0.64	73.22



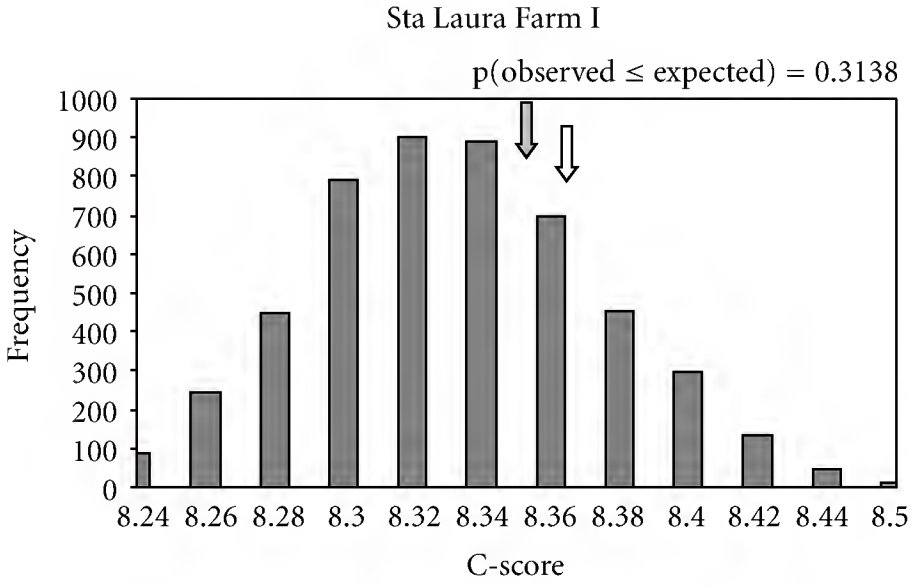
(a)

Sta Laura Farm I



(b)

Sta Laura Farm II



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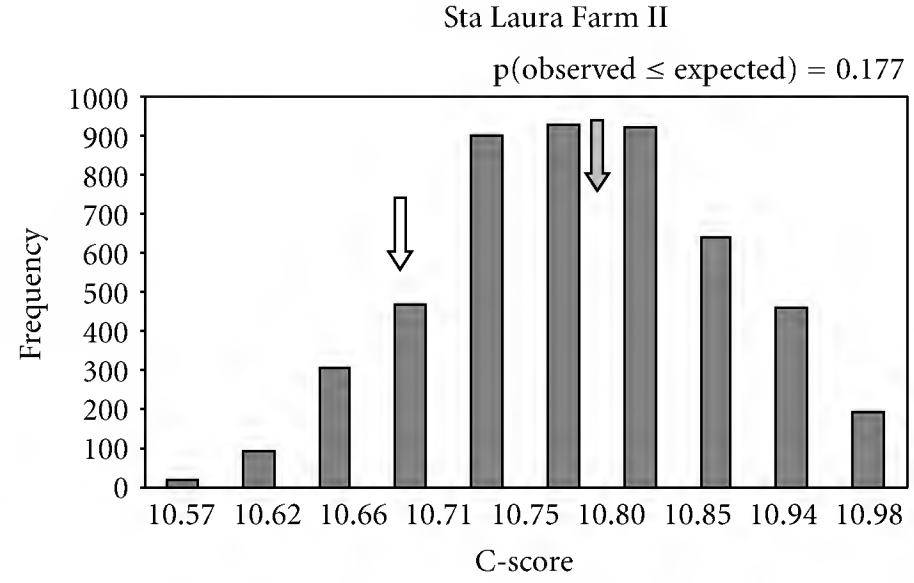
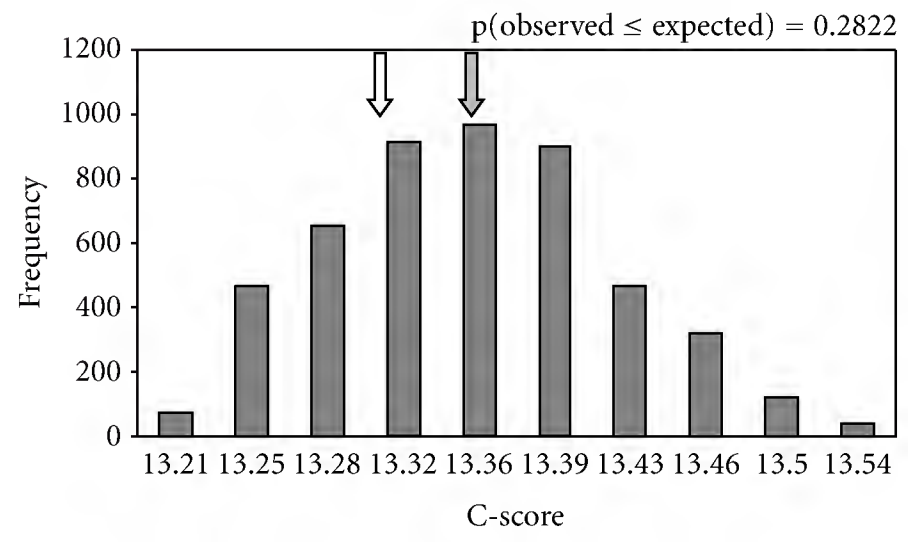


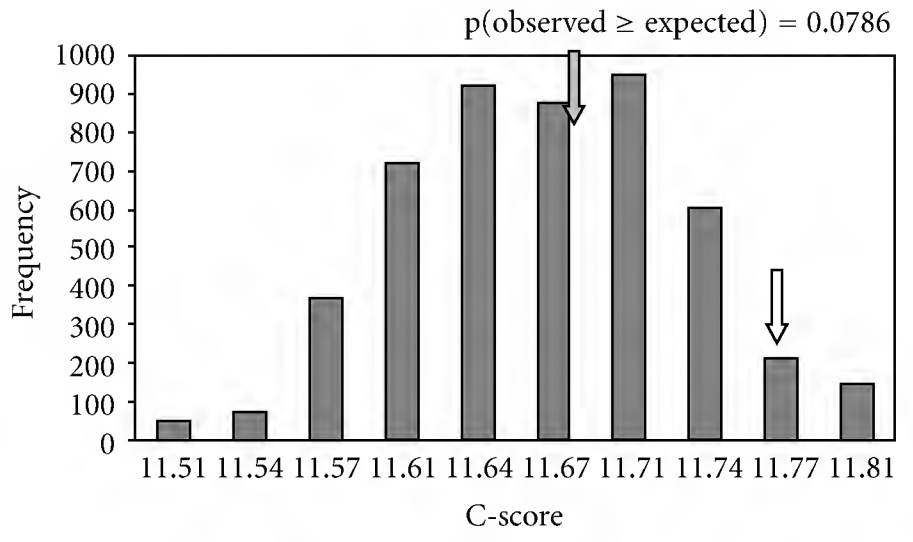
(d)

Harmonia Farm-Perdido River



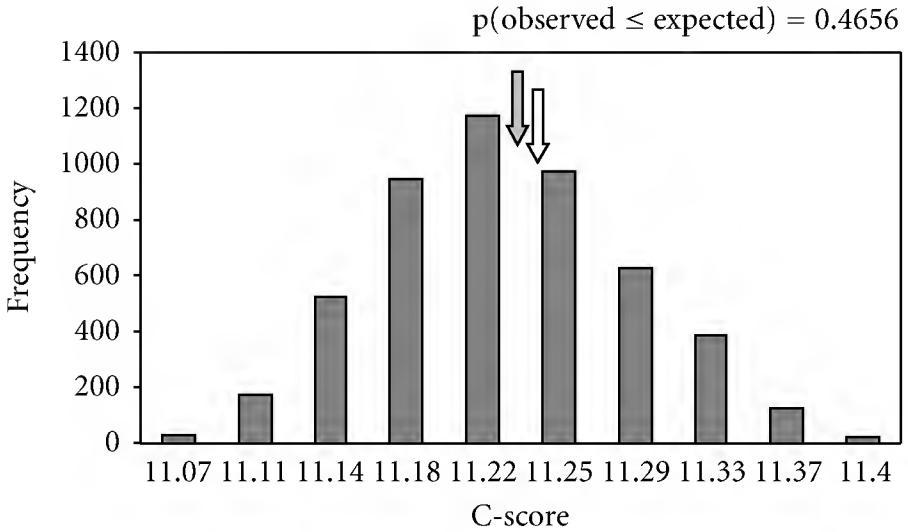
(e)

California Farm



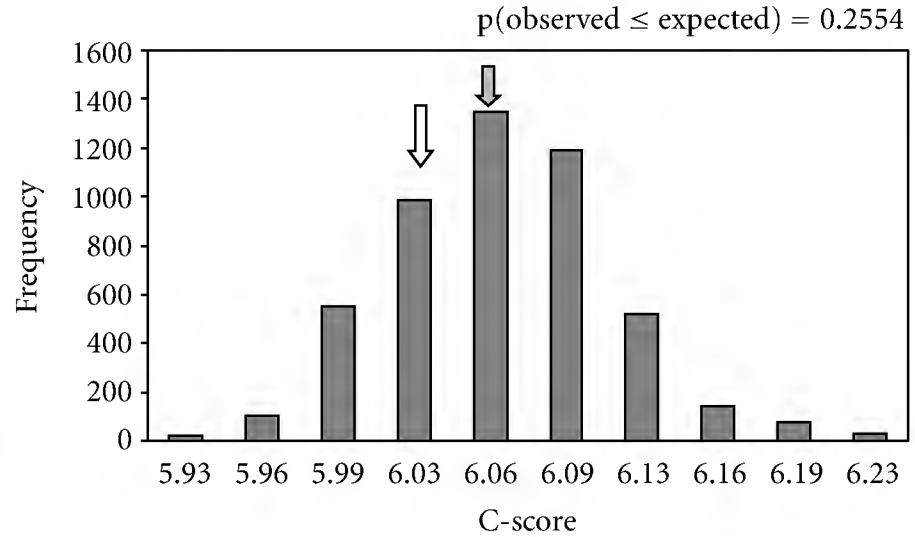
(f)

Kadiwéu reserve



(g)

Da Mata Farm



(h)

Figure 8: Continued.

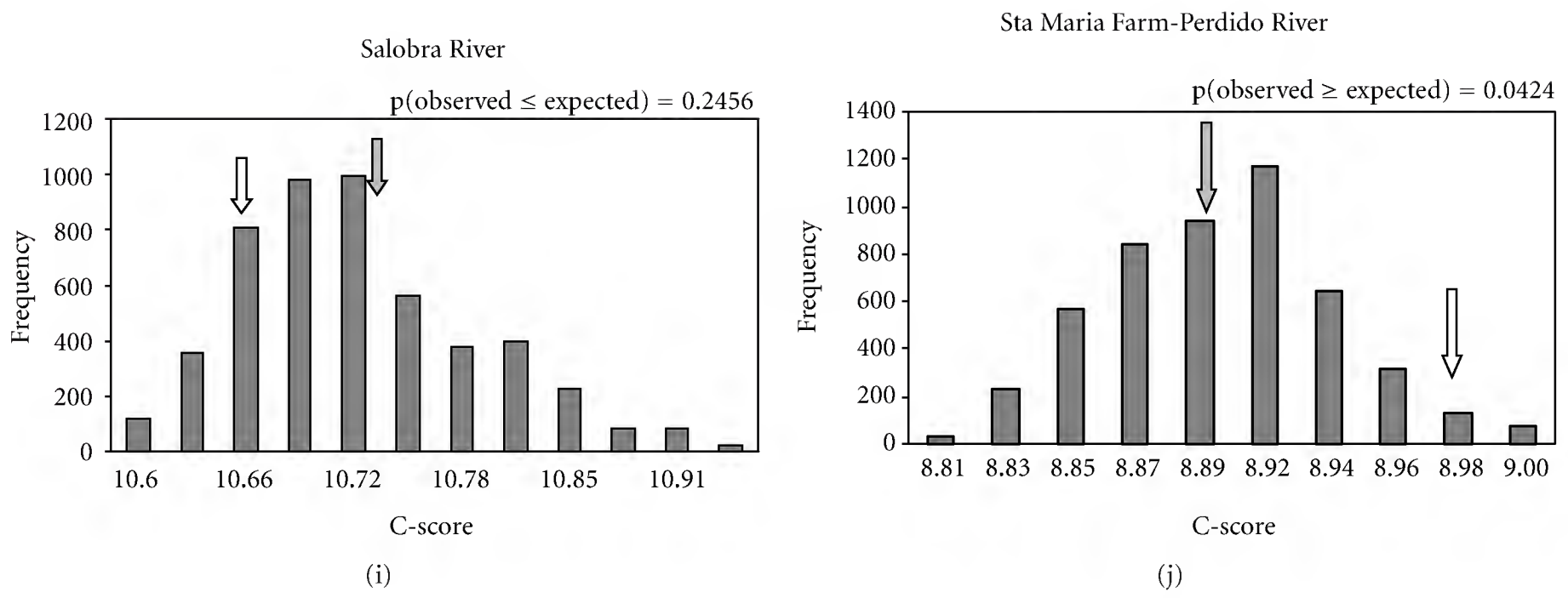


FIGURE 8: Co-occurrence analysis comparing 262 Winkler's samples of leaf-litter ants in Serra da Bodoquena. (Full arrow represents the expected values, and empty arrow represents the observed values for C-score measures.)

strongly associated with latitude, elevation, light availability, and vegetation composition, are important at regional spatial scales. Local and regional effects can mask or amplify larger-scale latitudinal patterns of species richness.

The differences in species diversity among the study sites could be related to Pleistocene events, such as biota interpenetration between two geologically distinct environments, and the phytophysionomic mosaic occurring in the region. According to Johnson and Ward [48], the topography of an ecotone and adjacent ecosystems is the most important factor affecting ant species richness. Here, each area could similarly allow the entrance of species coming from the surrounding matrix, affecting in turn the distribution of species in the core of the study sites.

There are several possible explanations for the inverse relationship between the observed low alpha diversity and high beta diversity, such as the particular characteristics of the forest fragments. The conservation status and potential connections between forest sites affect species persistence and colonisation in each site. These potential connections have a direct influence on the structure delimited by a buffer (considering the establishment of an influence zone for each area), which leads to an increase in species richness in interconnected forest fragments.

The absence of correlation between similarity and distance suggests that the ant species and assemblages are randomly distributed over the region. The low similarity between consecutive sampled sites suggests a strong formation effect and the influence of adjacent areas, which are evidenced by high beta diversity, with different arrangements of the ant fauna and a high turnover in species dominance across samples.

In forest fragments, ant richness depends on the diversity of local microhabitats and other factors acting at a local scale, such as physical and vegetation structure [33, 49], relief, humidity, and amount of leaf litter available at the location of the food resources and nesting sites used by ants [50, 51]. Significant variance in species composition can be explained by notable features that shape leaf litter ant communities, namely, litter biomass, soil stoichiometry, heterogeneous distribution of nutrients, soil moisture, invasive species, ecological disturbance at a small scale, and competition dynamics [52–56]. However, it is the ecological and historical biogeography that determines the current composition of the ant assemblages that colonize these micro-habitats (biotic and abiotic filters in the historical evolution of habitats), and also patchiness in space and time which are originated from different sources.

Our results suggest that estimated richness (Chao 2 = 231.7, Jackknife 2 = 250.4) is highly affected by the number of species that were only recorded once ("rare species" = 37). This pattern is in agreement with other studies in the Neotropical Region [5, 32, 57], which have found a high incidence of rare species in ant communities.

The Kadiwéu indigenous reserve, bordering the Pantanal plain, was the locality with lowest similarity conjunct dataset. The deciduous forest in this area forms an enclave of vegetation, influenced by the transition to Cerrado in this area. Transition zones are located at the boundaries between biogeographic regions and represent areas of biotic overlap, which are promoted by historical and ecological changes that allow the mixture of different biotic elements [9, 10].

The pattern observed suggests that the structure of the local community is directly affected by the landscape matrix in each region and that it is in fact an ecotone of the Chaco, Cerrado, Atlantic Forest, Amazonian Forest, and Pantanal.

The co-occurrence analysis of leaf-litter ant species indicates that competitive interactions are not the only factors responsible for organising ant assemblages. There is no reason to reject the null hypothesis that the number of checkerboard pairs in the samples is random.

Our results corroborate Andersen [16], agreeing that species coexistence is determined to a significant extent by processes operating during the colonization phase, rather than just by interactions between established colonies, and that competitive outcomes are highly conditioned by

environmental variation, which severely limits competitive exclusion.

In spite of changes in the extant ant species along a latitudinal gradient in the Cerrado biome [17], community functionality remains similar; this suggests a similar evolutionary ecological history in response to this matrix. In cases where the functionality of a community is distinct, we can assume that the evolutionary history of colonisation came from particular processes and not from a common process (monophyletic). Regarding the functional structure of the community, we suggest that further studies should investigate whether the same guilds are found in the northern and southern portions of Serra da Bodoquena.

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Research Article

The Ant Genus *Sphinctomyrmex* Mayr (Hymenoptera, Formicidae, Cerapachyinae) in the Neotropical Region, with the Description of Two New Species

Rodrigo M. Feitosa,[1] Carlos Roberto F. Brandão,[1] Fernando Fernández,[2] and Jacques C. H. Delabie[3,4]

[1] *Museu de Zoologia da Universidade de São Paulo, Avenida Nazaré 481, Ipiranga, 04263-000 São Paulo, SP, Brazil*
[2] *Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Carreira 30, No. 45-03, Apartado 7495, Bogotá, DC, Colombia*
[3] *Laboratório de Mirmecologia, Centro de Pesquisas do Cacau (CEPEC/CEPLAC), Caixa Postal 7, 45600-000 Itabuna, BA, Brazil*
[4] *Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Km 16 Rodovia Ilhéus-Itabuna, 45650-000 Ilhéus, BA, Brazil*

Correspondence should be addressed to Rodrigo M. Feitosa, rfeitosa@usp.br

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The ant genus *Sphinctomyrmex* has been represented in the Neotropical Region until now by a single species, *S. stali*, known only from sparse localities in southeastern Brazil. Two new neotropical species are herein described, *S. marcoyi* sp. n. and *S. schoerederi* sp. n. from workers collected in the Brazilian Amazon and Atlantic Forest, respectively. New records for *Sphinctomyrmex stali* are presented, and the species is redescribed together with discussions on its high morphological variation and the identity of its type specimen. A key for the neotropical *Sphinctomyrmex* workers, images of all species presently known, and a distribution map are supplied.

1. Introduction

Sphinctomyrmex is a pantropical and distinctive group of cerapachyine ants, originally described by Mayr [1] with *S. stali* as its type species by monotypy, based on a single gyne collected in Brazil. Borgmeier [2] provided taxonomic notes and described the worker caste of *S. stali*. Brown [3] revised the genus and provided an identification key for the species known at that time.

Morphologically, the genus is characterized by the unique arrangement of the gastric segments, which are nearly equal in length and separated from each other by distinct constrictions. *Sphinctomyrmex* is best represented in number of species in the Indo-Australian region compared to other parts of the world [4]. Until now, this genus remained known in the Neotropical region only by the rarely collected *S. stali*.

Very little is known on the natural history of *Sphinctomyrmex*. The few observations so far suggest that ants of this genus are nomadic predators of other ants [3, 5, 6].

Recent surveys of leaf litter ants in Brazilian biomes revealed several *Sphinctomyrmex* specimens, extending considerably the known distribution range of the genus. Moreover, these surveys have yielded specimens that do not fit the *S. stali* diagnosis and which are here described as two new species. In addition, we redescribe *S. stali* commenting on its extreme morphological variation and the identity of its type specimen. We hope that this paper will encourage further examination and revision of this biogeographically interesting ant genus.

2. Material and Methods

Observations were made at 60x magnification with a Leica MZ95 stereomicroscope. Measurements were made with a micrometer and recorded to the nearest 0.01 mm. Ranges between brackets are always presented as minimum–maximum values. All measurements are given in millimeters, and the abbreviations used are:

HL: head length—the maximum measurable length of head capsule excluding mandibles, measured in full-face view, in a straight line from the mid-point of the anterior clypeal margin to the midpoint of the vertexal margin;

HW: head width—the maximum width of the head capsule measured in full-face view, excluding the compound eyes;

SL: antennal scape length—the chord length of the antennal scape, excluding the basal condyle and its peduncle;

EL: eye length—the maximum measurable length of eyes in profile;

WL: mesosomal length (Weber's length)—the diagonal length of mesosoma in profile, from the midpoint of the anterior pronotal declivity to the posterior margin of the propodeal lobes;

PL: petiole length—in dorsal view, the maximum length of the petiole;

PW: petiole width—in dorsal view, the maximum width of the petiole;

GL: gaster length—the maximum length of gaster in lateral view, excluding the sting;

TL: total length—the summed length of HL, WL, PL, and GL;

CI: cephalic index—HW $\times$ 100/HL;

SI: scape index—SL $\times$ 100/HW;

OI: ocular index—EL $\times$ 100/HW;

Depository collections are referred to by the following acronyms.

CPDC: Centro de Pesquisas do Cacau, Itabuna, BA, Brazil;

MCZC: Museum of Comparative Zoology, Harvard University, Cambridge, Mass, USA;

MZSP: Museu de Zoologia da Universidade de São Paulo, São Paulo, SP, Brazil;

NHRS: Naturhistoriska Riksmuseet, Stockholm, Sweden;

UFV: Laboratório de Ecologia de Comunidades, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

High resolution digital images of *Sphinctomyrmex schoerederi* sp. n. and *S. stali* (workers) are here presented thanks to the kind permission of Dr. B. L. Fisher (California Academy of Sciences). These images are available at the AntWeb webpage (http://www.antweb.org/). Images of *Sphinctomyrmex marcoyi* sp. n. and *S. stali* (gyne) were obtained under a stereomicroscope Leica M205C attached to a video camera Leica DFC 295. The photos were then combined using the software Leica Application Suite V3.Ink. Combined photos were edited in PhotoShop (Adobe) to enhance parameters of brightness and contrast. The distribution map was generated by the software Quantum GIS 1.5.0 (Tethys) with coordinates imported from Google Earth (Google).

The terms for external morphology and surface sculpturing follow, respectively, [7], [8]. The reproductive females are here called "gynes" [9].

3. Results

3.1. Revised Diagnosis for the Neotropical Species of Sphinctomyrmex

3.1.1. Sphinctomyrmex *Mayr, 1866. Sphinctomyrmex* Mayr, 1866: 895. Type species: *Sphinctomyrmex stali*, by monotypy. (for the complete taxonomic synopsis, see [4]).

Worker. Size highly variable (TL 2.04–4.64). Body yellowish to black, commonly reddish-brown, with appendages slightly lighter. Pilosity relatively dense, composed mainly by long whitish hairs, which are suberect on head, mesosoma, and petiole, and subdecumbent on the gaster dorsum; appendages densely covered by short suberect hairs; antennal funiculi and leg tarsomeres with dense pubescence. Mandibular surface smooth, with sparse piligerous punctures; antennal scapes densely punctuate; body dorsum variably foveolate, with a single filiform hair projected from each fovea; declivous face of propodeum smooth to faintly areolate; dorsal surface of pygidium shallowly and densely areolate.

Head as long as to longer than broad; posterior margin mostly straight with a discrete median concavity; mandibles subtriangular, without intramandibular space when fully closed; mandibular dentition inconspicuous; antennae with 12 segments; antennal scapes clavate, distinctly curved at the base and relatively short; funicular segments length gradually increasing towards the apex; eyes strongly reduced to vestigial.

Mesosoma subrectangular in lateral view, relatively elongate, with a flat and continuous dorsal profile; inferior corners of pronotum rounded; anepisternum and katepisternum separated by a distinct suture; dorsal and posterior margins of propodeum meeting in a distinct angle, never projected as teeth or spines; propodeal spiracles set very low in the segment, bellow the midheight of propodeum in lateral view, with the opening directed posterad; propodeal lobes well developed and subquadrate; declivous face of propodeum straight in lateral view.

Petiole not pedunculate, slightly higher than long in lateral view, with dorsal face weakly convex; in dorsal view, petiole barely longer than broad; subpetiolar process developed, rounded apically, and in general with a conspicuous elliptical to rounded fenestra. In dorsal view, first gastral segment

(abdominal III) notably narrower than the posterior ones, separated from the second gastral segment (abdominal IV) by a wide and deep constriction; abdominal segment IV with a relatively large pretergite; segments V to VII nearly equal in length, separated from each other by distinct constrictions.

Gyne. See comments under *S. stali* description.

3.2. Identification Key to Neotropical Species of Sphinctomyrmex *(Workers).*

(1) Size relatively small (HW <0.40 mm); head notably elongate (CI <70); dorsum of mesosoma with a median longitudinal smooth stripe (Brazil: AM) ... *S. marcoyi* sp. n.

- Size relatively large (HW >0.40 mm); head moderately elongate to subquadrate (CI >70); dorsum of mesosoma without a median longitudinal smooth stripe . . . (2)

(2) Head slightly to considerably elongate; clypeus narrowly inserted between the frontal lobes, so that the frontal carinae are placed close to each other (Figures 2(a) and 2(d)); lateral lobes of the anterior margin of clypeus absent or vestigial; abdominal segments IV to VII with comparatively short pretergites; gastral dorsum devoid of appressed hairs (Brazil: BA, MG, RJ, SC, SP) ... *S. stali* Mayr

- Head distinctly subquadrate; clypeus broadly inserted between the frontal lobes, so that the frontal carinae are well separated from each other (Figure 1(d)); anterior margin of clypeus with two lateral lobes projecting over the mandibles; abdominal segments IV to VII with strongly developed pretergites; gastral dorsum with short appressed hairs (Brazil: MG) ... *S. schoerederi* sp. n.

3.3. Species Accounts

3.3.1. Sphinctomyrmex marcoyi *sp. n. (Figures 1 and 4).*

Holotype Worker. Brazil. Amazonas; Manaus, Rs 2206; in soil, 01.11.1993; A.B. Casimiro col. no. 6 (4832) [CDPC].

Diagnosis. Sphinctomyrmex marcoyi can be easily separated from other species in the genus by its comparatively diminutive size (HW <0.40 mm, TL <3.00 mm), conspicuously elongate head, comparatively short scapes, and by the presence of a smooth longitudinal stripe on the dorsum of mesosoma, which is otherwise covered by foveolae and subdecumbent to erect hairs.

Holotype Measurements. HL 0.53; HW 0.35; SL 0.21; EL 0.025; WL 0.61; PL 0.23; PW 0.21; GL 1.24; TL 2.04; CI 66; SI 60; OI 7.14.

Worker Description. Relatively small size (TL about 2.00 mm). Body reddish-brown with slightly lighter appendages. Body dull; dorsum of mesosoma and petiole shiny. Pilosity comparatively dense; dorsum of head with short suberect to subdecumbent hairs; dorsum of mesosoma with short suberect hairs and a few long, sparsely distributed erect hairs, except for a longitudinal median stripe devoid of pilosity; petiole densely covered by suberect hairs; gastral dorsum with short appressed hairs mixed with sparse, longer hairs. Posterior area of head (nuchal area) opaque; dorsum of body densely foveolate; dorsum of mesosoma with a longitudinal, smooth, and shining, median stripe; sides of mesosoma and petiole with faint irregular reticulation; declivous face of propodeum without discernible sculpture; dorsal surface of the apical segments of gaster finely foveolate, the foveolae separated by wide interspaces (wider than the foveolae).

Head elongate (CI 66), slightly broader anteriorly; lateral margins faintly convex; clypeus narrowly inserted between the frontal lobes; anterior margin of clypeus devoid of lateral lobes, with a distinct median incision; antennal scapes short, with the apices well below the level of eyes; antennal club formed only by the apical segment, which is longer than the four preceding segments combined; eyes strongly reduced, as small as or smaller than the adjacent foveae of the head surface, with two small facets at it's maximum diameter. In dorsal view, lateral margins of mesosoma subparallel; pronotum with evenly rounded humeral corners; promesonotal suture not impressed dorsally. In dorsal view, petiole slightly longer than broad, with lateral margins slightly divergent; subpetiolar process moderately developed. Abdominal segments IV to VII with short pretergites, separated from each other by deeply impressed, short constrictions.

Gyne. Unknown.

Male. Unknown.

Etymology. The specific epithet honors Laurent Saint-Cricq (1815–1888), who published several papers and books between 1853 and 1876 on his voyages to South America (under the pseudonym of Paul Marcoy or Paul de Carmoy). His writings were particularly humanistic and naturalistic; his most important book is "Voyage à travers l'Amérique du Sud, de l'Océan Pacifique à l'Océan Atlantique" published in 1869 and translated into different languages.

Comments. Sphinctomyrmex marcoyi is known only from the holotype. The specimen was collected from a soil sample (25 cm depth). Nothing is known about its biology. As far as we know this is the only *Sphinctomyrmex* species recorded in the Amazon Forest [10], extending the distribution range of the genus more than 2,500 km to the north-west.

3.3.2. Sphinctomyrmex schoerederi *sp. n. (Figures 1 and 4).*

Holotype Worker. Brazil. Minas Gerais; Viçosa; ii.1994; Sperber, Louzada and Lopes cols [MZSP].

Diagnosis. This species can hardly be confounded with other congeners given the combination of subquadrate head,

FIGURE 1: *Sphinctomyrmex marcoyi*. (a)–(c): Holotype worker from Manaus, AM, Brazil: (a) head in full face view, (b) lateral view, (c) dorsal view. Image by Ricardo Kawada, specimen CPDC 6(4832). *Sphinctomyrmex schoerederi*. (d)–(e): Holotype worker from Viçosa, MG, Brazil: (d) head in full face view, (e) lateral view, (f) dorsal view. Image by April Nobile, specimen CASENT 0178849.

anterior margin of clypeus with two lateral lobes projecting over the mandibles, abdominal segments IV to VII with strongly developed pretergites, and the presence of short appressed hairs on the dorsal surface of gaster.

Holotype Measurements. HL 0.72; HW 0.65; SL 0.43; EL 0.05; WL 1.01; PL 0.44; PW 0.41; GL 2.31; TL 4.48; CI 90.11; SI 65.81; OI 7.32.

Worker Description. Size comparatively large (TL 4.48 mm). Body reddish-brown with appendages slightly lighter. Pilosity dense; gaster covered by short appressed hairs.

Posterior area of head (nuchal area) smooth and shiny; body dorsum foveolate; space between the foveae mostly smooth, with fine longitudinal striation on the anterior portion of head; declivous face of propodeum shallowly punctuate-reticulate; sides of mesosoma and petiole strongly sculptured, with shallow foveae and irregular reticulation; sides of gaster predominantly smooth and shiny, with a few coarse punctures.

Head subquadrate (CI 90.11); lateral margins gently convex; clypeus broadly inserted between the frontal lobes, so that the frontal carinae are well separated from each other; anterior margin of clypeus with two lateral lobes projecting over the mandibles; antennal scapes reaching the level of eyes; apical segment of antennae longer than the three preceding ones together; antennal club formed only by the apical segment; eyes strongly reduced, as large as the adjacent foveae of head surface, with about three small facets at its maximum diameter. In dorsal view, lateral margins of mesosoma convex; pronotum with humeral corners rounded; promesonotal suture distinct in dorsal view but not impressed. In dorsal view, petiole a little longer than broad, with lateral margins slightly divergent; subpetiolar process

Figure 2: *Sphinctomyrmex stali*. (a)–(c): worker of morphotype 1 from São Bonifácio, SC, Brazil (a) head in full face view, (b) lateral view, (c) dorsal view. Image by Michele Esposito, specimen CASENT 0178866. (d)–(f): ergatoid from São Bento do Sul, SC, Brazil (d) head in full face view, (e) lateral view, and (f) dorsal view. Image by Ricardo Kawada.

moderately developed. Abdominal segments IV to VII with strongly developed pretergites, separated from each other by comparatively shallow and wide constrictions.

Gyne. Unknown.

Male. Unknown.

Etymology. The specific epithet honors our colleague Dr. José Henrique Schoereder, a prominent ant ecologist working at Universidade Federal de Viçosa, MG, Brazil. Dr. Schoereder kindly allowed us to describe this species formerly deposited in the ant collection of his laboratory.

Comments. Sphinctomyrmex schoerederi is known only from the holotype. The specimen was collected in a leaf litter sample from a forest remnant in the campus of Universidade Federal de Viçosa, MG, Brazil, where it occurs in sympatry with *S. stali*. Nothing is known about its biology.

3.3.3. Sphinctomyrmex stali *Mayr, 1866 (Figures 2, 3, and 4).* *Sphinctomyrmex stali* G. Mayr, 1866: 895, pl. 20, Figure 8. Holotype gyne: Brazil, Rio de Janeiro, F. Sahlberg coll., HEVA000000012 [NHRS] (high-resolution images examined); Borgmeier [2, page 105], (distribution records and worker description); Brown [3], (world revision and key to species).

Diagnosis. The distinctly elongate head, the narrow insertion of the clypeus between the frontal lobes, the absence of lateral lobes from the anterior margin of clypeus, and the absence of appressed hairs on the dorsum of gaster separate *S. stali* from *S. schoerederi*. This species can be separated from *S. marcoyi* by its much larger size and the absence of a median smooth longitudinal stripe on the dorsum of mesosoma.

Worker Measurements ($n = 20$). HL (0.60–0.79); HW (0.44–0.68); SL (0.31–0.50); EL (0.02–0.08); WL (0.71–1.12); PL (0.28–0.43); PW (0.29–0.41); GL (1.66–2.39); TL (3.33–4.64); CI (71.79–86.00); SI (68.42–77.50); OI (3.33–12.50).

FIGURE 3: *Sphinctomyrmex stali*. (a)–(c): worker of morphotype 2 from Ubatuba, SP, Brazil (a) head in full-face view, (b) lateral view, and (c) dorsal view. Image by Michele Esposito, specimen CASENT 0178865. (d)–(f): worker of morphotype 3 from Viçosa, MG, Brazil (d) head in full-face view, (e) lateral view, (f) dorsal view. Image by April Nobile, specimen CASENT 0178850.

Worker Description. Size highly variable (TL 3.33–4.64). Body yellowish to black, commonly reddish-brown with slightly lighter appendages. Pilosity dense; gaster devoid of appressed pilosity. Posterior area of head (nuchal area) smooth to coarsely striate; dorsum of body sparsely foveolate; space between the foveae predominantly smooth and shiny; declivous face of propodeum smooth and shiny to shallowly punctuate-reticulate; sides of meso- and metasoma predominantly smooth and shiny, with a few coarse punctures.

Head slightly to considerably longer than broad, (CI 71.79–86.00); lateral margins subparallel to gently convergent; clypeus narrowly inserted between the frontal lobes, so that the frontal carinae are close to each other; lateral lobes of the anterior margin of clypeus absent or vestigial; antennal scape apices not reaching the level of the compound eyes; apical segment of antennae as long as the three preceding sgments combined; antennal club formed by the apical segment or by the two apical segments; compound eyes strongly reduced to vestigial, as large as or slightly larger than the adjacent foveae of head surface. In dorsal view, lateral margins of mesosoma subparallel; pronotum with humeral corners angled but not forming teeth or spines; promesonotal suture distinct to vestigial in dorsal view, not impressed. In dorsal view, petiole as long as to gently longer than broad with lateral margins feebly divergent; subpetiolar process well developed. Abdominal segments IV to VII with relatively short pretergites, separated from each other by deeply impressed, short constrictions.

Gyne (Ergatoid) Measurements (n = 3). HL (0.66–0.68); HW (0.52–0.55); SL (0.36–0.38); EL (0.08–0.11); WL (0.91–0.98); PL (0.36–0.41); PW (0.32–0.36); GL (1.95–2.17); TL (3.89–4.20); CI (78.57–81.40); SI (65.71–72.73); OI (15.15–20.00).

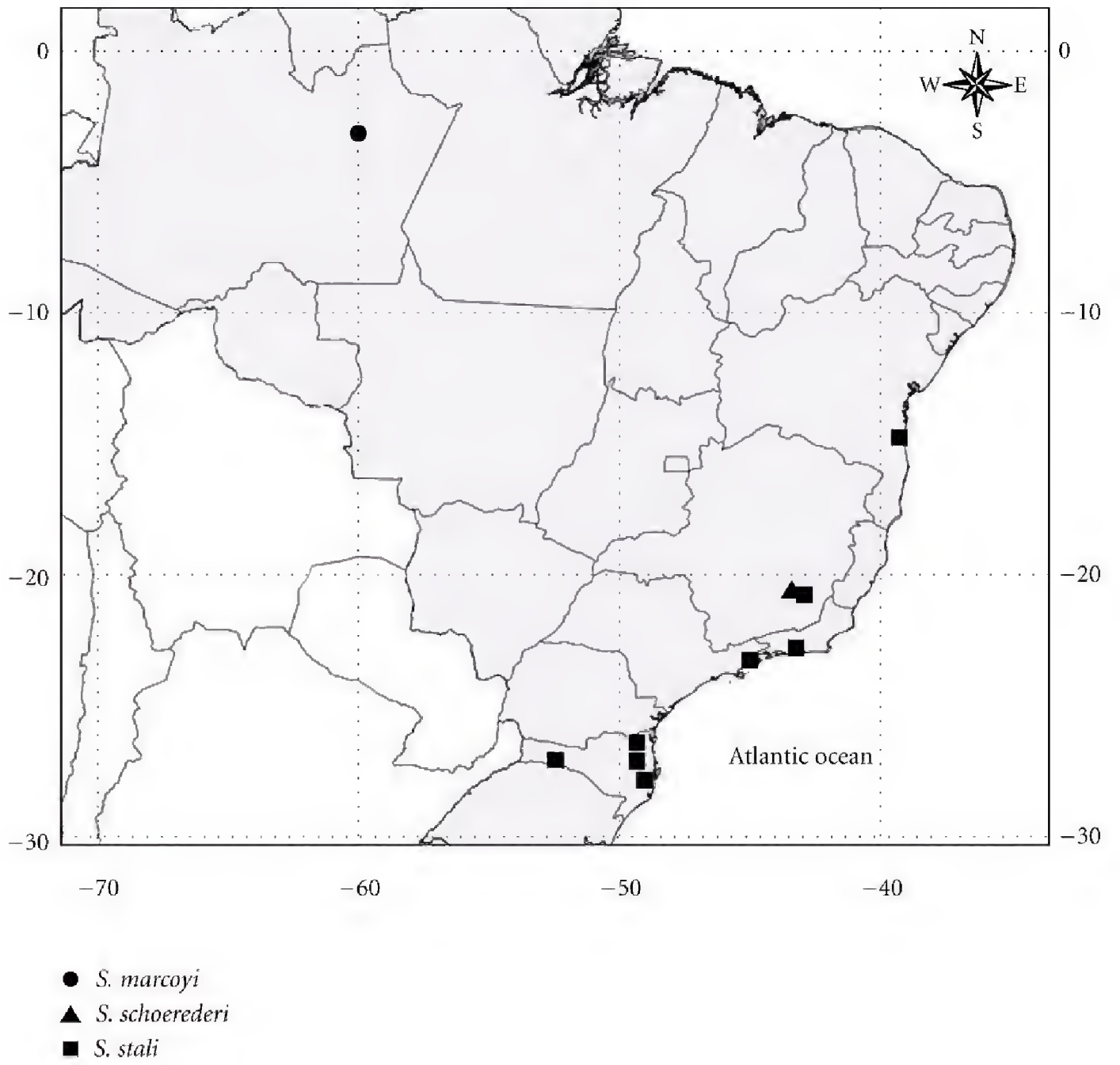

FIGURE 4: Distribution map for the neotropical species of *Sphinctomyrmex*.

Gyne Description. Two forms are recognized, alates and ergatoids (but see comments below). The alate form is known from a single specimen, the holotype. This gyne differs from workers by the typical characters expected for ant reproductive females: size significantly larger (TL *ca.* 6.00); ocelli well developed; compound eyes considerably large, occupying almost one third of the lateral margin of head. Pronotumis well developed, without projections; scutum large and trapezoidal; notauli shallow, almost indistinct; parapsidial lines feebly visible and convergent towards scutellum; scutoscutellar sulcus impressed; scutellum relatively narrow and set at the same level as the scutum, in lateral view; propodeum large in dorsal view, with dorsal face meeting the declivous face in a blunt angle; wings unknown. Petiole and gaster comparatively larger than in conspecific workers.

The ergatoids (Figures 2(d)–2(f)) differ from the conspecific workers only by the presence of three equally developed ocelli and by the compound eyes being comparatively well developed (OI 15.15–20.00).

Male. Unknown.

Etymology. Dr. Gustav Mayr named this species after the Swedish entomologist Dr. Carl Stål (1833–1878), who was a professor of the Zoological Department of the Royal Swedish Museum of Natural History in Stockholm. Although he published mostly on Hemiptera and was regarded as its world's foremost scholar, Dr. Stål also published on Orthoptera and to a lesser extent on Coleoptera and Hymenoptera.

Variation. At least three different morphotypes of this highly variable species can be distinguished. Categorization of these morphotypes is somewhat arbitrary as they are not entirely distinct from each other. Therefore, they are not to be recognized as distinct units, but rather as belonging to a gradient. The following comparison, however, simplifies the description of morphological variation and allows for the recognition of possible geographic patterns.

Morphotype 1 (Figures 2(a)–2(c)). Medium size (HW 0.48–0.55, TL 3.51–4.00). Color reddish-brown to blackish. Posterior area of head (nuchal area) predominantly smooth and shiny. Head distinctly longer than broad (CI 75.50–81.40), with lateral margins weakly convex; eyes reduced but distinctly larger than the adjacent foveae of head surface. Promesonotal suture distinct in dorsal view.

This morphotype conforms most closely with the first workers described for this species [2]. Despite its distribution being restricted to scattered localities in the state of Santa

Catarina, southern Brazil, it is the most common morphotype of *S. stali* in museum collections.

Morphotype 2 (Figures 3(a)–3(c)). Large size (HW 0.60–0.69, TL 4.05–4.64). Color reddish to dark brown. Posterior area of head (nuchal area) predominantly smooth and shiny. Head moderately longer than broad (CI 81.63–86.00), with lateral margins weakly convex; eyes reduced but distinctly larger than the adjacent foveae of head surface. Promesonotal suture variably impressed in dorsal view.

In his initial description of *S. stali* workers [2], the author stated: "*...The specimens collected in October 3, 1953 are somewhat larger than the other ...*" We examined these workers mentioned by Borgmeier; in fact, they are considerably larger than the other workers, and even larger than the ergatoids examined here. However, except for the exceptional size, the individuals of this morphotype are very similar to those of morphotype 1. Additional workers of morphotype 2 were collected in the Brazilian states of Bahia, Minas Gerais, and São Paulo.

Morphotype 3 (Figures 3(d)–3(f)). Small size (HW 0.44–0.47, TL 3.33–3.41). Color pale yellow to reddish-brown. Posterior area of head (nuchal area) irregularly striate, with sparse punctures. Head notably longer than broad (CI 71.79–75.00), with lateral margins subparallel; eyes vestigial, feebly convex; in some cases only discernable by a dark spot on the sides of head, of the same size as the adjacent foveae of the head surface. Promesonotal suture obsolete, almost indistinct in dorsal view.

This is the most distinctive morphotype of *S. stali*, known so far only from Viçosa, state of Minas Gerais, southeastern Brazil where it occurs in sympatry with *S. schoerederi*.

Comments. Mayr [1] described *Sphinctomyrmex* with *S. stali* as its type species, based on a single dealate gyne. However, except for the holotype, there are no records of normal (alate) gynes for *S. stali*. All reproductive females collected after the original description are ergatoids. Dr. H. Vårdal, Hymenoptera curator of the NHRS collection, kindly sent us images of the *S. stali* holotype. We confirm that it is a typical dealate ant gyne given the wing scars and the structure of mesosoma. Therefore, there are at least three possibilities: (1) *Sphinctomyrmex stali* can possess both forms of reproductive females, alates and ergatoids, as already recorded for other ant species [11, 12, Christian Peeters (pers.com.)]; (2) our current conception of *S. stali* includes more than a single species, not entirely distinguishable by morphology, and each species may present a different gyne form, or (3) the initial suspicion by Brown (see below) may prove correct, and the Brazilian locality record for the dealate gyne designated as holotype by Mayr may be in error. Hypotheses 1 and 2 seem to be the more plausible based on the label information of the type specimen. The collector of the type, the Finish entomologist Reinhold Ferdinand Sahlberg, probably captured this specimen while collecting insects in his visit to Rio de Janeiro in the middle 1800's [R.F. Sahlberg's field book; Hege Vårdal (pers.com.)]. Incidentally, this possibility is supported by Kempf's decision [13] to treat Rio de Janeiro as the type locality of *Sphinctomyrmex stali*. The nature of *S. stali* reproductive females will only be solved with the collection of additional material associated with workers.

Sphinctomyrmex stali is known from sparse localities along the southeastern portion of the Brazilian Atlantic Forest, from Santa Catarina to southern Bahia. Recent collections suggest that this species can be most commonly found in submontane forests (above 600 m) of the states of Santa Catarina and São Paulo, from whence come most of the specimens in collections. In correspondence between William Brown Jr. and Father Thomas Borgmeier in 1954 [2], Brown mentions: "*The thing that really surprised me about the paper was your mention of Plaumann's discovery of* Sphinctomyrmex *in Santa Catarina! As matter of fact, I have just finished examining the type of* S. ståli [SIC] *Mayr (Stockholm Museum), and just send it back to Sweden. I had concluded that the Brazilian locality must be in error, but if your specimens are the same, then I must revise my opinion! I could discover no characters of generic significance between* S. ståli *and the known winged females of certain Australian* "Notosphinctus"*, and I tentatively conclude that these two names are synonyms. I have seen the type of* furcatus *Emery, from Burma, and also a winged female of a species (undescribed?) marked as from "Sierra Leone/Afzelius" which is surely of the same genus as* ståli *on female characters alone, but which is blackish in color ...*."

In a single leaf-litter sample collected in São Bonifácio, Santa Catarina, six workers and two ergatoids, very similar to the workers, were captured, which suggests that *S. stali* is polygynous, as already described for other *Sphinctomyrmex* species [3, 6].

Material Examined. Brazil: *Bahia*: Ilhéus, Área Zoolog., CEPEC, Ilhéus-Itabuna km 22, x.1986, J. Delabie leg., no. 56 (1 worker) [MZSP]; *Minas Gerais*: Viçosa, Mata da Prefeitura. 07.i – 09.xi.1994, P.S.P. Ferreira col. (1 worker) [CDPC]; Mata do Paraíso, xii.1993–xii.1994, Ferreira, P.S.F. col. (3 workers) [CPDC]; same locality, 1997/1998, Soares, S.M. col., no. 5245 (1 worker) [CPDC]; same locality, Fragment P14, Winkler extractor, 08.iv.1998, Soares, S.M. col. (1 worker) [UFV]; same locality, same data, no. 116, Soares, S.M. col. (1 worker) [MZSP]. *Santa Catarina*: Blumenau, P.E. das Nascentes, 27°06′15″S 49°09′14″W, 20–27.x.2000, R.R. Silva & F. Eberhardt cols, no. 50 (1 worker) [MZSP]; Nova Teutônia [currently Seara], 27°11′S 52°23′W, x.1953, Fritz Plaumann col. (1 worker) [MZSP]; same locality, iv.1954, Fritz Plaumann col. (4 workers) [MZSP]; same data (1 worker) [MCZC]; same locality, xi.1957, Fritz Plaumann col. (2 workers) [MZSP]; same locality, v.1960, Fritz Plaumann col. (1 gyne) [MZSP]; same locality, iii.1971, Fritz Plaumann col., no. 7055 (1 worker) [MZSP]; same locality, iv.1972, Fritz Plaumann col., no. 7983 (1 worker) [MZSP]; same locality, xii.1974, Fritz Plaumann col., Kempf collection, no. 11627 (3 workers) [MZSP]; São Bento do Sul, A.P.A. Rio Vermelho, 26°21′51″S 49°16′16″W, 30.iii–4.iv.2001, R.R. Silva & F. Eberhardt cols, nos. 28/50 (6 workers and 2 ergatoid gynes) [MZSP]; São Bonifácio, P.E. da Serra do

Tabuleiro, 27°49′06″S 48°54′41″W, 8–13.iii.2004, R.R. Silva, B.H. Dietz & N.L. Albuquerque cols, nos. 2/6/23 (5 workers) [MZSP]. *São Paulo*: Ubatuba, P.E.S.M., N. Picinguaba, 600 m, 23°17′54.4″S 44°47′49.2″W, 23.i.2006, Scott-Santos, C.P. & Santos, E.F. cols, no. 4 (1 worker) [MZSP].

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Research Article

Subterranean Pitfall Traps: Is It Worth Including Them in Your Ant Sampling Protocol?

Renata Pacheco and Heraldo L. Vasconcelos

Instituto de Biologia, Universidade Federal de Uberlândia (UFU), C.P. 593, 38400-902 Uberlândia, MG, Brazil

Correspondence should be addressed to Heraldo L. Vasconcelos, heraldo@umuarama.ufu.br

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The use of subterranean traps is a relatively novel method to sample ants, and few studies have evaluated its performance relative to other methods. We collected ants in forests, savannas, and crops in central Brazil using subterranean pitfall traps and conventional pitfall traps placed on the soil surface. Sampling duration, soil depth, and sprinkling vegetal oil around traps all tended to affect the number of species found in subterranean traps. Sixteen percent of the species collected in subterranean traps were unique, and most of these had cryptobiotic morphology (i.e., were truly hypogaeic species). Surprisingly, however, subterranean and conventional traps were similarly efficient at capturing cryptobiotic species. Furthermore, subterranean traps captured far fewer species in total than conventional traps (75 versus 220 species), and this was true in all three habitats sampled. Sampling completeness increased very little using a combination of conventional and subterranean traps than using just conventional traps.

1. Introduction

Biodiversity inventories seek a characterization of the studied community or the elaboration of a complete species list [1]. In both cases a more efficient inventory is commonly achieved with the use of diverse and complementary sampling techniques, and this is especially true with regard to hyperdiverse groups such as terrestrial arthropods [1–3]. Ants are a particularly important group of arthropods as they are highly abundant and diverse, have a wide geographic distribution, and occupy a variety of niches [4]. Ants play important ecological roles, acting as herbivores, seed dispersers or, commonly, as predators and scavengers of other arthropods [4, 5]. Due to these characteristics, ants have been commonly used as a focal taxon in biodiversity studies or as bioindicators in studies of land management [6].

Diverse methodologies have been used to collect ants, and each of them has its own limitations given that no single method is able to collect all species inhabiting a given area (at least not in tropical and subtropical habitats where ant diversity is typically high), since these species commonly have a wide diversity of foraging and nesting habits [1, 7–9]. As a result, many ant inventories employ more than one sampling technique, as their use in combination often increases sampling efficiency [1, 9, 10]. Pitfall traps, for instance, tend to be more efficient for the collection of relatively large ants that are active on the soil surface, whereas the Winkler method favors the collection of smaller and often cryptic species that forage or nest in the litter layer [11]. The combined use of pitfall traps and the Winkler samples has been proposed in the Ants of Leaf Litter (ALL) sampling protocol, a protocol that has been employed successfully in ecological studies and inventories of tropical forest ants [7, 12, 13]. More recent studies, however, have indicated that many species with subterranean habits (i.e., hypogaeic species with cryptobiotic morphology) may not be collected with the use of the more traditional sampling methodologies such as the Winkler method, pitfall traps, baits, or direct search [12, 14, 15]. One way to collect hypogaeic species is to take soil-core samples and extract ants from soil manually or with the aid of the Berlese or the Winkler extractors [10, 16–18]. An alternative and increasingly used method is subterranean traps, such as the subterranean probe [15, 19] or subterranean pitfall traps [14, 20–23].

So far, inventories of ant diversity using subterranean traps have been performed in only a small number of sites and habitats including the rain forests of Ecuadorian Amazon [19], the Brazilian Atlantic Forest [22], and the *Eucalyptus*-dominated forests of northern Australia [14]. Therefore, there is a lack of information about the performance of subterranean traps in other types of vegetation. Also, there is only limited information about the best methods to improve the sampling efficiency of subterranean traps (but see [14]). Here we provide results of the first systematic survey of subterranean ants in the Cerrado region of central Brazil. The Cerrado is a biodiversity hotspot and is characterized as a mosaic of vegetation types, which include savannas of variable structure (the dominant vegetation), various types of forests, and grasslands [24, 25]. Most of the original Cerrado vegetation have already been converted to cattle pastures or crop fields, and as a consequence these human-managed ecosystems are now an important feature of the Cerrado landscape and thus have also to be taken into account when assessing diversity at the landscape level.

Pitfall trapping (i.e., pitfall traps placed on the soil surface or, hereafter, conventional pitfalls) is by far the most commonly employed method to sample ants, especially in savanna-dominated landscapes [9, 13, 26]. We thus compared the efficiency of subterranean traps relative to conventional pitfall traps and, most importantly, determined how complementary these two methodologies are. Trap efficiency was measured in terms of the total number of species collected, the number of unique species, and the number of species with cryptobiotic morphology (i.e., species with tiny or absent eyes and small body size). We also evaluated some simple methodologies designed to improve the sampling efficiency of subterranean traps. Finally, we evaluated the efficiency of subterranean traps in collecting hypogaeic ants in different types of ecosystems of the Cerrado region, including forests, savannas, and crop fields.

2. Material and Methods

2.1. Study Site. The study was conducted in 13 sampling sites located near the towns of Uberlândia (18°56′, 48°18′W) and Monte Alegre de Minas (18°52′S, 48°52′W) in the west region of Minas Gerais state in Brazil. The region is characterized by a tropical climate with two well-defined seasons: a dry winter (May–September) and a rainy summer (October–April). The mean annual temperature and precipitation are 22°C and 1650 mm, respectively. Sampling was conducted during the wet seasons of 2008 and 2009.

Soils at our study sites are primarily red latosols. The vegetation at these sites included savannas (locally known as *cerrado sensu stricto*; $n = 2$ sites), forests (semideciduous forests and the forest physiognomy locally known as *cerradão* [25]; $n = 5$ sites), and fields planted with annual crops (maize, sorghum or, most commonly, soybean) ($n = 6$). With a single exception, all sites with natural vegetation were adjacent to the crop fields.

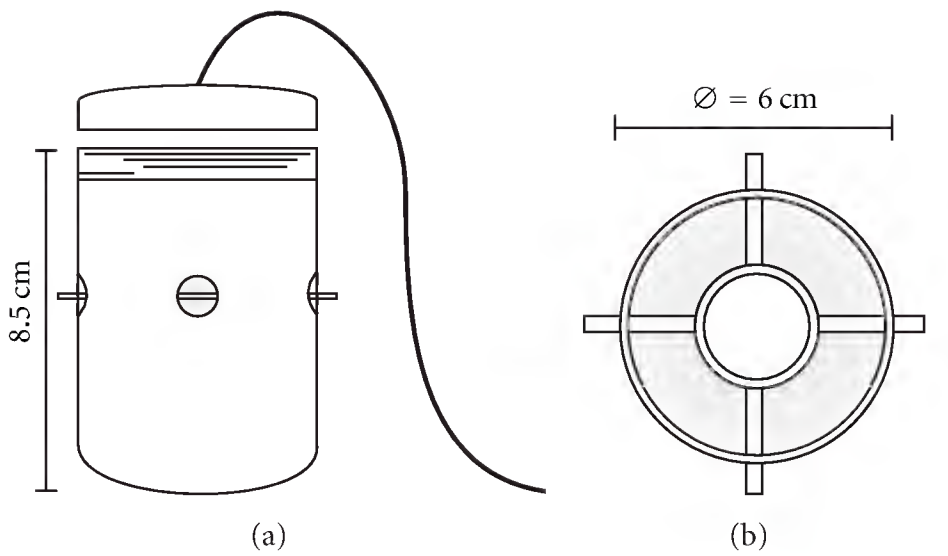

Figure 1: Schematic external (a) and internal (b) view of the subterranean pitfall trap.

2.2. Description of the Subterranean Trap. Our subterranean pitfall traps (Figure 1) were similar to those employed by other authors [14, 21, 22]. Each trap consisted of a closed plastic container (volume = 250 mL) with four 1 cm holes made in the side of the container (Figure 1). As done in a previous study [22], a 70 cm long rope was attached to the lid of each container to identify its location and facilitate removal. The traps were baited using sardine mixed with vegetable oil. About 5 mL of this mixture was poured onto a small lid (2.5 cm in diameter), and the lid was fixed in the interior of each container suspended by a plastic frame as detailed in Figure 1. About 50 mL of alcohol and glycerin was poured on the bottom of the traps to act as killing and preservative agents.

2.3. Factors Affecting Trap Efficiency. To evaluate if the number of species collected varied with soil depth, we simultaneously buried 60 traps to a depth of 20 cm and 60 traps to a depth of 50 cm. To prevent major alterations on soil structure, we buried traps by first making a cylindrical hole (ca. 8 cm in diameter) using a post hole digger [22] and then filled the holes with the excavated soil. These traps remained in operation for 7 days and were placed along transects in three sites (one forest, one savanna, and one crop field site), keeping a minimum distance of 20 m between traps and alternating the treatments (i.e., 20 or 50 cm). Twenty traps of each type were installed in each site.

We also evaluated if trapping duration affected the number of species collected. For this we compared the number of species collected in 40 of the 60 traps set at 20 cm for the experiment described above with the number of species collected in 40 other traps that were buried to a depth of 20 cm and were left out in the field for just two days. Traps that were left in the field for two days were installed in the same sites and at the same time as those that were left for 7 days. Finally, we evaluated if pouring vegetable oil around the hole made to bury each trap would increase the number of species collected as indicated in previous studies in Malaysia [20, 27, 28]. For this we sprinkled about 20 mL of a mixture consisting of 90% of soybean oil and 10% of palm (*Elaeais guineensis*, known as *dendê* in Brazil) oil on

Table 1: Efficiency of the subterranean pitfall traps as a function of soil depth, time of trap exposure, and addition of vegetable oil on soil around traps.

Factor	Treatment	Number of traps (natural areas + crops)	Observed number of species (SD)	Estimated number of species (SD)	Observed number of cryptobiotic species (SD)	Estimated number of cryptobiotic species (SD)	Total number of ant species records	Traps without ants (%)
Duration	2 days	20 + 20	13 (±3.1)	21.8 (±3.0)	2 (±0.0)	3.0 (±1.0)	27	50.0
	7 days	20 + 20	17 (±2.1)	24.8 (±2.5)	5 (±1.6)	8.9 (±1.9)	37	40.0
Depth	20 cm	40 + 20	20 (±2.6)	29.8 (±2.9)	6 (±1.7)	10.9 (±2.1)	43	53.3
	50 cm	40 + 20	16 (±3.3)	27.8 (±3.4)	3 (±1.9)	4.0 (±1.0)	27	63.3
Vegetable oil	without	37 + 11	9 (±1.9)	14.9 (±2.7)	2 (±0.6)	3.0 (±1.0)	24	79.2
	with	38 + 12	13 (±3.2)	22.8 (±3.1)	2 (±0.0)	3.0 (±1.0)	30	68.0

the soil around each trap. A total of 60 traps received this treatment while another 60 did not. These traps were buried to a depth of 20 cm and remained in operation for 7 days (20 of the 60 traps that received no oil treatment were the same traps used in the experiment about soil depth). As in the previous experiments, traps were spaced 20 m from each other (alternating treatments), with 40 of the traps from each treatment being placed in two forest sites and 20 in a crop field (Table 1).

2.4. Subterranean Traps versus Traps Placed on the Soil Surface. To determine if more species would be collected using a combination of conventional, and subterranean traps than using just conventional traps we installed these two types of traps in our sampling sites ($n = 13$). In addition to traps used in the previous experiments, we installed another 505 subterranean traps and 605 conventional traps in our sampling sites. Within each of these sites, traps were distributed along line transects, keeping a minimum distance of 20 m between traps and alternating the type of trap. Subterranean and conventional traps were installed simultaneously in each transect. Conventional pitfall traps consisted of plastic cups (300 mL volume), filled to one third of its volume with a mixture of alcohol (70%) and glycerin and placed on the ground so that the opening of the trap was leveled off with the soil surface.

All the additional 505 subterranean traps were set at 20 cm of depth (and vegetable oil was poured around the traps as described above) and remained in operation for 7 days. The conventional traps also remained in operation for 7 days, and both types of traps were baited using sardine mixed with vegetable oil.

2.5. Data Analysis. We built sample-based species accumulation curves [29], using the Mao Tau estimator in EstimateS version 8.2 [30], in order to compare the overall number of species collected and the number of cryptobiotic species collected at different depths, after different times of trap exposure, after applying or not vegetable oil around traps, and for comparing subterranean and conventional traps. We used the Jackknife 1 nonparametric richness estimator to determine the number of species expected to be found in different types of traps (or treatments) or in different habitats.

Nonmetric multidimensional scaling (NMDS) was used to evaluate the similarity in ant species composition among samples taken in different habitats or using different sampling methodologies. For this we first constructed a dissimilarity matrix (Sørensen index) using data on species presence or absence for species collected with each of the two sampling methodologies in each sampling site. The resulting ordination scores (two-dimensional solution) were then used in a multivariate analysis of variance (Manova) to test for differences in ant species composition (expressed as ordination scores) in relation to habitat and sampling method.

Of the 260 subterranean traps installed for the first three experiments, 22 were lost to digging animals (most of which in the crop fields) and, therefore, were excluded from our analyses. Of the 505 subterranean traps used in the subsequent experiment (when we protected the traps with a wire mesh fixed on the soil surface), only 6 were lost. Of the 605 conventional traps, 33 were lost and excluded from the analyses.

3. Results

3.1. Improving the Sampling Efficiency of Subterranean Pitfall Traps. The median number of species collected per trap was not significantly affected by soil depth (Mann-Whitney test, $U_{60,60} = 2058$, $P = 0.19$), time of trap exposure ($U_{40,40} = 675$, $P = 0.12$), or the addition of vegetable oil on soil around traps ($U_{48,50} = 1340$, $P = 0.20$). Similar results were obtained when data was analyzed considering only the species with cryptobiotic morphology (Mann-Whitney test, $P > 0.05$ in all cases). Nevertheless, when we compared the cumulative number of species collected (i.e., in all traps from each treatment), there was a clear trend towards finding more ant species, more species records, and less traps with no ants in traps that remained 7 days in operation, in those that were set at 20 cm of depth, and in those around which vegetable oil was poured (Table 1). Traps that remained in the field for 7 days also captured more cryptobiotic species in total than those that remained for two days. Similarly, more cryptobiotic species were found at 20 than at 50 cm of depth. The cumulative number of cryptobiotic species collected in traps with the addition of vegetable oil was equal to the number collected in traps with no oil (Table 1).

3.2. Subterranean Traps versus Traps Placed on the Soil Surface. We collected a total of 75 ant species from 27 genera in the 737 subterranean traps placed in 13 different sites and in three habitats (Table 2). Of all species collected with subterranean traps, 15 were cryptobiotic (20% of the total). Twelve of the 75 species (16%) captured with the subterranean traps were not found in the conventional traps, and most of these (8 out of 12 species) were cryptobiotic.

Using only the conventional traps ($n = 572$ traps), we collected a total of 220 species from 49 genera (a complete list of species/morphospecies is available from the authors upon request). Most of these species (157 species, or 71.3% of the total) were not found in the subterranean traps. Sixteen of the species collected using conventional traps (7.3%) were cryptobiotic (Table 2), and half of these were not captured with the subterranean traps.

The total number of species collected in the conventional traps was much greater than the number collected using subterranean traps, and this difference was detected in all three habitats sampled (forests: 4.3 more species, savannas: 3.7 more species, crop fields: 2.5 more species in the conventional than in the subterranean traps) (Figuers 2(a)–2(c)). When comparing the total number of species collected using just the conventional traps with that collected using both conventional and subterranean traps, we found that the number collected with the latter was only slightly greater than with the former (5.4% greater overall, 4.1% greater in forests, 5.8% greater in savannas, and 4.9% greater in crop fields). Considering only the species with cryptobiotic morphology, we found that conventional traps captured more species in the forest sites (Figure 2(d)), whereas in the crop fields subterranean traps tended to be more efficient (Figure 2(f)). Subterranean and conventional traps captured similar number of cryptobiotic species at the savanna sites (Figure 2(e)).

Overall (i.e., considering the two trapping methods), the proportion of species with cryptobiotic morphology varied a little among habitats, ranging from 9% of all species collected in forests to 8.4% in crops (savannas = 8.7%) (Table 3). The number of cryptobiotic species collected in each habitat represented 64 to 73% of the number of cryptobiotic species expected to be found in these same habitats (Table 3).

Most of the species collected only in the subterranean traps were rare species that were found in only one or two traps (Table 2). These included, for instance, two species of *Acanthostichus*, three species of *Hypoponera*, one species of *Carebara* (*lignata* group), *Oxyepoecus inquilinus*, and the exotic *Tetramorium simillimum* (Table 2). On the other hand, some of the species often collected with subterranean traps were not collected or were rare in the conventional traps. For instance, *Neivamyrmex punctaticeps* was collected 18 times with the subterranean traps but never with the conventional traps, while *Labidus mars* was collected 17 times with the subterranean traps but only once (and only a single individual) with the conventional traps (Table 2). As a result, differences in species composition resulting from collections using different types of traps tended to be even greater than differences in species composition between different habitats sampled with the same type of trap (Figure 3), even though differences in both habitat type (Manova, Pillai trace = 0.783, $F_{4,40} = 6.43$, $P < 0.001$) and trap type were significant (Manova, Pillai trace = 0.821, $F_{2,19} = 43.66$, $P < 0.001$).

4. Discussion

4.1. Increasing Trap Efficiency. Although during the past few years there has been a substantial increase in the use of subterranean traps in ant surveys [14, 15, 19–22], few studies have evaluated how to improve the efficiency of these traps. Our results suggest that extending the time of trap exposure from two to seven days increases trap efficiency in terms of total number of ant species records, total number of ant species, and number of cryptobiotic species. Similarly, Andersen and Brault [14] report that total ant records in subterranean traps were about 40% greater after four days than after just one. However, in their study, the number of species recorded did not change as a result of sampling duration (24 species after four as well as after one day) [14]. Although the number of additional ant species collected in subterranean traps appears to decline strongly as a function of sampling duration (with nearly 80% of the species being collected during the first 24 h of sampling) [19], we recommend that traps should remain in the field for more than two days and ideally for four to seven days. This is because installing the traps is a relatively time-consuming and labor intensive operation, and once the traps are set, few are lost (provided that some protection against digging animals is made). Therefore, unless there is the need to move to another and relatively distant sampling site quickly, there is no point in removing the traps after just one or two days.

Our results also suggest that sprinkling vegetal oil around the traps increases trap efficiency at least in terms of the overall number of species collected. Sprinkling vegetal oil on soil has been found to attract several hypogaeic ant species in Malaysia, especially army ants [20, 27, 28]. In the studies in Malaysia the oil used to attract ants was palm oil, while here we used a mix of palm (*dendê*) and soybean oil, given the elevated price of palm oil in Brazil. Palm oil has a very strong odor, and this odor is not lost after mixing it with soybean oil. Although vegetable oil has been found to increase trap efficiency, future studies should evaluate the use of other types of ant baits (in combination with oil), since vegetable oil is not attractive to all hypogaeic species [20].

As also found in other studies [14, 19], ant species tended to decline with soil depth. Furthermore, no evidence of a vertical stratification of the ant fauna was detected, as basically the same species were collected in different depths (Table 2). Wilkie et al. [19] suggested that in contrast to the situation they found in Ecuador, where the water table is particularly high, studies in dryer landscapes could detect a relatively unique deep-soil (>25 cm in depth) fauna. Our results suggest that this is may not be the case, as even though the water table at our sites is very deep, we found the same species foraging at different depths.

None of the previous studies that used subterranean traps provide data on the proportion of traps with no ant records. Our data indicate that, at least in our study region, this figure can be elevated and that 40% or more of the traps capture no

Table 2: List of all ant species collected with subterranean pitfall traps and list of all cryptobiotic species (marked with an asterisk) found during the study. Shown is the number of records (i.e., number of traps in which the species was present) of each species in different habitats, soil depths, and in total. The total number of records for each species in the conventional traps is also provided.

Species	Habitat			Depth		Total subterranean	Total conventional
	Forest	Savanna	Crops	20 cm	50 cm		
*Acanthostichus kirbyi**		1		1		1	
Acanthostichus sp. nr. *brevicornis**		1		1		1	
Acromyrmex sp.6	1			1		1	1
Acromyrmex subterraneus molestans			1	1		1	73
Acromyrmex subterraneus subterraneus	1			1		1	41
Atta laevigata	1			1		1	135
Brachymyrmex sp.2			1	1		1	100
Camponotus sp.10			1	1		1	26
Camponotus sp.40	1	1		2		2	43
*Carebara brevipilosa**	3		1	4		4	26
Carebara sp.1 (*lignata* gp.)*		1		1		1	
Carebara sp.2 (*lignata* gp.)*	1						1
*Carebara urichi**			1	1		1	5
Cephalotes pusillus	2			2		2	22
Crematogaster rudis		3	1	3	1	4	28
Dorymyrmex brunneus		1	13	14		14	196
Dorymyrmex sp.6			2	2		2	39
Dorymyrmex goeldii			3	3		3	60
Ectatomma sp.5	1		1	2		2	102
Ectatomma brunneum		1	6	6	1	7	96
Ectatomma opaciventri			2	2		2	117
Ectatomma planidens		2		1	1	2	34
Gnamptogenys sp.1			1	1		1	15
Gnamptogenys haenschi	4			4		4	1
Hypoponera sp.2	1				1	1	1
Hypoponera sp.5	1			1		1	
Hypoponera sp.6	1			1		1	
Hypoponera sp.8*	1						1
Hypoponera sp.11*			1				1
Hypoponera sp.1 (*punctatissima* gp.)*			1	1		1	
Hypoponera sp.3 (*punctatissima* gp.)*	1						1
Hypoponera cf. *trigona**	4						4
*Labidus coecus**	1	1	4	6		6	23
*Labidus mars**	3	7	7	15	2	17	1
*Labidus praedator**	1			1		1	7
Mycocepurus goeldii		1		1		1	122
Mycocepurus cf. *smithii*		1		1		1	14
Myrmicocrypta sp.3	1			1		1	
*Neivamyrmex bruchi**	7	21	4	27	5	32	7
*Neivamyrmex modestus**	3						3
Neivamyrmex cf. *pseudops**		2					2
*Neivamyrmex punctaticeps**	17		1	17	1	18	
*Octostruma jheringi**		1		1		1	1
Oxyepoecus inquilinus			1	1		1	
*Pachycondyla guianensis**	1			1		1	
Pachycondyla obscuricornis		1			1	1	76

Table 2: Continued.

Species	Habitat			Depth		Total subterranean	Total conventional
	Forest	Savanna	Crops	20 cm	50 cm		
Pheidole sp.1	1	6	28	34	1	35	203
Pheidole sp.2	2	4	2	7	1	8	76
Pheidole sp.3		3	7	8	2	10	38
Pheidole sp.A		1		1		1	2
Pheidole sp.4	1	3	8	11	1	12	36
Pheidole sp.5			2	1	1	2	8
Pheidole cf. *fowleri*	9	10	12	31		31	174
Pheidole sp.C (*diligens* gp.)		1		1		1	19
Pheidole sp.10			1	1		1	16
Pheidole sp.D	2			2		2	24
Pheidole sp.12		1	3	3	1	4	93
Pheidole sp.13	1			1		1	21
Pheidole sp.16		4	5	9		9	28
Pheidole sp.17		1	6	7		7	28
Pheidole sp.18			1	1		1	48
Pheidole sp.20	4	3	6	13		13	4
Pheidole sp.21			1	1		1	17
Pheidole sp.24	2			2		2	6
Pheidole sp.25	2			2		2	28
Pheidole sp.33	1			1		1	1
Pheidole sp.36*	2			2		2	
Pheidole sp.38	1			1		1	21
Pheidole sp.54			1	1		1	4
Pheidole fimbriata	33			33		33	27
Pheidole oxyops	8	1	32	41		41	281
Pogonomyrmex naegelli			2	2		2	90
Prionopelta cf. *antillana**			1	1		1	14
Sericomyrmex sp.1	1			1		1	15
Sericomyrmex sp.2	2			2		2	23
Solenopsis sp.1	3	1	1	4	1	5	101
Solenopsis sp.2		1	1	2		2	25
Solenopsis iheringi	3			3		3	50
Solenopsis saevissima	2	5	48	49	6	55	97
Tetramorium simillimum		1		1		1	
Trachymyrmex sp.7	1			1		1	3
Typhlomyrmex sp.1*	2						2
Wasmannia auropunctata	2	2	1	5		5	32

ants. This indicates that subterranean ants are relatively rare, with low density in the soil. In this sense, we may still need to develop methods to improve trap efficiency. Potential ways to improve trap efficiency include putting various types of baits on the soil around traps (as discussed above), making a large number of perforations in the traps (to facilitate ant access), and/or replacing baits periodically as done in some studies [19, 20].

4.2. Subterranean Traps versus Traps Placed on the Soil Surface. Sixteen percent of the species that we collected with subterranean traps were not collected with conventional pitfalls. Similarly, in Ecuador, 19.1% of the species collected with subterranean probes were not collected with other methods [15, 19], while the use of subterranean traps in Northern Australia resulted in the collection of 13.8% of unique species in one site and of 47% in another [14].

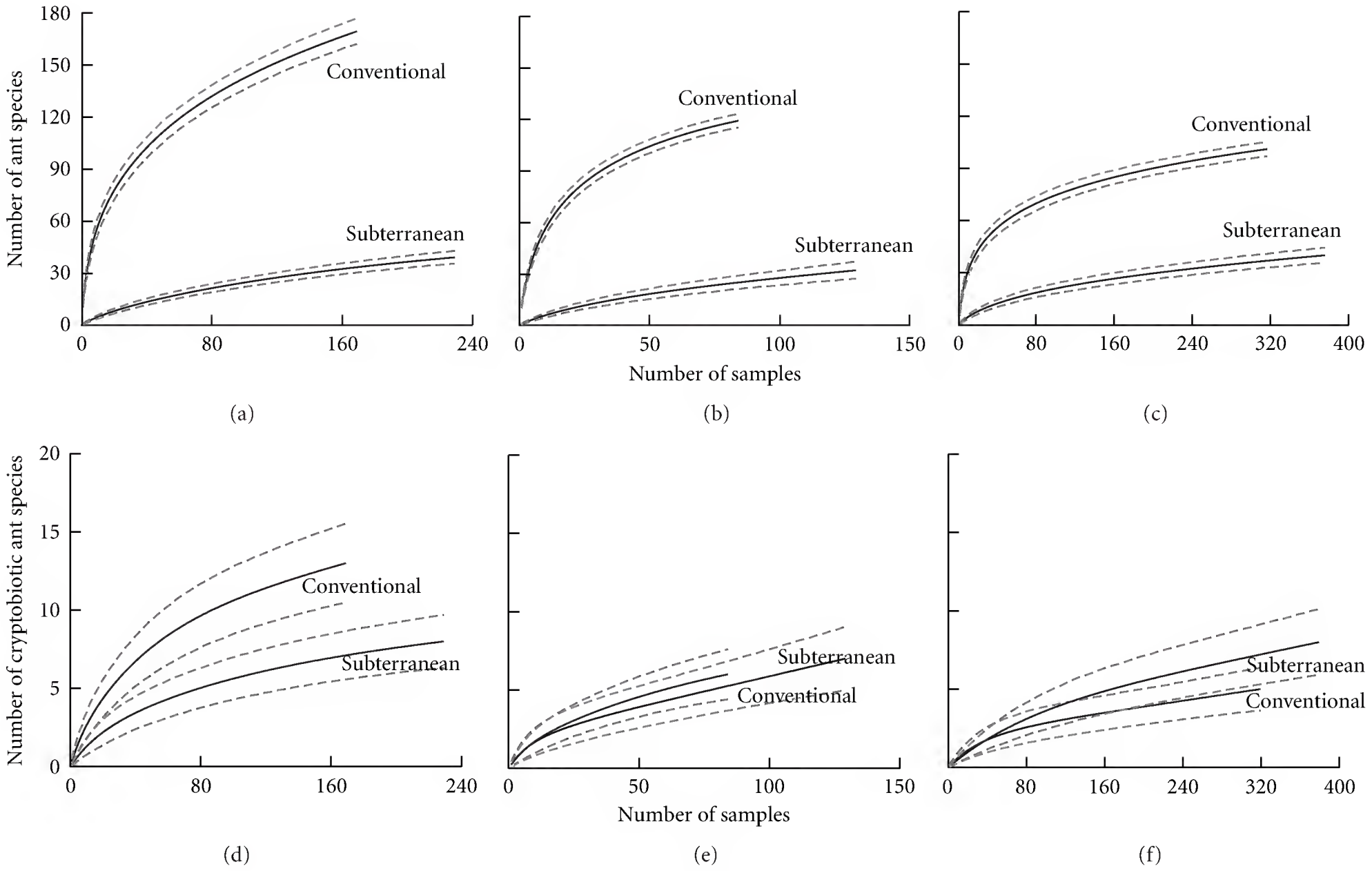

FIGURE 2: Sample-based species accumulation curves of the total number of ant species and the number of cryptobiotic species collected in subterranean or in conventional (i.e., on soil surface) pitfall traps placed in forests (a, d), savannas (b, e), or crop fields (c, f). Dotted lines represent one standard deviation around mean values.

TABLE 3: Overall ant species richness and richness of cryptobiotic species in three different habitats. Shown is the observed and estimated (±SD) number of species (Jacknife 1 species richness estimator).

	All species		Cryptobiotic species	
Habitat (no. of traps)*	Observed species richness	Estimated	Observed species richness	Estimated
Forest (398)	177	190.9 ± 6.1	16	21.0 ± 2.2
Savanna (213)	126	156.9 ± 6.0	11	15.0 ± 2.2
Crops (698)	107	133.0 ± 5.6	9	13.0 ± 2.0
Total (1309)	232	291.0 ± 7.9	23	32.0 ± 3.0

* Including subterranean and conventional traps.

Most of the species unique to our subterranean traps had cryptobiotic morphology (i.e., were truly hypogaeic species). However, surprisingly, subterranean traps were not more efficient than conventional traps in collecting cryptobiotic species. In fact, in the forest sites more cryptobiotic species were captured using conventional than subterranean traps (Figure 2). In addition, most of the cryptobiotic species found in the two types of traps (such as *Carebara brevipilosa*, *Carebara urichi*, *Labidus coecus*, *Labidus praedator*, and *Prionopelta* cf. *antillana*; Table 2) were more frequent in the conventional than in the subterranean traps. The only species that we can confidently say that are really more likely to be collected in our sites using subterranean than conventional pitfall traps are the army ants *Labidus mars* and *Neivamyrmex punctaticeps*. Nevertheless, it may well be possible that these two species could perhaps be more easily detected if we had used other methods designed specifically for finding army ants [23].

Overall (i.e., considering all species), subterranean traps collected far fewer species than conventional traps (see also [15] for a comparison between subterranean traps and other more traditional methods), indicating that ant diversity is much greater above- than below-ground. We collected two to four times more (depending on the type of habitat) species using conventional pitfall traps than using subterranean traps. Furthermore, when considering the overall number of species collected using both conventional and subterranean traps (232 species), only 5.2% were not collected using the conventional traps. In other words, the number of species collected by using a combination of subterranean and conventional traps was only slightly greater than the number those collected using just the conventional traps. This raises the question: is it worthwhile to include subterranean pitfall

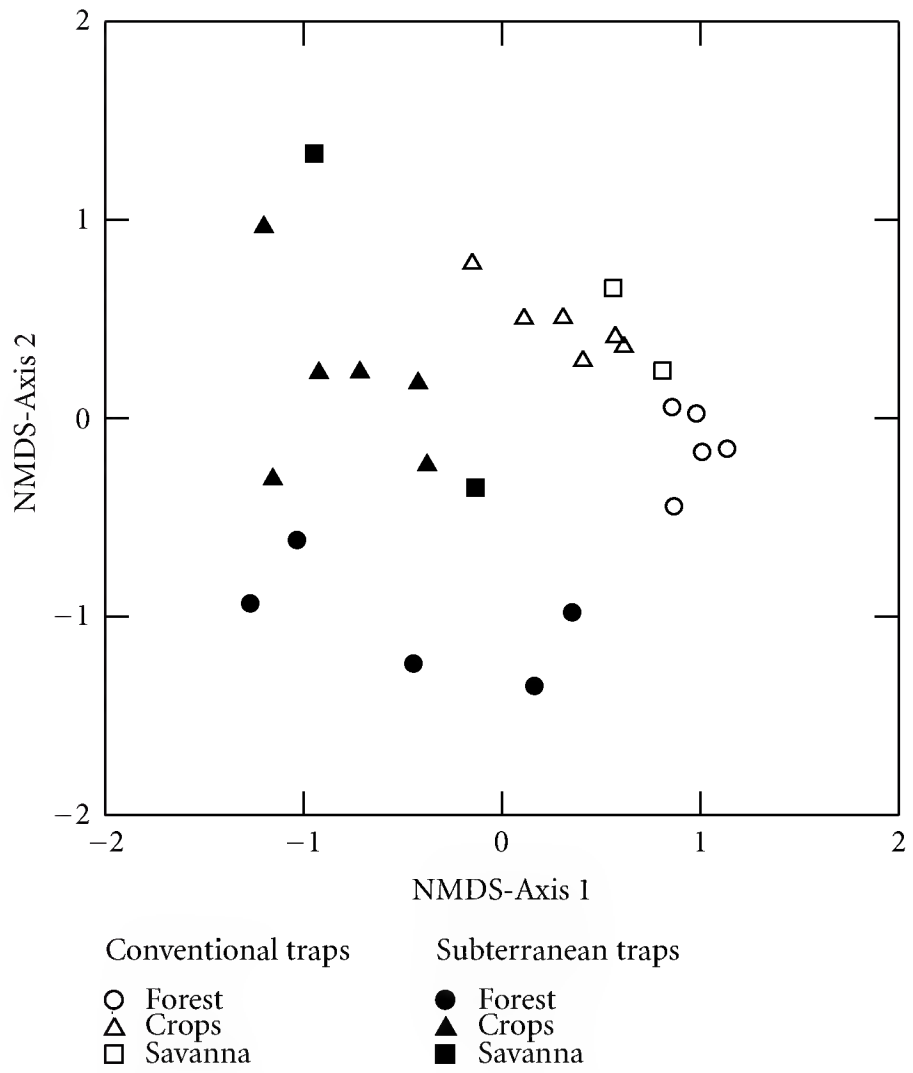

FIGURE 3: Nonmetric multidimensional scaling (NMDS) ordination of transects placed in different habitats based on the composition of ant species captured in subterranean or in conventional pitfall traps.

traps in your ant sampling protocol? In our view, if the purpose of the sampling is to provide just a general characterization of the studied ant communities, then the response is no. This is because many more species will be collected by using, say, 100 conventional pitfall traps than by using 50 conventional and 50 subterranean traps. In this way, even though a few cryptobiotic species would be missed (or their abundances underestimated), the completeness of the sampling would be much greater. Similarly, subterranean traps would probably not add much more information when the goal of the study is to compare the ant fauna of different habitats, since our results indicate that in most habitats conventional traps are at least as efficient as subterranean traps in collecting cryptobiotic species and, in addition, they collect much more species overall. On the other hand, if the purpose of the sampling is to evaluate the degree of vertical stratification of the ant fauna [31] or, especially, provide a relatively accurate species list (or a more reliable description of the functional composition of the studied community), then the response is yes. This is because some species are less likely to be recorded unless some kind of method to sample hypogaeic ants is employed. Examples include the first record of *Neivamyrmex punctaticeps* and *Labidus mars* in the Cerrado (this study), the rediscovery of *Simopelta minina* in the Atlantic forest [22, 32], the record of a new genus (*Pseudolasius*) for Western Australia [14], and the discovery of new species [19] and even new ant subfamilies [18, 33] by sampling subterranean ants. In this sense, we agree with previous claims that the subterranean ant fauna represents a novel and important frontier in biodiversity inventories [14, 19]. But to better explore this frontier we still need studies that compare the efficiency of the different methods developed so far, including traps, probes [15, 19], sieved buckets [20], and soil-core sampling [10, 16–18], taking into account the costs of each method and their efficiency in terms of the number and uniqueness of the species collected.

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Research Article

Evidence of Competition Between Two Canopy Ant Species: Is Aggressive Behavior Innate or Shaped by a Competitive Environment?

Nádia Barbosa do Espírito Santo,[1,2] Sérvio Pontes Ribeiro,[3] and Juliane Floriano Santos Lopes[1]

[1] *Programa de Pós-Graduação em Comportamento e Biologia Animal, Universidade Federal de Juiz de Fora, Campus Universitário Martelos, 36036-330 Juiz de Fora, MG, Brazil*
[2] *Programa de Pós-Graduação em Ecologia, Universidade Estadual de Campinas, 13083-970 Campinas, SP, Brazil*
[3] *Departamento de Biodiversidade, Evolução e Meio Ambiente, Programa de Pós-Graduação em Ecologia de Biomas Tropicais, Universidade Federal de Ouro Preto, Campus Morro do Cruzeiro, 35400-000 Ouro Preto, MG, Brazil*

Correspondence should be addressed to Nádia Barbosa do Espírito Santo, nadiabarbosa@yahoo.com.br

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Competition occurs in all ecological communities, although it has not always been experimentally tested as a structuring force in the distribution of species. We tested the hypothesis that the aggressiveness exhibited by *Camponotus rufipes* changes according to the pressures of a competitive environment. This is a dominant species in the montane forest of the Itacolomi State Park, Brazil, where *Camponotus sericeiventris* does not occur. Using bait traps in a field site where both species occur, ("Juiz de Fora" site) we showed that *C. sericeiventris* was able to remove *C. rufipes* workers at the same bait. In the laboratory, we used dyadic encounters to test workers from both species taken from colonies found in areas where both occur and where only *C. rufipes* was found. *Camponotus rufipes* from Itacolomi fought significantly less and was killed during the first few minutes in 60% of the events. On the other hand, the workers that co-existed with *C. sericeiventris* in the field were more aggressive, but less efficient fighters than the latter. This investigation demonstrated existence of competition between *C. rufipes* and *C. sericeiventris*, and also the lower aggressiveness of *C. rufipes*' individuals that did not co-exist in the field with *C. sericeiventris*.

1. Introduction

The importance of competition for structuring ecological communities is a matter for debate, and it has been extensively researched in ant assemblages [1]. Exploitation and interference competition in ants involve mutual aggression, which can frequently be observed, often resulting in injuries, death, and the avoidance of one colony by another [1]. The more similar are the species' morphology and niche breadths, the stronger is the competition [2, 3]. Species coexistence is possible when there are diversified strategies for resource usage, namely, time partitioning, feeding-source differentiation, or nesting locations [1, 3]. On the other hand, competition causes hierarchical dominance amongst the species through the use of aggression, food source exclusion, and different foraging strategies [4, 5]. Dominant ant species can influence the occurrence of other species and play a major role in ant assemblage structuring, in which they generate distribution patterns and a mosaic-like species coexistence, especially in forest canopies [6–9].

Territorial defence is related to dominance and occurs widely among canopy ant species in tropical rainforests [5]. Here, we observe the occurrence of a hierarchical competition that is based on their social organization and, ultimately, on foraging workers density. Hence, when a nonterritorial species encounter a territorial one, the former tends to run way from aggressive conflict, which may result in locally improbable species pairs [10, 11].

Camponotus (Myrmothrix) rufipes (Fabricius, 1775) is normally associated with ecotones between forests and open vegetation and is rarely found in great quantities in the canopy of lowland forests [12–18]. However, in the State Park of Itacolomi, a montane forest ecosystem in the borders between the Brazilian Cerrado and the Atlantic rainforest, this species is the most frequent member of this genus in the canopy [18]. In this particular forest the potential competitor, *Camponotus (Myrmepomis) sericeiventris* (Guérin-Menéville, 1838), which has a similar body size and uses the same kinds of food and nesting sites, was absent, unlike in the other areas of Atlantic rainforest and in most other Neotropical arboreal ecosystems [19]. On the contrary of *C. rufipes*, the latter is mostly frequent and dominant in the upper canopy [19, 20].

The present work investigates the degree of *C. rufipes* aggressiveness in contrasting competitive environments by means of direct observation in field and laboratory. We tested the hypothesis that *C. rufipes* shows different behaviours depending on the nature of the competitive environment. The prediction is that levels of aggressiveness of *C. rufipes* vary in response to presence or absence of competition from *C. sericeiventris*.

2. Methods

2.1. Field Experiments. Observations were carried out at two locations in the Atlantic rainforest. The first was Itacolomi State Park (Itacolomi), Minas Gerais State (20°22′30″S and 43°32′30″W), between 1000–1300 metres above sea level, within an area of 7.543 hectares, and belonging to the Espinhaço Mountain Range, which has a tropical montane climate, with rainfall varying from 1000 to 1500 mm per year and the temperatures between 4°C and 33°C [21]; The second was the campus of the Federal University of Juiz de Fora (UFJF) (21°46′47″S and 43°22′24″W), at 818 metres above sea level, also with a well-defined rainy and dry seasons, an annual average temperature of 19.3°C, and a annual precipitation of 1500 mm [22]. Both species, *C. rufipes* and *C. sericeiventris*, were encountered at the UFJF campus, but only *C. rufipes* was found in Itacolomi.

At Itacolomi, three trees were selected because of the high foraging activity of previously observed *C. rufipes* ants [18]. At the UFJF Campus, three trees were also selected in a territorial border where both *C. rufipes* and *C. sericeiventris* coexisted: in two of the trees there was an intense foraging by *C. rufipes* ants, due to the existence of nests closer than 10 metres to their trunk; in the third, there was a *C. sericeiventris* nest. The experiments were conducted during the months of October and November 2007.

Observations were made on one tree per day, thus in 3 days all trees were observed. Afterwards, two new rounds of observations, following the same order, were executed. Hence, in 9 days all trees were observed three times, with 2 days intervals between each observation. As the experiment was conducted within a short and continuous time interval, no relevant change in weather conditions was noticed. On the trunks of each of these trees a paper towel with attractant bait made of sardines (10 g) and honey (1 : 1; g : g) was placed. The behavioural recordings started with the arrival of the first *C. rufipes* ant in Itacolomi, whereas at the UFJF Campus it started when the first *C. rufipes* or *C. sericeiventris* appeared. From that moment on, we used the sequential sampling method [23]. For 4 hours, during five minutes at 10-minute intervals, all ant behaviours were recorded. These recordings include not only the behaviour of the two species in focus but also that of all the other species that appeared. Hence, this experiment was composed of six behavioural recordings per tree, constituting 18 repetitions, that provided 288 records. The observed behavioural acts were then divided into three categories: action, reaction, and nonaggressive.

Access to the tree crowns was achieved by tree-trunk climbing, either with or without a rope, and using safety equipment (see Ribeiro et al. [24]). Contingency tables were created to analyse species superiority in aggressiveness for both action and reaction types of behaviour. The analyses were done using Chi-square at a 5% significance level.

2.2. Laboratory Experiments. Experimental dyadic encounters were manipulated between *C. rufipes* and *C. sericeiventris* workers from different colonies collected in both Itacolomi and UFJF. Approximately 70 workers of *C. rufipes* from an Itacolomi colony were collected. Meanwhile, 50 workers of this species, along with 50 workers of *C. sericeiventris*, were collected from UFJF in an area where they coexisted. These collected ants were kept in a lidded plastic containers (12 cm × 9 cm) with a cotton ball soaked in water-diluted honey and remained isolated (at 25°C and 70% humidity) for 24 hours before performing the experiment. During this period, the samples were exposed to similar stress of collecting and travelling, and subsequent resting in the laboratory. The resting period and the experiments took place at the Myrmecology Laboratory of UFJF during the months of December 2007 and January 2008.

At each dyadic encounter, two ants of the same caste were placed in an arena (6 cm diameter) with Fluon at the edges to prevent their escape. To relieve the stress of transferring, the ants were separated by a partition in the arena for 10 minutes. Afterwards, the partition was removed, and their behaviour was registered during 5 minutes (*ad libitum* [23]). The observed behavioural acts were divided into three categories: action, reaction, and nonaggressive.

We manipulated six types of encounters (Table 1) and each one was repeated 10 times. After each repetition, the experimental arena was cleaned with alcohol 50% to eliminate any ant odour, so as to not interfere with the results of the next repetition. For the repetitions involving ants of the same species, the individuals were marked on the pronotum with nontoxic ink using an Edding 750 pen [25].

Aggression was calculated according to a modified index of aggressiveness from Errard and Hefetz [26]. The assigned values represent degrees of aggressiveness: 0 = "touched antennae and retreated", 1 = "on alert and charged", 2 = "bite", 3 = "torsioned gaster" and 4 = "fight". The resulting index for each treatment was subjected to the Kruskall-Wallis test, followed by the post-hoc Student-Newman-Keuls test, at 5% significance level (Table 2), using Biostat 4.0 software. This study was performed under licence permission from

Table 1: Dyadic encounters occurring at the laboratory of the Federal University of Juiz de Fora (UFJF) for all treatments and species involved.

	Treatment	Species
1	Control *C. rufipes* Itacolomi	*C. rufipes* Itacolomi × *C. rufipes* Itacolomi
2	Control *C. rufipes* UFJF	*C. rufipes* UFJF × *C. rufipes* UFJF
3	Control *C. sericeiventris* UFJF	*C. sericeiventris* UFJF × *C. sericeiventris* UFJF
4	Neighbours	*C. rufipes* UFJF × *C. sericeiventris* UFJF
5	Same species	*C. rufipes* UFJF × *C. rufipes* Itacolomi
6	Different species	*C. rufipes* Itacolomi × *C. sericeiventris* UFJF

Itacolomi = Itacolomi State Park; UFJF = Campus of the Federal University of Juiz de Fora.

Table 2: Student-Newman-Keuls ($H = 77.1648$) comparisions for each treatment of dyadic encounters listed in Table 1 (significative P-values in evidence).

		1 Control *C. rufipes* Itacolomi	2 Control *C. rufipes* UFJF	3 Control *C. sericeiventris* UFJF	4 Neighbours	5 Same species	6 Different species
1	Control *C. rufipes* Itacolomi	—	**0.0031***	0.7043	**<0.0001***	**<0.0001***	0.4172
2	Control *C. rufipes* UFJF		—	**0.0008***	0.0344	0.0355	**0.0002***
3	Control *C. sericeiventris* UFJF			—	**<0.0001***	**<0.0001***	0.6659
4	Neighbours				—	0.9891	**<0.0001***
5	Same species					—	**<0.0001***
6	Different species						—

Itacolomi: Itacolomi State Park; UFJF: Campus of the Federal University of Juiz de Fora.

the State Forestry Institute, and it followed university's ethic requirements for experiments with alive animals.

3. Results

3.1. Field Experiments. Nine morphospecies of ants were registered in experiments conducted in the Itacolomi State Park and 12 in the UFJF Campus. Fourteen types of behaviours were registered with the bait. The behaviour exhibited by the ants is categorized and described in Table 3.

The most frequent behaviours for the ants in the two experimental sites were "quietly feeding" and "exploring the surroundings" (Table 4). When observing interactive behaviour in Itacolomi, *C. rufipes* was the species that most engaged in the aggressive actions of "charge" and "bite" ($\chi^2 = 88.3$, $P < 0.001$). *Myrmelachista* sp.1 was the one that most engaged the reactions of "flee" and "gaster torsion" ($\chi^2 = 15.65$, $P < 0.05$) (Figure 1). For all the other species together, only two registers were recorded for the "charge" behaviour, while the "avoid", "flee", and "retreat" were the most frequent ones ($\chi^2 = 15.65$, $P < 0.05$), suggesting that they were submissive to the aggressiveness of *C. rufipes*.

In UFJF campus, *C. rufipes* and *C. sericeiventris* frequently showed aggressive behaviour through the actions of "charge" and "bite" ($\chi^2 = 45.78$, $P < 0.05$) with no statistical difference between these species in terms of the amount of these acts performed ($\chi^2 = 3.43$, $P > 0.05$). All other morphospecies showed significantly more defensive behaviour, especially "retreat" and "flee" reactions ($\chi^2 = 18.9$, $P < 0.05$) (Figure 2).

When comparing behaviour of *C. rufipes* workers from the two sites, the UFJF individuals bit more than the Itacolomi individuals, which showed more of "avoid" aggressors act ($\chi^2 = 19.34$, $P < 0.05$) (Figure 3). Concerning reaction behaviours, the *C. rufipes* from the UFJF colony tended to "retreat", especially in the presence of *C. sericeiventris* ($\chi^2 = 23.9$, $P < 0.05$) (Figure 2).

3.2. Laboratory Experiments. Fifteen types of behaviours were registered in the laboratory, which are categorized and described in Table 3. According to the calculated aggressiveness index, the more aggressive encounters were between *C. rufipes* workers from the two areas and between *C. rufipes* from Itacolomi and *C. sericeiventris*, thus, between workers whose colonies are far apart from each other (Figure 4). Colony workers had an average agonistic response significantly larger than that observed between themselves in the control experiment (Table 2).

Considering the mortality at the encounters, we verified that 70% of *C. rufipes* workers were dead in less than 2

Table 3: Description of behavioral acts displayed by the ants on Itacolomi State Park (Itacolomi) and the Campus of Federal University of Juiz de Fora (UFJF) during field experiments (**) and laboratory experiments (dyadic encounters) occurring at the lab of the Federal University of Juiz de Fora (*).

Action	Reaction	Nonaggressive
Charge—an ant approaches the other with its mandible open	(**) Remain on the bait—after any types of the listed actions, the ant remains in the area, eating the bait	(**) Quietly eating—when an ant is standing still, only eating the bait
Bite—grips part of the body of another individual with its mandibles	(**) Flee—after any types of these actions it flees not only from the area, but also from the bait	(**) Food transport—the individual carries part of the bait to the colony
Espontaneous gaster torsion—the ant curls its abdomen to emits formic acid	(**) Retreat—after the mentioned actions, it retreats from the other individual, but does not leave the bait and eats it	Autogrooming—cleaning itself
Avoid—when perceiving the proximity of another individual, the first moves away, avoiding the encounter	Defensive gaster torsion—after these actions, it exhibits aggressive behaviour by curling its abdomen to emit formic acid	Trophallaxis—exchange of regurgitated liquid from one individual directly into the crop of the other
(*) Antennal touching—an ant exchanges antennal touches with the other for identification	Fight—after mentioned actions, it grabs the other individual with its jaws and emits formic acid	Exploring the surroundings—walking around, touching its antennae on the whole extension of the paper where the bait was placed
(*) On alert—an ant stands still with its head and antenna raised, and with its gaster torsed in the posterior-anterior position ready to emit formic acid, if necessary	(*) Bite—grips part of the body of another individual with its mandibles in response to any action act	(*) Trying to escape—the ant stays on the border of the arena, trying to climb its wall
	(*) Charge—the ant advances in the direction of the other with its mandible open ready to bite back	

(*) Only in lab experiments; (**) Only in field experiments.

minutes (mostly in seconds) in the encounters between *C. rufipes* from Itacolomi and *C. sericeiventris*. (Kruskal-Wallis, Student-Newman-Keuls = 23.12, $P < 0.035$). For the remaining 30%, *C. rufipes* killed *C. sericeiventris* in one case, and two other times there were fights without deaths.

Unlike the encounters with the neighbouring *C. rufipes* and *C. sericeiventris* from UFJF, the fight lasted longer and all the repetitions severally resulted in death; 60% of the *C. sericeiventris* and 40% of the *C. rufipes* died. It is worth noting that when *C. rufipes* were killed, the trial was faster than when it was not killed. Finally, in the treatment between *C. rufipes* from the two studied sites, the ants from the Itacolomi colony died in half of the repetitions without causing a single death among the ants from UFJF (Table 1).

4. Discussion

4.1. Field Experiments. Regardless of the advantage of aggressiveness, high costs of competing may mean that its selective advantage is only sustainable if associated with the minimization of conflicts. An evidence is that the behaviours most frequently exhibited by all ants species from both sites were "quietly feeding" and "exploring the surroundings". According to ethograms found in the literature, the most common behavioural acts are into the categories of grooming, feeding, and exploring surroundings [19, 27–29].

Likewise, the "dear enemy" hypothesis [30] predicts that ant species are capable of recognizing and discriminating their neighbours (species/individuals), and then they are normally more aggressive with the "foreigners", saving energy by avoiding unnecessary conflicts with species or colonies with which they have already defined their boundaries [31, 32]. The species that live together adjust to the habitat and share resources, while foreigners may threaten this balance [30]. In addition, the constant contact among all coexisting ants followed by autogrooming results in a common Gestalt odour, that decreases the aggressiveness among all the species that share the same space [4]. Finally, for canopy ants, it has been reported that various species have the habit of foraging over great distances, presenting familiarity with the place, and tolerating the presence of neighbour species [33].

Both experiments suggest that these species tend to have a territory which is aggressively protected, and thus are able to dominate numerically in the canopies. Both species seem to have defensive behavior across the complete territory, instead of only at the nest or some particular feeding resource, as aggressive actions have been observed everywhere in the studied forest (pers.obs.) and have been corroborated experimentally. The dominant position could be taken by *C. rufipes* where *C. sericeiventris* does not occur, while in the presence of the latter, *C. rufipes* may still keep high abundance (mainly by combining foraging in different habitats, such as the canopy and in the litter) and codominate the assemblage.

Concerning other observed species, distinct behaviours at the bait reflected their recruiting and foraging strategies and, in many cases, their attack and defence tactics. Many species proved to be opportunists, eating rapidly until being

Table 4: Occurrence of behaviours for the morphospecies of ants in the Itacolomi State Park (Itacolomi) and the Campus of Federal University of Juiz de Fora (UFJF) during field experiments.

Local	Espécie	Quietly eating	Exploring the surroundings	Autogrooming	Food transport	Charge	Bite	Espontaneous torsion gaster	Avoid	Remain on the bait	Flee	Retreat	Fight	Trofalaxis
UFJF	*Acromyrmex* sp.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
UFJF	*C. rufipes*	42%	21%	14%	0%	9%	10%	0%	0%	1%	0%	2%	0%	0%
UFJF	*C. sericeiventris*	69%	8%	12%	0%	4%	4%	0%	0%	0%	1%	1%	0%	1%
UFJF	*Camponotus* sp.5	82%	8%	2%	0%	0%	0%	0%	1%	0%	3%	3%	0%	0%
UFJF	*Camponotus* sp.6	41%	19%	5%	0%	0%	0%	0%	0%	0%	27%	3%	5%	0%
UFJF	*Camponotus* sp.7	50%	8%	7%	0%	2%	1%	0%	7%	0%	9%	15%	2%	0%
UFJF	*Camponotus* sp.8	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
UFJF	*Cephalotes* sp.2	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
UFJF	*Crematogaster* sp.1	98%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
UFJF	*Pheidole* sp.2	75%	23%	0%	1%	0%	1%	0%	0%	0%	0%	0%	0%	0%
UFJF	*Pseudomyrmex* sp.2	69%	15%	3%	0%	3%	1%	0%	4%	0%	1%	5%	0%	0%
UFJF	*Solenopsis* sp.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Itacolomi	*C. rufipes*	29%	40%	14%	4%	6%	3%	0%	4%	0%	0%	0%	—	—
Itacolomi	*Camponotus* sp.4	86%	7%	0%	0%	0%	0%	0%	0%	0%	0%	7%	—	—
Itacolomi	*Camponotus* sp.2	76%	6%	12%	0%	0%	0%	0%	0%	0%	6%	0%	—	—
Itacolomi	*Camponotus* sp.3	50%	17%	33%	0%	0%	0%	0%	0%	0%	0%	0%	—	—
Itacolomi	*Camponotus* sp.1	65%	22%	8%	0%	1%	0%	0%	3%	0%	0%	0%	—	—
Itacolomi	*Cephalotes* sp.1	80%	20%	0%	0%	0%	0%	0%	0%	0%	0%	0%	—	—
Itacolomi	*Myrmelachista* sp.1	95%	4%	0%	0%	0%	0%	0%	0%	0%	1%	0%	—	—
Itacolomi	*Pheidole* sp.1	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	—	—
Itacolomi	*Pseudomyrmex* sp.1	37%	46%	10%	4%	0%	0%	0%	2%	0%	0%	2%	—	—

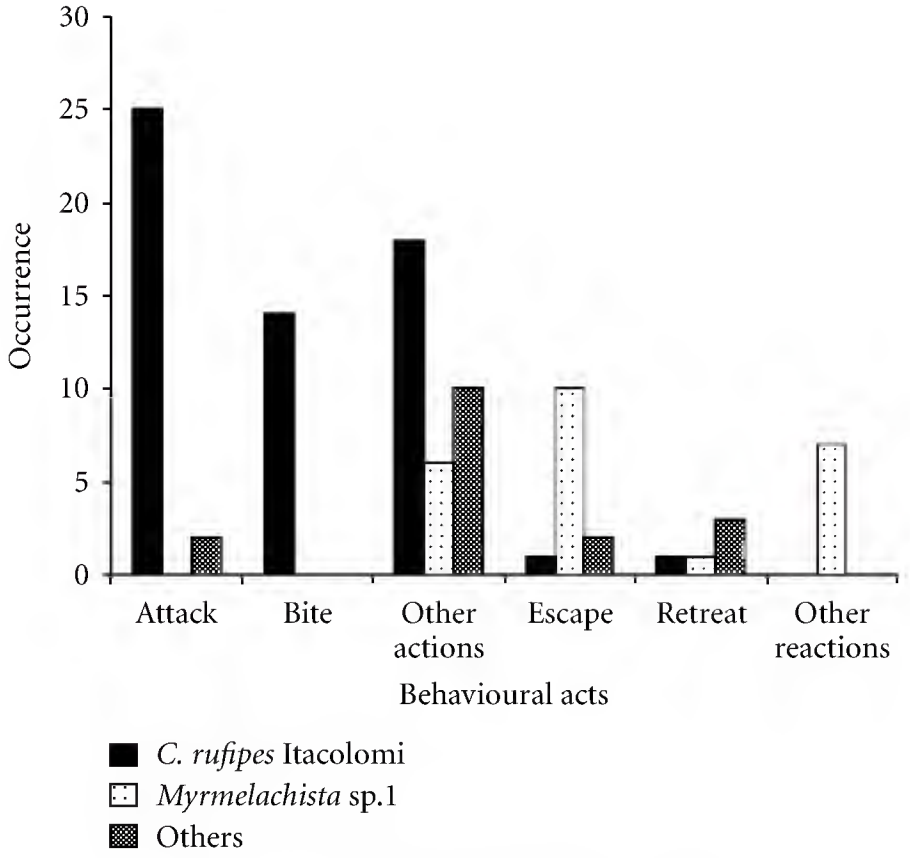

FIGURE 1: Action and reaction behavioural occurrences for *C. rufipes*, *Myrmelachista* sp.1 ants, and other morphospecies assemblages during field experiments in the Itacolomi State Park (Itacolomi). The "other actions" and "other reactions" are grouped together because of their low frequency.

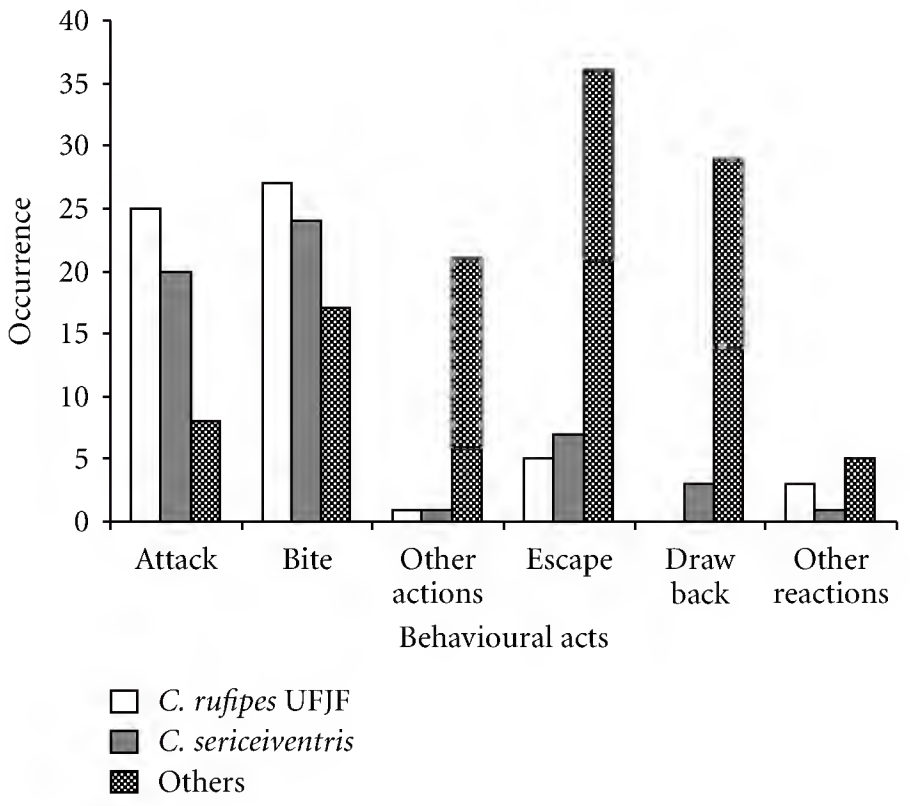

FIGURE 2: Action and reaction behavioural occurrences for *C. rufipes*, *C. sericeiventris*, and other morphospecies (grouped together) during field experiments at the Campus of Federal University of Juiz de Fora. The "other actions" and "other reactions" behaviours are grouped together because of their low frequency.

expelled by the dominant species, but avoiding interactive aggressive behaviour. In this study, the genera *Cephalotes* and *Pseudomyrmex*, along with some species of *Camponotus* (*C. crassus* and three nonidentified *Camponotus* species) exhibited this type of behaviour. The opportunistic or cowardly behaviour was previously recorded by *Cephalotes pusillus* in Byk and Del-Claro [34], as the species was never

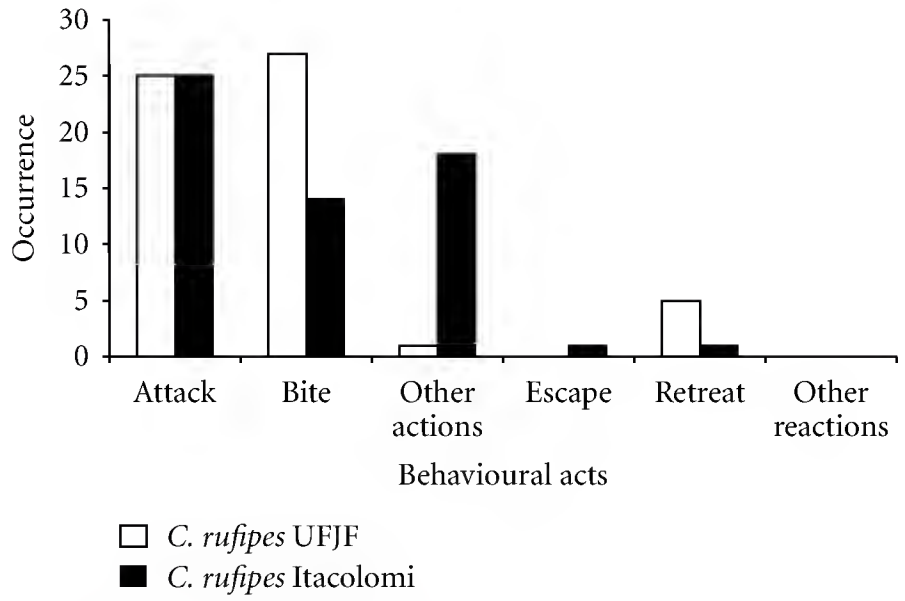

FIGURE 3: Action and reaction behavioural occurrences for the *C. rufipes* colonies at the Itacolomi State Park (Itacolomi) and campus of Federal University of Juiz de Fora (UFJF) during field experiments. The "other actions" and "other reactions" behaviours are grouped together because of their low frequency.

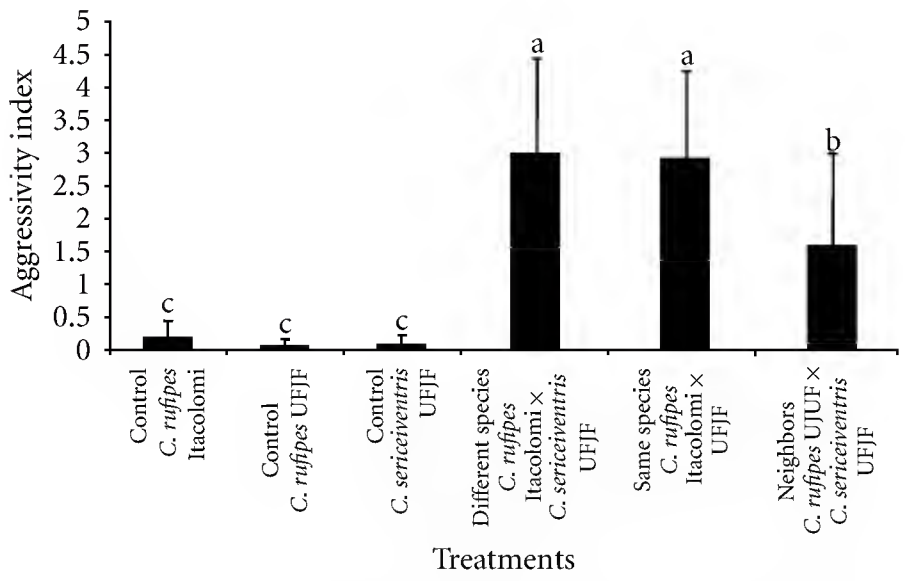

FIGURE 4: Aggressiveness index (mean and standard deviation) for each treatment of laboratory experiments (dyadic encounters) in which "control" is the manipulation with individuals from the same colony and species; "different species" between *C. rufipes* from Itacolomi State Park (Itacolomi) and *C. sericeiventris* from Campus of Federal University of Juiz de Fora (UFJF); "same species" between *C. rufipes* from both Itacolomi and UFJF; "neighbours" between *C. rufipes* and *C. sericeiventris* from UFJF.

observed attacking any insect, contrary to what is expected for a plan-ant-herbivore system.

To a certain extent, the coexistence of these species was possible due to behavioural diversity. For instance, we observed that different species place themselves almost opposite to the others when eating on the bait, thus avoiding conflicts while sharing the same resource at the same moment. In this study, at various times when the bait was exposed, there were around five ant species on it. An erroneous interpretation of this would be to say that there was no competition in place. However, upon behavioural observation *in locu*, it was evident that species placed themselves strategically on the bait, avoiding visual or chemical contact. Furthermore, when two species meet, there was aggressive behaviour, resulting in fleeing of one of them. It is worth noting, though, that

since baits were an energetic, unpredictable, and sufficiently abundant food source, it was possible for various species to eat at the same time without the need to expel or attack the others. Most of natural resources available ought to result in a tougher competitive environment than the one we manipulated.

4.2. Laboratory Experiments. *Camponotus rufipes* and *C. sericeiventris* were able to recognize individuals of their colony and to differentiate them from other colonies and species. For *C. rufipes,* this capacity has already been registered [35]. Most importantly, the present experiment measured the aggressiveness between these species, which was high for *C. sericeiventris*, but also stronger for those individuals of *C. rufipes* that coexisted in the field with *C. sericeniventris*, compared with the Itacolomi's individuals. Since Itacolomi's ants have grown without any substantial competition in nature, they become pacifists.

Similar results were previously described for other systems. Lucas [36] observed three species of *Pachycondyla* (*P. villosa, P. inversa*, and *P. subversa*) during dyadic encounters and found out that they were able to recognize members of the same colony, of the same species and of different species, increasing aggressiveness in the same order. For *C. rufipes* at UFJF, there was no differentiation of aggressiveness between intra- and interspecific encounters, which means that their defensive behaviour against *C. sericeiventris* or a potential invader colony of their own species was similar and may reflect their establishment in a hostile environment.

Combative behaviours are widely described in the myrmecological literature. In the laboratory, *Oecophylla longinoda* (a dominant arboreal genera) was observed while fighting for its territory with two foreign individuals that entered simultaneously the arena [37]. This species was also observed in its natural habitat fighting and excluding other ant colonies in Africa. Similar results were encountered for *Oecophylla smaragdina* in Australia [38, 39]. De Vita [40] measured the aggressiveness of *Pogonomyrmex californicus* populations in their natural habitat and observed that 81% of the encounters resulted in some type of aggression and, in some cases, led to the death of the individuals. In addition, there was evidence that ants of the same species from different colonies showed aggression against each other. Aggression was also shown against potential competitors of another species, which resulted in fights or death in all repetitions.

The lack of aggressiveness from Itacolomi's *C. rufipes* corroborates the assumption that aggressive behaviour is more likely learned than inherited, especially since the same species (*C. rufipes* from UFJF) that coexists with competitors presents a greater level of aggressiveness and a greater capacity to fight with foreign species of the same size (*C. rufipes* from Itacolomi). Also, the data from the encounters among *C. rufipes* from Itacolomi and *C. sericeiventris* show that *C. rufipes* from Itacolomi was almost always attacked and killed. On the other hand, when the encounters were between *C. rufipes* and *C. sericeiventris* that coexisted in the same area (UFJF), the battles were more evensided, registering 40% of the deaths for workers of *C. rufipes* and 60% for *C. sericeiventris*. In these cases, the aggressiveness index was higher; the ants fought for a longer time before one of them died. In the field experiments, there was no occurrence of a fight to death between *C. rufipes and C. sericeiventris,* as had occurred in the dyadic encounters. Nevertheless, a transposing experiment is needed to confirm whether such aggressiveness would be learnt or, otherwise, whether there could be a *C. rufipes* "pacifist genotype".

As generally observed in the field for all species, these two codominant species also avoid combative behaviour, in accordance with the prediction that a strategy of decreasing costs of combat is of great importance in behavioural evolution. This mutual avoidance during the field experiments could also be related to the abundance of food that the bait represents, which implies in no dispute. However, the dominant species was bothered by the presence of other species when they were encountered feeding at the same place in the bait. On the other hand, in the dyadic encounters in a small arena and with no escape route, the ants had no alternative other than to dispute that small space by attacking and killing the other individual.

In conclusion, direct contacts seem to be avoided between *C. rufipes* and *C. sericeiventris*, as is expected, since they are two species with similar feeding habits, nesting location, foraging strategy, and body dimensions. However, *C. rufipes* species from Itacolomi does not have a great fighting capacity, very likely because it does not coexist with any other similar competitor, showing that behavioural plasticity will always favour the cost-saving behaviour, namely avoiding conflict.

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Research Article

Poneromorph Ants Associated with Parasitoid Wasps of the Genus *Kapala* Cameron (Hymenoptera: Eucharitidae) in French Guiana

Jean-Paul Lachaud,[1,2] Philippe Cerdan,[3] and Gabriela Pérez-Lachaud[2]

[1] *Centre de Recherches sur la Cognition Animale, CNRS-UMR 5169, Université de Toulouse UPS, 118 Route de Narbonne, 31062 Toulouse Cedex 09, France*
[2] *El Colegio de la Frontera Sur, Entomología Tropical, Avenida Centenario km 5.5, Chetumal 77014, Quintana Roo, Mexico*
[3] *Laboratoire Environnement de Petit Saut (HYDRECO), BP 823-97388 Kourou Cedex, France*

Correspondence should be addressed to Jean-Paul Lachaud, jlachaud@ecosur.mx

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Eucharitid wasps are specific, specialized parasitoids of ants. The genus *Kapala* Cameron is the most common in the Neotropics but few species are described, and information dealing with their biology, behavior and host associations is scarce. Numerous poneromorph ant colonies were inspected over 4 collection surveys in French Guiana. A diverse fauna of parasites and parasitoids was found, including mermithid nematodes, flies, eucharitids, and another gregarious endoparasitoid wasp. Five new host associations for *Kapala* are reported, all of them involving medium- to large-size poneromorph ant species from 4 genera: *Ectatomma brunneum* Fr. Smith, *Gnamptogenys tortuolosa* (Fr. Smith), *Odontomachus haematodus* (L.), *O. mayi Mann*, and *Pachycondyla verenae* (Forel). Three other associations involving *O. hastatus* (Fabr.), *P. apicalis* (Latreille), and *P. stigma* (Fabr.), already reported for other countries but new for French Guiana, are confirmed. The data extend the number of hosts for *Kapala* to 24 ant species from 7 genera. The high diversity of the ant host genera associated with *Kapala,* combined with the fact that these ant genera are the most widely distributed among Neotropical poneromorph ants, could account for the dominant status of the genus *Kapala* among the eucharitine wasps of Central and South America.

1. Introduction

Within Hymenoptera, the family Eucharitidae (subdivided in three subfamilies: Oraseminae, Eucharitinae, and the Indo-Pacific Gollumiellinae) is the most numerous and diverse group of ant parasitoids [1, 2]. All of the members of this family have a highly modified life cycle [3–6]. Unlike most parasitic wasp species, eucharitid females deposit their eggs away from hosts, in or on plant tissue [2, 7]. The active first-instar larva, termed planidium, is responsible for gaining access to the host ant larvae by using various phoretic behaviors including either attachment to an intermediate host (most often a potential ant prey) or to foraging ant workers, with on occasion the presence of attractive substances in or on the eggs [2, 8]. Within the nest, planidia attach themselves to ant larvae, but development is only completed when the host pupates [6, 7, 9, 10]. In almost all of the cases, adults emerge among ant brood (but see [11]) and have to leave the host nest to reproduce. Ants show only moderate aggression to newly emerged eucharitids [7, 12–17] and transport them outside as if they were refuse [11, 15, 17], ultimately enhancing wasp dispersal. Parasitism is very variable and localized in time and space [13, 18, 19]. A very high local prevalence may lead to only a very low impact at the regional scale, suggesting that these parasitoids do not have a major influence on the dynamics of their host population [19]. However, they constitute a remarkable example of both host-parasitoid coevolution and host behavior manipulation.

The eucharitine genus *Kapala* Cameron is widespread in the New World, with only one species, *K. ivorensis* Risbec, found in the Old World (Ethiopian region and Malagasy). This is one of the eucharitid genera most commonly collected by traps and aerial nets in the Neotropical region [1].

However, taxonomic and systematic studies of the species belonging to this genus have proved difficult because of the high degree of morphological variability both within and among species [20]. Only 17 species have been described up to now, but more than 60 are estimated to exist [1, 10]. This genus is presently under revision (E. Murray and J. M. Heraty, pers. comm). Information dealing with the biology, ecology, and behavior of *Kapala* wasps is still very scarce (but see [10, 16, 18]), even though the number of known associations with ant hosts significantly increased in the past ten years. To date, all of the ant species which have been recorded as reliable hosts for the genus *Kapala* apply to medium to large poneromorph ant genera belonging to two subfamilies: Ponerinae and Ectatomminae [1, 4, 11, 21–24]. As all of the ant larvae attacked by *Kapala* pupate inside a protective cocoon, immature stages of the parasitoids are not easily spotted unless under close scrutiny. Furthermore, accurate host records can only be obtained by direct rearing of the parasitoids from ant brood and need laborious target sampling and collection. This could explain in part why so few associations have been reported before the late 90s. Here we both summarize the results of several collection surveys in French Guiana aimed to contribute to the knowledge of the diversity and distribution of the *Kapala* species in the Neotropics and provide a comprehensive review of ant-host associations for this highly variable genus.

2. Materials and Methods

Several dozen colonies (or portions of colonies) of poneromorph ants were collected in French Guiana, during 4 extensive surveys between 2002 and 2010. Collecting surveys were performed during both dry and rainy seasons and at several biotopes, mainly lowland rainforest fragments but also second growth vegetation. Ants were collected from several forest fragments along the road leading to the Hydroelectric complex at Petit Saut, Sinnamary (5°03′39″N; 53°02′36″W). Samples were obtained by systematically breaking up all of the fallen rotten logs found on the ground or were visually detected in the case of tree-inhabiting ants. Colonies of *Ectatomma brunneum* Fr. Smith were common in a ruderal area running along Route No. 1 from Kourou to Sinnamary (kilometric point 101) and were collected from the soil by excavation. Complete colonies or significant parts of colonies containing cocoons and larvae were taken to the laboratory. Most ants were identified to species level with available keys. Nest composition (presence and number of dealate females, alate females, males, workers, cocoons, and larvae), including the presence of adult eucharitids, was determined, and all of the pupae (cocoons) were dissected under a stereomicroscope and checked for the presence of eucharitids or for evidence of their attack. In particular, we looked both for wasp remains (exuvia) within empty ant cocoons denoting previous eucharitid emergence and for the presence of any abnormal pupa (phthisergate, phthisogyne, or phthisaner, according to the caste) indicating an unsuccessful eucharitid attack [12]. Larvae were also checked for the presence of planidia attached to their cuticle or for the presence of round melanized scars, an evidence of the previous attachment of a planidium [19]. Voucher specimens of ants were deposited in the Arthropod Collection at El Colegio de la Frontera Sur-Chetumal. Eucharitid specimens were sent to the specialist of this group, Dr. John M. Heraty (UCR).

3. Results

A total of 161 complete colonies of poneromorph ants or colony fragments with pupae, representing 26 species from 3 subfamilies, were collected and their contents examined (Table 1). No evidence of eucharitid attack to larvae was found for any of the poneromorph ant species examined, but several species of *Kapala* were found parasitizing the pupae of 8 different ant species. Of these, 5 represent new host associations (Table 1): one for the genus *Ectatomma* Fr. Smith (*E. brunneum* Fr. Smith), one for the genus *Gnamptogenys* Roger (*G. tortuolosa* (Fr. Smith)), and two for the genus *Odontomachus* Latreille (*O. haematodus* (L.), *O. mayi* Mann). The fifth new host record involves the genus *Pachycondyla* Fr. Smith and concerns a *P. verenae* (Forel) colony which was collected by Ronara de Souza Ferreira in the Southwestern part of French Guiana, at Camp Patawa, about 40 km from Roura in the direction of Raw. In addition, the host status of *O. hastatus* (Fabr.)—already reported for Ecuador [1]—and of *P. stigma* (Fabr.) and *P. apicalis* (Latreille)—already reported for Mexico [10, 22, 24]— were confirmed, and this constitutes the first report for French Guiana.

Due to the very reduced number of sampled nests for *O. mayi* and *P. apicalis*, percent parasitism figures (number of infested nests/number of revised nests) for these species are given only as indicative of eucharitid attack. For those species where a significant number of nests could be revised, prevalence of parasitism was generally low to medium (from 14.3% for *P. stigma* to 27.3% for *G. tortuolosa*) but reached up to 50% in the case of *O. hastatus*. However, parasitism rate within parasitized colonies was very low for all of the species, and only few eucharitid specimens were retrieved (Table 1).

Apart from eucharitids, some other parasites were found attacking ant colonies. Pupae of *P. goeldii* (Forel) were parasitized by a gregarious endoparasitoid wasp, and those of *P. commutata* (Roger) and *Paraponera clavata* (Fabr.) were parasitized by two gregarious dipteran species. Finally, a mermithized worker was found in a *P. stigma* colony, and this constitutes the first report of mermithid nematode parasitization for this species. In the absence of any post-parasitic juvenile, the mermithid species identification was not possible. Voucher specimens of both the ants and the mermithid nematode were deposited in the authors' collection.

4. Discussion

A diverse fauna of parasites and parasitoids attacked the different poneromorph ant species present in French Guiana near Petit Saut, including nematodes, flies, a gregarious

Table 1: Poneromorph ants of French Guiana and parasitism by *Kapala*. Ant species revised (n = number of nests containing pupae), presence of eucharitid wasps, parasitism rate by eucharitids (in %), and number and developmental stage of specimens of Eucharitidae retrieved (F: female; M: male; A: adult, damaged specimen; Lif: first-instar larva feeding on host; Lf: last-instar larva; Pht.: phthisergate; Ex.: presence of eucharitid exuvia within an empty cocoon).

Ant species	Parasites and parasitoids
Ectatomminae	
Ectatomma brunneum ($n = 46$)	*Kapala* sp. (21.7%): 7 F, 1 M, 1 A, 2 Lif, 1 Pht., 1 Ex.
Gnamptogenys pleurodon ($n = 5$)	—
Gnamptogenys tortuolosa ($n = 11$)	*Kapala* sp. (27.3%): 1 F, 1 Lf, 1 Lif, 1 Pht.
Gnamptogenys sp. ($n = 1$)	—
Ponerinae	
Anochetus sp. 1 ($n = 1$)	—
Anochetus sp. 2 ($n = 3$)	—
Centromyrmex sp. ($n = 1$)	—
Hypoponera sp. 1 ($n = 4$)	—
Hypoponera sp. 2 ($n = 1$)	—
Leptogenys sp. 1 ($n = 2$)	—
Leptogenys sp. 2 ($n = 1$)	—
Odontomachus haematodus ($n = 20$)	*Kapala* sp. (15.0%): 1 F, 3 M, 1 Pht.
Odontomachus hastatus ($n = 6$)	*Kapala* sp. (50.0%): 2 F, 1 M, 2 Lf, 1 Lif
Odontomachus mayi ($n = 2$)	*Kapala* sp. (50.0%): 1 F
Odontomachus sp. 1 ($n = 1$)	—
Pachycondyla apicalis ($n = 2$)	*Kapala* sp. (50.0%): 1 F, 2 M, 1 Ex.
Pachycondyla commutata ($n = 2$)	—[(a)]
Pachycondyla constricta ($n = 6$)	—
Pachycondyla crenata ($n = 1$)	—
Pachycondyla goeldii ($n = 2$)	—[(b)]
Pachycondyla harpax ($n = 6$)	—
Pachycondyla obscuricornis ($n = 3$)	—
Pachycondyla stigma ($n = 21$)	*Kapala* sp. (14.3%)[(c)]: 1 F, 1 M, 1 Lif
Pachycondyla verenae ($n = 6$)	*Kapala* sp. (16.7%): 1 M
Platythyrea sinuata ($n = 6$)	—
Paraponerinae	
Paraponera clavata ($n = 1$)	—[(a)]

(a) Presence of dipteran parasitoids;
(b) Presence of unidentified, gregarious parasitoid wasps;
(c) Presence of a mermithid nematode.

endoparasitoid wasp, and, above all, eucharitids. Several authors have recorded the presence of eucharitids in ant nests, but the information is scattered in the literature and concerns only few ant species. Neotropical eucharitine wasps are represented by approximately 160 species from 16 genera [1], but information on the host plant(s) used for oviposition is extremely scarce, and the identity of the ant host has been clearly established for only a few species belonging to 5 genera (*Dilocantha* Shipp, *Isomerala* Cameron, *Kapala* Cameron, *Obeza* Heraty, and *Pseudochalcura* Ashmead). These ant hosts concern exclusively formicine, ponerine, and ectatommine ants, whose larvae pupate inside a protective cocoon.

With about 60 estimated species, the genus *Kapala* is by far the most diverse and dominant eucharitine genus of Central and South America [1]. All of the hosts reported until now for this genus belong to 7 genera of ectatommine and ponerine ants (Table 2). Only *K. floridana* (Ashmead) has been reported associated with a host from another subfamily, the myrmicine ant *Pogonomyrmex badius* (Latreille) (according to Ashmead, in [12]), but, considering that *Pogonomyrmex* larvae do not spin a cocoon, such an association seems very doubtful [1, 10]. Even if the presence of an undetermined species of eucharitid was already signaled for *Gnamptogenys annulata* (Mayr) and also for *G. horni* (Santschi) in Venezuela [26], and, very likely concerned a *Kapala* species, reliable associations between the genus *Kapala* and the ant genera *Ectatomma* and *Gnamptogenys* have previously been reported only for Mexico [10, 22] and for Colombia [25]. The new associations with these two ectatommine genera reported here for French Guiana, as well as the new associations or confirmations of record for the ponerine genera *Odontomachus* and *Pachycondyla*, support the outstanding importance of these four genera

Table 2: Known ant host species associated with the eucharitid genus *Kapala*.

Host species	Associated *Kapala* species
Ectatomminae	
Ectatomma Fr. Smith	
E. brunneum Fr. Smith	*Kapala* sp. [this work]
E. ruidum Roger	*K. iridicolor* (Cameron) [10, 18, 25], [16, 22]*, *K. izapa* Carmichael [10, 18]
E. tuberculatum (Olivier)	*Kapala* sp. [23]
Gnamptogenys Roger	
G. regularis Mayr	*K. iridicolor* (Cameron) [10], [22]**
G. striatula Mayr	*K. iridicolor* (Cameron) [10], [22]**
G. sulcata (Fr. Smith)	*K. iridicolor* (Cameron) [10], [22]**, *Kapala* sp. [24]
G. tortuolosa (Fr. Smith)	*Kapala* sp. [this work]
Typhlomyrmex Mayr	
T. rogenhoferi Mayr	*Kapala* sp. [24]
Ponerinae	
Dinoponera Roger	
D. lucida Emery	*Kapala* sp. [11]
Hypoponera Santschi	
H. nitidula (Emery)	*Kapala* sp. [24]
Odontomachus Latreille	
O. bauri Emery	*Kapala* sp. [1]
O. brunneus (Patton)	*Kapala* sp. [10, 22]
O. haematodus (L.)	*Kapala* sp. [this work]
O. hastatus (Fabr.)	*Kapala* sp. [1], [this work]
O. insularis Guérin-Méneville	*K. terminalis* Ashmead [4]
O. laticeps Roger	*Kapala* sp. [10, 22, 24]
O. mayi Mann	*Kapala* sp. [this work]
O. meinerti Forel	*Kapala* sp. [24]
O. opaciventris Forel	*Kapala* sp. [10, 22]
Pachycondyla Fr. Smith	
P. apicalis (Latreille)	*Kapala* sp. [22, 24], [this work]
P. crassinoda (Latreille)	*K. cuprea* Cameron [21]
P. harpax (Fabr.)	*K. atrata* (Walker) [1]***, *Kapala* sp. [24]
P. stigma (Fabr.)	*K. iridicolor* (Cameron) [10], [22]**, *Kapala* sp. [24], [this work]
P. verenae (Forel)	*Kapala* sp. [this work]
Myrmicinae	
Pogonomyrmex Mayr	
P. badius (Latreille)	*K. floridana* (Ashmead) [12] (doubtful record) [1, 10]

* Referred to as *Kapala sulcifacies*;
** Referred to as *Kapala* sp.;
*** Referred to as *Kapala surgens*.

of medium- to large-size poneromorph ants as potential hosts for *Kapala* wasps, a hypothesis previously formulated on the basis of more limited data [10, 22]. In contrast with other eucharitid genera which have a limited range of potential hosts and a marked specificity towards the host as far as genus is concerned [4–6], the diversity of host genera and species attacked by *Kapala* is impressive. The data reported here for French Guiana extend the number of reliable host species for *Kapala* to 24 (see Table 2), a diversity of host species only comparable to that found for the worldwide distributed orasemine genus *Orasema* Cameron [1, 6]. Such a broad range of potential hosts for *Kapala* is also observed at the species level. For example, *K. iridicolor* (Cameron) (Table 2) parasitizes no less than five different species from three genera and two ant subfamilies [10, 22], and a similar phenomenon is likely to concern also some of the other species reported here as undescribed under the label "*Kapala* sp.". Furthermore, *Kapala* species are known to parasitize ants in very diverse habitats including both highly anthropic modified environments (roadsides, college campus, agroecosystems, pastures) and well-preserved forests [1, 10, 22, 24, 25], [this work]. Combined with the fact that *Ectatomma*, *Gnamptogenys*, *Odontomachus,* and *Pachycondyla* are probably the four most widely distributed

genera among the Neotropical poneromorph ants [27–29], the wide host range of *Kapala* species and their ability to attack ants in diverse habitats could account, at least in part, for the dominant status of this genus among the eucharitine wasps of Central and South America. Moreover, though the sampling of ant nests in general is not really adequate to confidently discuss rates of parasitism, for four species (*E. brunneum*, *G. tortuolosa*, *O. haematodus*, and *P. stigma*), the number of collected nests is significant enough (see [18, 19] for a comparison) to give a good idea of the rates of parasitism at the population level. The rate of parasitism is low to medium (from 14.3% to 27.3%, see Table 1) in all of these four cases, but for certain associations, parasitism rate can reach significant values. Half of the nests were parasitized in the case of the association *Kapala* sp. and *O. hastatus* in French Guiana [this work] (but our sample limitation—only 6 nests—could result, in this specific case, in rates of parasitism relatively far from the natural figures) and up to 63% of the nests for the association between *K. iridicolor*/*K. izapa* (Carmichael) and *E. ruidum* Roger in Mexico [18]. Such high parasitism rates certainly contribute to explain why *Kapala* is one of the eucharitid genera most commonly collected by traps and aerial nets in the Neotropical region [1].

All of the eucharitids are parasitoids of ants, but the exact relationship between these wasps and their host is far from being understood, and the scenario might be more complicated than once thought. For example, from the 17 species already described in the genus *Kapala*, the complete life cycle is known for only one of them, *K. iridicolor* [10]. However, even for this species, the way planidia manage to enter ant nests is unknown. Instead of a direct attachment to a foraging ant worker, some *Kapala* planidia have been recently reported attached to different species of scorpions, suggesting the use of alternative phoretic transport [30]. However, such occasional phoretic attachments to scorpions more likely correspond to random attachment to any nearby living object and likely have nothing to do with getting into the ant nest. They would just give evidence in favour of the high mobility of the planidia. Eucharitids are known to attack five subfamilies of ants, and a correlation between the phylogenetic relationships of the parasitoid and host is suggested by both morphological and molecular evidence ([1], E. Murray and J. M. Heraty unpubl. data). Additional information about ant hosts and within-nest biology as well as about oviposition habits throughout the geographical range of *Kapala* is critical for further resolving the limits and phylogenetic relationships of the species of this genus.

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Research Article

Tree-Dwelling Ants: Contrasting Two Brazilian Cerrado Plant Species without Extrafloral Nectaries

Jonas Maravalhas,[1] Jacques H. C. Delabie,[2] Rafael G. Macedo,[1] and Helena C. Morais[1]

[1] *Departamento de Ecologia, Instituto de Biologia, Universidade de Brasília, 70910-900 Brasília, DF, Brazil*
[2] *Laboratório de Mirmecologia, Convênio UESC/CEPLAC, Centro de Pesquisa do Cacau, CEPLAC, Cx. P. 07, 45600-000 Itabuna, BA, Brazil*

Correspondence should be addressed to Jonas Maravalhas, jmaravalhas@gmail.com

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Ants dominate vegetation stratum, exploiting resources like extrafloral nectaries (EFNs) and insect honeydew. These interactions are frequent in Brazilian cerrado and are well known, but few studies compare ant fauna and explored resources between plant species. We surveyed two cerrado plants without EFNs, *Roupala montana* (found on preserved environments of our study area) and *Solanum lycocarpum* (disturbed ones). Ants were collected and identified, and resources on each plant noted. Ant frequency and richness were higher on *R. montana* (67%; 35 spp) than *S. lycocarpum* (52%; 26), the occurrence of the common ant species varied between them, and similarity was low. Resources were explored mainly by *Camponotus crassus* and consisted of scale insects, aphids, and floral nectaries on *R. montana* and two treehopper species on *S. lycocarpum*. Ants have a high diversity on cerrado plants, exploring liquid and prey-based resources that vary in time and space and affect their presence on plants.

1. Introduction

Foliage-dwelling ants are an important component in tropical environments [1–3], affecting locally the composition and abundance of other insect communities [4–6] and directly or indirectly driving mutualistic and trophic interactions in plant-herbivore-predator/parasite interactions [7–11]. High abundance and richness of ants on this stratum are due to a highly energetic liquid diet, mainly extrafloral nectaries (EFNs) and hemipteran honeydew [12–14]. Ants use a variety of resources from plants and their herbivores and these associations are facultative and vary temporally and spatially [1].

In the cerrado, a savanna-like vegetation in central Brazil, there is a high proportion of plants bearing EFNs, representing up to 31% of the plant individuals surveyed [15, 16] and a rich fauna of ants exploiting them [2, 17–19]. A vast literature about direct and indirect associations of ants and plants in this biome is available [20], but there are few studies comparing ant faunas and their resources between plant species, especially those without EFNs. Results presented by Schoereder et al. [2] indicate that the presence of EFNs does not affect ant species richness within a given tree and there is no particular ant species composition typical of plants with EFNs.

To link the richness and seasonal variation of ants to attractive resources available on a particular plant species, we compared ant assemblages on two species that do not bear EFNs and are common of the cerrado region of central Brazil: *Roupala montana* Aubl. (Proteaceae) and *Solanum lycocarpum* St. Hill. (Solanaceae). In our sampling area, both plants have similar stature and structure, are consumed by myrmecophilous hemipterans [21, 22], and were found in different environments: *R. montana* occurring in native cerrado vegetation, where *S. lycocarpum* is rarely found, being common in altered areas at the borders of roads and agropastoral fields. The ability of *S. lycocarpum* to establish itself in a wider range of environments supposedly leads to bigger ant richness, in contrast to *R. montana*. On the other hand, impoverished areas, where *S. lycocarpum* occurs, can sustain weaker ant diversity, which can affect the ant fauna foraging on this species. We expect that differences

on the area of occurrence and resource availability between these plant species may lead to important differences in the composition of ant species.

2. Material and Methods

2.1. Study Area. This study was conducted in the Fazenda Agua Limpa (15°57′S, 47°54′W), Federal District, Brazil. This 4,500 ha farm belongs to the University of Brasilia and includes mainly undisturbed cerrado vegetation and agro-silvo-pastoral experimental areas. The region has altitudes around 1,050 m a.s.l., average annual temperature of 22°C, and average annual rainfall of 1,417 mm (series from 1980 to 2004, data from RECOR Meteorological Station, http://www.recor.org.br/), and a marked seasonality, with a lengthy dry season ranging from May to September and a wet season from October to April.

2.2. Plant Species. Roupala montana is widely distributed in the Brazilian cerrado [23] and is abundant in cerrado remnants of the Federal District. It is an evergreen shrub that simultaneously sheds leaves and produces new ones, reaching up to three meters height, blooms for a long period during the year, and is pollinated by moths [24–26]. It hosts ant-tended hemipterans like scale insects (Coccoidea), aphids (Aphidoidea), and, especially a leafhopper species, *Rotundicerus* sp. (Cicadellidae and Idiocerinae), which forms large aggregates of nymphs feeding on new leaves at the beginning of the rainy season [21]. A rich fauna of caterpillars, including *Hallonympha paucipuncta* (Spitz, 1930) (Riodinidae) and at least 10 species of Lycaenidae, consumes its leaves and inflorescences [27–29].

Solanum lycocarpum is an evergreen shrub with maximum height of about two meters, being extremely common in disturbed environments [30, 31]. New leaves and flowers grow throughout the year, but flowers do not reward nectar to insects, and pollen is the floral resource collected by bumblebees through buzz pollination behavior [30]. Leaf surfaces are covered with simple glandular and nonglandular trichomes and stellate trichomes [32]. The treehopper *Enchenopa brasiliensis* Strümpel, 2007 (Membracidae) is a common species feeding on apical meristems and inflorescences [22]. In the study area, two species of Membracidae, one of Aetalionidae and an inderterminated number of species of scale insects were tended by ants on new leaves of this plant. One Cicadelidae species was very abundant too on the same plants, but it was not associated with ants. *Solanum lycocarpum* leaves are eaten by several microlepidoptera, especially *Symmetrischema chloroneura* (Meyrick, 1923) (Gelechiidae) [33], its stems are consumed by gall-forming weevil, *Collabismus clitellae* Boheman, 1837 (Curculionidae) [34] and its leaves and fruits are eaten by several species of mammals [31, 35–37]. Attini nests (Formicidae and Myrmicinae) favor the establishment of seedlings and enhance nutrient in the leaves of *S. lycocarpum* [38].

2.3. Samples and Data Analysis. Individuals of both plant species were carefully examined always in the morning period. Every ant observed on the plant was collected and the occurrence of myrmecophilous hemipterans registered. *Roupala montana* plants ($n = 327$) were examined between April and September (dry season) of 2007 in a typical cerrado vegetation area of 2 ha. Previous surveys in the same study area were made on this species during the wet season of 2006, when the focus was to collect the ants tending nymphs of *Rotundicerus* sp. ($n = 116$). *Solanum lycocarpum* plants ($n = 431$) were inspected between March 2007 and March 2008 along dirt roads that cross a mosaic of environments, including typical and "campo sujo" (a physiognomy dominated by herbaceous vegetation) native cerrado areas, pastures and cultures of coffee, sorghum, and pine. The sampling area of *S. lycocarpum* was more widely spread than that of *R. montana.*

The mean similarity of ant species composition between the two plant species was calculated by grouping the samples of each month from April and October ($n = 7$). The similarity indexes and rarefaction curves were generated using EstimateS [39]. The frequency tests were made using BioEstat 5.0 [40].

Table 1: Frequency of occurrence of ants and myrmecophilous hemipterans on *Roupala montana* (Proteaceae) and *Solanum lycocarpum* (Solanaceae), in Fazenda Agua Limpa, Federal District, Brazil. Comparisons made with contingency tables.

Caracteristics	Roupala	Solanum	χ^2	P
Examined plants	327	431		
Plants with ants	218	226	15.517	0.0001
Plants with myrmecophilous hemipterans	139	188	0.094	0.816
Co-occurrence of ants and hemipterans	115	143	0.328	0.620
Plants with hemipterans without ants	24	45	2.161	0.141
Plants with ants without hemipterans	103	83	15.046	0.0001

3. Results

Ants were more frequent on *R. montana* (67%) as compared to *S. lycocarpum* (52%) and this difference was due to higher occurrence of ants on *R. montana* plants without myrmecophilous hemipterans (Table 1). Along the study we collected a total of 45 ant species from 11 different genera. We recorded 35 species on *R. montana* and 26 on *S. lycocarpum*, with estimated richness (first order Jackknife ± standard deviation) of 40 (± 2.6) and 29 (± 2.3), respectively, (Figure 1; Table 2).

The frequency of occurrence of the most common ant species were different on the two plant species (Table 2) and the mean similarity (±sd) of the ant assemblages for the dry season was low (Sorensen Index = 0.419 ± 0.078), especially when the frequency of occurrence of ant species was considered (Morisita-Horn Index = 0.372 ± 0.167).

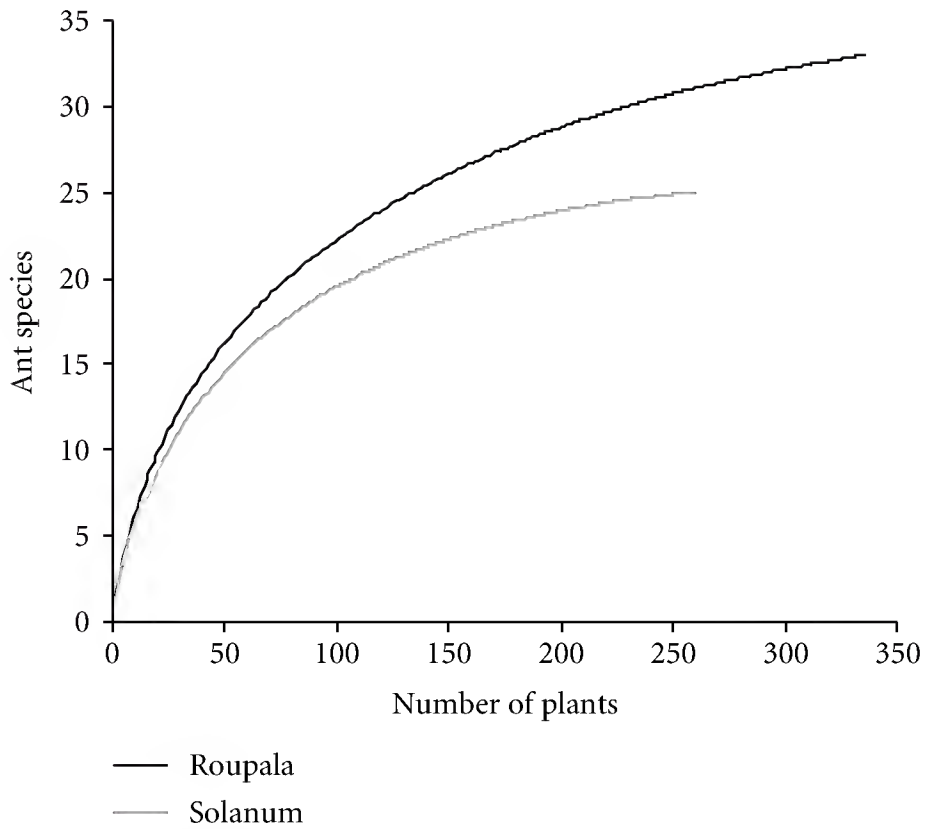

FIGURE 1: Rarefaction curves of ant species collected on *Roupala montana* (Proteaceae) and *Solanum lycocarpum* (Solanaceae), in Fazenda Agua Limpa, Federal District, Brazil.

Scale insects were an abundant resource in the leaves of *R. montana*, being found throughout the sampling period and 21% of ants in this plant were exploring this resource. We found 15 species of ants attending scale insects, and the most frequent were *Cephalotes pusillus* (Klug, 1824) (17 occurrences), *Camponotus crassus* (Mayr, 1862) (8), *Brachymyrmex* sp. (7), *Crematogaster evallans* (Forel, 1907) (6), and *Solenopsis* sp. (5). Some individuals of *R. montana* were flowering between April and July 2007 and developing inflorescences were explored by aphids, associated to four ant species, mainly *Cr. evallans* (4) and *Camponotus* (*Myrmaphaenus*) sp.2 (3). Seven species were recorded exploring mature floral nectaries, mostly *Ce. pusillus* (5) and *Camponotus* (*Myrmaphaenus*) sp.2 (4).

The frequency of occurrence of the commonest ant species was different on *R. montana* with and without *Rotundicerus* sp. A large proportion (67%) of the *R. montana* individuals examined during the dry season had ants, and 68% of the groups of *Rotundicerus* sp. on the same plant species and in the same area where tended by ants during the rainy season of 2006. Although the occurrence of the leafhopper did not enhance the frequency of visits of ants, it altered the composition of the ant fauna. The most notable cases were *Ca. crassus* and *Azteca instabilis* (Smith, 1862), which increased their frequency on the plants when *Rotundicerus* sp. was present, from 4 to 28% and 0 to 10%, respectively. *Cephalotes pusillus* and *Crematogaster stollii* Forel, 1885, on the other hand, decreased their frequency from 19% to 8% and from 7% to 0%, respectively.

Major resources explored by ants in *S. lycocarpum* were two species of Membracidae, each one present in 23% of the examined plants and *Ca. crassus* was the most frequent ant found in association with them. One species of Aetalionidae and scale insects were infrequent on this plant, being seen three and seven times, respectively. We lack nutritional information of glandular trichomes of *S. lycocarpum*, but small ants, like some species belonging to the genera *Brachymyrmex*, *Dorymyrmex*, *Pheidole*, and *Solenopsis*, possibly use this resource. *Pseudomyrmex* spp. were generally present (68%) on plants without myrmecophilous hemipterans.

4. Discussion

We found a high frequency of plants visited by ants, showing the prevalence of ants foraging on cerrado plants, even during the dry season, when *R. montana* plants were examined. The high availability of potential resources for ants [2, 13, 41], especially myrmecophilous hemipterans in the cerrado vegetation [21, 22, 42, 43], makes this stratum attractive to ants. Even with the predominance of liquid diet, it is important to stress that various species of ants forage for preys on plants, including myrmecophilous hemipterans [44]. In an urban area of Campinas (SP), for example, 70% of the diet of *Pseudomyrmex gracilis* (Fabricius, 1804) was based on arthropods, primarily a Psyllidae species (Hemiptera and Sternorryncha) [45]. This can explain the high frequency of *Pseudomyrmex* spp. in the samples, as well as the presence of *Pachycondyla* spp., a genus of predator ants [46]. Besides, some genera (e.g., *Cephalotes* and *Pseudomyrmex*) nest in dried plant branches, and this too can affect the ant assemblage that forage on an individual plant.

Richness of ants was high in both plant species, especially considering that the surveys were conducted only in the morning. Studies including day and night time observations, showed an average of 21 ant species visiting EFNs on six species of cerrado plants, with a minimum of nine on *Qualea multiflora* Mart. (Vochysiaceae) and a maximum of 34 on *Caryocar brasiliense* Camb. (Caryocaraceae) (Appendix 6.1 in [1]). Sequential samplings on *Schefflera vinosa* (Cham. & Schltdl.) Frondin and Fiasch (Araliacae) revealed that *Guayaquila xiphias* (Fabricius, 1803) treehoppers are attended day and night by 21 species of honeydew-gathering ants [42], whereas shrubs of *Solanum lycocarpum* hosting *Enchenopa brasiliensis* treehoppers are regularly visited by 10 ant species [22]. Campos et al. [19], using pitfall traps in a cerrado area of the state of Goias, found 16 species of ants on the shrub stratum (height between 0.5 and 1.5 m) and 28 on the arboreal stratum (dominated by taller, mature trees).

As expected, ant fauna between the two plants species investigated showed small similarity. The different habitats of occurrence of the plants undoubtedly had an effect on this result [47–50], but variation on resource availability might have an important influence on the composition of ant species [41]. So there must be a particular ant species composition typical of plants with these kinds of resources (e.g., big groups of myrmecophilous hemipterans and active EFNs) even though for a limited period of time. Probably the fidelity of dominant ants plays a key role in structuring assemblages of tending ants on this rich food resource. Hence, comparisons on the frequency of occurrence and composition of species of ants on plants with and without these resources need to be done when they are present

Table 2: Ant species and its occurrence on *Roupala montana* (Proteaceae) and *Solanum lycocarpum* (Solanaceae), in Fazenda Agua Limpa, Federal District, Brazil. The most frequent species are highlighted.

Formicidae	Roupala	Solanum
Myrmicinae		
Cephalotes adolphi Emery	2	0
Cephalotes atratus Linnaeus	0	2
Cephalotes betoi De Andrade	3	0
Cephalotes depressus (Klug)	5	10
Cephalotes grandinosus (Smith)	6	0
Cephalotes liepini de Andrade & Baroni Urbani	2	0
***Cephalotes pusillus* (Klug)**	**147**	**18**
Crematogaster distans Mayr	3	6
Crematogaster evallans Forel	11	3
Crematogaster stollii Forel	14	0
Crematogaster victima (Smith)	1	0
Crematogaster sp.	3	0
Nesomyrmex pleuriticus (Wheeler)	1	0
Nesomyrmex tristani Emery	1	0
Pheidole capillata Emery	10	7
Pheidole sp.1 grupo *fallax*	2	1
Solenopsis substituta (Santschi)	0	9
Solenopsis sp.	12	0
Formicinae		
Brachymyrmex sp.	14	9
Camponotus arboreus (Smith)	4	0
Camponotus atriceps (Smith)	2	0
Camponotus blandus (Smith)	1	2
***Camponotus crassus* Mayr**	**64**	**105**
Camponotus fastigatus Roger	0	2
Camponotus melanoticus Emery	1	5
Camponotus novogranadensis Mayr	3	0
***Camponotus rufipes* Fabricius**	**0**	**38**
Camponotus (*Myrmaphaenus*) sp.1	1	3
***Camponotus* (*Myrmaphaenus*) sp.2**	**34**	**2**
Camponotus (*Myrmaphaenus*) sp.3	0	1
Camponotus (*Myrmaphaenus*) sp.4	0	3
Camponotus (*Myrmobrachys*) sp.1	1	0
Camponotus (*Myrmobrachys*) sp.2	2	0
Camponotus (*Tanaemyrmex*) sp.	4	2
Dolichoderinae		
Azteca instabilis (Smith)	19	0
Azteca sp.	14	0
Dorymyrmex sp.1	0	6
Pseudomyrmecinae		
Pseudomyrmex gracilis (Fabricius)	0	14
Pseudomyrmex pupa (Forel)	0	2
Pseudomyrmex termitarius (Smith)	0	1
Pseudomyrmex tenuissimus (Emery)	7	2
Pseudomyrmex rufiventris (Forel)	1	4
Pseudomyrmex sp. gp. *Pallidus*	1	4
Ponerinae		
Pachycondyla inversa (Smith)	1	0
Pachycondyla villosa (Fabricius)	3	0

(e.g., active EFNs) and, preferentially, carefully choosing the species of plants, as phylogeny and genetic distance have a known influence in herbivore communities [51, 52].

Most studies about ant-hemipteran associations do not take into account records of ants on plants during periods of absence of these sap-sucking insects. Nevertheless they feed on young tissues of the host plant [9, 21, 22, 42, 43], so plant phenology has a direct effect on their occurrence and their potential association with ants. *Solanum lycocarpum* continuously produces leaves that are consumed by treehoppers practically throughout the year, while *R. montana* produces leaves roughly around September and November, period when its main sap-sucking insect, the myrmecophilous hemipteran, *Rotundicerus* sp., achieves its development and leaves the host plants. In any case, both plant species are patrolled by ants in the absence of their main hemipteran resources and other apparent ones. Plant phenology and seasonality have an effect on availability of resources exploited by tree-dwelling ants, producing a dynamics of niches occupied by a turnover of ant species. This yearly variation can reveal the strength of mutualistic associations between ants and myrmecophilous hemipterans and plants with or without EFNs, being important to understand the patterns of ant-plant-herbivore interactions.

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Research Article

Removal of Nonmyrmecochorous Seeds by Ants: Role of Ants in Cattle Grasslands

Selene Escobar-Ramírez, Sebastián Duque, Natalia Henao, Alejandra Hurtado-Giraldo, and Inge Armbrecht

Department of Biology, University of Valle, A.A., 25360 Cali, Colombia

Correspondence should be addressed to Selene Escobar-Ramírez, escobar.selene@gmail.com

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Livestock production models prevailing in Colombian Andes are simplified treeless pastures for extensive ranching, with the consequent reduction of environmental services, such as seed dispersal, due to lack of primary dispersers, scarcity of adequate sites for seedling establishment and competition with grasses. This study evaluated if, in these harsh environments, ants can promote the colonization of arboreal species through directed dispersion of seeds towards the nests. Ten seeds of each species were offered to ants in six grazing pastures. Ants removed 25% of the seeds (1827) in 48 hours. Preference for arillated and small-to-medium sized seeds, such as *Pithecellobium dulce*, and *Guazuma ulmifolia*, was observed. *Cyphomyrmex major, Ectatomma ruidum, Solenopsis geminata* and *Atta cephalotes* were the key ant species in seed removal. It was concluded that functional ant groups present in the pastures could contribute to secondary dispersion of seeds with potential for restoration.

1. Introduction

In Latin America, cattle raising is perhaps the productive activity that most contributes to simplification of the landscape. For instance, in Colombia, cattle raising is predominant in more than 80% of the Andean Region, an area inhabited by 70% of the population [1]. The concept of functional diversity relates ecosystemic processes to species diversity through patterns of resource use [2, 3]. Thus, reduction in plant heterogeneity caused by traditional production systems of cattle raising leads to a reduction of both ecosystemic processes and the environmental services they provide [1].

Vegetal regeneration is limited in cattle pastures due to the absence of dispersers, little availability of viable propagules and of adequate sites for germination, and establishment of seedlings [4, 5]. Also, competition for the few nutrients with introduced grasses under rude environmental conditions reduces establishment options for arboreal species.

Ants could play a role in the recovery of disturbed systems potentiating recolonization of plant species in agro ecosystems by removing seeds towards their nests [6, 7]. This interaction could affect the local abundance and distribution of myrmecochorus and nonmyrmecochorous species [8–10]. In spite of the growing information on the interaction between ants and seeds in neotropical forests [11–14], few studies document the process in neotropical agroecosystems (but see [15–17]). Thus, the role of ants as dispersers of nonmyrmecochorus seeds in pastures is unknown.

The use of diaspores by opportunistic ground dwelling ants can affect the biology of seeds and seedlings of plant species dispersed mainly by vertebrates [18]. The ants can rearrange the rain of seeds produced by primary disperser, facilitate their germination [19, 20], and promote the establishment of their seedlings [21, 22]. The seeds introduced into the nests are not found by predators and remain protected from adverse environmental events, such as burning in ecosystems that experience regular fires [8]. Although nonmyrmecochorous plants produce seeds without specialized adaptations for dispersal by ants, the presence of arils or nutritious tissues is not indispensable for transport to occur [16, 23].

Events of seed removal by ants are influenced by both morphological factors of the seeds and morphological and behavioral traits of the ants. The presence of a nutritious

tissue or aril attracts the attention of ants thereby increasing probability of an encounter [24]. Once the ant locates the seed, removal will depend on its weight and the carrying capacity of the ant [25, 26]. In general, large ants possess a greater carrying capacity in their jaws and can remove seeds greater distances [27, 28]. Nevertheless, small ants usually massively recruit workers towards the seeds and then displace them towards the nests [17, 29].

Characterization of ant-seed interaction under pasture conditions is a first step towards understanding the potential of ants as functional agents in the ecological recovery of cattle pasture by the dispersal of seeds of plants useful for ecological restoration and seeds of interest to peasant farmers (i.e., forage). This study predicted that ants associated with grasslands act as functional agents by removing nonmyrmecochorous seeds from trees that are useful for ecological rehabilitation. Specifically, the following questions were addressed. Do ants in open pastures actively remove seeds of nonmyrmecochorous plant species with a potential for the ecological recovery of pastures? Do these ants exhibit a preference for a certain kind of seed? Which ant species are most frequently involved in removal events? In addition, the implications of the observed interactions were discussed.

2. Materials and Methods

2.1. Study Site. Fieldwork was carried out between September and October 2009 in six cattle pastures located in the flat inter-Andean Cauca River Valley area in the Departments of Valle and Cauca (Southwestern Colombia) covering an area of approximately 627 km^2. The farms and geographic locations are as follows: I. Department of Cauca, (1) Limonar (03°08′10.1″N; 76°27′42.2″W), (2) La Josefina (3°5′17.3″N; 76°28′18.5″W), (3) Cachimbalito 3°9′1.00″N; 76°27′46.00″W); II. Department of Valle del Cauca, (4) Sachamate (3°16′27.49″N; 76°33′28.00″W), (5) Lituania (3°20′48.5″N; 76°30′30.6″W), (6) Marañón (3°20′48.30″N; 76°31′23.91″W). Over a century ago, dry tropical forest dominated the region [30], but at present only 2.7% of the original forest remains, the remainder having been replaced with great expanses of sugarcane fields and cattle farms [31]. Average annual temperature is 24°C, and average annual precipitation fluctuates between 1000–15000 mm [30] with peaks in March-May and October-December [32]. During the sampling, the pastures averaged temperatures of 29.4 ± 3.7°C and an average relative humidity of 63.4 ± 11%. Each pasture was open, with an average extension of at least two hectares and an arboreal density inferior to 4%. Dominant vegetation consisted of *Cynodon plectostachyus*, an introduced grass, as well as other African grasses like *Brachiaria decumbens*, accompanied by weeds from Malvaceae and Asteraceae families. Inside the pasture, some isolated trees of *Guazuma ulmifolia*, *Pithecellobium dulce,* and *Albizia saman* are allowed by the farmers to provide shade to the cattle and supplement its feed. During the experiments, the cattle were removed from the lot.

Orthodox seeds from five arboreal species commonly found in pastures were used: (1) *Guazuma ulmifolia* Lam. (Sterculiaceae), seeds with hydrophilic mucilage; (2) *Pithecellobium dulce* (Roxb.) Benth. (Leguminosae-Mimosoideae), arillated seeds; (3) *Senna spectabilis* (DC.) H. S. Irwin & Barneby (Leguminosae-Caesalpinioideae), nonarillated seeds; (4) *Leucaena leucocephala* (Lam.) de Wit. (Leguminosae-Mimosoideae), seeds without arils, and (5) *Albizia saman* (Jacq.) Merr. (Leguminosae-Mimosoideae) which seeds are usually impregnated with a sweet, oily substance (seeds of *A. saman* used here lack of this substances as they become from a certified seed company provider). Seeds used have a potential for the restoration of livestock systems and with exception of *L. leucocephala* represent part of the native vegetation of open areas. These trees also serve as forage for cattle as they consume the foliage and/or fruit, provide shade for the cattle, protect the ground from erosion, and offer new habitats for other animals. Seeds of *Passiflora ligularis* Juss. (Passifloraceae) was employed as a positive control. Its seeds are intermediate in size, and they are neither orthodox nor recalcitrant [33]. Their removal by ants was verified in previous studies in disturbed habitats such as pastures [16, 34] and mining areas undergoing rehabilitation [17].

In order to sample the ant community, a lineal transect of 190 m with 10 sampling units (SUs), separated 20 m from one another, was established. Each SU consisted of a circle of white paper 12 cm in diameter with 5 g of tuna in oil and approximately 0.5 mL of honey. These were left on the ground for a period of 2 hours. The ants attracted to baits were collected in alcohol (96%), identified to morphospecies, and conserved in a reference collection deposited in the Entomology Museum of the Universidad del Valle (Cali, Colombia). The functional guilds (*sensu* Silvestre et al. [35]) to which the collected ants belonged were identified. Hill N1 and N2 Number Series were calculated [36] for establishing the number of abundant and very abundant species, respectively.

Three transects were simultaneously established in each pasture: a transect that allows ants access to the seeds ("Ant Transect"), a transect that excluded the ants ("Exclusion transect"), and a transect with seeds of *P. ligularis* to which the ants had access (*P. ligularis* Transect). Each transect consisted of 20 seed depots located 10 meters apart. A depot consisted of a disk of white paper 12 cm in diameter containing 10 seeds of each of the five species used in the study and covered by netting to exclude vertebrate activity. In the excluded transect, each depot was isolated by encircling it within a PVC arum, 12 cm in diameter and 6 cm high, the upper edge impregnated with an adhesive substance (tangle foot). In order to guarantee independence, transects were 5 to 10 m apart. Seeds were served at 0700 hours, and the number of seeds of each species removed at 2, 4, 8, 24, and 48 hours after serving the depots was registered. A seed removed out of the depot was considered a removal event. The species of ants observed removing seeds were identified. A total of 2400 seeds of each species in the study and 1200 *P. ligularis* seeds were offered, for a total of 13200 seeds offered during the entire experiment. The response variable was the proportion of seeds removed per depot (P_i). For purposes of analysis, the response variable was transformed by the function *arcsine* $\sqrt{P_i}$. To evaluate ant preference for seeds,

a two-factor analysis of variance under a mixed effect model [37] of the following type was carried out:

$$P_{ij}(\text{Removal proportion}) = \mu + \alpha_i(\text{plant species}) + \beta_j(\text{cattle pasture}) + E_{ij}(\text{error}), \quad (1)$$

where types of seeds represent fixed effects and the pastures the random effects. To establish whether the removal events were associated with removal by ants and not other factors, the proportion of seeds removed in Ant Transect and Exclusion Transect were compared. Removal dynamics over time was described for all species. For the two most preferred species, 0, 25, 50, 75, and 100% percentiles were calculated to estimate removal percentages at 2, 4, 8, 24, and 48 hours after offer, respectively.

3. Results

A total of 21 morphospecies distributed in 13 genera and 5 subfamilies for a total of 3471 individuals were collected (Table 1). The most representative genera were *Pheidole* (6 morphospecies and 176 individuals) and *Solenopsis* (four morphospecies and 1315 individuals) (both Myrmicinae). *Crematogaster abstinens* (Myrmicinae), present in six pastures, was the most abundant species with 1774 individuals. The number of ant species found in baits in each pasture fluctuated between 6 and 11. Diversity (H' index between 0.52 and 1.75) and equitability (J' index between 0.29 and 0.51) were also consistently low in all the lots, there being from two to three very abundant species in each pasture (averages values of N1 and N2 estimated as 3 and 2 species, respectively).

The composition of ants attracted to tuna baits was characterized by a predominance of generalist species native to open and degraded habitats. In spite of low richness and equitability, the genera collected represent 8 of the 15 functional guilds described by Silvestre et al. [35] for the Cerrado, Brazil.

Based on ant species composition, the lots do not form agglomerations corresponding to their spatial proximity. In spite of the spacial scale, important variations in ant composition were found (Table 1). But, on a functional level, the pastures contained the same functional guilds.

Ants removed 25% of the seeds, for a total of 1827 seeds removed in 48 hours period. In general, ant preference for seeds with attractive external tissues was found ($F = 17.6$; $P < 0.01$; $gl = 5$). Of the five species of interest to the cattleman, the most preferred was *G. ulmifolia* with 56% removal, followed by *P. dulce* with 25% removal while removal did not surpass 10% for the other species (Table 2).

The presence of ants notably favored the removal of *G ulmifolia* and *P. dulce*. In the absence of ants (Exclusion Transect), the removal of these species was drastically reduced. Actually, in the case of *P. dulce*, removal was null when the ants were excluded. On the other hand, access of ants to the depots (Ant Transect) did not significantly favor the removal of *S. spectabilis* ($F_{1.5} = 4.35$; $P = 0.09$),

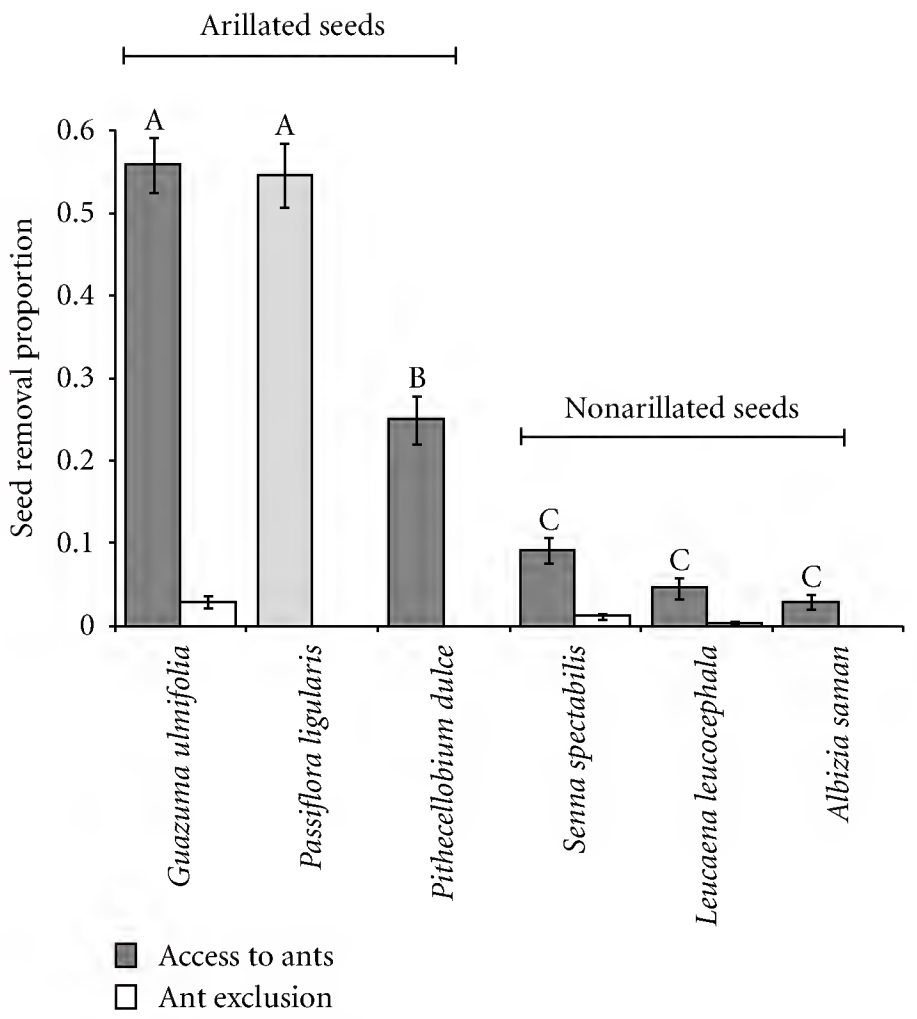

FIGURE 1: Mean (±SE) removal of nonmyrmecochorous seeds by ants in cattle grasslands. Letters above columns show different seed preference groups by ants after a Newman-Kewls test ($\alpha = 0{,}05$).

L. leucocephala ($F_{1.5} = 5.17$; $P = 0.07$), and *A. saman* ($F_{1.5} = 4.37$; $P = 0.09$) (Figure 1). The seeds preferred by the ants were separated into three main groups in the following order of preference: Group A with small *G. ulmifolia* seeds with mucilage, which were preferred as much as the arillated *P. ligularis* seeds in the positive control (*P. ligularis* Transect) ($t = 0.28$; $P = 0.78$). Group B consisted of arillated but heavier *P. dulce* seeds. Group C was made up of smooth covered, nonarillated *S. spectabilis, L. leucocephala,* and *A. saman* seeds (Figure 1).

In general, a greater seed removal rate was observed from 8 to 24 hours after initiating the offer (Figure 2), a pattern maintained in each of the cattle pastures sampled. The removal dynamic overtime of the two most preferred seeds suggests an increase in transporting *G. ulmifolia* and *P. dulce* seeds during the night (Table 3).

Very low percentages of removed seeds of *G. ulmifolia* and *P. dulce* seeds were re-located: only 2% and 24%, respectively. The majority of the *G. ulmifolia* and *P. dulce* seeds were removed an average of 20 cm from the depot, and an important part of the seeds were removed a distance of at least 10 cm. However, the final distance of these seeds could not be established. A *Cyphomyrmex major* worker transported a *G. ulmifolia* seed 1.20 m before entering the nest in the ground while an *Ectatomma ruidum* worker transported a *P. ligularis* seed 4.6 m from the depot.

The ant species that most frequently, and in greatest number, approached the tuna baits were, also, those most frequently involved in seed removal events. The ants observed transporting seeds in grasslands belong to three functional groups: (1) a dominant group of small-sized myrmicines

Table 1: Composition and total abundance of ant species attracted to tuna and honey baits in each locality. (Sa: Sachamate, LJ: La Josefina, Ca: Cachimbalito, Lit: Lituania, Ma: Marañón, Li: Limonar).

Morphospecies	Sa	LJ	Ca	Lit	Ma	Lim	Total abund.
Myrmicinae							
Atta cephalotes						1	1
Cardiocondyla gr. *minutior*	7						7
Crematogaster abstinens	73	644	75	56	822	104	1774
Cyphomyrmex major		4	5			3	12
Pheidole ebenina		22					22
Pheidole susannae		26	85	7	8	4	130
Pheidole sp1	1		1				2
Pheidole sp2				5			5
Pheidole sp3			3			2	5
Pheidole sp5						12	12
Solenopsis geminata	213	206	298	72	160	321	1270
Solenopsis sp1	4			35		1	40
Solenopsis sp2	4						4
Solenopsis sp3	1						1
Wasmannia auropunctata	27	43		10		41	121
Formicinae							
Brachymyrmex sp1	6						6
Camponotus sp1				4			4
Nylanderia sp1				8	3		11
Ectatomminae							
Ectatomma ruidum		11	6	11	1	9	38
Ponerinae							
Hypoponera sp1		1					1
Pseudomyrmecinae							
Pseudomyrmex sp1			2	1	1	1	5

Table 2: Mean seed removal of nonmyrmecochorous seeds by ants in cattle grasslands. Standard errors are shown for seed weights.

Type of seed	Species	Seed weight (g)	*Ant access*		*Ant exclusion*	
			Seeds removed	Removal percentage	Seeds removed	Removal percentage
Arillated	*G. ulmifolia*	0.005 ± 0.00	672	56.0%	35	2.9
	P. dulce	0.307 ± 0.10	300	25.0%	0	0.0
	P. ligularis	0.16 ± 0.02	656	54.7%	N/A*	N/A
Nonarillated	*S. spectabilis*	0.026 ± 0.00	109	9.1%	14	1.2
	L. leucocephala	0.062 ± 0.01	55	4.6%	4	0.3
	A. saman	0.233 ± 0.03	35	2.9%	0	0.0
	Total		1827	25.4%	53	4.4%

* N/A: Not Applicable. Ant exclosures were not performed in *P. ligularis* transect.

Table 3: Percentiles for the total removal percentage of *Pithecellobium dulce* and *Guazuma ulmifolia* nonmyrmecochorous seeds in six cattle pastures in Cauca and Valle.

Percentile	Observation period	Hour of the day	Percentage of seeds removed	
			P. dulce	*G. ulmifolia*
0%	2 hours	0800 hours	2.17	1.08
25%	4 hours	1200 hours	2.92	1.33
50%	8 hours	1600 hours	16.6	1.92
75%	24 hours	0800 hours	44.8	15.7
100%	48 hours	0800 hours	56.3	25.0

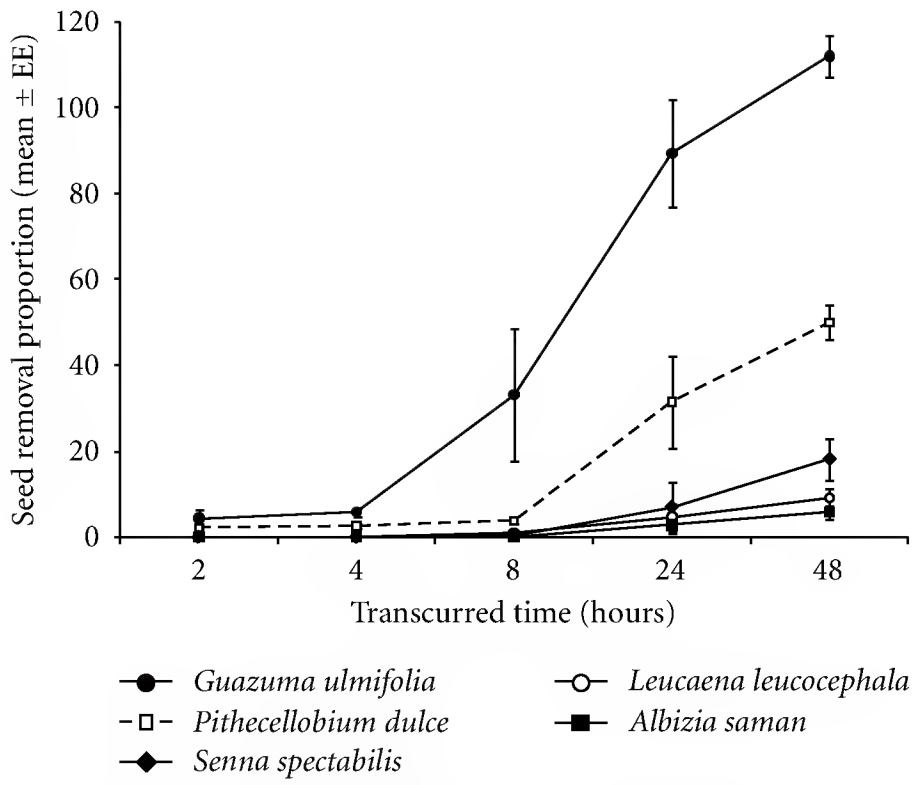

FIGURE 2: Removal of seeds by ants overtime. Average number of seeds removed from depots 2, 4, 8, 24, and 48 hours, after initiating offer, are showed ($n = 6$).

with generalist habits (*Pheidole* spp., *Solenopsis geminata,* and *C. abstinens*); (2) two species of attine, a leaf-cutter (*Atta cephalotes*), and a cryptic fungus grower (*Cyphomyrmex major*) which were the main seed transporters of *P. dulce* and *G. ulmifolia*, respectively; (3) a large epigeal ponerinae (*E. ruidum*), very common and abundant in disturbed lowland habitats (0 to 1500 m.a.s.l.) (Table 4).

Removal activity seems to be occurring throughout the day, with great activity of generalist species during the morning hours such as *E. ruidum* and other small myrmicine, and great activity of attines after 17:00 hours, the activity of *C. major* and *A. cephalotes* increased.

4. Discussion

The ant community associated to cattle pastures in the study area is not very diverse and has a great predominance of only a few species and a relatively predictable composition. The simplification of plant structure in pastures and the surrounding matrix could explain the limited ant diversity [38]. The pattern of diversity found is that expected for the highly fragmented landscape of the biogeographical valley of the Cauca River where pastures and sugar cane fields dominate, and there is only 2% forest cover immersed in a low-quality matrix [39, 40]. The results of this study are consistent with Armbrecht and Ulloa-Chacón [41] who, one decade earlier, found that ant diversity reduced drastically in the pasturelands with respect to forest fragments. In the same area of this study, they found only 21 species in the productive systems, using six capture methods. In spite of limited diversity, generalist ants in pastures rapidly located and displaced the seeds offered in the depots. Twenty-four percent of the seeds were removed for a total of 1827 removal events during a 48-hour period in treeless pastures with cattle activity and compact, ecologically degraded soil. These removal values are similar to those found by Escobar et al. [16] who reported a 26% total removal in open grasslands and silvopastoral systems in Valle del Cauca and Quindío, while Zelikova and Breed [15] reported 38.3.% removal in open pastureland in Costa Rica. Although total seed removal did not exceed 40%, each removal event as such is of biological importance because it potentiates a possibly effective dispersion event. This is particularly critical in highly disturbed habitats rarely visited by primary dispersers.

Results in the Excluded Transect and direct observation indicate that ants are important seed dispersers of trees useful in ecological recovery of degraded ecosystems. Ants have morphological adaptations which allow them to carrying seeds. Also, their social behavior promotes seed transport from the foraging point to their nests, where conditions may be more favorable for the germination and growth of seedlings. Tendency towards territoriality and the stability of their colonies in the tropics makes removal by ants a permanent ecological service throughout the year. These aspects allow to consider ants as key functional agents, facilitating the distribution of seeds of interest in pasturelands.

Ant preference for seeds with an attractive, nutritious tissue in *G. ulmifolia, P. dulce,* and *P. ligularis* was found. This result coincides with the pattern identified for myrmecochorus and nonmyrmecochorous seeds in multiple habitats worldwide [14, 18]. The *G. ulmifolia* species was preferred as much, or more than the *P. ligularis* (positive control) seeds. However, while in the present study, the ants removed 56% of the *G. ulmifolia* seeds, this species was the less preferred in mining areas undergoing rehabilitation [17] in a subxerophytic area where the ants removed only 5% of its seeds. Instead, in the same experiment, ants preferred larger arillated seeds such as *Capparis* sp. (5 mm in diameter) and *Seguieira* sp. (4 mm in diameter). In this experiment, the second most preferred seeds (*P. dulce*) are 60 times heavier than *G. ulmifolia* seeds, the lightest in this study. This suggests that the context is an important factor in modeling the way ant-seed interactions occur: the presence of other arillated species that compete for the "transporting energy" of foraging ants as well as the presence of key ant species.

Removal occurred with a great degree of activity of key species such as *E. ruidum, S. geminata, C. abstinens,* and *Pheidole* spp. during the day while, after 17:00, removal was continued by *C. major* and *A. cephalotes*. The genera observed transporting seeds in the pasturelands (Table 5) coincide with the functional ant guilds exploiting diaspores in flat, sandy forests, and humid tropical forests in the Brazilian lowlands [14, 42]. In these habitats, small myrmicines as well as attine actively interacted with diaspores. Dominguez-Haydar & Armbrecht [17] identified species of these same functional groups removing seeds. *E. ruidum, S. geminate,* and *Acromyrmex octospinosus* were the species that removed the greatest number of seeds from mining lands in early rehabilitation, suggesting that they are key species in the recovery of the ecological function of disturbed areas.

Morphological seed traits, like weight and presence of arils, and foraging strategies in the different ant functional groups are ecologically relevant because they determine how the transport process occur [28]. For example, the foraging of the small-sized myrmicines with generalist habits

Table 4: Description of ant species carrying seeds. Body length of workers and its incidence in baits and seed depots (SDs) were recorded, as the seed species they removed. The body length of workers was measured from the posterior margin of clypeus to the posterior end of the last petiole.

Ant species	Workers body length (mm)	Presence		Seed species removed		
		In baits %	In SD %	*P. dulce*	*G. ulmifolia*	*P. ligularis*
Atta cephalotes	12.36 ± 10.9	1.7	1.7	X		
Ectatomma ruidum	5.52 ± 0.12	36.7	35.0		X	X
Solenopsis geminata	2.12 ± 0.01	41.7	16.7		X	
Cyphomyrmex major	1.85 ± 0.11	6.7	36.7		X	
Pheidole susannae	1.67 ± 0.01	20.0	10.0		X	X
Crematogaster abstinens	1.58 ± 0.21	41.7	40.8	X		X

Table 5: Ant genus classification of ants attracted to baits into the functional groups established by Silvestre et al. [35].

Item	Functional guild (*sensu* Silvestre et al. [35])	Genus attracted to baits
(1)	Omnivorous soil dominants	*Crematogaster, Pheidole, Solenopsis*
(2)	Large epigean predators	*Ectatomma*
(3)	Cryptic fungus growers attines	*Cyphomyrmex*
(4)	Leaf-cutter attines	*Atta*
(5)	Agile pseudomyrmecinae	*Pseudomyrmex*
(6)	Soil and vegetation opportunistics	*Nylanderia*
(7)	Small arboreal ants with massive recruitment	*Wasmannia*
(8)	Cryptic ponerinae, specialized predators	*Hypoponera*

is characterized by mass recruiting of workers towards the food resource [35]. The carrying capacity of these ants is limited by their small size, and those seeds that surpass their carrying capacity are foraged on the ground without being displaced [43]. This was observed in the field with species of the *Pheidole, Solenopsis,* and *Wasmannia* genera (body length less than 3 mm) that foraged the aril of *P. ligularis* and *P. dulce* seeds without removing them. Nevertheless, small myrmicines also team up to transport heavy seeds. In this study, dozens of *C. abstinens* workers succeeded in moving heavy *P. dulce* seeds from the depots, suggesting that this species is an important functional agent for seed dispersal too.

This study also emphasizes on species of generalist ants considered pests such as *S. geminata, E. ruidum,* and attines. The tropical fire ant (*S. geminata*) could be playing a dual role as both predator and seed disperser because, although it is a regular grain collector and eater [44, 45], some seeds could survive and germinate in garbage dumps or soils near the nests [29]. On the other hand, *E. ruidum* has already been identified in Colombia as one of the main seed transporter species in pastures and mining areas under rehabilitation [16, 17, 34]. In Costa Rican agroecosystems, *E. ruidum*, together with *Pheidole fallax*, were responsible for 92% of all observed removal events, *E. ruidum* being the species that removed most seeds in pastures [15]. The greater size of this ant allows a single worker to carry seeds of different sizes thus increasing the range of seeds that can be dispersed by this species.

Finally, attines such as *C. major* and *A. cephalotes* were key transporters of arillated *G. ulmifolia* and *P. dulce* seeds, respectively. They also acted during evening and nocturnal hours (obs. pers.) thus relaxing competition with other generalist species. These results coincide with the pattern of seed attention described by Rico-Gray & Oliveira [18] and Bas et al. [46] where attines are usually attracted by nonmyrmecochorous diaspores with large arils and low lipid content (<8%). According to the authors, if these diasporas are also light in weight (<0.1 g), they can be transported greater distances (to 10 m), something that could be significant for the lighter *G. ulmifolia* seeds. The hypothesis of directed dispersion [47–49] can be reinforced when seed removal involves species of attines because the seeds are directed towards the nest where appropriate conditions for germination can be provided. Under conditions of dehydration, aridness, and high temperatures, *A. cephalotes* can concentrate foraging activity at night, and its workers can obtain foraging distances of up to 235 m. Genera of cryptic attine, such as *Cyphomyrmex,* usually collect seeds during the dry months, and it is when greater foraging distances are reached by its workers [50].

Despite the low removal distances reported for some functional groups, ants can act as complementary dispersing agents [51] bringing up to their nests seeds that have been dispersed long distances (meters, even kilometers) by primary seed dispersers such as cattle, birds, bats, and rodents [52].

In summary, this study presents evidence supporting the idea of ants as functional agents for the ecological recovery of degraded pastures. The preference pattern of the ants for small-sized seeds with attractive structures was confirmed. In spite of the limited diversity of persistent

ants in the pastures, key species were identified that could provide dispersion service for nonmyrmecochorous species in pastures where barriers to the advance of plant succession exist. The interaction of the different foraging strategies of the participating ants can contribute to the dispersion of seeds through different mechanisms; leaf cutters offer the greatest carrying capacity (quantity and size), and the great majority of seeds will be taken to nests thereby contributing to the reduction of s competence and depredation by aggregation. Large hunters such as *E. ruidum* removed seed greater distances and feed on the aril instead of the seed. Ant-seed interaction was facultative and generalist instead of an obligatory mutualism specific to one species in particular. Far from being an inconvenience, this generalism opens the way to the use of grassland ants as dispersers of plant species ecologically important. This would also be of interest to producers if seed hauling of economically valuable seeds by ants could be induced, for example, through the simulation of artificial arils in those seeds (Henao-Gallego et al. [53]). Ant-seed interaction in grasslands can be taken into account for ecological rehabilitation plans by directing this functional diversity. For the rehabilitation of degraded habitats, these facts could mean that, with an abundant although not very diverse ant biota, rehabilitation actions can be initiated, and the plant species chosen will depend on ant preferences and the ecological context of the pasture to be rehabilitated.

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Research Article

Behavioral Differentiation and Ovarian Development of Unmated Gynes, Queens, and Workers of *Ectatomma vizottoi* Almeida 1987 (Formicidae, Ectatomminae)

Alexsandro Santana Vieira,[1] Wedson Desidério Fernandes,[2] and William Fernando Antonialli-Junior[3]

[1] *Programa de Pós-graduação em Entomologia e Conservação da Natureza, Universidade Federal da Grande Dourados. 241, 79804-970 Dourados, MS, Brazil*
[2] *Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados. 241, 79804-970 Dourados, MS, Brazil*
[3] *Laboratório de Ecologia do Centro de Análise Integrado e Monitoramento Ambiental, Universidade Estadual de Mato Grosso do Sul, 351 79804-907 Dourados, MS, Brazil*

Correspondence should be addressed to Alexsandro Santana Vieira, alexsvieira@yahoo.com.br

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Behavioral differentiation and ovarian development of unmated gynes, queens, and workers of *Ectatomma vizottoi* were investigated in laboratory conditions. Forty-one behavioral acts were identified and quantified for workers, 19 for queens and 24 for unmated gynes, for an overall species repertoire of 42 different behavioral acts. Ovipositing reproductive eggs was an exclusive task of the queen, whereas workers showed 15 caste-specific behaviors. The most important (frequent) behaviors for the queens were brood care, immobility, and reproduction, and for workers were immobility, grooming/interaction, brood care, and foraging. Unmated gynes (not winged) primarily showed immobility, brood care, grooming/interaction, and foraging. Analysis of ovarian development showed that unmated gynes had little-developed ovarioles, in contrast to queens. Queens and unmated gynes showed a clear behavioral differentiation, in which queens played the role of reproducers and unmated gynes performed activities belonging to the worker repertoire. Despite the presence of several breeding queens in the colony, functional monogyny was the rule.

1. Introduction

The basic characteristic responsible for the reproductive success of the social insects is the division of labor among the individuals of the colony [1, 2]. This is an important topic for understanding the evolution of social behavior [3, 4]. The behavior of different individuals in a colony of ants is controlled by a sophisticated system of transfer of information, which allows the entire colony to work as a single superorganism [1].

As discussed by Bolton [5] the group of poneromorph ants is very diverse, and the individuals show a mixture of basal and derived morphological and social characters. For example, morphologically and behaviorally, *Amblyopone* Erichson shows basal characteristics, while other genus of the Amblyoponinae subfamily show derived characteristics, for example *Prionopelta amabilis* Borgmeier [6–8]. There is wide variation of behavior among the species of the poneromorph group, ranging from generalist predators that make use of a well-developed sting to dominate their prey, such as the Ponerinae *Pachycondyla harpax* Fabricius and Ectatomminae *Ectatomma ruidum* Roger, to others that are more limited in their feeding habits, such as *Proceratium* Roger, Proceratinae subfamily, which feeds on arthropod eggs, or *Leptogenys* Roger, Ponerinae subfamily, which feeds on isopods. Others, such as *Ectatomma tuberculatum* Olivier, add to their diet sweet substances secreted by hemipterans, secretions from the extrafloral nectary, or fallen fruit pulps [9, 10]. The foraging pattern in this group is termed "social facilitation" [1] and is typical of species of small ant colonies such

as *E. ruidum*, where the workers forage individually (each forager in its own hunting area) [9, 10].

There are two reproductive systems in ants, monogyny and polygyny. Hölldobler and Wilson [6] defined monogyny as the situation in which several queens are present in the colony, but only one is inseminated and able to produce fertile eggs. Polygyny refers to the occurrence of several queens in the same colony, all able to produce fertile eggs. This characteristic is present in many species of ants, and the number of queens can vary among and within the species [4]. As discussed by Brandão [11] there is a clear division of labor between queen and workers in monogynic colonies, in which the workers are responsible for most of the tasks; some examples are *Ectatomma permagnum* Forel [12] and *Ectatomma planidens* Borgmeier [13] (erroneously identified as *Ectatomma edentatum* Roger [14]). The subfamily Ectatomminae also includes several polygynous species, such as *E. ruidum* [15], *Gnamptogenys striatula* Mayr [16], and *E. tuberculatum* [17]. Also, social organization has been studied in some species of *Ectatomma* Smith, including *E. tuberculatum* [18], *E. permagnum* [12], *Ectatomma brunneum* (=*quadridens*) Smith [19], *E. ruidum* [20, 21], *E. planidens* [13], and *Ectatomma opaciventre* (Roger) [22, 23].

The nest of *Ectatomma vizottoi* has ellipsoid entrance and exit openings that lead to a wide tunnel, similar to a hall, which is connected to other chambers deeper in the nest. The nests are up to 360 cm deep and contain three to ten chambers. This species builds more elaborate nests than other species of Ectatomminae, and its colonies can have up to 10 queens [24]. This study investigated whether colonies of this species can also show behavioral and ovarian development that differentiates it from other members of the genus *Ectatomma*.

2. Methods

Three colonies of *E. vizottoi* were collected according to the methodology described by Antonialli-Junior and Giannotti [26], on the campus of UEMS—Universidade Estadual de Mato Grosso do Sul (22°12′S 54°48′W), in August 2006. Behavioral observations were conducted, and the ovarian development of unmated gynes, queens, and workers was also evaluated. The behavioral observations continued from December 2006 until July 2007. The population composition of the colonies is described in Table 1.

2.1. Division of Labor. Ethograms of different species of ants have contributed to the quantification and qualification of the ways in which the tasks are divided among individuals [27, 28]. Drogoul et al. [29] introduced the concept of the Structural Ethomodel (EMF), defining a method to model complex organizations such as social insect societies.

The reproductive and sterile castes were marked individually on the thorax with model-airplane paint, similarly to the method used by Nakata [30]. The colonies were kept in laboratory conditions at a temperature of 26° ± 2°C, relative humidity 55–81%, and a natural light cycle, in artificial nests (28 × 18 cm), made of molded plaster with three chambers and tunnels connecting them in the horizontal plane [13]. Each nest was maintained in a glass box (68 × 38 × 30 cm), which functioned as a foraging arena, and the ants were offered larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and a 1 : 1 honey-water solution as food, in addition to water.

Table 1: Number of adult and immature individuals from three colonies of *Ectatomma vizottoi* collected on the campus of Mato Grosso do Sul State University, in August 2006.

Colonies	Queen	Unmated gyne	Workers	Males	Immature (larvae and pupae)
colony 1	1	1	92	—	7
colony 2	1	2	82	—	55
colony 3	1	4	123	11	222

We carried out 20 hours of qualitative observation, with the help of a hand lens, in sessions of 60 minutes each. We used the method of all occurrences ("*ad libitum*" *sensu* [31]) to define the main categories and behavioral acts of all unmated gynes, queens, and workers in the three colonies.

After this step, we carried out 121 hours of quantitative observation, in 60-minute sessions with intervals of 2 minutes after every 5 minutes, using the scanning sample method [31], totaling 43,270 reports. After the quantification of the behavioral acts, the sample coverage value was calculated, estimated according to Fagen and Goldman [32], calculated by the expression: $\theta = 1 - (N_1/i)$, with "N_1" the number of behavioral acts observed only once and "i" the total number of behavioral acts. The closer θ is to 1.0, the better is the sample coverage. The study can be considered complete when θ ranges between 0.90 and 0.99. The behavioral repertoires of queens, workers, and unmated gynes were compared through Morisita-Horn cluster analysis (multivariate analysis), which is most appropriate for percentage data [33]. The daily 24-hour cycle was divided into three periods (06:00–12:00 h, 12:00–18:00 h, and 18:00–06:00 h) to determine the peak foraging period.

All behavioral acts shown by *E. vizottoi* also occur similarly in other species. These acts have been described in detail for other species, for example *E. planidens* [13] and are therefore not described here.

2.2. Ovarian Development. For the comparative analysis of the ovarian development, 12 workers and 10 queens from the 3 colonies were anesthetized by thermal shock (3 to 5 min. at 4°C) and dissected in a Petri dish in a saline solution for insects, with the aid of a stereomicroscope. The ovaries were removed, and morphological data were obtained, as well as the number of ovarioles in each case, and then schematized.

3. Results

3.1. Ethogram. The sample cover value of the behavioral repertoire was $\theta = 0.98$, showing 42 behavioral acts of which 41 were performed by workers, 19 by queens, and 24 by unmated gynes. The "ovipositing reproductive eggs"

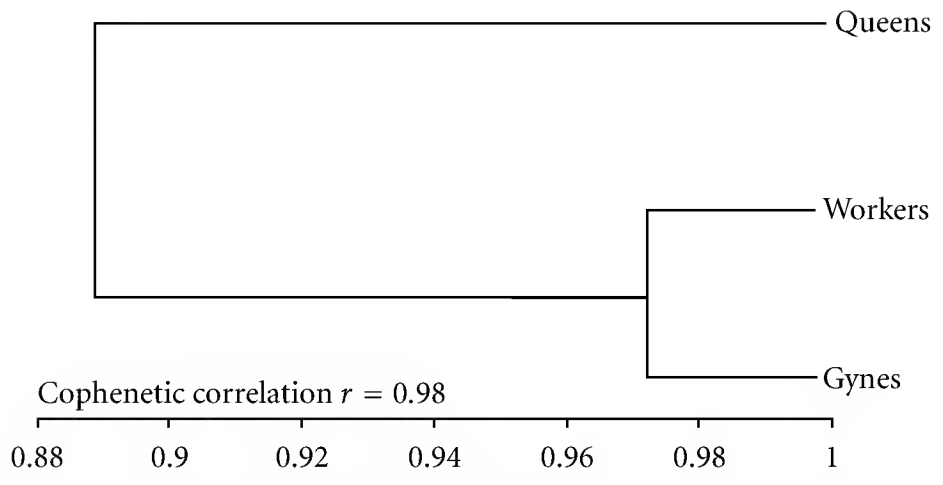

FIGURE 1: Dendrogram of similarity (Morisita-Horn) of the behavioral repertoires among the queens, workers, and unmated gynes of *Ectatomma vizottoi*.

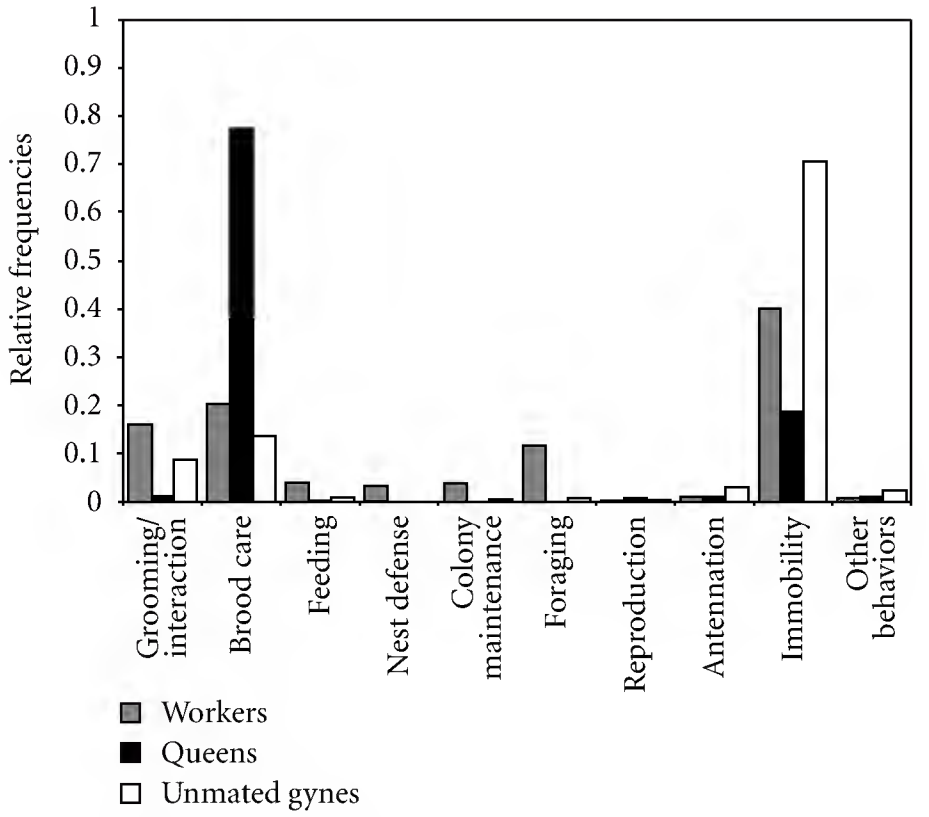

FIGURE 2: Relative frequency with which workers, queens, and gynes performed ten behavioral categories, in colonies of *Ectatomma vizottoi* under laboratory conditions.

was exclusive to only one queen in each colony, while the workers showed 15 exclusive behaviors (Table 2). Cluster analysis revealed that the unmated gynes showed a behavioral repertoire similar to the workers (Figure 1).

The behavioral tasks were grouped to 10 distinct categories. The queens were more effectively involved with the categories "brood care", "immobility", and "reproduction" (Table 2, Figures 1 and 2). Unmated gynes performed mainly tasks in the categories "immobility", "brood care", "grooming/interaction", and "foraging" (Table 2, Figures 1 and 2), while the repertoire of the workers was distributed in all the categories, mainly "immobility", "grooming/interaction", "brood care", and "foraging" (Table 2, Figures 1 and 2).

(A) Grooming/Interaction. In this behavioral category, eight behaviors were observed, of which the most significant were: "self-grooming of the 1st, 2nd, and 3rd pairs of legs", "self-grooming of the gaster extremity", and "*allogrooming* in workers", most frequently performed by the workers; while the task "touch workers with antennas" was performed more frequently by unmated gynes (Table 2, Figure 2).

(B) Brood Care. Fourteen behavioral acts were observed in this category, which includes the tasks that are mostly performed by the queens, in comparison to unmated gynes and workers (Figure 2, Table 2), for example, "standing on or beside the pile of eggs", "standing on or beside the pile of larvae", and "standing on or beside the pile of pupae". On the other hand, the tasks "inspecting larvae", "licking larvae", and "inspecting pupae" were performed more frequently by the workers (Table 2).

(C) Feeding. Seven behavioral acts were included in this category, most of them were performed more frequently by workers and unmated gynes (Figure 2, Table 2). The tasks of "feeding larva with solid food" (pieces of prey), "feeding on solid food", and "worker-larva transfer of food" were notable. "Ovipositing trophic eggs" was performed only by workers and unmated gynes, and these eggs were whitish.

(D) Nest Defense. This category includes only the task of "guarding the entrance of the nest", which was performed by workers and unmated gynes (Figure 1, Table 2).

(E) Colony Maintenance. Four behavioral acts are included in this category, and they were performed only by workers and unmated gynes (Table 2, Figure 1). The queen performed only the task "inspecting the nest" (Table 2).

(F) Foraging. "Foraging" (Table 2, Figure 2) was defined as any activity outside the nest, including the search for any resource or transportation of detritus from the nest to the foraging arena. No forms of recruitment were observed for workers and unmated gynes in this activity. The peak of the "foraging" activity was during the morning, between 06:00 and 12:00. During the other periods the activity was continuous, decreasing gradually.

(G) Reproduction. Only the queens performed the task "ovipositing reproductive eggs" (Table 2, Figure 2).

(H) Antennation. This category involved the acts of antennating and being antennated by workers and queen and unmated gynes from the colony (Table 2, Figure 2).

(I) Immobility. Remaining immobile without performing any other task was the most frequent act among the unmated gynes, followed by workers and queens (Figure 2, Table 2).

(J) Other Behaviors. In this category the acts of "moving inside the nest", carrying workers and queens were observed, and the last three acts were performed only by the workers (Figure 2, Table 2).

3.2. Ovarian Development. The workers possess 1 or 2 ovarioles per ovary (Figure 3, IA–C). Three degrees of

Table 2: Relative frequency of behavioral acts from the behavioral repertoire of workers, queens, and unmated gynes of *Ectatomma vizottoi*.

Categories and behavioral acts	Workers	Queens	Unmated gynes
(A) Grooming/Interation			
(1) Self-grooming; 1st pair of legs	0.05014	0.00672	0.04898
(2) Self-grooming; 2nd pair of legs	0.01732	0.00134	0.01503
(3) Self-grooming; 3rd pair of legs	0.01727	0.00134	0.01503
(4) Self-c grooming; extremity of the gaster	0.00818	0.00134	0.00436
(5) Self-grooming of mandibles	0.00010	—	—
(6) *Allogrooming* in workers	0.04616	—	0.00436
(7) *Allogrooming* in queens	0.01685	—	—
(8) *Allogrooming* in males	0.00297	—	—
(B) Brood Care			
(9) Standing on or beside the pile of eggs	0.01957	0.31720	0.00048
(10) Inspecting eggs	0.00497	0.01613	—
(11) Carrying eggs	0.00049	0.00403	—
(12) Licking eggs	0.00163	0.00269	0.00048
(13) Standing on or beside the pile of larvae	0.04540	0.38575	0.03055
(14) Inspecting larvae	0.01819	0.00538	0.00194
(15) Carrying larvae	0.00121	—	—
(16) Licking larvae	0.03057	0.00134	0.00194
(17) Helping larvae to pupate	0.00816	—	—
(18) Standing on or beside the pile of pupae	0.04537	0.04032	0.08972
(19) Inspecting pupa	0.01792	0.00269	0.00970
(20) Carrying pupa	0.00304	—	—
(21) Licking pupa	0.00529	—	—
(22) Helping individual to emerge	0.00007	—	—
(C) Feeding			
(23) Feeding larvae with solid food	0.01221	—	0.00048
(24) Worker-larva food of transfer	0.00094	—	0.00048
(25) Feeding with solid	0.02054	—	0.00533
(26) Cannibalism of immature	0.00289	0.00134	0.00097
(27) Feeding of detritus	0.00049	—	—
(28) Ovipositing trophic eggs	0.00079	—	0.00048
(D) Nest Defense			
(29) Guarding the entrance to the nest	0.03349	—	—
(E) Colony Maintenance			
(30) Carrying material in the nest	0.00175	—	—
(31) Carrying detritus	0.00796	—	—
(32) Licking walls of the chambers	0.00042	—	0.00048
(33) Inspecting the nest	0.02568	0.00134	0.00630
(F) Foraging			
(34) Foraging	0.11702	—	0.00533
(G) Reproduction			
(35) Ovipositing reproductive eggs	—	0.00672	—
(H) Antennation			
(36) Touch workers with antennas	0.00786	0.00941	0.02861
(37) Touch queen with antennas	0.00079	—	0.00048

Table 2: Continued.

Categories and behavioral acts	Workers	Queens	Unmated gynes
(I) Immobility			
(38) Immobility	0.39858	0.18817	0.70514
(J) Other Behaviors			
(39) Moving inside the nest	0.00472	0.00806	0.02328
(40) Carrying workers	0.00133	—	—
(41) Carrying males	0.00010	—	—
(42) Carrying queens	0.00002	—	—

Table 3: Number of acts from behavioral repertoires in different species of poneromorph ants (W = worker; Q = queen; G = gyne).

Species	Subfamily	Number of behavioral acts	References
Ectatomma brunneum	Ectatomminae	?	Overal [19]
Ectatomma tuberculatum	Ectatomminae	?	Lachaud and Fresneau [18]
Ectatomma permagnun	Ectatomminae	40?	Paiva and Brandão [12]
Ectatomma ruidum	Ectatomminae	?	Corbara et al. [20]
Ectatomma opaciventre	Ectatomminae	27 W; 7 Q	Pie [22]
Ectatomma planidens	Ectatomminae	42 W; 28 Q	Antonialli-Junior et al. [13]
Ectatomma opaciventre	Ectatomminae	47 W; 12 Q	Miguel and Del-Claro [23]
Ectatomma vizottoi	Ectatomminae	42 W; 19 Q; 24 G	This work
Gnamptogenys horni	Ectatomminae	31?	Pratt [25]

ovarian development were found (Figure 3, IA–C): 67% of the analyzed workers had two filamentous ovarioles, without oocytes (Figure 3, IA); 25% had more defined ovaries, with oocytes in the initial stage (Figure 3, IB); 8% had two ovarioles, with more developed oocytes in comparison to the previous case (Figure 3, IC).

The results showed that only one queen of each colony was inseminated (Figure 3, IIA), and therefore the other queens were confirmed as unmated gynes. All queens and unmated gynes possess 7 and 5 ovarioles in the left and right ovaries, respectively, totaling 12 ovarioles (Figure 3, IIA–D). The queens of all colonies had developed ovaries, with mature oocytes, a corpus luteum and spermatheca filled with sperm (Figure 3, IIA).

For unmated gynes, three different degrees of ovarian development were found. In the first type, most of the ovarioles were little developed, each with one large and several smaller oocytes, with the presence of a corpus luteum and empty spermatheca (not sperm) (Figure 3, IIB). In the second type, there are two developed ovarioles, each with one large and another smaller oocyte, with a corpus luteum and empty spermatheca (Figure 3, IIC). In the third type, all 12 ovarioles were filamentous, with a few small oocytes and empty spermatheca (Figure 3, IID).

4. Discussion

The sample coverage value shows that the behavioral repertoire can be considered complete, according to Fagen and Goldman [32]. The number of behavioral tasks was very close to those described for other species of Ectatomminae ants (Table 3).

The tasks related to "grooming/interaction" (Table 2) were also described for *E. planidens* [13], *E. permagnum* [12], and *E. opaciventre* [22, 23]. *Allogrooming* is defined by Hölldobler and Wilson [6] as the act of grooming the body of another individual. One of the functions of grooming the body is to impregnate the cuticle of the nestmate with the odor of the colony, which is related to the content of cuticular hydrocarbons or surface pheromones present in the exoskeleton [6]. Studies performed in *E. vizottoi* have shown that the cuticular hydrocarbons can be involved in the process of recognizing nestmates and distinguishing the castes within the colony [34]. Also, the grooming of the body is a way to inhibit the proliferation of microorganisms; studies such as that by Vieira et al. [35] have revealed that derived ants (*Atta laevigata*) produce antibiotic secretion by metapleural glands.

Queens of *E. vizottoi* remain most of the time standing near the pile of immatures, while unmated gynes and workers spend most of the time performing other tasks. In fact, activities involving "brood care" were significant in the repertoire of the queens (Table 2) as previously described for *E. planidens* [13] and *E. opaciventre* [23]. Still, unmated gynes in *E. vizottoi* performed this activity least often.

According to Hora et al. [36] queens of *E. tuberculatum* spend more time protecting the eggs, because they invest a large amount of energy in their production. However, in other members of Ectatomminae, such as *E. permagnum* [12], only workers were seen protecting the eggs. In colonies of *E. permagnum* [12] and *Gnamptogenys horni* Santschi [25] workers were never seen protecting pupae. In addition, in *E. planidens* [13] and *E. opaciventre* [23] workers were seen cannibalizing immatures.

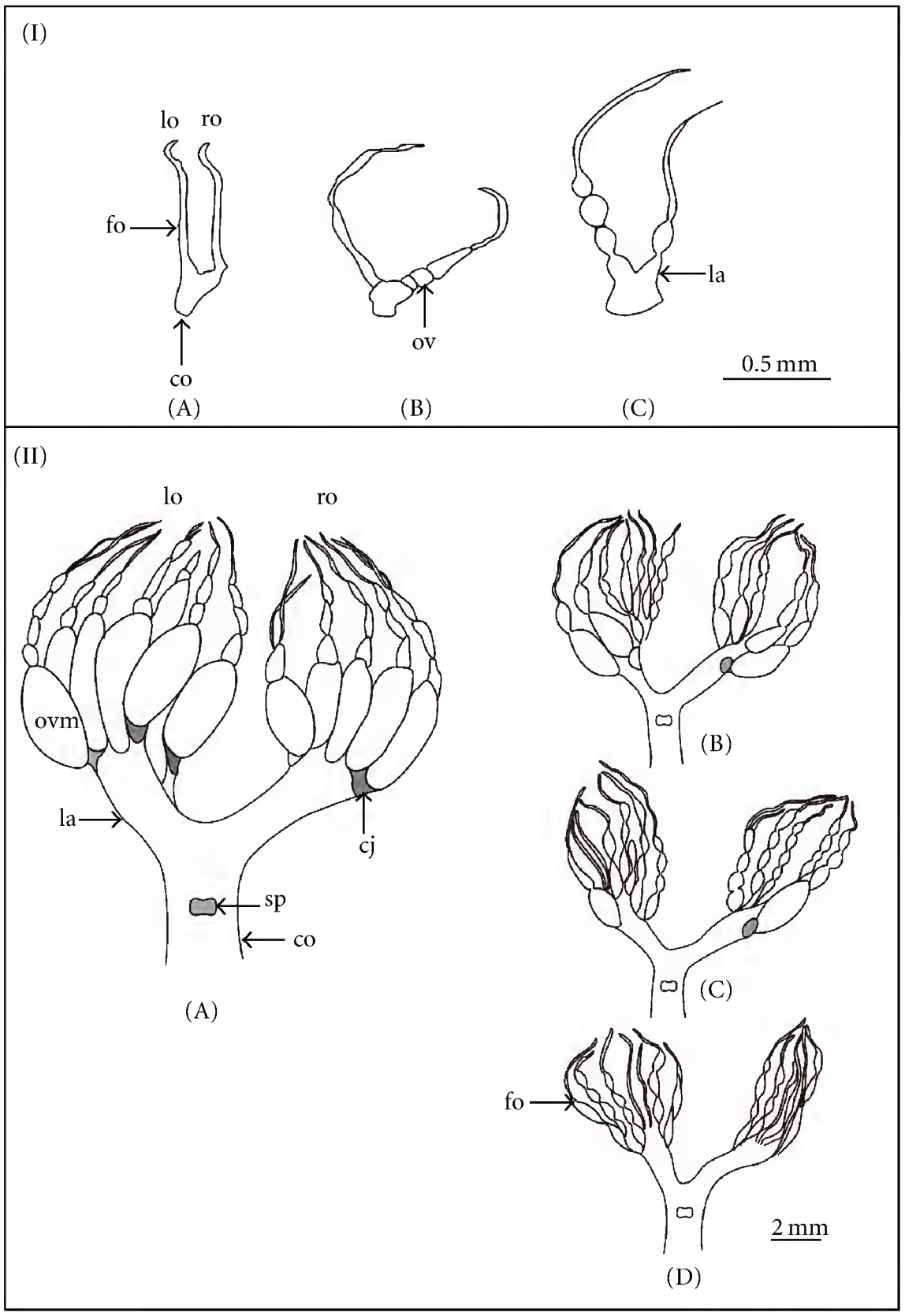

Figure 3: Morphological condition of the different degrees of ovarian development in workers, queens, and unmated gynes of *Ectatomma vizottoi*. (I) three types of ovarian development in workers (A–C), lo: left ovariole, la: lateral ovariole, co: common oviduct, ro: right ovariole, fo: filamentous ovariole, ov: oocyte. (II) A: ovaries of queens. (B–D): three degrees of ovarian development of unmated gynes. Ovm: mature oocyte, cj: corpus luteum, sp: spermatheca.

The task "worker-larva food transfer" was performed by both unmated gynes and workers (Table 2). However, in colonies of *E. opaciventre* [22, 23], *E. tuberculatum* [36] only workers fed the larvae. According to Wilson [1], ants from the subfamilies Myrmicinae, Aneuretinae, Dolichoderinae, and Formicinae exchange regurgitated food, and their intestine is modified to store and distribute food for nestmates, a process called trophallaxis. In poneromorph ants such as *E. tuberculatum* [37] this behavior is defined as pseudo-regurgitation, in which an individual transfers previously collected liquid food to the open jaw of a nestmate. Feeding larvae with solid items without trophallaxis is a "primitive" characteristic [38, 39], as is pseudo-trophallaxis, which is present in the poneromorph group [36].

Both workers and unmated gynes were involved in the activity "foraging", differently from *E. planidens* [13] and *E. opaciventre* [22, 23], in which only the workers performed this task. However, Hora et al. [17] also observed unmated gynes of *E. tuberculatum* foraging.

During the foraging activity by the workers of this species, no recruitment of nestmates was observed, in contrast to *E. brunneum* [19] and *G. horni* [25]. The peak of foraging occurred during the morning. However, in *E. planidens* [13] and *E. tuberculatum* [40] this activity is more intense during the night.

Workers, unmated gynes, and queens of *E. vizottoi* performed the activity "inspecting the nest" (Table 2). In the colonies of *E. planidens* [13] and *E. opaciventre* [22, 23], this activity is performed only by workers.

Although there was more than one individual with reproductive potential present, "ovipositing reproductive eggs" was performed by only one inseminated queen. The appearance of these eggs was similar to those described by Hora et al. [36] for *E. tuberculatum*, in which they change color from white to dark some hours after oviposition. Eggs of *E. planidens* [13] and *E. opaciventre* [22, 23] show a yellowish color. On the other hand, the trophic eggs, such as those of *E. planidens* [13], *E. opaciventre* [22, 23], and *E. tuberculatum* [36] are whitish. In ants, there is an inverse relationship between the existence of either trophallaxis or trophic eggs as a means of food exchange within the society [1]. Trophic eggs are efficient to furnish proteins that are necessary for vitellogenesis for reproductive eggs, and they also enable colony members to store and distribute nutrients and to survive seasonal food shortages [41, 42]. Trophic eggs produced by workers are common in many ants [6]. Although trophallaxis is absent in the poneromorphs, trophic eggs are known in five queens of the poneromorph group [8, 43].

The most frequent activity among the workers, queens, and unmated gynes (Table 2) was "immobility" as described for *E. planidens* [13]. According to Hölldobler and Wilson [6], all poecilotherms spend most of the time doing nothing in particular, except for carrying out physiological functions.

The same number of ovarioles (1-2) in the workers of *E. vizottoi* also occurs in the ovaries of workers of *E. brunneum* [44] and *E. planidens* [13]. The three degrees of ovarian development and the presence of developed oocytes (Figure 3, IA, B, and C) in *E. vizottoi* also occur in workers of *E. brunneum* [44]. However, in workers of *E. planidens* [13] only filamentous ovarioles were found, with no trace of developed oocytes. Probably the workers with a greater degree of ovarian development are those that show trophic oviposition, as observed in workers of *E. brunneum* [44]. The presence of oocytes in the reabsorption stage may indicate previous production of reproductive or trophic eggs by workers [45]. However, workers of *E. vizottoi* possess atrophied spermatheca (Figure 3), similarly to *E. brunneum* [44] and *E. planidens* [13]. Therefore, in *Gnamptogenys menadensis* Mayr [46] virgin workers may lay trophic eggs, and only mated workers produce reproductive eggs.

Queens and unmated gynes of *E. vizottoi* contained 12 ovarioles (Figure 3 IIA, B, C, and D), while queens of *E. planidens* [13] contained 4 and queens of *E. brunneum* [44] 7 to 8 ovarioles. The ovaries of the queens contain mature oocytes and sperm-filled spermatheca, in contrast to unmated gynes which do not contain sperm and also have little-developed ovarioles. This corroborates the behavioral observations, in which it was possible to identify only one queen laying reproductive eggs. Unmated gynes have never been observed laying fertilized eggs and performing activities related to "colony maintenance", and therefore show a repertoire which is more similar to the workers' than to the queen's. However, even if there are several reproductive castes in a colony, a functional monogyny occurs, in which only one queen lays fertilized eggs [17].

The presence of several queens may be the result of adoption, as described for *E. permagnum* [12], *E. ruidum* [15], and *E. tuberculatum*, which, in this case, show facultative polygyny [17]. However, queens and unmated gynes showed a clear behavioral differentiation, in which queens played the role of reproducers and unmated gynes performed activities belonging to the worker repertoire. Analysis of ovarian development showed that unmated gynes had little-developed ovarioles, in contrast to queens. Despite the presence of several breeding queens in the colony, functional monogyny was the rule.

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Research Article

Effects of the Heterogeneity of the Landscape and the Abundance of *Wasmannia auropunctata* on Ground Ant Assemblages in a Colombian Tropical Dry Forest

Rafael Achury,[1] Patricia Chacón de Ulloa,[1] and Ángela Arcila[2]

[1] *Grupo de Investigación en Biología, Ecología y Manejo de Hormigas, Departamento de Biología, Universidad del Valle, Calle 13 No. 100-00, AA 25360 Cali, Colombia*
[2] *Corporación Colombiana de Investigación Agropecuaria (Corpoica), E.E Caribia, Km 6 vía Sevilla, Zona Bananera, Magdalena, Colombia*

Correspondence should be addressed to Rafael Achury, rafaelachury@gmail.com

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To evaluate the response of the ant assemblages to different management practices in the tropical dry forests of southwestern Colombia, 10 sites that conserve forest fragments surrounded by pastures and sugarcane crops were sampled. Tuna-fish baits placed on the ground in the three habitats captured 100 ant species (41 genera). The greatest number of species was found in the forests in contrast with a significant loss of richness and diversity in the productive habitats, the pastures being richer than the cane fields. Species richness was negatively correlated with the abundance of the little fire ant *Wasmannia auropunctata*. Ant species composition was related to soil temperature and percent ground cover, as well as being partially determined by location and the abundance of *W. auropunctata*. The forests had a significantly different species composition from the other two habitats, but there were no consistent differences between the pastures and the cane fields.

1. Introduction

The commonest consequence of converting tropical forests on a large scale is a mosaic of fragments of relictual vegetation surrounded by productive habitats such as pastures for cattle, sugarcane crops, and oil palm plantations [1, 2]. Modifications of the natural habitat generally result in loss of species and changes in the composition and diversity of different animal groups [3–6] including insects [7–9]. To understand the effects of transforming the natural habitat, it is important to examine how the richness and composition of species change in areas that contain different-sized fragments as part of one large disturbed matrix [10].

The tropical dry forest is one of the most transformed and threatened ecosystems in the world [11, 12]. Of the 519,597 km^2 of remnants of this forest distributed throughout the Americas, half the area (51%) is found in South America [13], where conversion to agriculture represents a substantial risk to the highly fragmented patches of dry forest [14]. Of the 30,713 km^2 of dry forest existing in Colombia today, 1977 km^2 [13], are found in the geographic valley of the Cauca River, spread out in more than 1600 fragments with an average size of 6 ha [15]. This situation is the consequence of the expansion of the sugarcane monocrop and pastures [16].

Given the urgency of determining the biodiversity in the relictual forests of the Cauca River Valley, studies have been conducted on ants and Staphylinidae (Coleoptera) [17], highlighting the need for obtaining information about the structure and dynamics of the biotic communities in these forest remnants and their adjacent matrixes.

Ants have a large number of attributes, offering many possibilities for monitoring, inventorying, and basic ecology [18, 19]. In addition to their high diversity and dominance in terms of biomass and numbers, particularly in tropical forests [20, 21], the ground-foraging ant assemblage is especially sensitive to impacts due to transformation of the habitat [22], because of this, they have been widely

used as indicator species [23]. Many studies report that such disturbances promote changes in the structure and composition of the ant assemblages [24, 25], loss of species richness [26], reduced density of nests within the fragments [27, 28], and a high number of tramp species in fragments as compared with continuous forests [29].

In the dry forests of southwestern Colombia, where some 200 ant species have been recorded [17], the forest fragments conserve greater species richness than their adjacent matrixes [30]. Moreover, the richest forests have a greater number of rare species [31], and the richness and relative frequency of some groups (e.g., legionary or army ants) are correlated positively with the area [32]. Although the actual composition of ant species varies significantly among sampling sites [33], the little fire ant (*Wasmannia auropunctata* R.) is numerically dominant; thus, it has been proposed as an indicator of low diversity in the dry forest [34].

This study compared the richness and composition of the ground ant community at 10 sites in the three different habitats: forest fragments, pastures for cattle-raising, and sugarcane crops. The objective was to determine the effect of the different sites and habitats on the richness and composition of ant species, taking into account a gradient of disturbance and environmental and biotic variables. In addition, the study aimed to determine whether the changes in species richness and composition were conditioned by the presence of dominant ants. Finally, based on the results, a broader view for conserving forest fragments and using sustainable management practices for the associated habitats can now be provided.

2. Materials and Methods

2.1. Study Area. Located in southwestern Colombia, the area corresponds to the inter-Andean floodplain formed by the upper watershed of the Cauca River, with an area 230 km long by 10–20 km wide and at an altitude of 900–1100 m. The climate is typical of a tropical dry forest, with an average annual temperature of 24°C and 1000–2000 mm of rainfall, distributed in two periods (April-May and October-November), during which 70% of the total annual rainfall occurs [35].

Ten sites were selected, distributed from South to North, covering three states in southwestern Colombia (Table 1). Each site comprises a forest fragment whose arboreal vegetation reaches a canopy of 30 m, with prominent species of wild cashew (*Anacardium excelsum*), *Xylopia ligustrifolia*, *Laetia americana*, cow or wild fig (*Ficus glabrata*), *Cecropia* sp., and kapok (*Ceiba petandra*) [36], frequently mixed with clumps of *Guadua angustifolia* (Tribe Bambuseae). The forest fragments differ in shape, area, and type of matrix [37], in which pastures for cattle raising (36% of the area) and intensive sugarcane production (52%) predominate [15].

2.2. Sampling of Ants. Sampling was performed during the rainy season, from October to December, 2005 and February to May, 2006. Three habitats were identified at each site: forest, sugarcane fields, and pastures. All the pastures and cane fields surveyed were adjacent to the forest. Linear transects were marked off at random in each habitat, and 40 sampling stations were placed every 20 m to guarantee independence of the samples based on the ants' foraging distances [38]. All transects were placed parallel to the forest edges at a minimum distance of 50 m. At each sampling station, coordinates were taken (GPS Garmín 12XL); and four variables were measured: soil moisture, soil pH (Kelway meter), soil temperature (Weksler thermometer), and canopy cover (spherical concave densitometer, Forestry Suppliers, Inc.).

The ants were collected using tuna-fish baits, one per sampling station. This method is useful for estimating the composition and richness of the ant fauna that forage actively on the ground [39] and has been widely used in dry forests [30, 31, 34]. The baits, previously evaluated by Achury et al. [33], consisted of a piece (4 × 4 cm) of white bond paper, on which was placed an average of 9 g (±1.7) of tuna fish conserved in oil. They were then put on the ground and left there for 3 hours. The ants that were found directly on the tuna fish were collected, and the rest of the bait, including part of the soil beneath, was also picked. This was done because on underside of the paper, there were often very small or less aggressive ant species.

The samples were cleaned in the laboratory and conserved in ethanol at 80%. Their identification was done to the genus level according to Palacio and Fernández [40] and Bolton [41]. At the species level, Longino's key [42] was used, as well as to comparing ants with specimens from the Museum of Entomology at the Universidad del Valle (MEUV) and the Museum of Zoology at the Universidade de São Paulo (MZUSP). The reference collection was deposited in the MEUV.

2.3. Data Analyses. For each sample (1 tuna-fish bait), the number of morphospecies of ants that were attracted to the bait and their respective abundances were counted. A descriptive analysis was done of the percent occupation of the baits by the different subfamilies, genera, and certain species.

To determine the differences with respect to ant richness and composition, two scales were analyzed: sites (total 10) and habitats (total 3, represented by forests, cane fields, and pastures). Correlations were sought between species richness per site versus three variables: latitude, total number of captures, and abundance of the commonest species (*W. auropunctata*). A correlation was also run between latitude and abundance of *W. auropunctata*. The richness per habitat was compared using rarefaction curves based on samples [43, 44], using the Estimates S program v. 8.2 [45]. Data on species density were used because they provide a better indicator of the differences in structure within the habitats [24].

The three habitats were compared with respect to four variables: abundance of ants per bait (natural logarithm), species richness per bait, the Shannon diversity index, and the numeric dominance index (ratio between number of workers of the most abundant species and total abundance of all species in the habitat). A one-way analysis of variance (the Kruskal-Wallis test) was applied, and multiple comparisons

Table 1: Description of the location and characteristics of the sites within fragments of tropical dry forest in the upper watershed of the Cauca River; sites ordered geographically from North to South.

Site	Adjacent matrix	Municipality and state	Coordinates	Altitude (masl)	Area (ha)
Miralindo I	Pastures	La Virginia, Risaralda	4°54′19.89″N 75°51′30.5″W	900	6.7
Aguas Claras	Pastures and sugarcane	Pereira, Risaralda	4°53′23.1″N 75°55′56.6″W	940	13.0
Alejandría	Pastures	La Virginia, Risaralda	4°49′58.6″N 75°53′2.4″W	900–940	15.3
Las Pilas	Pastures	Zarzal, Valle	4°26′25.7″N 75°59′23.1″W	1000	12.4
El Medio	Sugarcane	Zarzal, Valle	4°20′13.8″N 76°5′0.1″W	950	13.1
Las Chatas	Pastures and sugarcane	Buga, Valle	3°51′20.8″N 76°20′5.35″W	950	10.8
El Vínculo	Pastures	Buga, Valle	3°50′2.38″N 76°17′19.7″W	980–1150	15
El Hatico	Pastures and sugarcane	El Cerrito, Valle	3°38′34.48″N 76°19′40.52″W	980	12.6
Colindres	Pastures and sugarcane	Jamundí, Valle	3°16′25.8″N 76°29′31″W	975	10.0
San Julián	Pastures and sugarcane	Santander de Quilichao, Cauca	3°06′38.8″N 76°31′41.2″W	950	3.5

were made using the Tukey test and Bonferroni correction [46].

Species composition was described using the data on frequency of capture for all the species, and the similarity among the sampled sites was determined by means of hierarchical cluster analysis [47]. Then, the PC-ORD program v. 4 [48] was used to compare the groups formed, using the nonparametric multivariate technique MRPP (Multiresponse permutation procedure), which tests the null hypothesis of there being no difference between two or more groups of entities, based on previously defined groups [47, 49]. This technique was also used to compare the ant assemblages among habitats.

To examine whether the environmental variables (soil moisture, pH, temperature, and percent ground cover) and biotic variables (species richness and abundance of *W. auropunctata*) are related to the ant assemblage structure (frequency of capture per habitat), a canonical correspondence analysis (CCA) [48] was performed, followed by the Monte Carlo significance test (999 permutations). These analyses were done with the PC-ORD program v. 4.

3. Results

3.1. General Data on Ants Collected. Of the 1062 baits placed, 93.03% scored positive for ants, with an average per site of 92.83% (± 6.41) (Table 2). A total of 194,347 ants were attracted to the tuna-fish baits, classified into 100 species, 41 genera and 8 subfamilies (see the appendix). Myrmicinae was the subfamily with the most genera and species (24 and 66, resp.), followed by Dolichoderinae (5 genera, 7 species), Formicinae (4, 16), Ponerinae (2, 4), Ecitoninae (2, 2) Ectatomminae (2, 2), Pseudomyrmecinae (1, 2), and Heteroponerinae (1, 1). The richest genera were *Pheidole* (19 species), *Crematogaster* (9), *Solenopsis* (8), and *Camponotus* (4). The dominant species in the study were *W. auropunctata* (130,757 workers) and *Solenopsis geminata* (29,565), together representing 82.5% of the captures.

3.2. Richness and Abundance. The average number of ant species per site was 30.4 (minimum 17, maximum 46), with higher values toward the North of the study area (Table 2). Except for two sites (Colindres and San Julián), where the pastures or cane fields, respectively, had the greatest species richness, more species were recorded in the forest habitat (Figure 1). The rarefaction curves showed that the forests had the greatest richness compared with the pastures and cane fields ($F_{2,12} = 789.34$; $P < 0.001$), which had the lowest number of species (Figure 2).

A highly significant positive correlation was found between richness and latitude ($r = 0.8453$; df = 8; $P = 0.002$). In contrast, a highly significant negative correlation was found between abundance of *W. auropunctata* and latitude ($r = -0.9581$; df = 7; $P < 0.001$). Species richness per site varied inversely with the total captures of *W. auropunctata* ($r = -0.8062$; df = 7; $P = 0.008$) and its respective abundance ($r = -0.8569$; df = 7; $P = 0.003$).

Significant differences were found among the habitats for the variables ant abundance per bait ($F_{2,985} = 12.31$; $P < 0.0001$) and species richness per bait ($H = 25.86$; df = 2, $n = 988$; $P < 0.001$), as well as for the Shannon diversity index ($F_{2,22} = 21.13$; $P < 0.001$). Abundance was greater in the cane fields and the forest than in the pastures (Tukey: $P < 0.001$) (Figure 3(a)), while richness was greater

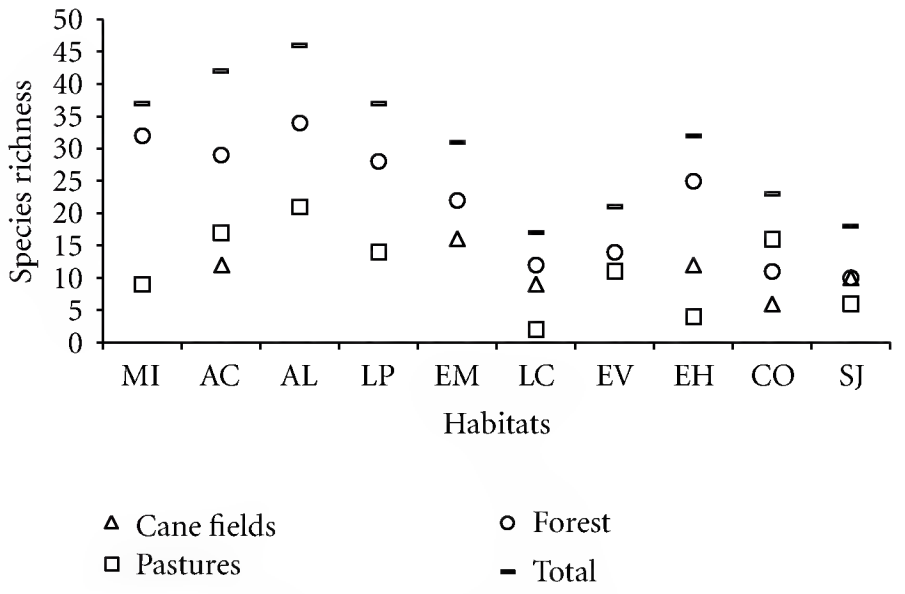

Figure 1: Richness of ant species broken down by habitat at 10 sites ordered geographically from North to South. MI (Miralindo), AC (Aguas Claras), AL (Alejandría), LP (Las Pilas), EM (El Medio), LC (Las Chatas), EV (El Vínculo), EH (El Hatico), CO (Colindres), and SJ (San Julián).

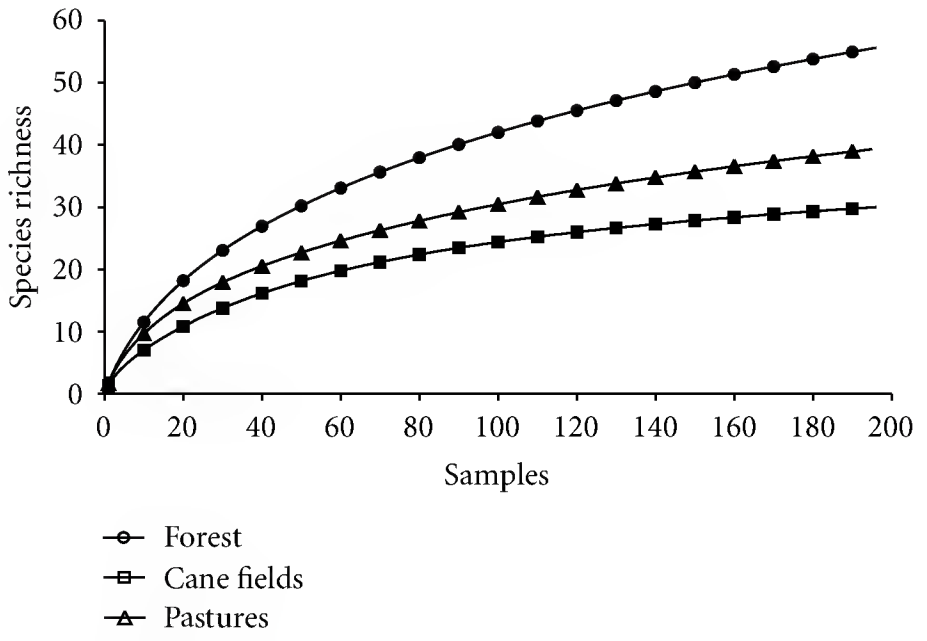

Figure 2: Rarefaction curves, based on the Coleman method, for species of ants captured at tuna-fish baits in three habitats within the tropical dry forest region. For purposes of clarity, the confidence intervals (95%) and the SD are omitted in the figure.

in the pastures and forest (Bonferroni correction: $P < 0.001$) (Figure 3(b)). The diversity index was significantly greater in the forest (Tukey: $P < 0.001$) (Figure 3(c)). No significant differences were found for the dominance index ($F_{2,19} = 0.13$; $P = 0.87$) given that in the three habitats it was higher than 60% (Figure 3(d)). *Wasmannia auropunctata* ant was dominant in the forest and cane fields, whereas in the pastures its index was low (36%), the dominant species being *S. geminata*.

3.3. Species Composition. Based on the dendrogram, four groups were differentiated with a level of retention of over 50% (Figure 4). There were significant differences in ant composition among sites (MRPP: $A = 0.129$; $T = -4.408$; $P = 0.0002$), which were determined by geographic location and the abundance of *W. auropunctata*. The first group was formed by the four sites in the North, which also had the lowest abundance of *W. auropunctata* (Aguas Claras, Alejandría, Miralindo, and Las Pilas). In Las Chatas, which was separate from the other sites, no *W. auropunctata* ants were captured. The third group covered the midzone of the geographic valley of the Cauca River (El Hatico, El Vínculo, and El Medio). Lastly, the fourth group was formed by the two sites in the South (Colindres and San Julián), where the greatest abundance of *W. auropunctata* was found.

Table 2: Effectiveness of tuna-fish baits and total richness of ant genera and species in the study area.

Site	Bait attraction (%)	No. of genera	No. of species
North			
Miralindo	95.6	23	37
Aguas Claras	96.8	21	42
Alejandría	87.2	22	46
Las Pilas	96.7	18	37
Midzone			
El Medio	97.5	15	31
Las Chatas	86.7	13	17
El Vínculo	78.9	15	21
Hatico	95.0	21	32
South			
Colindres	95.0	17	23
San Julián	98.7	12	18
Average ± SD	92.8 ± 6.41	17.7 ± 3.92	30.4 ± 10.24

The CCA showed that the habitats formed separate groups that varied with respect to the composition of ants (Figure 5), which was significant globally (MRPP: $A = 0.116$; $T = -5.847$; $P = 0.0001$). However, the differences were between the forest and pastures (MRPP: $A = 0.124$; $T = -6.263$; $P = 0.0003$) and the forest and cane fields (MRPP: $A = 0.067$; $T = -3.475$; $P = 0.007$); but not between the pastures and cane fields (MRPP: $A = 0.052$; $T = -1.757$; $P = 0.063$). Moreover, the canonic regression (Table 3) shows that that there is a strong relationship between the community structure and the variables soil temperature, percent ground cover, abundance of *W. auropunctata*, and richness of ant species, of which the first three were strongly associated with the second axis, while the last one was associated with the first axis. The variables pH and soil moisture had weak relationships with the composition of ants. The proper values (eigenvalues) for the first two axes explained the highest percent of cumulative variance (21.7%) and were significant (Table 3).

4. Discussion

Sampling methods in this study did not include collecting litter, so it would be expected that species richness would not be as high; however, the 100 ant species collected with the tuna-fish baits in the 10 dry forest fragments represent 50% of the species recorded in the study area [17]. Myrmicinae was the most diverse subfamily, with five times more genera

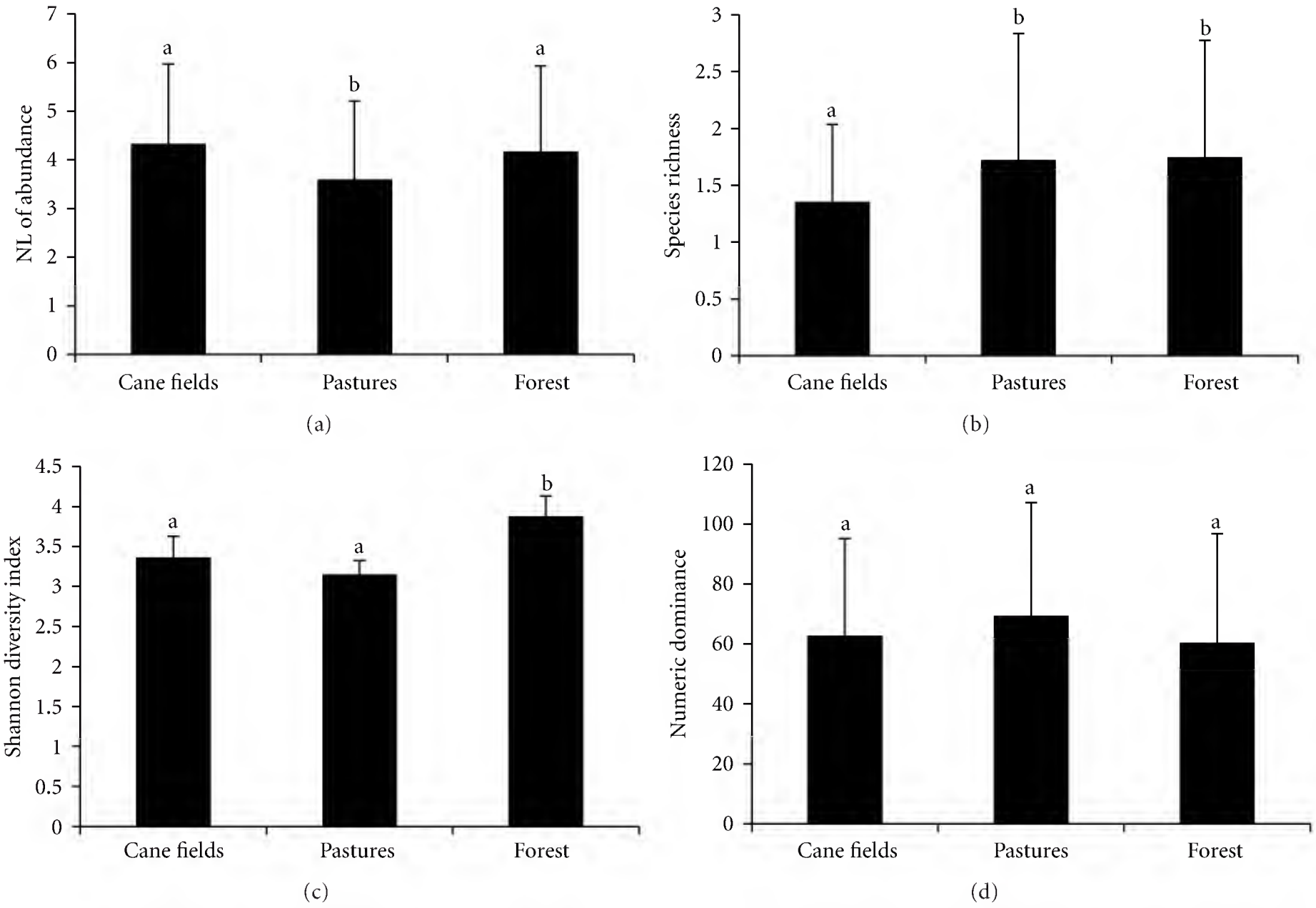

Figure 3: Average values and SD for (a) natural logarithm of the abundance of ants per bait; (b) species richness per bait; (c) shannon diversity index, and (d) numeric dominance of the most abundant species in each habitat. Bars with different letters are statistically different.

Table 3: Summary of the CCA statistics for each axis and the Monte Carlo significance test for the proper values (eigenvalues) based on 999 permutations.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.538	0.319	0.233
Monte Carlo probability test	0.001	0.006	0.015
% cumulative variance in species data	13.7	21.7	27.6
Variables of the canonic multiple regression			
Soil moisture	−0.038	0.104	−0.172
pH	0.080	−0.170	−0.141
Soil temperature	−0.141	0.314	−0.086
% ground cover	0.304	−0.480	−0.784
Abundance of *W. auropunctata*	0.170	−0.268	0.984
Species richness	0.713	0.787	0.482

and four times more species than the other subfamilies. This preeminence can be explained by the great adaptive radiation, range of foraging behaviors, nesting habits, and colony structure of this subfamily [20], which includes the genus *Pheidole*, which had the greatest richness (19 species) and which is represented by 651 species in the New World [50].

In the study area, agricultural intensification has occurred from the edges of the Cauca River in the flatlands toward the foothills [16]. Species' richness increased toward the North of the study area (Figure 1), where the sampled sites are found close to the foothills of the central and western Andean mountain ranges; by contrast, the sites in the South are found in the flatlands closer to the edge of the Cauca River. Arcila-Cardona et al. [37] showed that the sites located to the North are more interconnected than the midzone and South of the geographic valley and are surrounded by gallery forests and patches of *Guadua angustifolia*, which increases

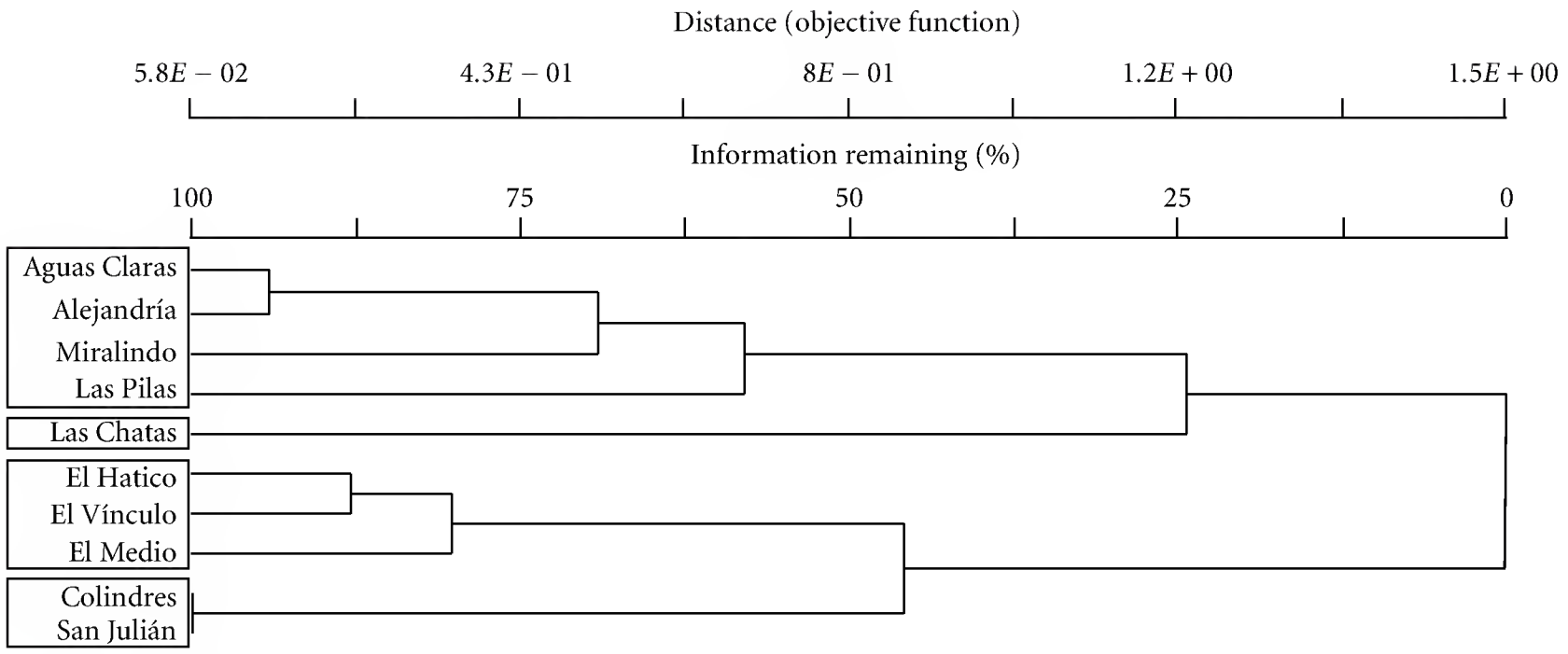

FIGURE 4: Clustering of the ant assemblages at the 10 sampled sites, based on the Sorensen (Bray-Curtis) index of dissimilarity and the Beta-flexible clustering method ($b = -0.25$). Percent concatenation: 10%.

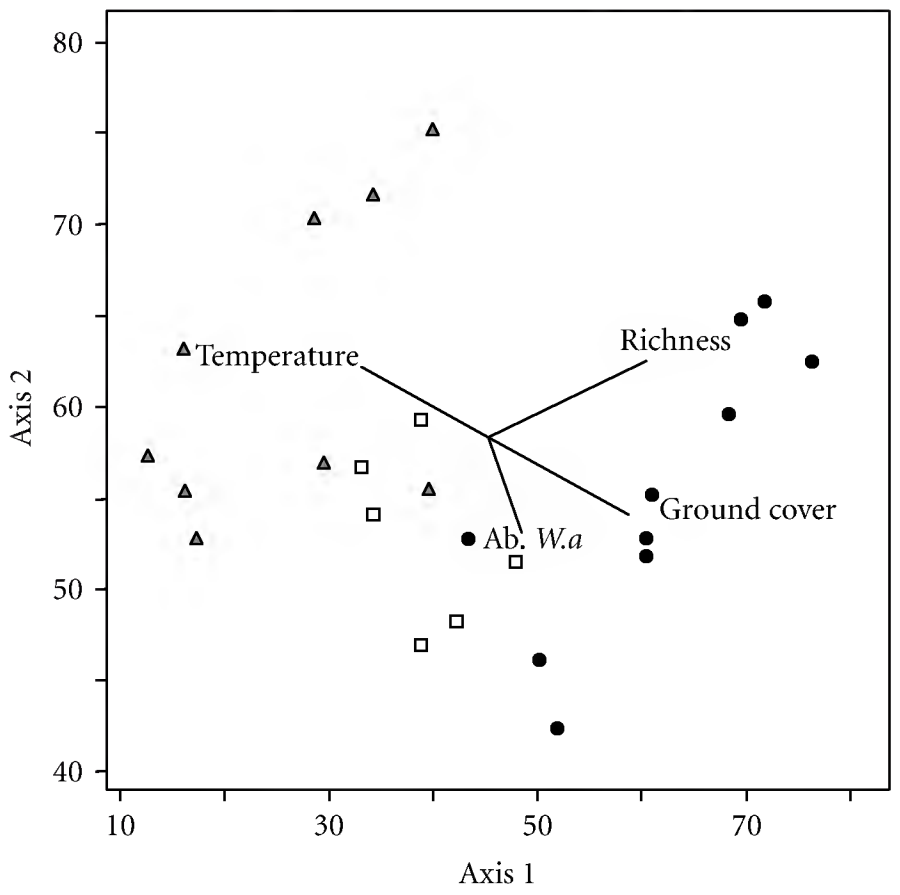

FIGURE 5: Canonical correspondence analysis (CCA) of the composition of ants in three habitats (●: Forest; ▲: Pastures; □: Cane fields). The lines show the direction and strength of the relationship of the environmental and biotic variables with respect to the structure of the ant assemblage per habitat.

the heterogeneity of the landscape and favors the movement of ant species among sites. This result is related to what Cook et al. found [51], where small or isolated fragments maintain high species richness due to the availability of colonizers from adjacent heterogeneous matrixes. Another phenomenon that can affect the species richness of ground ants is the periodic flooding that affects the sites in the South with greater frequency [16]. This stochastic event could alter the availability and quality of the habitat dramatically [22], resulting in a reduction in the richness of ants [52] when they are forced to move their nesting sites regularly [53].

The inverse correlation between species richness and the abundance of *W. auropunctata* reinforces the idea that the little fire ant can be used as an indicator of low diversity in ant assemblages [34]. This species is 10 times more abundant toward the South of the region sampled, where the process of fragmentation has been more aggressive [16]. Under these circumstances, *W. auropunctata* has largely displaced the ant fauna in some areas of the tropical forest in southwestern Colombia, accounting for up to 84% of the total captures. The increase in invasive or aggressive species is one of the effects of fragmentation that has been reported with greatest frequency [28, 29, 54–56] and has had negative effects on the native fauna, decreasing the utilization of resources for other species of ants and other arthropods, thereby affecting plants and associated arthropods, either directly or indirectly [22]. Although *W. auropunctata* is a very common native species of neotropical forests that does not usually dominate entire assemblage [57–59], in disturbed areas such as agricultural and forestry systems, as well as in regions outside its native range, the little fire ant commonly has enormous populations [60, 61] and is capable of exterminating ant populations over large areas [20]. That result is in agreement with those found in this study.

In agreement with the rarefaction curve (Figure 2), there are significant differences in the number of species per habitat, which shows that management has an important effect on the ant fauna in these tropical dry forests. Species richness is greatest in the forests and lowest in the cane fields. The comparison among habitats indicates a significant loss of species between the forest and productive habitats, given that the forests have 51 species that were not recorded in either the cane fields (only 3 exclusive species) or the pastures (8 species). This pattern of species richness is common in tropical zones [24, 27, 62], where the richness declines along a gradient of succession or disturbance. Majer et al. [63] showed that the permanent transformation of forests to crops dramatically reduces species richness. Perfecto et al. [64] reviewed 22 studies, of which 18 reported that ant diversity decreased with the intensification of agriculture. In

Table 4: List of ant species sampled and their abundance at baits along a tropical dry forest region of southwestern Colombia.

Species	Forest	Cane fields	Pastures	Total abundance	% occupation of baits
Dolichoderinae					
*Azteca instabilis**	1324	26	0	1350	5.36
Azteca sp. 1	29	0	0	29	0.10
*Dolichoderus bispinosus**	1279	0	0	1279	3.74
Dorymyrmex brunneus	0	0	1	1	0.10
*Linepithema iniquum**	135	14	1	150	1.32
Linepithema sp. 1*	66	0	0	66	0.61
*Tapinoma melanocephalum**	3	0	1	4	0.40
Ecitoninae					
Eciton burchelli	5	0	0	5	0.10
Labidus coecus	774	228	294	1296	0.91
Ectatomminae					
*Ectatomma ruidum**	0	8	42	50	3.24
*Gnamptogenys annulata**	1	0	0	1	0.10
Formicinae					
*Acropyga exsanguis**	1	0	0	1	0.10
*Brachymyrmex heeri**	0	1	57	58	1.52
Brachymyrmex sp. 1*	7	0	8	15	0.51
Brachymyrmex sp. 2*	11	0	0	11	0.61
Brachymyrmex sp. 3	5	0	2	7	0.20
Brachymyrmex sp. 4*	0	0	1	1	0.10
Brachymyrmex sp. 5*	11	0	73	84	0.91
Brachymyrmex sp. 6*	4	0	0	4	0.20
Camponotus claviscapus	0	5	0	5	0.10
*Camponotus novogranadensis**	248	2	9	259	4.25
Camponotus sp. 1*	0	2	45	47	2.63
Camponotus sp. 2	1	0	0	1	0.10
Nylanderia fulva	390	0	0	390	1.72
Paratrechina longicornis	0	3	1	4	0.20
Paratrechina sp. 1*	19	4	7	30	1.42
Paratrechina sp. 2*	11	11	20	42	1.62
Heteroponerinae					
Heteroponera sp. 1*	1	0	0	1	0.10
Myrmicinae					
Acromyrmex octospinosus	3	0	0	3	0.10
*Apterostigma pilosum**	1	0	0	1	0.10
*Atta cephalotes**	27	0	0	27	0.81
*Cardiocondyla minutior**	0	2	42	44	1.42
*Cardiocondyla obscurior**	0	3	131	134	1.92
Carebara brevipilosa	31	0	0	31	0.10
*Cephalotes minutus**	0	0	1	1	0.10
*Crematogaster carinata**	6994	0	0	6994	5.26
*Crematogaster curvispinosa**	343	0	0	343	0.61
Crematogaster distans	172	0	0	172	0.20
Crematogaster erecta	0	0	57	57	0.10
*Crematogaster evallans**	4922	35	0	4957	2.63
*Crematogaster limata**	284	0	51	335	1.01

Table 4: Continued.

Species	Forest	Cane fields	Pastures	Total abundance	% occupation of baits
*Crematogaster nigropilosa**	448	0	0	448	0.71
*Crematogaster sotobosque**	3751	0	0	3751	3.04
Crematogaster sp. 1	178	0	0	178	0.51
*Cyphomyrmex costatus**	1	0	0	1	0.10
*Cyphomyrmex rimosus**	5	0	2	7	0.71
Hylomyrma reitteri	3	0	0	3	0.10
Megalomyrmex sp. 1	4	0	0	4	0.10
*Megalomyrmex wallacei**	3	0	0	3	0.10
*Monomorium florícola**	88	992	197	1277	3.04
Mycocepurus smithii	3	0	0	3	0.30
Myrmycocrypta sp. 1*	3	0	0	3	0.20
Myrmycocrypta sp. 2*	19	0	0	19	0.30
Octostruma balzani	2	0	0	2	0.20
Octostruma sp. 1	4	0	0	4	0.10
Pheidole radoszkowskii	0	0	27	27	0.20
*Pheidole rugiceps**	57	0	0	57	2.33
*Pheidole sabella**	79	534	4	617	2.43
*Pheidole scalaris**	359	19	132	510	8.20
Pheidole sp. 1	228	0	0	228	0.10
Pheidole sp. 2	0	325	80	405	0.40
Pheidole sp. 3	4	0	0	4	0.20
Pheidole sp. 4*	1124	0	7	1131	4.15
Pheidole sp. 5	4	0	0	4	0.20
Pheidole sp. 6	354	0	0	354	0.40
Pheidole sp. 7*	1	407	5	413	0.91
Pheidole sp. 8	778	9	0	787	0.61
Pheidole sp. 9*	52	0	0	52	0.20
Pheidole sp. 10	3	0	0	3	0.10
Pheidole sp. 11	4	0	0	4	0.30
Pheidole sp. 12	4	0	0	4	0.10
Pheidole sp. 13*	0	0	3	3	0.10
*Pheidole subarmata**	5	0	151	156	0.91
*Pheidole susannae**	507	518	38	1063	7.09
*Pheidole synarmata**	644	71	0	715	2.43
*Pyramica denticulata**	22	0	1	23	0.51
*Rogeria belti**	1	0	0	1	0.10
*Solenopsis geminata**	2087	11653	15825	29565	18.02
Solenopsis picea	259	0	0	259	0.30
*Solenopsis pollux**	1592	469	54	2115	10.53
Solenopsis sp. 1	6	0	0	6	0.10
Solenopsis sp. 2*	16	0	0	16	0.30
Solenopsis sp. 3	67	0	2	69	0.30
Solenopsis sp. 4*	37	0	5	42	1.72
Solenopsis sp. 5*	40	36	25	101	2.13
Strumigenys sp. 1	1	0	0	1	0.10

Table 4: Continued.

Species	Forest	Cane fields	Pastures	Total abundance	% occupation of baits
Strumigenys trieces	1	0	0	1	0.10
*Temnothorax subditivus**	0	470	0	470	0.30
Tetramorium bicarinatum	0	147	21	168	0.51
Tetramorium simillimum	0	1	4	5	0.20
Trachymyrmex opulentus	1	0	0	1	0.10
Trachymyrmex sp. 1	2	0	0	2	0.20
*Tranopelta gilva**	0	0	138	138	0.20
Wasmannia auropunctata	101864	22718	6176	130758	38.36
Ponerinae					
Hypoponera sp. 1*	12	0	0	12	0.40
Hypoponera sp. 2	0	7	0	7	0.10
*Pachycondyla constricta**	25	0	0	25	0.91
*Pachycondyla impressa**	5	0	0	5	0.51
Pseudomyrmecinae					
Pseudomyrmex boopis	7	0	0	7	0.71
Pseudomyrmex sp. 1*	0	0	20	20	1.82

* Species sharing the resource with the little fire ant (*W. auropunctata*).

the tropics, a large part of the anthropogenic disturbance is due to pastures for cattle raising [22], and this intensification can result in the loss of richness, especially of cryptic or specialized predator species [65]. The habitat with the lowest number of species was the cane fields, which can be related to common cultural practices in this monocrop, such as burning, application of agrochemicals, and removal of litter from the ground [66]. These factors, in contrast with management of the pastures, impact species richness more intensely by eliminating nesting and food resources.

In this study, the composition of the ant assemblage at the scale of the site and habitat could be partially structured by the abundance of *W. auropunctata*. Some studies provide evidence that the dominant species is a key factor in structuring the ant assemblages [25, 67], given that such species partially control the competitive interactions in tropical forests [33, 68].

Significant differences were found in ant composition among habitats. The forests are more diverse (Figure 3(c)) and have a composition separate from the other two habitats. In the study area, some mechanisms that explain the changes in species composition are related to microenvironmental variables, such as soil temperature and percent ground cover (Table 3). The loss of arboreal vegetation has a significant effect on the assemblage of ants, given that it changes microclimatic conditions, including temperature regimes and relative humidity gradients [22]. These disturbances and conversions to productive agroecosystems limit the nesting sites, generating changes in species composition [69]. For example, *W. auropunctata* is the dominant species in the forest and cane fields, whereas in the pastures *S. geminata* dominates (Figure 3(d)). The pastures have a higher temperature and little ground cover in the form of litter and pieces of bark, conditions that are adverse for the nesting of *W. auropunctata*. In contrast, *S. geminata* dominates sites where the intensification of agriculture has generated open systems with high solar radiation [70]. Although there is a clear distinction between the assemblages of ants associated with the cane fields and pastures, the differences were not significant ($P = 0.063$). One possible reason is that the cane fields in the study area are adjacent to the pastures, so there could be a movement of species between habitats. Our results agree with other studies conducted in tropical forests [24], which found that in a regeneration gradient, soil generalists ant fauna can move between adjacent areas with low complexity. Thus, they propose the existence of spatial self-correlation as an explanation, given that low-complexity habitats most likely present a more traversable surface at the scale of a foraging ant.

5. Conclusions

The results obtained confirm that the structure and composition of the ground ant assemblage in the tropical dry forest of southwestern Colombia differ at both site and habitat levels. These changes are related to the abundance of dominant species (primarily *W. auropunctata*), as well as to geographic position, microclimatic conditions, and the complexity of the habitats and sampling sites. Moreover, it is important to take into account the conditions of each site and the gradient of isolation and disturbance that there is from South to North. The sites in the South, in contrast with those from the North, are more isolated and have been submitted to greater disturbance (e.g., cattle entering the forest fragments, cutting down trees, and greater use of agrochemicals in the cane crops).

Moreover, it is hypothesized that the indirect effect of loss of species due to isolation and fragmentation can favor

colonization by species such as *W. auropunctata*, which takes advantage of the freeing up of resources and niches due to the disappearance of other species and manages to build up a high population density. Given that agricultural conversion has favored the excessive abundance of *W. auropunctata* to the detriment of the diversity of ants associated with the dry forest, it is important to maintain the heterogeneity of the landscape. Despite the aggressive transformation of the dry forest for agriculture, these relicts conserve a large number of species whose composition depends on the site. Consequently, the loss of some of the forest fragments would theoretically mean the disappearance of some species at the local and regional scale, resulting in the homogenization of the ant fauna. Accordingly, it would be important to increase the structural connectivity between sites, which would also serve to prevent the continued degradation of the forest and improve management of the matrixes. Finally, in line with other studies [71, 72], this work reinforces the idea of how vulnerable ant assemblages are to environmental disturbance.

Appendix

For more details, see Table 4.

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Research Article

Effects of Habitat and Human Activities on Species Richness and Assemblages of Staphylinidae (Coleoptera) in the Baltic Sea Coast

Ulrich Irmler

Department of Applied Ecology, Institute for Ecosystem Research, Christian Albrecht University, 24098 Kiel, Germany

Correspondence should be addressed to Ulrich Irmler, uirmler@ecology.uni-kiel.de

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In 2009, the staphylind fauna was studied in six habitats of the Baltic Sea coast of Schleswig-Holstein (northern Germany). The following habitats lagoon, sandy beach, shingle beach, primary dune, wooded cliff, and woodless cliff were significantly separated by their species composition. Vegetation and soil moisture were the most important factors separating the assemblages. Lagoons exhibited the most species-rich habitat. Sandy beaches provided the highest number of endangered species. Both sandy beaches and woodless cliffs showed the highest number of exclusive species. A loss of species was determined in the gradient from sandy to shingle beaches. Few species preferred shingle beaches; abundance of *Cafius xantholoma* increased with the increasing amount of shingle. More species preferred the sandy conditions, for example, *Polystomota grisea, P. punctatella*, and *Phytosus spinifer*. *Anotylus insecatus* and *Bledius defensus* require distinct mixtures of sand and silt on woodless cliffs. Tourist impact on sandy beaches accounts for approximately 50% loss of species.

1. Introduction

In general, staphylind beetles are rarely taken into consideration in ecological investigations [1, 2]. This is due to the fact that the identification is difficult, and little information is available on species ecology. Moreover, in several investigations, the differentiated assemblages corresponded only weakly to environmental parameters [3, 4]. Nevertheless, Staphylinidae are one of the most species-rich coleopteran families and, thus, might give more information about biodiversity than any other arthropod group. This is particularly relevant as many species are associated with other animals, for example, with birds, mammals, or as parasitoids of flies. Staphylinid diversity not only informs about the abiotic heterogeneity of the habitat, but also about the heterogeneity produced by animal species.

Very few studies are available concerning the biodiversity of coastal habitats along the Baltic Sea. More is needed since activity by tourists on beaches has increased dramatically in the last decades [5] and coastal habitats are included in the list of endangered habitats in the Fauna-Flora-Habitat (FFH) directive of the European Union (EU). Coastal lagoons are listed in a priority class [6]. Most information exists concerning salt marshes which also include studies on Staphylinidae [7–9].

Even though the fauna of beaches is well known in general in many European countries (e.g., [10, 11]), there is very little information about the fauna of other coastal habitats and the influence of environmental parameters from the coasts of the Baltic Sea. In particular, little knowledge is available about sandy beaches and cliffs. In contrast to tidal coasts, the Baltic Sea coast has little or no tides. This is reflected by a wide distribution of terrestrial organisms towards the sea line.

Thus, the following study mainly focuses on sandy and shingle beaches and cliffs. In the present study, the relationships between staphylinid assemblages and habitat factors, the relationships between individual species and soil factors, and the effect of tourism on species richness are highlighted. We address the following questions in this study: (i) which environmental parameters control the composition of staphylinid assemblages at the coast? (ii) are soil parameters responsible for the occurrence of different

staphylinid species? and (iii) where are the most species-rich coastal habitats and are they influenced by beach tourism?

2. Sites and Methods

The investigation was performed in 2009, from April 9 to August 20 at nine locations along the Baltic Sea coast in Schleswig-Holstein, northern Germany, having an extremely low tidal range of less than 10 cm (Figure 1). At these 9 locations, different sites were selected representing the following habitat types: woodless and wooded cliffs, sandy and shingle beaches, primary dunes, and lagoons (Table 1). Six sandy beaches were selected in contrast to three or four sites of the other habitat types since beaches were the focus of the investigation. Two of the six beaches were open to tourists. Staphylinidae were collected by means of pitfall traps. Up to eight replicate pitfall traps were installed at each site to compensate loss by tourist damage. Only four pitfall traps were included in the final analysis in order to have equal numbers of traps for the different sites. Pitfall traps with an opening of 5.6 cm diameter were filled with 10% vinegar and a tension-reducing agent and covered by a transparent shelter to shield against direct precipitation.

To compare the environmental conditions between the habitat types, the following environmental parameters were determined: soil moisture by difference between wet weight and dry weight of soil as mean of 11 sampling intervals; pH in deionised water using a WTW pH-Meter; organic matter after combustion of a dried soil sample; shingle content by sieving a larger soil sample in the field; sand content by sieving using a 0.063 mm sieve after oxidising the organic matter by H_2O_2; finer silt and clay material was determined by subtracting sand content [12].

The statistical analysis was performed using the program STATISTICA [13]. Data were tested according to parametric or nonparametric distribution using the Kolmogorov-Smirnov test. The data of environmental factors of assemblages and species richness (normal distribution) were compared using ANOVA with subsequent LSD post hoc test. Differences between two habitats were tested by U test or t-test, correlations by Pearson correlation. To get an idea of total species richness in the habitats, Jackknife II species richness was calculated using the program PAST version 2.04 [14]. The second order of Jackknife estimator seems to be most accurate to estimate total species richness [15]. Detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) were executed using the program CANOCO [16]. According to ter Braak [17], a distinct ordination of assemblages can be expected at eigenvalues higher than 0.5. Monte Carlo Permutation test was performed to find the significance of environmental parameters.

In the beach habitats, sand and shingle contents were closely correlated: sand content = 97 + 0.96* shingle content ($r = 0.99$; $P < 0.001$). Therefore, either sand content or shingle content was used to analyse the occurrence of species in the sand-shingle gradient. For some comparisons, the sand-shingle gradient was subdivided into the following three classes: shingle: >70% shingle and <30% sand; medium: 10–60% shingle and 40–90% sand; sand: >90% sand and <10% shingle. To analyse the abundance in these gradients, Kruskal-Wallis ANOVA was used with subsequent U test and Bonferroni correction using the program STATISTICA. The status of endangered species refers to the red list of Schleswig-Holstein [18].

3. Results

3.1. Environmental Parameters. The highest soil moisture contents were found at the lagoons and wooded cliffs, while all beach habitats have very low soil moistures without significant differences (Table 2). Sand content was high at the sandy beaches and the primary dunes and lowest at the wooded cliffs and the shingle beaches. The shingle content differs between shingle beach with 77% on average and all other habitats. Soil pH was on a high level in a narrow range. Nevertheless, significant differences were also found for this parameter. The highest soil pH was found at the woodless cliffs and at the shingle beaches, and the lowest pH at the primary dunes. Thus, sandy beaches and primary dunes that show no differences in all other parameters vary significantly in their soil pH. Organic material was low in all habitats, but highest in wooded cliffs and lagoons. Overall, each habitat type reflected a specific combination of soil parameters. They could be significantly separated by at least one of the measured parameters.

3.2. Species Composition. From a total of 4324 specimens collected, 165 species have been identified. The highest number of species was found at lagoons, and the lowest number of species on shingle beaches (Table 3). Jackknife species richness was also highest at lagoons and lowest at shingle beaches. The highest number of endangered species was found on sandy beaches. Only primary dunes have no endangered species.

In correspondence to the environmental differences between the habitats, the detrended correspondence analysis revealed assemblages of rove beetle that are clearly separated by their habitat characterisation (Figure 2). Only the shingle beach at Weissenhaus was attributed to the sandy beaches and not to the other three shingle beaches. According to the canonical correspondence analysis, 3 of the 6 factors have a significant effect on the species composition of the assemblages and account for 76% of the total variance. The most important factor was soil moisture ($F = 3.6$) which accounted for 34% of the total variance. Wooded or non-wooded situation accounted for 23% ($F = 2.8$), and soil pH accounted for 21% ($F = 2.7$). Neither sand content nor shingle content nor content of organic matter were significant for the separation of the assemblages.

The composition of dominant species in the 6 habitat types shows that several species are widely distributed along the different habitat types (Table 4). The widely distributed *Aleochara sparsa* was found highly dominant in the cliff habitats. While several species frequently found in Schleswig-Holstein revealed highest dominance in the wooded cliffs,

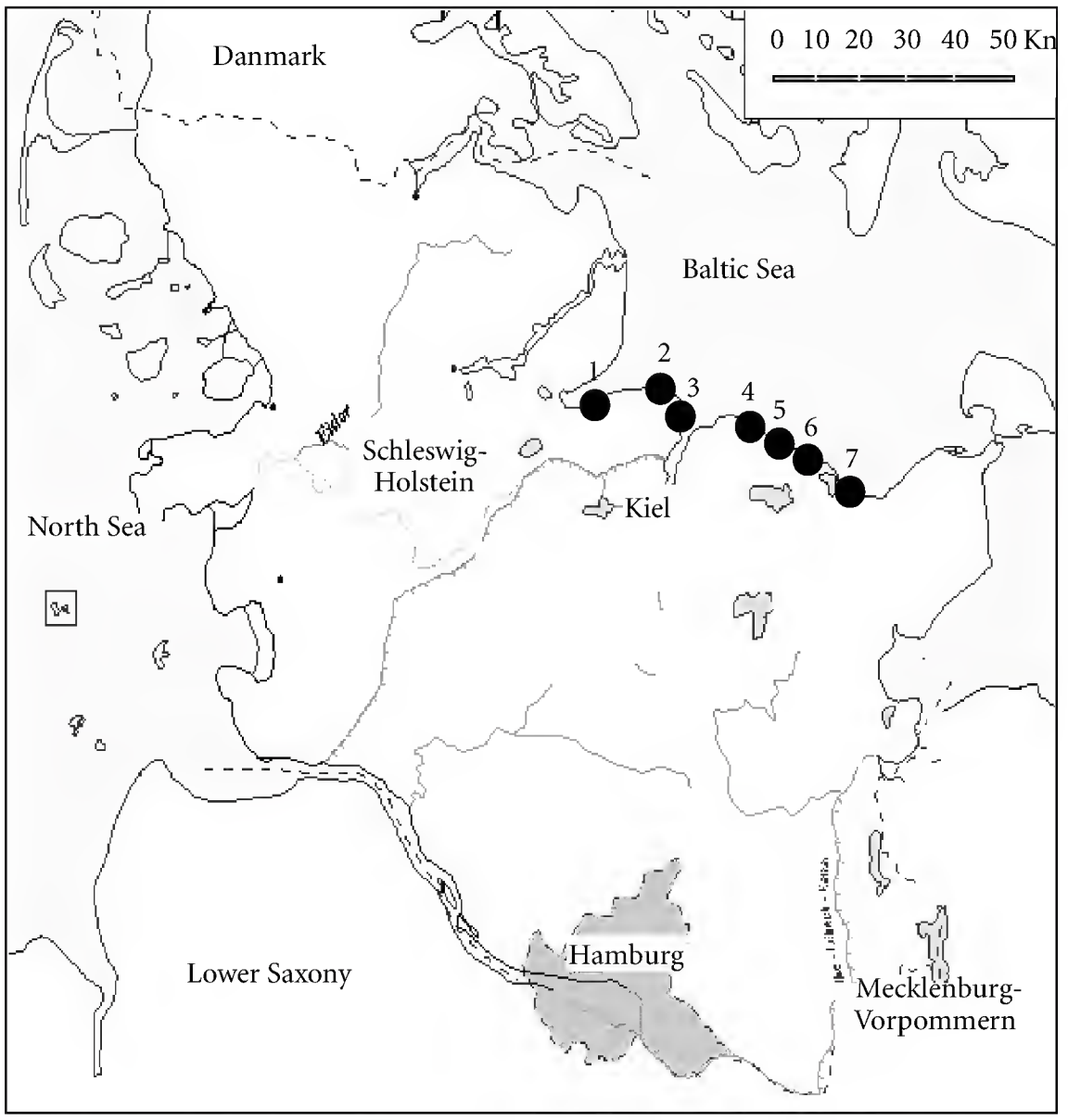

Figure 1: Investigated locations at the Baltic Sea coast: Lindhöft (1), Dänisch-Nienhof (2), Stohl (3), Stakendorf and Hohenfelde (4), Hubertsberg (5), Behrensdorf and Lippe (6), and Weißenhaus (7).

Table 1: Habitats at the investigated locations and abbreviations of locations and habitats; x indicates a number of 4 replicate pitfall traps.

Location/Habitat (abbreviation)	Tourism on sandy beach	Lagoon (L)	Sandy beach (SB)	Shingle beach (GB)	Primary dune (PD)	Woodless cliff (Wl)	Wooded cliff (Wc)
Behrensdorf (BD)	Closed	x	x	—	—	—	—
Dänisch-Nienhof (DN)	—	—	—	x	—	x	x
Hubertsberg (HB)	—	—	—	x	—	x	—
Hohenfelde (HF)	Open	x	x	—	x	—	—
Lippe (KB)	Closed	—	x	—	x	—	—
Lindhöft (LH)	Open	—	x	—	—	—	x
Stakendorf (SD)	Closed	x	x	—	x	—	—
Stohl (ST)	—	—	—	x	—	x	—
Weißenhaus (WH)	Closed	—	x	x	x	x	x

Table 2: Mean values of environmental parameters for the differentiated staphylinid assemblages; different exponents indicate significant differences by ANOVA and consecutive LSD test.

Parameter	Wooded cliffs	Woodless cliffs	Lagoon	Sandy beach	Primary dune	Shingle beach
Soil moisture (%)	$^{a}18 \pm 3.0$	$^{b}8 \pm 1.1$	$^{a}31 \pm 5.9$	$^{c}1 \pm 0.5$	$^{c}1 \pm 0.5$	$^{c}1 \pm 0.6$
Wood	yes	no	no	no	no	no
Sand content (%)	$^{b}36 \pm 6$	$^{c}46 \pm 3$	$^{c}51 \pm 16$	$^{a}89 \pm 9$	$^{a}92 \pm 9$	$^{b}22 \pm 21$
Shingle content (%)	$^{b}5 \pm 2$	$^{b}8 \pm 2$	$^{b}21 \pm 20$	$^{b}8 \pm 10$	$^{b}6 \pm 8$	$^{a}77 \pm 20$
Soil pH	$^{b}7.4 \pm 0.1$	$^{a}7.9 \pm 0.2$	$^{b}7.5 \pm 0.2$	$^{b}7.3 \pm 0.2$	$^{c}7.2 \pm 0.1$	$^{a}7.9 \pm 0.3$
Organic content of soil (%)	$^{a}6.6 \pm 2.6$	$^{b}1.1 \pm 0.1$	$^{a}5.6 \pm 0.9$	$^{b}0.2 \pm 0.2$	$^{b}1.4 \pm 2.2$	$^{b}0.3 \pm 0.1$

Table 3: Species richness in the investigated habitats and Jackknife II species richness; RL species richness of endangered species (only status 1 and 2).

Habitat type	Localities									Species richness		
	BD	DN	HF	HB	KB	LH	SD	ST	WH	Total	Per trap	Jackknife II	RL
Lagoon	37	—	47	—	—	—	51	—	—	85	13.9 ± 2.5	154 ± 6.5	2
Sandy beach	36	—	10	—	27	18	21	—	22	70	10.0 ± 4.2	116 ± 6.6	5
Shingle beach	—	9	—	21	—	—	—	13	5	33	4.4 ± 3.3	61 ± 5.5	2
Primary dune	—	—	21	—	17	—	28	—	28	75	9.4 ± 4.0	88 ± 5.3	0
Woodless cliff	—	18	—	38	—	—	—	27	20	58	10.7 ± 3.5	99 ± 7.1	1
Wooded cliff	—	22	—	—	—	26	—	—	19	43	8.7 ± 3.4	72 ± 4.8	1

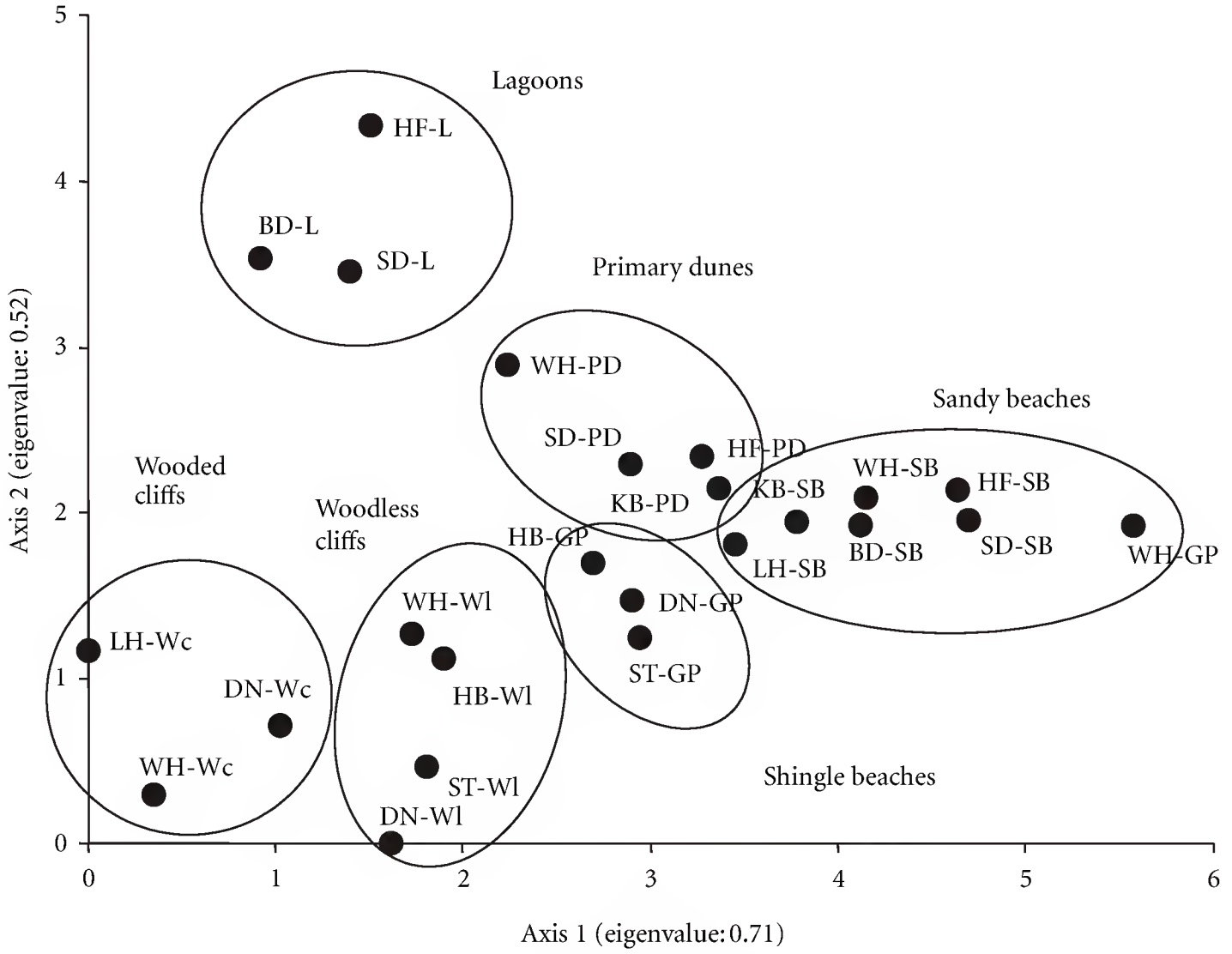

Figure 2: Results of the detrended correspondence analysis showing the separation of the 6 habitat types along the first two axes.

a higher number of specialised species were found in the woodless cliffs. In particular, rare species of bare soils, for example, *Stenus fossulatus*, *Bledius erraticus*, *Bledius defensus*, and *Anotylus insecatus*, were almost exclusively recorded there. Lagoons as wooded cliffs have no remarkable species that are restricted to the specific lagoon situation. All dominant species are frequently found also on mainland lake shores. Primary dunes showed only one specific species, that is *Ocypus brunnipes*, in contrast to the two beach habitats, where a number of 8 species can be regarded as typically independent from the soil situation. Shingle beaches thus seem to have no specific species in comparison to sandy beaches. In contrast, 3 species, that is, *Phytosus balticus*, *Bledius subniger*, and *Atheta vestita*, seem to prefer the sandy beaches.

3.3. Relationships between Individual Species and Environmental Parameters. Although the differences between shingle beaches, sandy beaches, and primary dunes are minor in rove beetle assemblages, individual species show significant differences concerning their occurrence. *Cafius xantholoma* only inhabits the beach habitats and was never found on the adjacent primary dunes (ANOVA: beach versus primary dune: $F = 6.7$, $P < 0.01$). Along sand gradient of beaches, the species significantly prefers the shingle beaches; a significant linear increase along the sand-shingle gradient was found (abundance $= 0.02 \cdot$ shingle content $+ 0.36$, $r = 0.66$, $P = 0.04$). A similar restriction to the beach habitats was found for *Polystomota grisea* (ANOVA: beach versus primary dune: $F = 17.2$, $P < 0.001$) and *Polystomota punctatella* (ANOVA: beach versus primary dune: $F = 10.9$, $P < 0.002$). In contrast

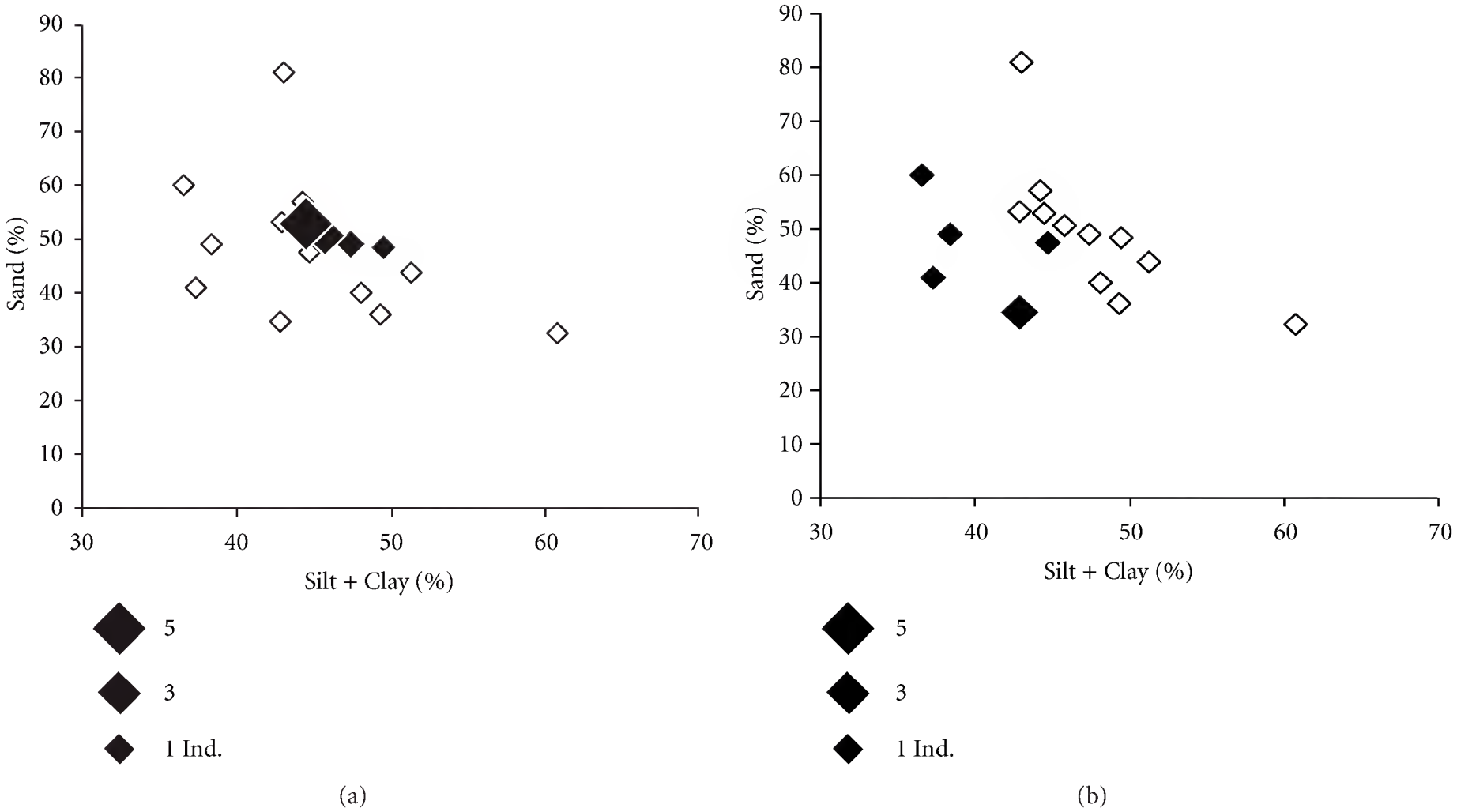

FIGURE 3: Occurrence of *Bledius defensus* (a) and *Anotylus insecatus* (b) in the sand-silt/clay relationship of woodless cliffs.

to *C. xantholoma*, both species significantly preferred the sandy beaches: *P. grisea* abundance = 0.03 · sand content − 0.27, $r = 0.73$, $P = 0.02$; *P. Punctatella* abundance = 0.02 · sand content + 0.19, $r = 0.67$, $P = 0.03$. Furthermore, *Phytosus spinifer* also occurred only in the beach habitats and preferred significantly the beaches of the sand class (Kruskal-Wallis ANOVA using the three classes: $\text{Chi}^2 = 7.8$, $P = 0.02$). The species was absent on beaches with a shingle content greater than 10% and on beaches with a sand content lower than 80%. In contrast to the four species restricted to beaches, *Aleochara bipustulata* was found on sandy beaches and primary dunes in similar abundance (ANOVA: $F = 2.1$, $P = 0.1$, not significant). However, the species significantly preferred the sandy habitats. Using all sites of beaches and primary dunes for the correlation between abundance and sand content, abundance increases with an increase in sand content of the habitat (abundance = 0.53 · sand content − 6.18, $r = 0.67$, $P = 0.006$).

For three species of the woodless cliffs, preference for specific mixtures of silt/clay and sand is assumed in regard to their occurrence in the silt/clay-sand relationship (Figure 3). *Anotylus insecatus* was mainly found on woodless cliffs between 30% and 40% silt/clay content, while it was absent from cliffs with silt/clay contents higher than 50% (Kurskal-Wallis ANOVA: $\text{Chi}^2 = 8.8$, $P = 0.01$). *Bledius defensus* was found in a silt/clay range between 40% and 50% (Kurskal-Wallis ANOVA: $\text{Chi}^2 = 5.8$, $P = 0.04$). The species was not found at cliffs with silt/clay contents lower than 40%. Only 1 specimen was found at cliffs with silt/clay contents higher than 50%. The species seems to occur in a very restricted range having a sand content between 45% and 50% and silt/clay content between 35% and 52%. Another typical species of woodless cliffs, that is *Stenus fossulatus*, showed no significant preference of specific sand-silt/clay mixtures. It occurred in woodless cliffs with a range of sand content between 42% and 50% and a wide range of silt/clay content between 36% and 81%. However, soil moisture on woodless cliffs was important. Abundance increased with increasing soil moisture (abundance = 0.39 · soil moisture − 2.21, $r = 0.96$, $P = 0.03$).

3.4. Effect of Tourism. Sandy beaches closed to tourists have significant higher species richness than beaches open to tourism (Table 5). The closed beaches revealed nearly twice as many species as the beaches open to tourists. No species was found exclusively on beaches open to tourists. Thus, half of the species of closed beaches are absent from open beaches. The difference between the two beach types is still more obvious if Jackknife II species richness is considered. Three species of the aleocharinae subfamily were abundant enough to analyse their occurrence in the two beach types. Both *Aleochara bipustulata* and *Polystomota grisea* showed significantly higher abundance on the beaches closed to tourists. A significant difference between both beach types concerning their sand content was not found (U test: $Z = 8.8$, $P = 0.06$). Thus, the differences found can be referred to the tourist impact. Only in *Polystomota punctatella* was the difference not significant.

4. Discussion

Unfortunately, no other studies concerning staphylinids from beach habitats considering environmental parameters are available for comparison. Rose [3] investigated

Table 4: Dominance of species that contribute to the species characterisation of the habitats.

Species	Wooded cliff	Woodless cliff	Lagoon	Primary dune	Shingle beach	Sandy beach
Tasgius morsitans	**5.0**	0.4	0.1	0.2	—	—
Omalium rivulare	**4.3**	—	0.6	—	0.8	0.1
Atheta crassicornis	**3.2**	0.1	0.8	0.2	0.8	—
Lathrimaeum unicolor	**2.8**	0.4	0.2	0.2	—	0.1
Quedius fuliginosus	**2.8**	0.3	1.0	—	—	—
Atheta atramentaria	**2.1**	0.4	0.1	0.4	—	2.2
Aleochara sparsa	**50.2**	**13.5**	0.8	1.8	2.3	1.7
Stenus fossulatus	0.7	**2.2**	—	—	—	—
Tachyporus dispar	1.1	**5.4**	—	0.3	—	0.1
Bledius erraticus	—	**1.6**	—	—	—	0.1
Bledius defensus	—	**1.3**	—	—	—	0.1
Tasgius winkleri	—	**0.9**	0.1	—	—	—
Anotylus insecatus	—	**0.9**	—	—	—	—
Tachyporus nitidulus	1.8	**27.1**	0.4	0.1	12.5	0.1
Drusilla canaliculata	1.1	**24.6**	**18.3**	**13.2**	**16.4**	1.1
Stenus pallipes	—	—	**3.3**	—	—	—
Stenus juno	0.4	—	**4.1**	0.2	—	—
Atheta graminicola	—	0.3	**8.9**	—	—	0.1
Stenus canaliculatus	—	0.4	**3.3**	—	—	—
Pachnida nigella	—	—	**2.6**	—	—	0.1
Ocypus brunnipes	—	—	0.2	**1.6**		0.4
Aleochara bipustulata	0.7	2.8	2.9	**72.5**	**19.5**	**56.7**
Polystomota grisea	—	—	0.1	—	**7.0**	**5.4**
Polystomota punctatella	—	—	—	—	**1.6**	**5.1**
Cafius xantholoma	—	—	—	—	**7.8**	**4.7**
Omalium riparium	—	—	—	0.1	**0.8**	**0.8**
Phytosus spinifer	—	—	—	—	**0.2**	**0.8**
Phytosus balticus	—	—	—	—	—	**0.3**
Bledius subniger	—	—	—	0.2	—	**0.8**
Atheta vestita	—	—	0.1	0.3	—	**2.2**

Table 5: Species richness and median abundance of three species in four sandy beaches closed to tourists and two beaches open to tourists with results of *t*-test or *U* test; significant differences are underlined.

Situation of beach	*n* traps	Species richness				*Aleochara bipustulata*	*Polystomota grisea*	*Polystomota punctatella*
		Species per trap	S.D	Jackknife II	S.D.	Median	Median	Median
Closed	16	<u>11.6</u>	4.2	109	6.3	<u>28.0</u>	<u>2.0</u>	1.0
Open	8	6.8	1.8	56	3.4	2.5	0.0	0.0
t/Z		3.1				3.7	2.5	0.6
P		0.005		<0.001		<0.001	0.01	0.5

the staphylinid fauna of three North Sea islands of Lower Saxony, but beaches were not included. In his study of dunes, salt marshes, and bushy vegetations, the eigenvalues of the first axes ranged between 0.26 and 0.41. This is remarkably lower than the value of 0.71 in the present study, which indicates a greater dissimilarity between the habitats of the Baltic Sea coast. The higher similarity of the species compositions on the North Sea islands might be referred to the island situation, where a lower diversity might have developed than in mainland habitats. However, the high species richness on the three North Sea islands, ranging between 227 and 269 species, does not support this hypothesis; the number of species was higher than the 165 species found in the present study. It is more likely that the similar sandy soil conditions of the three North Sea islands are responsible for the higher similarity of the rove beetle assemblages. In the present study, clay, sand, and shingle soils were included. Similar results concerning environmental parameters were found

Table 6: List of staphylinid species (total number of individuals) at the investigated sites.

Species	Lindhof	Dänisch-Nienhof	Stohl	Stakendorf	Hohenfelde	Hubertsberg	Behrensdorf	Weißenhaus
Acidota crenata	—	—	—	—	—	—	1	—
Aleochara bilineata	—	—	1	—	—	3	5	—
Aleochara binotata	—	—	—	—	1	—	4	—
Aleochara bipustulata	—	12	5	141	363	29	878	221
Aleochara brevipennis	—	—	—	—	—	—	1	—
Aleochara sparsa	26	104	28	14	1	22	25	65
Aleochara verna	—	—	—	—	1	—	3	4
Aloconota gregaria	1	2	1	8	7	3	11	13
Amischa analis	—	—	—	4	2	2	10	9
Amischa decipiens	—	—	—	—	—	—	2	—
Amischa soror	—	—	1	1	1	3	2	—
Anotylus insecatus	—	2	—	—	—	3	—	1
Anotylus rugosus	2	1	1	28	23	2	15	3
Anotylus sculpturatus	1	1	1	1	—	3	—	3
Anotylus tetracarinatus	—	3	—	2	11	—	2	—
Atheta amicula	—	—	1	1	—	1	1	—
Atheta atramentaria	4	4	—	5	8	1	18	3
Atheta cauta	—	—	—	1	—	—	1	—
Atheta celata	—	—	—	1	—	1	1	—
Atheta crassicornis	7	2	—	5	2	2	—	2
Atheta elongatula	—	—	—	—	1	—	—	—
Atheta fungi	1	8	—	9	136	1	12	19
Atheta gagatina	—	—	—	—	—	—	—	1
Atheta graminicola	—	—	—	11	52	2	19	—
Atheta ischnocera	—	—	—	—	—	—	1	—
Atheta laticollis	—	—	—	5	—	—	—	—
Atheta liliputana	—	—	—	—	—	—	1	1
Atheta luteipes	—	—	—	—	4	—	1	—
Atheta marcida	—	—	—	—	—	—	2	—
Atheta melanaria	—	—	—	—	—	—	1	—
Atheta nigricornis	1	2	2	—	—	—	—	1
Atheta oblita	—	—	—	1	—	—	1	1
Atheta palustris	—	—	1	—	—	8	—	1
Atheta sodalis	2	—	—	—	—	1	—	—
Atheta sp.	3	—	—	—	—	—	—	—
Atheta triangulum	1	1	—	—	2	6	2	2
Atheta vestita	3	—	—	13	1	—	4	1
Atheta volans	1	—	1	1	1	1	1	—
Bledius defensus	1	—	1	—	—	—	—	8
Bledius erraticus	1	11	—	1	1	—	—	1
Bledius opacus	—	—	—	1	—	—	—	—
Bledius pallipes	—	1	—	—	—	—	—	—
Bledius subniger	—	—	—	7	—	—	1	—
Brundinia marina	—	—	—	—	—	1	1	2
Brundinia meridionalis	—	—	—	—	—	—	1	—
Cafius xantholoma	—	4	3	7	—	3	15	14
Callicerus obscurus	—	1	—	—	—	—	—	—
Calodera aethiops	—	—	—	2	2	—	3	—
Carpelimus corticinus	—	—	1	5	13	3	7	—
Carpelimus elongatus	1	—	—	—	—	—	—	

Table 6: Continued.

Species	Lindhof	Dänisch-Nienhof	Stohl	Stakendorf	Hohenfelde	Hubertsberg	Behrensdorf	Weißenhaus
Carpelimus foveolatus	—	—	—	1	—	—	—	—
Carpelimus gracilis	—	—	—	—	—	—	1	—
Carpelimus rivularis	—	—	—	—	—	—	—	1
Cordalia obscura	—	—	—	—	—	—	—	1
Cryptobium collare	—	—	—	—	1	—	—	—
Cypha discoideus	—	—	—	—	1	—	—	—
Dacrila fallax	—	—	—	1	—	—	7	—
Dinaraea angustula	—	5	3	5	6	13	5	4
Drusilla canaliculata	1	4	6	59	9	115	123	218
Euaesthetus bipunctatus	—	—	—	—	1	—	—	—
Euaesthetus laeviusculus	—	—	—	—	—	—	1	—
Falagria thoracica	—	—	1	5	—	1	—	—
Gabrius breviventer	—	1	—	2	8	—	7	—
Gabrius vernalis	—	—	—	—	—	—	2	—
Geostiba circellaris	—	—	—	1	—	—	—	—
Gnypeta carbonaria	—	—	—	—	—	—	1	—
Gyrohypnus angustatus	—	—	—	—	—	1	—	5
Habrocerus capilaricornis	—	1	—	—	—	—	—	—
Halobrecta puncticeps	2	—	—	6	5	—	2	1
Haploglossa nidicola	—	—	—	—	—	—	—	1
Heterothops dissimilis	—	—	—	2	—	—	—	—
Hydrosmecta longula	3	—	—	1	—	2	1	1
Ilyobates bennetti	—	—	—	—	1	—	—	—
Ischnosoma splendidus	—	—	1	1	3	—	1	—
Lathrimaeum unicolor	6	—	—	1	—	2	1	6
Lathrobium brunnipes	1	—	—	1	—	—	—	—
Lathrobium fulvipenne	—	—	1	1	—	—	—	—
Lathrobium longulum	—	—	—	—	1	—	—	—
Lathrobium rufonitidum	—	—	—	2	—	—	—	—
Lesteva longoelytrata	—	3	10	3	—	3	1	4
Lesteva sicula	—	—	—	—	1	—	—	—
Liogluta alpestris	—	—	—	1	—	—	—	1
Lordithon thoracicus	—	—	—	—	—	—	1	—
Myllaena intermedia	—	—	—	—	1	—	1	—
Myllaena minuta	—	—	—	—	2	—	3	—
Myrmeocephalus concinnus	—	—	—	—	—	1	—	—
Nehemitropia lividipennis	—	—	—	—	—	—	2	—
Neobisnius procerulus	—	—	—	1	—	—	—	—
Ocalea picata	4	—	—	—	—	—	—	—
Ocypus brunnipes	—	—	—	2	19	—	1	4
Ocypus similis	—	—	—	2	1	—	—	—
Omalium littorale	—	—	—	1	—	—	3	2
Omalium riparium	5	—	1	1	—	—	1	1
Omalium rivulare	5	1	1	6	—	—	—	6
Omalium rugatum	—	—	—	—	—	—	—	3
Othius punctulatus	2	—	—	—	—	—	—	—
Oxypoda acuminata	—	1	—	1	—	—	—	2
Oxypoda brachyptera	—	1	2	—	—	2	—	—
Oxypoda brevicornis	—	—	—	—	—	—	2	—
Oxypoda elongatula	—	—	—	4	3	—	8	—
Oxypoda haemorrhoa	—	—	—	—	—	—	—	1

Table 6: Continued.

Species	Lindhof	Dänisch-Nienhof	Stohl	Stakendorf	Hohenfelde	Hubertsberg	Behrensdorf	Weißenhaus
Oxypoda procerula	—	—	—	2	8	—	5	—
Oxytelus fulvipes	—	1	—	—	—	—	—	—
Oxytelus sculptus	—	—	—	—	1	—	—	—
Pachnida nigella	—	—	—	18	6	—	1	—
Paederus riparius	—	—	—	8	—	—	—	—
Philonthus cognatus	—	—	—	—	—	1	—	—
Philonthus decorus	1	—	—	—	—	—	—	—
Philonthus fumarius	—	—	—	6	—	—	—	—
Philonthus fuscipennis	—	—	—	—	—	—	—	1
Philonthus micans	—	—	—	2	—	—	—	—
Philonthus quisquiliarius	—	—	—	—	—	—	1	—
Phytosus balticus	—	—	—	2	—	—	—	—
Phytosus spinifer	—	1	—	1	—	—	6	—
Placusa depressa	—	—	—	—	—	—	1	—
Placusa pumilio	—	1	—	—	—	—	—	—
Plataraea brunnea	2	—	—	—	—	—	—	—
Platydracus stercorarius	—	—	—	1	—	—	—	1
Polystomota grisea	2	—	9	21	1	—	17	3
Polystomota punctatella	10	1	1	5	—	—	26	—
Quedius fuliginosus	5	—	2	4	3	—	2	3
Quedius fumatus	1	—	—	—	—	—	—	—
Quedius molochinus	—	—	2	—	—	—	—	—
Quedius picipes	1	—	—	—	—	—	—	—
Quedius xanthopus	1	—	—	—	—	—	—	—
Rugilus rufipes	1	—	—	—	—	1	—	2
Scopaeus minutus	—	—	—	—	—	1	—	—
Sepedophilus marshami	—	—	—	1	—	—	—	—
Stenus atratulus	—	—	—	—	1	—	—	—
Stenus bimaculatus	—	1	—	12	9	—	13	—
Stenus boops	—	—	—	—	—	—	1	—
Stenus brevipennis	—	—	—	—	—	—	1	—
Stenus brunnipes	—	—	—	—	—	—	1	—
Stenus canaliculatus	—	—	—	3	—	3	28	—
Stenus clavicornis	1	—	—	6	6	2	6	2
Stenus formicetorum	—	—	—	3	21	—	—	—
Stenus fossulatus	—	2	4	—	—	3	—	8
Stenus juno	—	—	—	1	30	—	6	3
Stenus nigritulus	—	—	—	—	—	—	3	—
Stenus nitens	—	—	—	—	3	—	—	—
Stenus pallipes	—	—	—	5	3	—	22	—
Stenus palustris	—	—	—	—	—	—	1	—
Stenus pusillus	—	—	2	—	2	—	—	—
Stenus solutus	—	—	—	1	—	—	—	—
Tachinus corticinus	—	—	1	—	1	—	—	—
Tachinus signatus	1	—	—	3	7	2	—	1
Tachyporus atriceps	—	2	—	1	—	—	—	—
Tachyporus chrysomelinus	—	—	—	1	—	—	—	—
Tachyporus dispar	1	4	1	1	—	34	2	2
Tachyporus hypnorum	—	4	—	2	—	1	2	6
Tachyporus nitidulus	3	44	12	2	4	110	—	36
Tachyporus obtusus	—	—	—	—	—	1	—	1

Table 6: Continued.

Species	Lindhof	Dänisch-Nienhof	Stohl	Stakendorf	Hohenfelde	Hubertsberg	Behrensdorf	Weißenhaus
Tachyporus pusillus	—	—	—	—	1	1	—	—
Tachyporus quadriscopulatus	—	—	1	—	—	—	—	—
Tachyporus solutus	—	—	—	2	—	—	—	—
Tasgius ater	—	—	—	—	—	1	—	—
Tasgius compressus	—	2	—	—	—	—	—	—
Tasgius melanarius	1	5	1	3	—	—	1	1
Tasgius morsitans	10	4	1	2	1	1	—	1
Tasgius winkleri	—	5	—	1	—	1	—	—
Thinonoma atra	—	—	—	1	1	—	—	3
Tinotus morio	—	—	—	—	—	—	1	—
Xantholinus linearis	1	—	1	—	2	3	—	5
Xantholinus longiventris	3	1	—	3	7	3	1	—
Zyras limbatus	—	—	—	—	—	—	—	2

in both investigations. Factors connected with wooded or nonwooded situations and moisture conditions were mainly responsible for the different species compositions of the habitats in both studies. Moreover, in a similar analysis of the spider fauna from the same sites analysed in the present investigation, the wood situation and moisture content were the main environmental parameters responsible for the separation of spider assemblages [19].

In specific investigations of North Sea salt marshes, Schaefer [20] found two habitat types: one from 80 cm to 130 cm above NN and one from 20 cm to 60 cm above NN. Consequently, elevation and frequency of inundation were the most important environmental factors. This was also true for a comprehensive study of staphylind assemblages in Baltic Sea salt marshes that included sites from the states of Schleswig-Holstein (north-western Germany) and Vorpommern (north-eastern Germany) [9]. In the salt marshes, soil conditions were less important than in the investigations on the sandy islands of Lower Saxony and in the present study of the Baltic Sea coast habitats.

The vertical gradient from sea level to higher elevations certainly affects the staphylinid assemblages of beaches and primary dunes. There has been no increase in species richness of Staphylinidae found between sandy beaches and primary dunes. However, specific species occurred on beaches that were not found in primary dunes, for example, *Polystomota grisea* and *Phytosus spinifer*. In contrast to Staphylinidae, a significant increase of species richness from beaches to primary dunes was found for spiders [7, 19]. Schaefer [7] also found an increase in species richness in the adjacent habitats of dunes and dry grassland, representing a gradient of increasing elevation. The decrease of species richness from higher elevated sites to lower elevated sites in coastal habitats was referred to the higher instability of habitats exposed to the sea [20]. This effect of instability was also supposedly responsible for the decrease of species richness of other organisms such as meiofauna and macrofauna [21, 22] and might also account for the elevation gradient of species richness in salt marshes [23]. Since the high-energy input by wind and waves combined with high erosion is greater on shingle beaches than on sandy beaches, the low species richness of shingle beaches might be referred to the effect of instability. Nevertheless, several species are adapted to such unstable habitats as could be shown for the aleocharine species, for example, *Aleochara bipustulata*, *Polystomota grisea*, and *P. punctatella* that all live as parasitoids in flies without host specificity except host size [24]. The first species occurs in many sandy habitats of the mainland, but the abundance decreases from high elevated dune sites to low elevated beach sites at coasts. On the other hand, the two other species are restricted to beach habitats only although host species are the same as in *A. bipustulata*. It can be assumed that the flies live off the rich wrack debris on beaches which exhibit rich food resources for parasitoid staphylinids and can compensate for the loss in species richness found in other animal groups.

When considering all investigated parameters, lagoons are certainly the most species-rich habitats for staphylinids in this study. However, sandy beaches also reveal high numbers of species, particularly the endangered species. 5 species on the red list of endangered species status 1 and 2 have been found on sandy beaches, whereas only 2 were found at lagoons. Moreover, total species richness on sandy beaches as estimated by the Jackknife II method is only slightly lower than at lagoons, but distinctly higher than in all other habitat types. The eminent status of sandy beaches becomes still more relevant concerning exclusive species for the habitat. According to this investigation, only one exclusive species has been found at lagoons, *Stenus pallipes*. However, this species is abundant at most lake margins in northern Germany. In comparison, 5 exclusive species have been found at beaches. Overall, beaches support a higher number of endangered species and a much higher number of exclusive species compared to lagoons. Therefore, from a regional point of view, sandy beaches are the hot spots of species richness.

Another habitat also seems to be of great value for the coastal species richness at the Baltic Sea: the woodless cliffs. Species richness is distinctly lower than on beaches, but many exclusive and rare species live there. In particular, *Stenus fossulatus*, *Bledius defensus* (RL, 2), and *Anotylus insecatus*

seem to be adapted to open, bare soil habitats on clay or silt soils. The distribution of the two species *A. insecatus* and *B. defensus* also indicates that the specific mixtures of sand and silt material are responsible for the occurrence of these species. Regarding their occurrence in the sand-silt gradient, they prefer completely different mixtures of sand and silt/clay. *B. defensus* seems to be restricted to a very narrow range of the mixture gradient. Thus, not only the conservation of the cliff situation is needed but also the whole range of sand and silt mixtures is necessary to preserve habitats for the different demands of species.

The present results show that tourism significantly decreases species richness on sandy beaches. According to both species per trap and Jackknife II species richness, beaches open to tourists reveal approximately half of their potential species richness. The loss of species on beaches has been attributed to tourist activities for several species and animal groups. Unfortunately, this ecological problem has not been investigated at the species level in Germany. In South Africa, Moffett et al. [25] quantified the damage by tourist trampling for intertidal macrofauna and found a loss of 5% to 70% depending on the species. The negative effect of trampling on *Talitrus saltator* (Crustacea) has been determined in different studies [26–28]. According to their results, it is not possible to compensate for the losses during short periods of tourist activity during summer. The loss of the ground beetle *Cicindela maritima* to near extinction on Baltic Sea beaches has also been referred to tourist activity [29]. Discussions about this species considered not only the sensibility of larvae against trampling but also the large home range to be the cause for the high losses. The high sensibility of beach species to trampling can certainly be referred to the porous sandy soils. The existing instability of the sandy soils caused naturally by wind and waves increases dramatically if tourist trampling is added. It can be assumed that the increase of instability triggering the species loss from sandy to shingle beaches also causes the species loss from closed to open beaches. At present, no studies investigating the degree of loss under different intensities of tourist activity are available. According to Kammer and Schernewski [30], tourist activity fluctuates in relation to weather, season, and week day. In their study, they found tourist densities between $7\,m^2$ person^{-1} and $84\,m^2$ person^{-1}. However, no studies are available that provide information about the level of tourist density which can be tolerated while preserving the species richness of beaches. Nevertheless, the present study documents that beach conservation is needed in order to preserve the species richness of coasts, not only for birds.

Appendix

For more details, see Table 6.

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Research Article

The Ant Genus *Dorymyrmex* Mayr (Hymenoptera: Formicidae: Dolichoderinae) in Colombia

Fabiana Cuezzo[1] and Roberto J. Guerrero[2,3]

[1] *CONICET, INSUE, Facultad de Ciencias Naturales e IML, Miguel Lillo 205, T4000JFE San Miguel de Tucumán, Argentina*
[2] *Grupo de Investigación en Insectos Neotropicales, Instituto de Investigaciones Tropicales (INTROPIC), Universidad del Magdalena, Carrera 32 No. 22-08, Santa Marta, Colombia*
[3] *Instituto de Zoología y Ecología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela*

Correspondence should be addressed to Fabiana Cuezzo, cuezzof@yahoo.com.ar

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The aim of this paper is to actualize the taxonomy of *Dorymyrmex*, by addressing problems at both the genus and the species levels. We also explore the taxonomy and distribution of *Dorymyrmex* in Colombia. We list, diagnose, and key nine species in the country, including three new species: *Dorymyrmex amazonicus* n. sp. Cuezzo & Guerrero, *Dorymyrmex xerophylus* n. sp. Cuezzo & Guerrero, and *Dorymyrmex tuberosus* n. sp. Cuezzo & Guerrero. We provide a detailed description of these new species based on the worker caste and, where possible, other castes. All localities where *Dorymyrmex* was collected or cited in the literature were mapped to provide a graphical view of its range.

1. Introduction

Dorymyrmex Mayr [1] is one of the most diverse and complex genera of the ant subfamily Dolichoderinae from a taxonomical and biogeographical point of view. In a recent study, Ward et al. [2] provided a detailed phylogeny of Dolichoderinae based on molecular data and proposed an internal arrangement of this subfamily in four tribes, based in one unrooted topology: (((Dolichoderini, Leptomyrmecini), Bothriomyrmecini, Tapinomini)). *Dorymyrmex* is considered by these authors as a monophyletic member of Leptomyrmecini and sister group of *Forelius* Emery, 1888 [3].

This genus has a strictly American distribution, inhabiting in the Nearctic and Neotropical regions and containing more than 90 species, several undescribed. Reasons for considering as an especially difficult group of ants include variability within species in color, pilosity, sculpture, and size. The majority of species are actually poorly defined, often distinguished only on the basis of color. No broader modern taxonomic key exists at species level. The most recent contribution to solve the taxonomic jungle of *Dorymyrmex* was Snelling [4], who built on work by Trager [5] to clarify the taxonomy of the Nearctic species.

Despite being considered by many ant collectors as "road side weeds", several species of *Dorymyrmex* shown a high degree of endemicity, specialized habitat preferences, and varied population structure. Some species may serve as potential agents of biological control of annual crop pests [5, page 12]. Species of *Dorymyrmex* nest preferentially in dry or disturbed habitats, generally in soil without vegetation cover. Several species are known to attend aphids and other hemipterous insects. Such behavior is common in other Dolichoderinae genera and related subfamilies.

The main purposes of this paper are to provide a redefinition of *Dorymyrmex* using morphological characters from worker, queen, and male and to make a revision of the genus in Colombia. We describe three new species and provide a key to workers of all nine species found in Colombia. This is the first contribution of a series of systematic studies about this still poorly known ant genus.

2. Materials and Methods

2.1. Studied Material Belongs to the Following Institutions

CASC: California Academy of Sciences, San Francisco, California, USA.

CEUM: Insect Collection, Universidad del Magdalena, Santa Marta, Magdalena, Colombia.

IAvH: Insect Collection Instituto Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia.

ICN: Insect Collection, Instituto de Ciencias Naturales, Universidad Nacional, Bogotá D.C., Colombia.

IFML: Instituto Fundación Miguel Lillo, Tucumán, Argentina.

LACM: Los Angeles County Museum of Natural History, Los Angeles, California, USA.

MCZC: Museum of Comparative Zoology, Cambridge, Massachusetts, USA.

MHNG: Muséum d'Histoire Naturelle, Geneva, Switzerland.

MZSP: Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil.

NHMB: Natural History Museum of Basel, Switzerland.

USNM: United States National Museum of Natural History/ Smithsonian Institution, Washington D.C., USA.

2.2. Primary Type Material Examined

2.2.1. Dorymyrmex amazonicus **Holotype**. Amazonas, Leticia, 4°13′08″S 69°56′29″W, 06 Jun 1976, COLOMBIA (ICN). Paratypes: 2w, Amazonas, Leticia, 4°13′08″S 69°56′29″W, 06 Jun 1976, COLOMBIA: 1w (CASC), 1w (IFML).

2.2.2. Dorymyrmex bicolor **Syntypes**. 1w and 1q, Phoenix, USA (MCZC); 2w, Tucson, Arizona, USA (MHNG).

2.2.3. Dorymyrmex biconis **Syntypes**. 2w, Sierra Nevada de Santa Marta, San Antonio, Guajira Prov., COLOMBIA (MHNG).

2.2.4. Dorymyrmex brunneus **Lectotypes**. 2w, São Paulo, BRAZIL (MHNG) designed by Kempf 1975: 375 [6].

2.2.5. Dorymyrmex goeldii **Syntypes**. 2w, Pará, BRAZIL (MHNG).

2.2.6. Dorymyrmex insanus **Neotype**. 1w, Interstate 20, 12 mi E Big Spring, Howard Co., Texas, USA, 16 April 1981, coll. by W. F. Buren, (USNM) designed by Snelling 1995: 4 [4].

2.2.7. Dorymyrmex tuberosus **Holotype**. 1w, Santander: Bucaramanga, UIS, 906 m., 7°21′0.12″N 73°20′1.22″W, COLOMBIA, Jun 2003, N. Ruiz & F. Fernández coll., (ICN). Paratypes: 7w, Bolivar, San Juan Nepomuceno, 24.Oct.1990 F. Bekker leg. Ex *Manihot esculenta*, COLOMBIA: 2w, (USNM); same data and loc. than holotype, 4w, (ICN), 1w (CASC).

2.2.8. Dorymyrmex xerophylus **Holotype**. 1w, Magdalena, Santa Marta, Vereda Mosquito, 11°10′23.6″N 74°10′45″W 96 m, manual collection; 03 Jan 2008, R. Guerrero, coll., COLOMBIA (ICN). Paratypes: 1w, La Guajira, Zona El Cerrejón, 11°1′59.88″N 72°39′0″W, 240 m, COLOMBIA, pitfall trap, 19 Dec 2006, R. Guerrero, coll., (ICN); 1w, Magdalena, Santa Marta, Vereda Mosquito, 11°10′23.6″N 74°10′45″W 96 m, COLOMBIA, manual collection; 03 Jan 2008, R. Guerrero, coll., (CEUM).

2.3. Measurements and Indexes. Measurements were taken with micrometer ocular to 40x–80x attached to a stereoscope. All measurements are expressed in mm. The measurements and indexes used were the following:

HL: head length, in full-face view, the maximum length of the head capsule,

HW: head width, in full-face view, the maximum width of the head capsule above the eyes,

EL: eye length, the maximum length of the eye in full-face view,

EW: eye width, the maximum width of the eye in full-face view,

SL: scape length, the length of the scape from the apex to the basal flange, not including the basal condyle,

WL: Weber's length, measured in perfect lateral view of the mesosoma, diagonally from the posteroventral corner of the mesosoma to the farthest point on anterior face of the pronotum, excluding the neck.

Indexes

CI: Cephalic Index = HW × 100/HL.

SI: Scape Index = SL × 100/HL.

REL: Relative Length of the Eye = EL × 100/HL.

OI: Ocular Index = EW × 100/EL.

TLI: Thorax Length Index = WL × 100/HL.

Holotypes will be deposited in IAvH, ICN and paratypes in CASC, CEUM, IFML, and MZSP. All the species recorded were re-described based in all castes (worker, gyne, and male) when material was available.

Male terminology follows Ward [7].

3. Results

3.1. Synopsis. Genus *Dorymyrmex* Mayr, 1866 [1].

Type species: *Dorymyrmex flavescens*, by monotypy.

Region: Neotropical, Nearctic.

Dorymyrmex Mayr (1866a: 494 [1]).

Psammomyrma as subgenus of *Dorymyrmex*: Forel, 1912: 43 [8]. Type species: *Dorymyrmex planidens*, by subsequent designation of Wheeler, 1913: 82 [9]. Kempf, 1972: 100 [10].

Psammomyrma as junior synonym of *Dorymyrmex*: Forel, 1913: 350 [11]; Santschi, 1922: 365 [12]; Wheeler, W. M. 1922: 689 [13]; Snelling and Hunt, 1976: 93 [14]; Shattuck, 1992: 77 [15]; Bolton, 1994: 27 [16]; Bolton, 2003: 86 [17].

Conomyrma as subgenus of *Dorymyrmex*: Forel, 1913: 350 [11]. Type species: *Prenolepis pyramica*, by subsequent designation of Santschi, 1922: 365 [12]; Forel, 1917: 248 [18]; Wheeler, W. M., 1922: 689 [13]; Gallardo, 1930: 147 [19]; Smith, M. R., 1951: 837 [20]; Smith, M. R., 1958: 140 [21].

Conomyrma as a genus: Kusnezov, 1952: 429 [22]; Kusnezov, 1959: 51 [23]; Kusnezov, 1964: 66 [24]; Kempf, 1972: 78 [10]; Snelling, 1973: 1 [25]; Smith, D. R., 1979: 1419 [26]; Holldöbler and Wilson, 1990: 17 [27]; Jaffé, 2004: 9 [28].

Conomyrma as junior synonym of *Dorymyrmex*: Brown Jr., 1973: 179 (provisional) [29]; Shattuck, 1992: 77, [15]; Bolton, 2003: 86, [17].

Araucomyrmex as genus: Gallardo, 1919: 249 [30]. Type species: *Dorymyrmex tener*, by original designation; Wheeler, 1922 [13]: 689; Kusnezov, 1956: 28 [31]; Kusnezov, 1959: 51 [23]; Kusnezov, 1964: 66 [24]; Kempf, 1972: 25 [10]; Snelling, 1975: 9 [32]; Snelling and Hunt, 1976: 93 [14]; Dlussky and Fedoseeva, 1988: 77 [33].

Araucomyrmex as junior synonym of *Conomyrma*: Snelling, 1981: 402 [34].

Araucomyrmex as junior synonym of *Dorymyrmex*: Brown Jr., 1973: 178 (provisional) [29]; Shattuck,1992: 77 [15]; Bolton, 2003: 87 [17].

Ammomyrma as a subgenus of *Dorymyrmex*: Santschi, 1922: 365 [12]. Type species: *Dorymyrmex exanguis*, by original designation. Gallardo, 1930: 147 [19]; Kempf, 1972: 100 [10].

Ammomyrma as junior synonym of *Araucomyrmex*: Snelling and Hunt, 1976: 93 [14].

Ammomyrma as junior synonym of *Dorymyrmex*: Shattuck, 1992: 77 [15]; Bolton, 2003: 87 [17].

Biconomyrma as a subgenus of *Conomyrma*: Kusnezov, 1952: 429 [22]. Type species: *Dorymyrmex pyramicus* var. *brunneus* (now *Dorymyrmex brunneus*), by subsequent designation of Kusnezov, 1959: 51 [23].

Biconomyrma as genus: Kusnezov, 1959: 51 [23]; Kusnezov, 1964: 67 [24].

Biconomyrma as junior synonym of *Conomyrma*: Smith, M. R., 1958: 140 [21]; Kempf, 1972: 78 [10].

Biconomyrma as junior synonym of *Dorymyrmex*: Shattuck, 1992: 78 [15]; Bolton, 2003: 87 [17].

Spinomyrma as subgenus of *Dorymyrmex*: Kusnezov, 1952: 429 [22]. Type species: *Dorymyrmex alboniger*, by subsequent designation of Kusnezov, 1959: 51 [23]; Kempf, 1972: 100 [10].

Spinomyrma as genus: Kusnezov, 1956: 30 [31] (in key); Kusnezov, 1959: 51 [23]; Kusnezov, 1964: 66 [24].

Spinomyrma as junior synonym of *Dorymyrmex*: Kempf, 1972: 100 [10]; Snelling and Hunt, 1976: 93 [14]; Shattuck, 1992: 78 [15]; Bolton, 2003: 87 [17].

The characters used here to identify *Dorymyrmex* are based on the diagnosis proposed by Shattuck [15, page 78], with some differences. Characters mentioned below, with**, are redefined and based in the Shattuck proposal; characters with* are new.

We have not used the length of curved hairs placed in the dorsal clypeal margin proposed by Shattuck [15], because it is quite variable along the genus. Some species have these setae shorter, not reaching the distal edge of closed mandibles.

3.2. Diagnostic Characters Common to All Castes. Apical teeth of mandible elongate, at least twice longer than preapical**. This character was used by Shattuck [15] only for workers but is also a constant in all known queens and males. Psammophore is present as a discrete group of elongated hairs, uniform in length, arranged in a definite pattern, on the ventral face of head**. Third segment of the maxillar palp elongate, longer than segments 4 + 5 + 6 joined together.

3.3. Worker Diagnosis. Monomorphic to slightly polymorphic ants. Mandibles with 5-6 teeth and 2–4 denticles on masticatory margin and several denticles on basal margin*. Dorsal surface of mandible longitudinally striated*. Pair of erect setae on the dorsal face of pronotum present or absent*. A well-defined spine, cone, or tubercle always present between dorsal and declivitous faces of propodeum**.

3.4. Queen Diagnosis. Forewing with close radial cell*. Forewing with 1-2 cubital cell and 0-1 discoidal cells*. Hindwing with only 0–3 closed cells placed in the basal part of the wing*.

3.5. Male Diagnosis. Antennal scape relatively short, at most only slightly longer than the length of funicular segments 1 + 2 + 3*. Second funicular segment with a lateral bend**. Mandible with 2–4 teeth (sometimes with 2 or more denticles)**. Forewing with close radial cell*.

4. Dorymyrmex in Colombia

Only four species of *Dorymyrmex* have been mentioned in the most recent list of Neotropical ants [35] in Colombia. We record 9 species, two new records and three new for science: *Dorymyrmex amazonicus* n. sp., *Dorymyrmex tuberosus* n. sp., and *Dorymyrmex xerophylus* n. sp.

Table 1: Major characters differing among worker, queen, and male of *Dorymyrmex* and *Forelius*.

Character	*Dorymyrmex*	*Forelius*
Psammophore	Present	Absent
Third maxillary palp segment	Elongated	Subequal in length to the remaining segments
Apical tooth of mandible	Greatly elongated	Slightly larger than the subapical
Queen and male forewing	With a close radial cell	With an open radial cell

Major characters used to separate *Dorymyrmex* from *Forelius,* the closest Dolichoderinae ant genus found in South America are given in Table 1.

4.1. List of Dorymyrmex in Colombia

D. amazonicus n. sp. Cuezzo & Guerrero

D. bicolor Wheeler, 1906 [36]

D. biconis Forel, 1912 [8]

D. brunneus Forel, 1908 [37]

D. goeldii Forel, 1904 [38]

D. insanus (Buckley, 1866) [39]

D. pyramicus Roger, 1863 [40]

D. tuberosus n. sp. Cuezzo & Guerrero

D. xerophylus n. sp. Cuezzo & Guerrero.

4.2. Key to Dorymyrmex Workers in Colombia. This key is based on worker caste of all valid species of *Dorymyrmex* found in Colombia.

(1) Mesosomal profile with two well-developed tubercles, one on the posterodorsal margin of mesonotum and the other on the dorsal face of propodeum (Figures 3(b) and 11(b)) ... (2).

(1′) Mesosomal profile lacking a mesonotal tubercle (Figures 1(b), 8(b), and 9(d)); in some specimens, we can see an angle in the posterior end of mesonotum (Figures 2(b), 7(b), 7(d), 9(b), and 12(b)) but never a well-differentiated knob (= tubercle). Mesosomal profile bearing only a well-developed tubercle placed between the dorsal and declivitous faces of propodeum. ... (3).

(2) Body concolorous light reddish brown to yellowish. Pubescence sparse. Dorsal face of propodeum, anterior to the tubercle, convex. Propodeal tubercle stout and higher than the promesonotal profile in lateral view (Figure 3(b)) ... *D. biconis.*

(2′) Body concolorous dark brown, pubescence dense. Dorsal face of propodeum, anterior to the tubercle, straight. Propodeal tubercle thin and lower than or at the same level than the promesonotal profile in lateral view (Figure 11(b)) ... *D. tuberosus* n. sp.

(3) Body bicolored, head and mesosoma always yellow-reddish, gaster dark brown to black; some specimens could be lighter but always with the gaster darker than the rest of the body ... (4).

(3′) Body concolorous orange or medium to dark brown ... (5).

(4) Promesonotal profile continuous (Figure 9(d)). Propodeal tubercle pointed backward. Petiolar scale short, sharp apically, forward directed. Subpetiolar process feebly developed, convex, and covering all the ventral surface of petiole ... *D. pyramicus.*

(4′) Promesonotal profile interrupted in posterior end of mesonotum, forming an angle and determining a clear mesosomal dorsal and declivitous face (Figure 2(b)). Petiolar scale tall, rounded apically, upward directed. Subpetiolar process not well developed, only conspicuous in the ventral end of petiole ... *D. bicolor.*

(5) Posterior end of mesosoma, in lateral view, forming an angle but not a tubercle (Figures 7(b), 7(d), 9(b), 12(b), and 12(c)) ... (6).

(5′) Posterior end of mesosoma straight, without a differentiated dorsal and declivitous face (Figures 1(b) and 8(b)) ... (8).

(6) Small ants, TLI: <117. Scape short, not surpassing the posterior margin of head more than twice its maximum diameter (Figure 12(a)). Posterior margin of head strongly convex ... *D. xerophylus* n. sp.

(6′) TLI >117. Scape longer, surpassing the posterior margin of the head more than three times its maximum diameter (Figures 7(a), 7(c), and 9(a)). Posterior margin of head straight to concave in the middle but never convex ... (7).

(7) Posterior margin of head always medially concave (Figure 9(a)). Promesonotal profile uniformly convex. Dorsal face of propodeum, anterior to the tubercle, straight (Figure 9(b)) ... *D. insanus.*

(7′) Posterior margin of head straight to slightly concave in the middle. Promesonotal profile straight to feebly convex. Dorsal face of propodeum, anterior to the tubercle, sinuous (Figures 7(b) and 7(d)) ... *D. brunneus.*

(8) Lateral margin of head, in full-face view, strongly convex, with compound eyes placed far inside the head capsule (Figure 1(a)). Posterior margin of head concave in the middle. Propodeal tubercle well developed and upward directed (Figure 1(b)). ... *D. amazonicus* n. sp.

(8′) Lateral margin of head slightly convex (Figure 8(a)). Posterior margin of head strongly convex (Figure 8(a)). Propodeal tubercle poorly developed (Figure 8(b)) ... *D. goeldii.*

(a) (b)

(c)

FIGURE 1: *Dorymyrmex amazonicus* n. sp. worker, holotype. (a) Head in full-face view; (b) body in lateral view; (c) body in dorsal view (CASENT0192703). Pictures and labels of localities of each photographed specimen are available at http://www.antweb.org/.

5. Species Account

5.1. Dorymyrmex amazonicus Cuezzo & Guerrero n. sp. *(Figures 1(a)–1(c) and 13)*

5.1.1. Diagnosis

Worker. Large ant, TLI: 133–135. Head with lateral margins broadly convex and posterior margin of head concave medially. Short scape not surpassing the posterior margin of the head more than three times its apical width.

5.1.2. Description

Worker

Measurements. Holotype (Paratypes = 2). HL: 1.00 (1.02–1.04). HW: 0.92 (0.94–0.96). EL: 0.30 (0.28–0.30). EW: 0.22 (0.22). SL: 1.06 (1.06–1.08). WL: 1.32 (1.36–1.40). CI: 92 (92). SI: 106 (104). REL: 30 (27–29). OI: 73 (73–79). TLI: 132 (133–135).

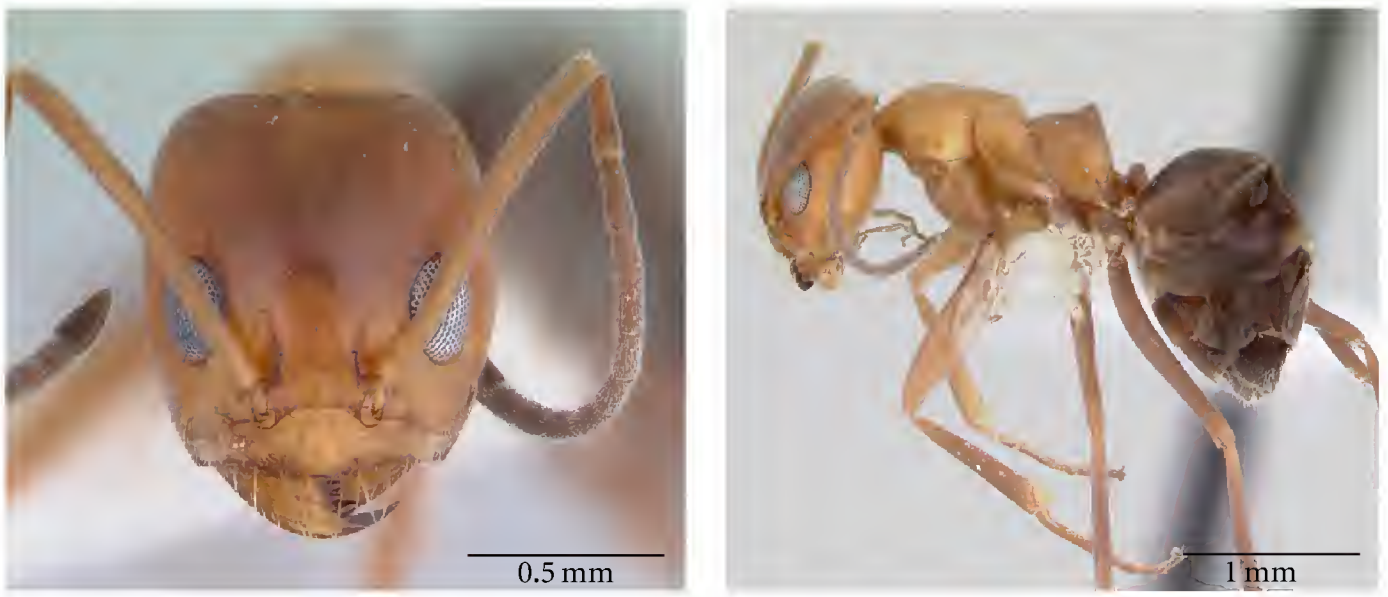
Head and scapes reddish-brown; lateral clypeal region and dorsal face of mandibles testaceous yellow; masticatory mandibular margin dark brown; mesosoma, legs, petiole, and gaster yellowish-brown. Whitish, short, and appressed pubescence covering all the body tagma. *Head* (Figure 1(a)): head slightly longer than wide, with lateral margins strongly convex and posterolateral corners rounded. Posterior margin of the head concave in the middle. Mandibles strongly striate, with apical tooth four times longer than others. Masticatory margin with four denticles. Compound eye well developed and placed far inside the head capsule. Psammophore with short hairs disposed in a semicircle; the hairs on the top line are close to the foramen magnum and not reaching the oral cavity. Scape short (SI = 104–106) not surpassing the posterior margin of the head more than three times its apical width. *Mesosoma* (Figure 1(b)): dorsal face of pronotum with no erected setae, pronotum and mesonotum in profile forming a continuous line, not interrupted (Figure 1(b)), metanotal suture not impressed. Dorsum of propodeum weakly sinuate. Propodeal cone acute, upward directed with wide base. Apical point of the propodeal cone reaches the same level of the highest point of pro-mesonotum in lateral view. Declivitous face of propodeum convex. *Metasoma*: petiolar scale wide, thin and rounded in the apex.

Queen and Male. Unknown.

Examined Material. Type series.

Geographic Distribution. Colombia, Amazon rainforest. Only known from its type locality (Figure 13).

Etymology. The name *amazonicus* refers to the apparently unusual distribution of this species, the Amazon rainforest in Colombia. It is a noun in apposition and invariant.



(a) (b)

(c)

FIGURE 2: *Dorymyrmex bicolor* worker. (a) Head in full-face view; (b) body in lateral view; (c) body in dorsal view (CASENT0179517). Pictures and labels of localities of each photographed specimen are available at http://www.antweb.org/.

Natural History. The type series was collected in the vicinity of an Amazonian forest relict, outside Leticia (Colombia). All the specimens were collected in open deforested habitat, probably this is an indication of the preference of this species to nest in highly anthropic or disturbed environments.

5.1.3. Comments. At first view, this species could be confused with *D. brunneus,* but a greater TLI, shorter scapes, eyes placed deep inside the head capsule, and a continuous mesosomal profile in *D. amazonicus* are the best characters to separate it from *D. brunneus*. Other *Dorymyrmex* species found in Colombia share with *D. amazonicus* the shape of mesonotal profile (i.e., *D. pyramicus*), but all the characters, given in the key and in the diagnosis above, are useful to separate *D. amazonicus* from other species of *Dorymyrmex* found in Colombia.

5.2. Dorymyrmex bicolor Wheeler, 1906 [36] *(Figures 2(a)–2(c), 6(a), and 13)*

Dorymyrmex pyramicus var. *bicolor* Wheeler, 1906: 342 [36]. Description of worker.

Dorymyrmex pyramicus var. *bicolor* Wheeler: Gallardo, 1916: 63 [41]. Description of queen.

Conomyrma (*Biconomyrma*) *bicolor* (Wheeler): Kusnezov, 1952: 430 [22].

Conomyrma bicolor Wheeler: Snelling, 1973: 4 [25]; Johnson, 1989: 192 [42].

Dorymyrmex bicolor Wheeler: Cole Jr., 1957: 130 [43]; Crozier, 1970: 114 (karyotype) [44]; Shattuck, 1992: 85 [15]; Shattuck, 1994: 75 [45]; Snelling, 1995: (key) [4]; Bolton et al. 2006 (catalog) [46].

5.2.1. Diagnosis

Worker. CI equal or over 90. Worker bicolored: head, mesosoma, and petiole, dark reddish; gaster black (Figures 2(b) and 2(c)). Same pattern of color is found in queen. Posterior margin of the head slightly concave in frontal view. Dorsal face of pronotum with no erect setae. Mesonotal profile continuous with pronotum, with a distinct dorsal and declivitous face before mesopropodeal suture.

Queen. Head slightly wider than long with the posterior margin of head strongly concave (Figure 6(a)). Maximum diameter the head behind of compound eyes.

Male. Unknown.

(a) (b)

(c)

FIGURE 3: *Dorymyrmex biconis* worker. (a) Head in full-face view; (b) body in lateral view; (c) body in dorsal view (CASENT0179483). Pictures and labels of localities of each photographed specimen are available at http://www.antweb.org/.

5.2.2. Descriptions

Worker

Measurements. ($n = 25$): HL: 0.78–1.1; HW: 0.7–1; EL: 0.18–0.28; EW: 0.15–0.2; SL: 0.78–1.05; WL: 1.25–1.53; CI: 90-91; SI: 105–111; REL: 23–25; OI: 73–86; TLI: 139–161.

Worker bicolored: head, mesosoma, and petiole, dark reddish; gaster black. Funicle and legs darker than rest of the body in some specimens. *Head* (Figure 2(a)): square in frontal view, almost as wide as long, sides slightly convex. Posterior margin of head feebly convex medially. Scape short (SI: 105–111). Psammophore with short hairs disposed in a triangle, far from the foramen magnum, slightly reaching the oral cavity. *Mesosoma* (Figures 2(b) and 2(c)): pro-mesonotum in profile, forming a continuous convexity; end of mesonotum with well-differentiated dorsal and declivitous faces, anterior to metanotal suture. Propodeal tubercle short, upward directed, with wide base. *Metasoma*: petiolar scale forward directed.

Queen

Measurements. ($n = 7$): HL: 1.18–1.23; HW: 1.25–1.33; EL: 0.38–0.4; EW: 0.18–0.23; SL: 1.08–1.13; WL: 2.43–2.6; CI: 106–108; SI: 91-92; REL: 32-33; OI: 47–56; TLI: 206–212.

Similar to worker in color. Whitish pubescence covering all body tagma. *Head*: Wider than long, with convex sides, in frontal view. Posterolateral corner rounded, posterior margin of head slightly concave (Figure 6(a)). Masticatory margin of mandible with six teeth and two or three denticles; basal margin completely dentate with a well-differentiated angle between both margins. Scape surpassing posterior margin of the head by more than twice its maximum diameter. *Mesosoma*: Parapsidal furrow well developed, diverging forward, axilla not divided. Anepisternum and katepisternum incompletely divided by a short pleural suture. Wings: forewing with only one close radial cell, one cubital cell, and no discoidal cell; pterostigma well developed, longer than wide. Hindwing with three cells closed in basal area; hamuli with 12 hooks. *Metasoma*: petiolar scale tall, stout, forward directed, and rounded apically. Ventral face of petiole slightly convex. Gaster with dark brown tergites and covered with whitish pubescence.

Male. Unknown.

Examined Material. COLOMBIA: La Guajira, Riohacha, Corregimiento Camarones, SFF Los Flamencos, 1w (CASC), 4w (CEUM); Magdalena, 1w (ICN), 1w (LACM) (see Figure 12).

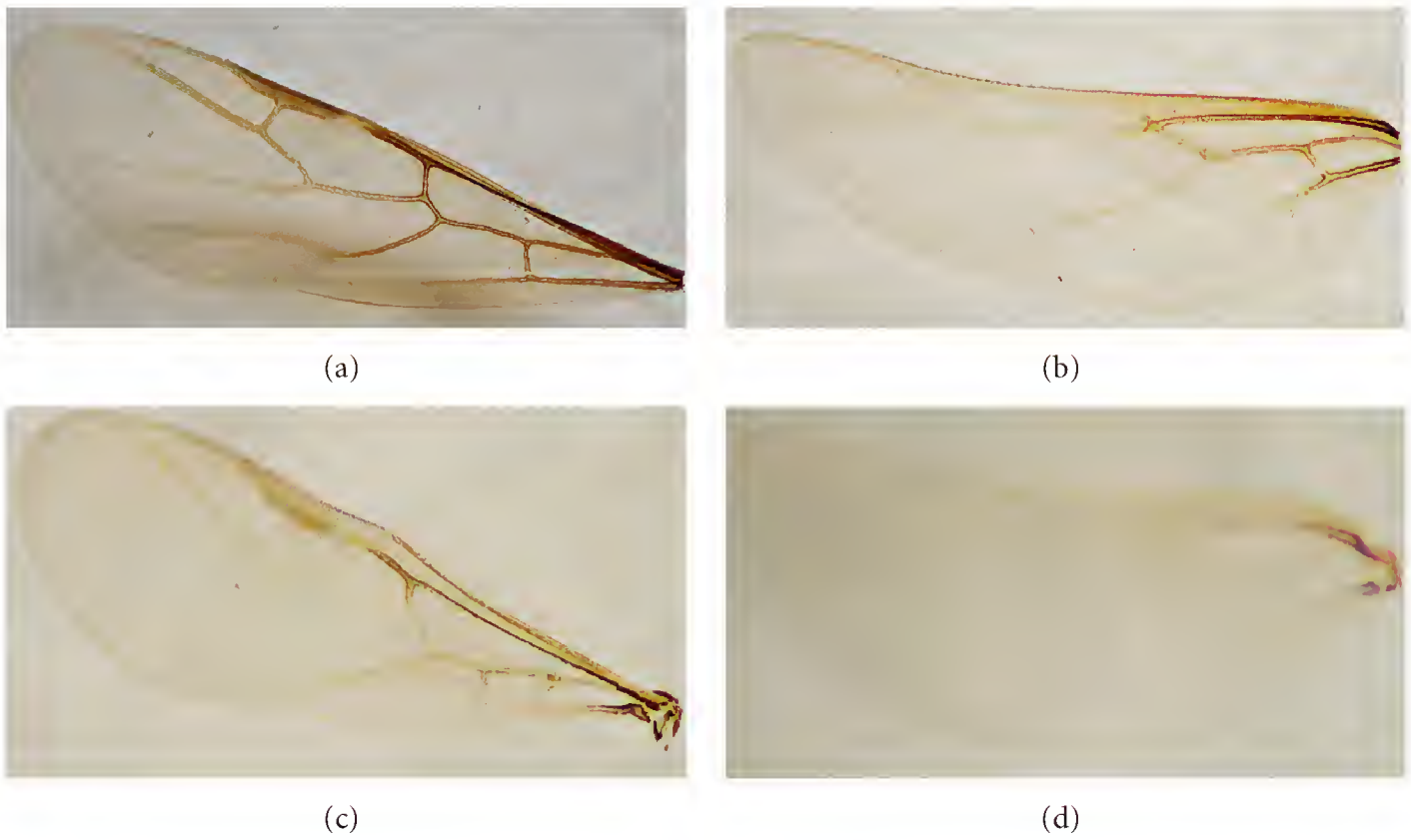


FIGURE 4: Wings of *Dorymyrmex biconis*. (a) Queen forewing; (b) queen hindwing; (c) male forewing; (d) male hindwing.

Additional Examined Material. Outside COLOMBIA: Syntypes and no type material: USA (Western Texas to Southern Nevada, and California), Mexico, Belize, El Salvador, Grenada, Honduras, Jamaica, and Perú. MEXICO: Acapulco, 2w (USNM); Chihuahua, 1w, (LACM); Cordoba, 1w, (USNM); Guerrero, 2w, (USNM); Ixtapa, 8w, (LACM); Revolcadero, 4w, (USNM); Zihuatanejo, 4w, (LACM); Uruapan, 2w, (USNM); San Blas, Playa Nayarit, 2w (LACM); Sinaloa, 30.5mi N. Los Mochis, 1w, (LACM); Guaymas, 11w, (LACM); Nogales, 4w, (USNM); Rio Yaqui, 1w, (LACM); San Bernardo, 3w, (LACM); Tecamachalco, 7 mi. NW Puebla, 2w, (LACM); Veracruz, 2 mi. S. Mocambo, 3w, (USNM); Veracruz, Boca del Rio, 2w, (USNM); Cordoba, 1w, (USNM); Veracruz, Fortin, 3w, (USNM); Jalapa, 3w, (USNM); Itze ChiChen, 1w, (USNM); BELIZE: 9w, (USNM); EL SALVADOR: San Salvador, 2w, (USNM); GRENADA: BWI, St. George's, 8w, (USNM); HONDURAS: Tegucigalpa, 1w, (USNM); JAMAICA: no further data, 1w, (USNM); PERÚ: Lima, 10w, (USNM).

Geographic Distribution. Southwest of USA to Peru.

Etymology. The name of *bicolor* is referred to the particular pattern of colors found in all known castes (worker and queen).

Natural History. Nest, briefly described by Wilson [47], has a small entrance hall with more regularly formed craters than *D. insanus*. *D. insanus* and *D. pyramicus* are sympatric in the northern part of its distributional range. Both species mentioned above are very active in open areas between 11:00 a.m. and 3:30 p.m and share similar habits of foraging according wilson's observations [47].

5.2.3. Comments. Several species of *Dorymyrmex* (*D. pyramicus, D. thoracicus,* etc.) have the same pattern of colors and could be confused with *D. bicolor* s. str. In some papers, *D. bicolor* was confused with *D. pyramicus*, because of its pattern of colors (orange head, mesosoma and petiole with dark gaster), but two main characters are useful to identify and to differentiate both species: head width (larger in workers and queens of *D. bicolor*) and mesonotum interrupted in lateral view, with a well-defined dorsal and declivitous faces, often descending vertically or nearly so, into mesopropodeal suture, (as described below, *D. pyramicus* has a promesonotal profile continuous, convex in lateral view). Apparently, *D. bicolor* belongs to a complex of species, as observed by Forel [48]. The identity of this complex could be solved with a more detailed and extensive research, specially comparing nest series from the west part of USA.

5.3. *Dorymyrmex biconis* Forel, 1912 [8] *(Figures 3(a)–3(c); 4(a)–4(d); 5(a)–5(d); 6(c)-6(d); 13)*

Dorymyrmex biconis Forel, 1912: 37 [8]. Description of worker.

Conomyrma (Biconomyrma) biconis Forel: Kusnezov, 1952: 430 [22].

Dorymyrmex biconis Forel: Shattuck, 1992: 85, [15]; Shattuck, 1994: 75 [45]; Bolton et al. 2006 [46] (catalog).

5.3.1. *Diagnosis*

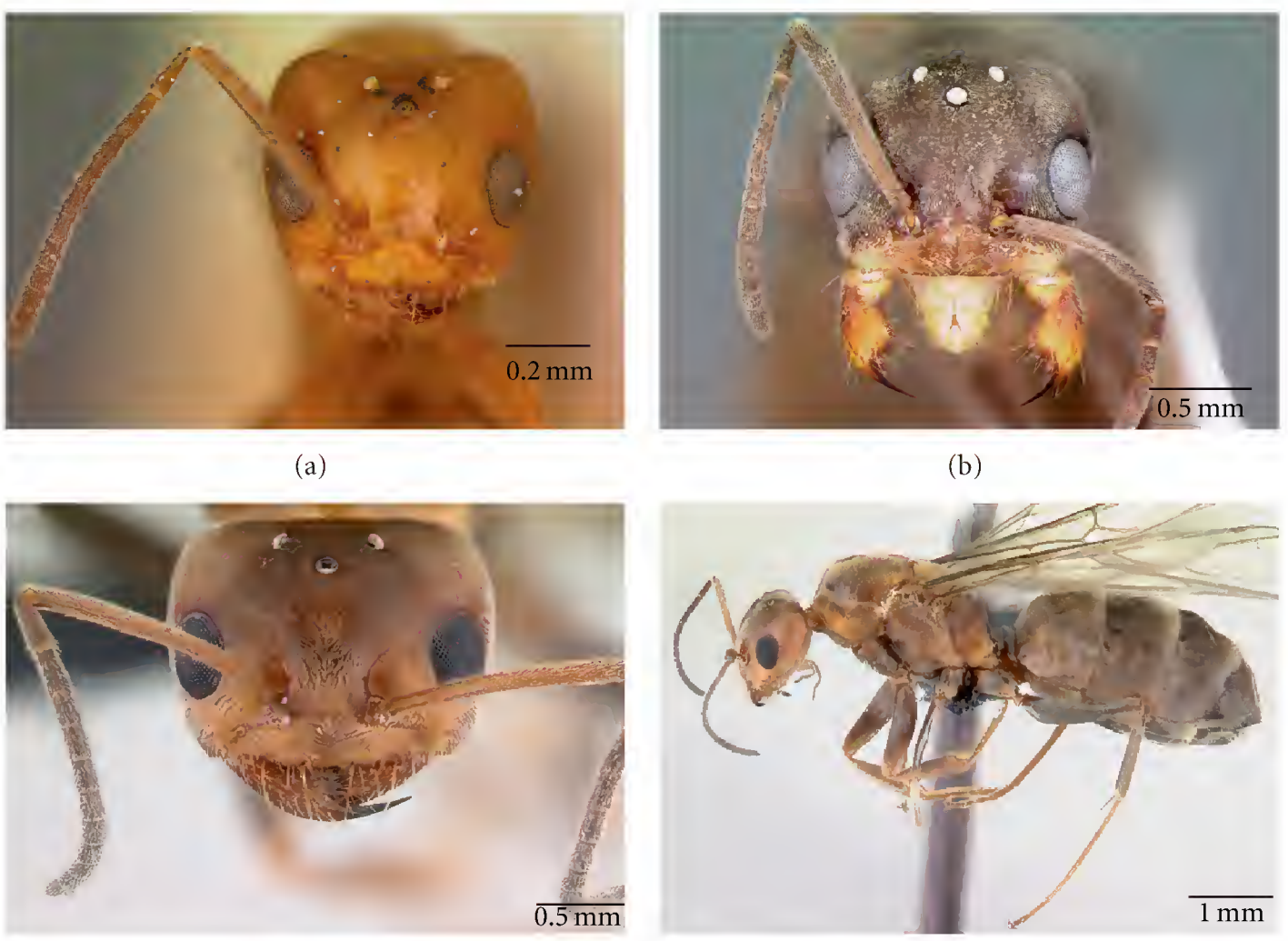
Worker. Concolorous reddish brown. CI: 84–107. Posterior margin of head straight to slightly convex. Mesosomal profile interrupted by the presence of two tubercles: one stout short metanotal tubercle, posteriorly directed, and another placed in apical corner of the propodeum, dorsally directed.

Queen. Color similar to worker but darker. Head subquadrate with a straight posterior margin. Compound eyes

(a) (b)

(c) (d)

FIGURE 5: *Dorymyrmex biconis* male. (a) Head in frontal view; (b) profile of mesosoma; (c) dorsal view; (d) male genitalia in dorsal view (CASENT0192695). Pictures and labels of localities of each photographed specimen are available at http://www.antweb.org/.



(a) (b)

(c) (d)

FIGURE 6: Queens of three *Dorymyrmex* species. (a) and (b) Heads of (a) *Dorymyrmex bicolor*; (b) *Dorymyrmex brunneus*; (c)-(d) *Dorymyrmex biconis*: (c) head and (d) body profile.

FIGURE 7: *Dorymyrmex brunneus* workers. (a) and (c) Head in full-face view; (b) and (d) body in lateral view. (a)-(b) CASENT0192705; (c)-(d) CASENT0192698. Pictures and labels of localities of each photographed specimen are available at http://www.antweb.org/.

notably developed, longer than wide, placed in middle of lateral margin of head.

Male. Mandible thin, falcate, with only three teeth: one long apical tooth, one subapical, and one denticle. Masticatory and basal margin well differentiated, basal margins completely devoid of teeth or denticles.

5.3.2. Descriptions

Worker

Measurements. ($n = 62$): HL: 0.56–1.16; HW: 0.62–1.20; EL: 0.18–0.34; EW: 0.16–0.32; SL: 0.78–1.10; WL: 1.00–1.12; CI: 84–107; SI: 93–127; REL: 24–33; OI: 55–88; TLI: 128–191.

Concolorous reddish brown, whitish pubescence covering all body tagma. Some specimens have some segments of gaster darker than rest of the body. Worker length 2.8–3.0 mm. *Head* (Figure 3(a)): subquadrate, longer than wide, with lateral sides straight to slightly convex, posterior margin of the head straight to slightly convex. Scape surpassing the posterior margin of head by no more than 1/3 of its length. Compound eyes placed far from posterior clypeal margin but in the first half of the head. Psammophore with short hairs disposed in a triangle; the hairs in the top line are near to the foramen magnum and do not reach the oral cavity. *Mesosoma*: mesonotum with a stout cone, rounded apically, and shorter than propodeal tubercle. Mesonotal tubercle directed posteriorly. Metanotal suture well developed and located inside a very pronounced concavity posterior to mesonotal tubercle (Figure 3(b)). Dorsal face of propodeum anterior to tubercle sinuate (Figure 3(b)). *Metasoma*: petiolar scale directed dorsally.

Queen (Figures 6(c) and 6(d))

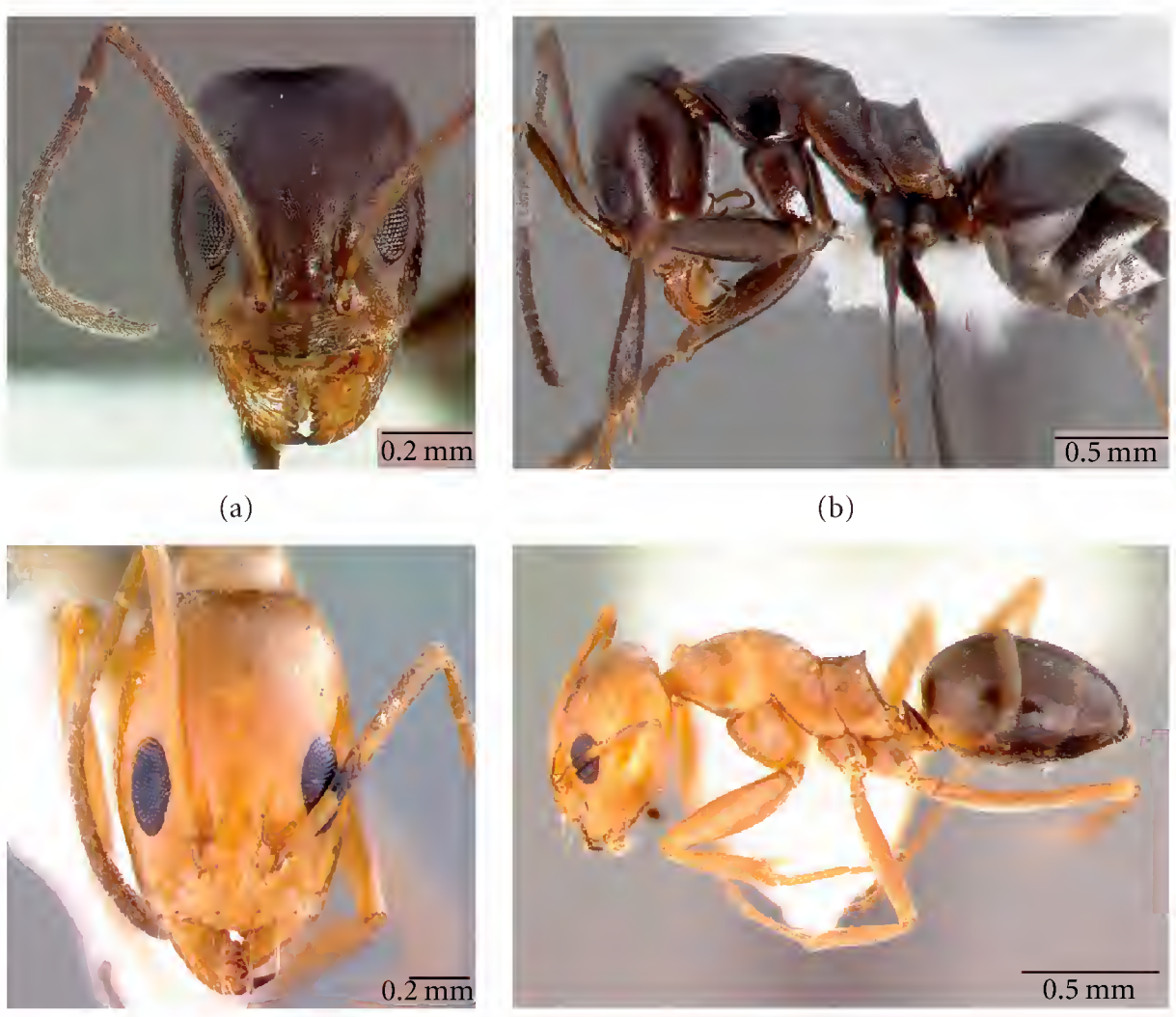
Measurements. ($n = 7$): HL: 1.10–1.16; HW: 1.18–1.20; EL: 0.40–0.46; EW: 0.28–0.32; SL: 1.06–1.12; WL: 2.04–2.32; CI: 102–107; SI: 93–102; REL: 36–40; OI: 61–76; TLI: 179–200.

Body reddish brown, darker than worker. Pubescence dense, with long, decumbent hairs covering all body tagma. *Head*: subquadrate. Head capsule with lateral margins parallel to slightly convex, especially in their posterior half. Posterior margin of head straight, with occipital corners rounded. Mandible with a long and sharp apical tooth, three additional teeth and four denticles along the masticatory margin; basal margin completely denticulate without any

(a) (b)

(c)

FIGURE 8: *Dorymyrmex goeldii* worker. (a) Head in full-face view; (b) body in lateral view; (c) body in dorsal view (CASENT0192699). Pictures and labels of localities of each photographed specimen are available at http://www.antweb.org/.



(a) (b)

(c) (d)

FIGURE 9: *Dorymyrmex* workers. (a) and (c) Heads of (a) *Dorymyrmex insanus*; (c) *Dorymyrmex pyramicus*; (b) and (d) body profiles of (b) *Dorymyrmex insanus*; (d) *Dorymyrmex pyramicus*.

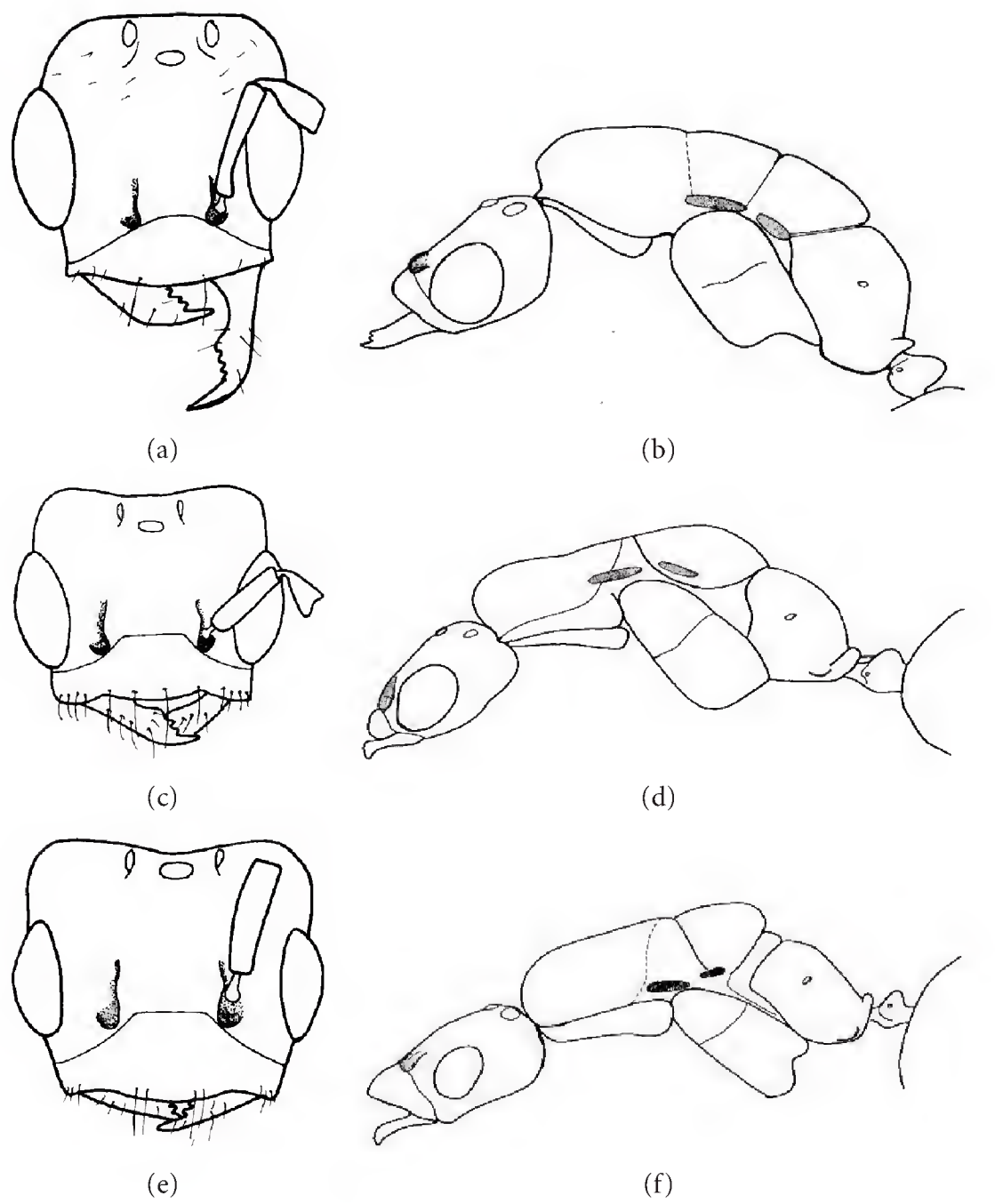


FIGURE 10: Males of three *Dorymyrmex* species. (a), (c), and (e) Heads of (a) *Dorymyrmex brunneus*; (c) *Dorymyrmex insanus*; (e) *Dorymyrmex pyramicus*; (b), (d), and (f) body profiles of (b) *Dorymyrmex brunneus*; (d) *Dorymyrmex insanus*; (f) *Dorymyrmex pyramicus*.

angle between both margins. Compound eyes notably large, longer than wide, placed in the middle of lateral sides. Scape surpassing the posterior margin of head by twice its maximum diameter. *Mesosoma*: parapsidal furrow slightly developed, visible in dorsal view as a tenuous line reaching posterior half of pronotum, diverging forwards, axilla not divided. Anepisternum and katepisternum incompletely divided by a short pleural suture. Pleural suture with long and abundant hairs (better observed in specimens preserved in EtOH). Wings (Figures 4(a) and 4(b)): forewing with one close radial cell, only one cubital cell, no discoidal cell; pterostigma well developed, longer than wide. Hindwing with three closed cells, hamuli with 12 hooks. *Metasoma*: petiolar scale tall, directed dorsally, and apically obtuse. Petiolar ventral face straight with posterior end slightly convex. Gastral tergites dark brown, especially in posterior 2/3; sternite 1 and 2 lighter than the rest of the body.

Male (Figures 5(a)–5(d))

Measurements. (n = 5): HL: 0.56–0.60; HW: 0.58–0.62; EL: 0.30–0.34; EW: 0.24–0.28; SL: 0.22–0.24; WL: 1.24–1.36; CI: 100–107; SI: 38–41; REL: 50–59; OI: 75–88; TLI: 217–234.

Body dark brown to black, mandibles yellowish brown except masticatory margin which is reddish brown; legs, except femora, lighter than rest of the body. Head and mesosoma strongly pointed. Pilosity with dense, decumbent, thin, and whitish hairs covering all tagma. Katepisternum and metapleural area with only a few hairs. Anterior face of petiolar scale with 2-3 long hairs. *Head* (Figure 5(a)): square. Posterior margin of head weakly concave medially; occipital corners rounded; dorsal face of head with a weak, middle furrow. Mandible thin and falcate, with parallel sides; one long apical tooth, three times longer than subapical one, one denticle, and a diastema before the angle with the basal margin. Masticatory and basal margin well differentiated; basal margins completely devoid of teeth or denticles. Posterior part of clypeus wide and reaching the toruli; anterior clypeal margin convex, with a subclypeal border thin, anteromedial part of clypeus straight. Antenna with exposed condyle; long scape (< to EL) reaching posterior margin of compound eye; pedicel as long as each flagellomere. Compound eye large, maximum length more than 2/3 of cephalic length. Median ocellus hyaline, well developed; lateral ocelli close to posterior margin of head. *Mesosoma* (Figure 5(b)): pronotum long, comprises more than a half of mesosoma, projecting forward as an

(a) (b)

(c)

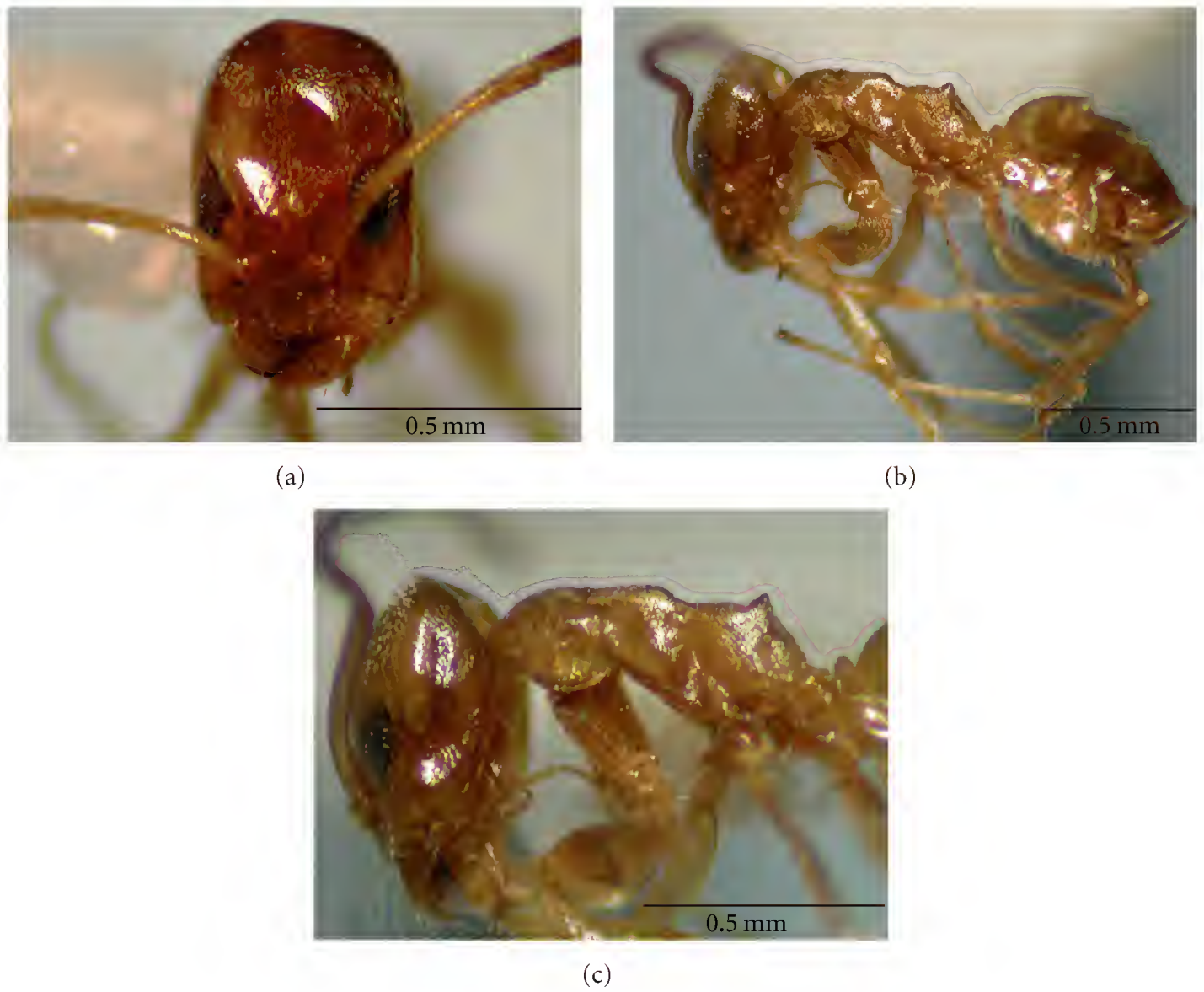
FIGURE 11: *Dorymyrmex tuberosus* n. sp. worker. holotype. (a) Head in full-face view; (b) body in lateral view; (c) body in dorsal view (CASENT0179518). Pictures and labels of localities of each photographed specimen are available at http://www.antweb.org/.

elbow with a strong depression medially; parapsidal furrows running parallel, reaching the middle part of pronotum, placed inside the depression. Mesonotum twice longer than wide. Anepisternum and katepisternum completely divided by a mesopleural sulcus. Dorsal face of propodeum not well differentiated form posterior face, with a strong declivitous face; propodeal spiracles strongly protruded. Wings (Figures 4(c) and 4(d)): forewing with close radial cell, no close cubital nor discoidal cell. Hindwing without closed cells, hamuli with 12 hooks. *Metasoma*: petiolar scale low and apically rounded, without ventral petiolar process. Pygostyle stout and short, well developed; paramere stout, covered with long dark setae; digitus curved ventrally, longer than volsella (Figure 5(d)); aedeagus serrate ventrally.

Examined Material. COLOMBIA: Atlántico: Puerto Colombia, 1w (IAvH). Bolívar: Zambrano, Hda. Monterrey, 15w (IAvH); same locality above, 8w (IAvH); same locality above, 3w (IAvH-E 90487); same locality above, 3w (IAvH-E 90486); same locality above, 3w (IAvH-E 90485); same locality above, 3w (IAvH-E 90484). Boyacá: Moniquirá, 8w (IFML); Ráquira, Desierto de la Candelaria, 10w (IAvH); Villa de Leyva—Plaza central, 4w (IAvH and IFML). Caldas: Mpio. Aguadas, La Nubia, 7w (IAvH); Mpio. Aguadas, La Nubia, Cañón Río Arma, 3w (IAvH). Cesar: Chiriguaná, 10w (CEUM). Córdoba: Ciénaga de Oro, 2w (IAvH). Cundinamarca: Fusagasugá, 1w (IAvH). Huila: Altaminar [Altamira], 1w (ICN-MHN # 2847); Altamira, 1w (IAvH); 10 Km. N San Agustín, 2w (IAvH); 10 Km. W Palermo, 1w (IAvH). La Guajira: Cabo de la Vela, 4w (CEUM, ICN); Maicao, 6w (LACM); Riohacha, 24w (LACM); SFF Los Flamencos, 6w (CEUM, ICN). Magdalena: Ciénaga, 1w (LACM); PNN Tayrona, Sector Neguanje, 131w and 15q (CAS, IAvH and IFML); Pivijay, 7w (IAvH); Santa Marta, San Antonio, 2w (IAvH), Santa Marta, Valenera, 9w (LACM), Santa Marta, 4w (LACM); Santa Marta, 5m (ICN).

Additional Examined Material. Outside COLOMBIA: Brazil, Peru and Venezuela. BRAZIL: Bahia, Planalto Casimiro Andrade, 10w (MZSP); ES, Santa Tereza, 2w (MZSP); PA, Pindobal, 2w (MZSP). PERU: Puerto Pizarro: #1091, 5w (MZSP). VENEZUELA: Lara: Barquisimeto, 2w (IFML).

Geographic Distribution. Brazil (Bahia, Pará, and Espírito Santo States), Colombia (Atlántico, Bolívar, Boyacá, Caldas, Córdoba, Cundinamarca, Huila, La Guajira, Magdalena, and Valle del Cauca Departments), Perú (Puerto Pizarro), Venezuela (Lara State: Barquisimeto).



(a) (b)

(c)

FIGURE 12: *Dorymyrmex xerophylus* n. sp. worker. (a) Head in full-face view; (b) and (c) body in lateral view; (c) body in dorsal view. These pictures were made by RJG using Nikon Coolpix digital camera and COMBINE Z5 software.

Etymology. The name of this species refers to the main diagnostic character of *D. biconis*: the presence of a pair of tubercles interrupting the mesosomal profile (Figure 3(b)).

Natural History. In Colombia, *Dorymyrmex biconis* is spread throughout, from sea level to more than 2300m. It is more common in lowlands (i.e., Colombian Caribbean region, North of Colombia) and quite common in anthropic environments, with higher abundance in urban places. Nevertheless, workers of *D. biconis* have been collected in primary dry forests and in mangroves. *D. biconis* builds nests in soil devoid of vegetation, in very warm areas. Exceptions are arid but extremely cold places such as Boyacá, Colombia. As in other species of *Dorymyrmex*, nests are superficial, with simple architecture, and no more than 10–15 cm in depth. Excavated nests by RJG in Santa Marta (Magdalena, Colombia) share a similar architecture: only one circular entrance (no more than 5 mm in diameter) surrounded by a mound of sand or other soil particles with a diameter of 9-10 cm. The nest consist of one chamber with queens and males, a second one with larvae and worker pupae, and a third chamber with food and insects remains, such as several Bruchidae (Coleoptera). In one nest, RJG collected some dead Thysanoptera in the food storage camera.

One nest of *D. biconis* can keep between 10 to 15 alate queens, 8 to 10 males and several tens of workers. Sometimes there are no more than 100 to 200 workers in a single nest. No dealate queens have been found in explored nests. Colonies of *D. biconis* are probably polydomous, and queen may fly outside of its colony to build satellite nests. In some cases, RJG has found two or three nests in an area of 10 m^2; only one of these nests had queens.

5.3.3. Comments. The first description of *D. biconis* was based in workers collected in San Antonio (St. Antonio), Santa Marta, Colombia [8]. Three species of *Dorymyrmex* (*D. bituber*, *D. pulchellus*, and *D. tuberosus* n. sp.) share a few characters with *D. biconis*. This is a group of species easy to recognize by the presence of two well-developed mesosomal tubercles: one in the last part of the metanotum and the other one between the dorsal and declivitous faces of propodeum. Considering its geographical distribution, *D. biconis* seems to be confined to the north and central parts of South America, from Venezuela to Peru. This species was included in the subgenus "*Biconomyrma*" [19] based on characters of wing venation of queen.

5.4. Dorymyrmex brunneus Forel, 1908 [37] *(Figures 6(b); 7(a)–7(d); 10(a)-10(b); 13)*

Dorymyrmex pyramicus var. *brunnea* Forel, 1908: 385 [37]. Description of worker.

Dorymyrmex pyramicus subsp. *brunneus* Forel: Forel, 1911: 306 [48]. Description of queen.

Dorymyrmex pyramicus subsp. *brunnea* Forel: Emery, 1913: 37 [49].

Dorymyrmex (*Conomyrma*) *pyramicus* race *brunneus* Forel: Forel, 1913: 244 [50].

Dorymyrmex pyramicus subsp. *brunneus* Forel: Santschi, 1912: 53 [51], Gallardo 1916: 59 [41] (queen redescribed).

Dorymyrmex (*Conomyrma*) *brunneus* var. *spurious* Forel: Santschi, 1929: 305 [52].

Conomyrma (*Biconomyrma*) *brunnea* (Forel): Kusnezov, 1952: 430 [22].

Conomyrma brunnea (Forel): Kempf, 1975: 375 [6].

Dorymyrmex brunneus Forel: Shattuck, 1992: 85 [15]; Shattuck, 1994: 77 [45]; Bolton et al. 2006 (catalog) [46].

5.4.1. Diagnosis

Worker. Head slightly longer than wide. Posterior margin of head straight to feebly concave medially (Figure 7(a)). Psammophore with short hairs disposed in a triangle, not reaching the posterior end of hypostome. Pro-mesonotum depressed in lateral view, always lower than the apex of propodeal tubercle. Mesonotal profile with a well-defined dorsal and declivitous face in the posterior end. Metanotal suture well impressed forming a concavity anterior to the propodeum.

Queen. Maximum diameter of head behind the compound eyes. Posterior margin of head feebly concave medially. Forewing with only one close cubital cell.

Male. Dark brown. Scape long, reaching the posterior margin of compound eyes. Mandible with only three teeth. Forewing with no discoidal and no cubital cells, hindwing with only two closed cells. Pygostyle poorly developed, paramere stout and covered with long hairs.

5.4.2. Descriptions

Worker

Measurements. Lectotype: HL: 0.95; HW: 0.8; EL: 0.23; EW: 0.125; SL: 0.875; WL: 1.175; CI: 84; SI: 92; REL: 24; OI: 54; TLI: 124.

Other Examined Material. w (n = 98): HL: 0.70–1.04; HW: 0.64–0.92; EL: 0.22–0.30; EW: 0.10–0.20; SL: 0.80–1.14; WL: 1.00–1.46; CI: 83–117; SI: 98–129; REL: 24–34; OI: 45–77; TLI: 122–171.

Concolorous dark brown; whitish pubescence covering all body tagma. 0–2 erected setae on the dorsum of pronotum. *Head* (Figures 7(a) and 7(c)): subquadrate, with lateral margins strongly convex, maximum head width at the compound eye level. Mandibles strongly striate, reddish brown. Compound eyes in central 1/3 of the head as seen in frontal view. Scape long (SI: 92–129), surpassing the posterior margin of head by more than twice its maximum width. Posterior margin of head usually straight but sometimes feebly concave in the middle. Psammophore with a few extremely short hairs disposed in a triangle, the hairs in the top line are near to the foramen magnum and do not reach the oral cavity. Upper setae of psammophore close to the anterior margin of foramen magnum. *Mesosoma*: promesonotal profile sinuate to straight and, in lateral view, always lower than the apex of propodeal tubercle (Figures 7(b) and 7(d)). Posterior end of mesonotum forming two faces, one dorsal and one declivitous but not conforming a well-developed tubercle (Figures 7(b) and 7(d)). Propodeal tubercle stout, with wide base, and slightly directed dorsally. Declivitous face straight to slightly convex (Figure 7(d)). *Metasoma*: petiole forward directed, included in a concavity placed in the anterior face of the first gastral segment. Scale apically thin and rounded.

Queen

Measurements. (n = 5): HL: 1.13; HW: 1.15; EL: 0.38; EW: 0.18; IOD: 0.78; SL: 1.1; WL: 2.3.

Color and pubescence as in worker; *head*: subquadrate, maximum diameter after the compound eyes (Figure 6(b)). Clypeal sides lighter than the rest of the head; scape surpassing the posterior margin of head by more than twice its maximum width; mandibles striated with four teeth and two denticles; posterior margin of head feebly concave medially. *Mesosoma*: parapsidal furrows well developed, parallel, axilla not divided. A short, incomplete suture divides anepisternum from katepisternum. Forewing with only one closed cubital cell; radial cell long and close. *Metasoma*: low and stout petiole, apically rounded.

Male (First Description)

Measurements. (n = 3): HL: 0.58–0.6; HW: 0.58–0.63; EL: 0.28–0.33; EW: 0.15–0.2; SL: 0.25; WL: 1.3–1.38.

Color of the body similar to worker and queen; *head* (Figure 10(a)): subquadrate with round occipital corner; mandibles thin with only three teeth, the apical more than twice longer than the others. Scape long, reaching the posterior margin of compound eyes. *Mesosoma*: parapsidal furrows parallel, axilla not divided medially; forewing with one close radial cell and no cubital nor discoidal cell. Hindwing with only two closed cells. *Metasoma*: petiolar scale (Figure 10(b)) low, round, and stout, ventral process round, feebly developed. Pygostyle poorly developed; paramere stout covered with long, erected setae; aedeagus serrate ventrally.

Examined Material. COLOMBIA: Amazonas: Araracuara, 9w (IAvH). Antioquia: Sonsón, Quebrada La Violeta, 6w (IAvH-E 90491); same data as above, 1w (IAvH-E 90502); same locality, 3w (IAvH-E 90476); same locality, 2w (AvH-E 90492). Boyacá: El Infiernito, 14w (IAvH); Ráquira, Desierto

de la Candelaria, 10w (IAvH), one of these specimens was photographed; Arcabuco, 1w (IAvH). Caldas: Aguadas, 2w (IAvH); Aguadas, Arenillas, 9w (IAvH); same data, #CES292, 3w (IAvH-E 90503), same data, #CES292, 3w (IAvH-E 90496); same data, #CES292, 3w (IAvH-E 90461); Aguadas, Puente Albania, 1w (IAvH-E 90501); Aguadas, Cañón Río Arma, 3w (IAvH); same data, #CES069, 12w (IAvH), same data, #CES068, 1w (IAvH-E 90504); Aguadas, Quebrada Pito, 1w (ICN-MHN 022550); Manizales, Vereda El Dorado, Finca El Placer, 2w (IAvH); La Nubia, 7w (IAvH). Cauca: Mercaderes, Mojarras, rivera del río Guachicano, 7w + 1 queen (CEUM). Caquetá: Puerto Solano, PNN Chiribiquete, río Curañé-Amú, 1w (IAvH). Cundinamarca: Fusagasugá, 2w (ICN); Nariño: RN La Planada, 6w (IAvH). Meta: 7w (LACM); 65 Km. E Puerto López, 2w (LACM). Quindío: Circasia, Vereda Buena Vista, Finca Calamar, 12w (IAvH); Calarcá, Vereda Pradera Baja, Finca La Holanda, 3w (IAvH); same data, 9w [2w (IAvH-E 90473), 2w (IAvH-E 90474), 3w (IAvH-E 90470), 2w (IAvH-E 90472)]. Risaralda: La Virginia, Finca Miralindo, 6w (CEUM). Santander: Barrancabermeja, [2w (IAvH-E 90490), 2w (IAvH-E 90498), 2w (IAvH-E 90493)]; Socorro, Vereda Altos de Reina, Finca San Luis, [1w (IAvH 25099), 1w (IAvH 25097), 1w (IAvH 25098)]; same locality, Finca El Clavelino, [1w (IAvH 25162), 1w (IAvH 25163)]. Tolima: locality not recorded, 8w (LACM). Valle del Cauca: Buenaventura, Bajo Anchicayá, [1w (IAvH-E 90499), 1w (IAvH-E 90500)]. Vichada: Cumaribo, Selva de Matabén, 7w (IAvH).

Additional Examined Material. Outside COLOMBIA: Several series from Argentina, Bolivia, Brazil, Guatemala, and Paraguay. ARGENTINA: Chaco: Las Palmas, #989, 6m, (MACN); Entre Ríos, 5w, (MACN); Misiones: Esperanza, 5w and 1m, (MZSP); San Luis2w, (MZSP); #236, C. Bruch coll, identified as "cotypus", 2w, (MACN). BOLIVIA: Cochabamba, 10w, #9479, 4w, (IFML); Depto. Santa Cruz, Prov. Andrés Ibáñez, 12 km E Santa Cruz, 5w, (IFML). BRASIL: BA: Bom Jesus da Lapa, 6w, (CEPEC); MT: Araguai, 2w, (MZSP); Cáceres, 1w, (MZSP); Campo Grande, 6w, 1q (MZSP); Carmo da Cachoeira, 5w, 1q, (MZSP); same loc, 3w, (MZSP); Colonia Vicentina, Dourados, 1w, (MZSP); 3w, (MZSP); Cuiabá, 22w, 2m, (MZSP); Chapada, 8w, (MZSP); same loc, 2w, (MZSP); Diamarum, Parque Nac. Xingu, 12w, (MZSP); Fátima, 7w, (MZSP); Faz. Beija Flor, 4w, (MZSP); Faz. Sta. Blanca, Corumbá, 4w, (MZSP); Itaum, 1w, (MZSP); Jardim, 9w, (MZSP); Mons. Paulo, V. dos Santos, 6w, (MZSP); Paconé, 8w, (MZSP); same loc, 3w, (MZSP); same loc, 5w, (MZSP); Porto Murtinho, 4w, (MZSP); Rondonópolis, 2w, (MZSP); same loc, 3w, (MZSP); Santa Bárbara, 2w, (MZSP); Serra Caraça, 16w, (MZSP); same loc., 4w, (MZSP); Serra do Urucum-Corumbá, 24w, (MZSP); S. Lourenço, 1w, (MZSP); Tiradentes, 1w, (MZSP); Três Lagoas, 4w, (MZSP); Utiariti, Río Papagaio, 6w, (MZSP); same loc, 3w, (MZSP); PE: Caruara, 2w, (MZSP); Diapoque, 5w, (MZSP); João Pessoa, 9w, (MZSP); Prado, Recife, 3w, (MZSP); Recife, 4w, (MZSP); same loc, 3w, (MZSP); Tapera, 2w, (MZSP); PR: Castro, 3w, (MZSP); Foz do Iguazú, Cataratas, 4w, (MZSP); Marienthal, 2w, (MZSP); Rio Negro, 3w, (MZSP); same loc, 1w, (MZSP); Rolandia, 3w, (MZSP); RD: Assis, 2w, (MZSP); RGS: Erechim, Campinas, 14w, (MZSP); Três Arroios, 5w, 2m, (MZSP); RJ: Fábrica Nacional de Motores, 2w, (MZSP); Itaipava, 2w, (MZSP); Jardim Primavera, 2w, (MZSP); Macaé, 4w, (MZSP); Marambaia, 2w, (MZSP); Rio de Janeiro, 5w, (MZSP); RS: Morretes, 6w, (MZSP); SC: Blumenau, 1w, (MZSP); Camboriu, 12w, (MZSP); Canoinhas, 3w, (MZSP); Forquilhinha, 5w, (MZSP); Ituporanga, 3w, (MZSP); Poço Grande, 3w (MZSP); Rio do Sul, 9w, (MZSP); Rodeio, 1w, (MZSP); SP: Agudos, 14w, (IFML); Amparo, 3w, (MZSP); Anhembi, 11w, 2q, (MZSP); Assis, Rd 333, km 44, 2w, (MZSP); Avaré, 5w, (MZSP); Barueri, 31w, (MZSP); Butantan, 1w, (MZSP); Campinas, 5w, (MZSP); Campo dos Jordão, 6w, 3m (MZSP); Campo Limpo, 1w, (MZSP); Caraguatatuba (Res. Flor. 40m), 4w, (MZSP); Curitiba, 6w, (MZSP); Embú, 1w, (MZSP); Faz. Itaqueri, Rôa Esperança do Sul, 18w, (MZSP); Alhambra, 6w, (MZSP); Ilha da Vitória, 16w, 5q, 8m, (MZSP); Ilha dos Buzios, 1w, (MZSP); Interlagos, 12w (cotype), (MZSP); Monte Mor, 7w, 2m, (MZSP); Piracicaba, 12w, (MZSP); Rio Claro, Bairro Saudade, 9q, (MZSP); Rio Claro, Horto Forestal, 3w, (MZSP); Rod. S.Paulo-Curitiba, Km 300, M.Iporanga, 15w, (MZSP); S.Sebastião, 4w, (MZSP); Teod. Sampaio, 2W, (MZSP); Ubatuba, 2w, (MZSP); Venceslau, 3w, (MZSP); 20 km W Conchas, 4w, (MZSP); Boraceia, 6w, (MZSP); Piracicaba, 9w, (MZSP); S. Sebastião, Bairro S. Francisco, 4w, (MZSP); Orlândia, 3w, (MZSP); Holambra, 6w, (MZSP); Praia Grande 3w, (MZSP); Interlagos, 12w, (MZSP); Anhembi, Faz. B. Rico, MANAUS, Amazonas, 4w, (IFML); PARAGUAY: Caacupé, 3w, (IFML); Pastoreo, 5w, (MZSP); GUATEMALA: Antigua, 3w, (USNM).

Geographic Distribution. Argentina, Bolivia, Brazil, Colombia (Amazonas, Antioquia, Boyacá, Caldas, Caquetá, Cundinamarca, Nariño, Meta, Quindío, Santander, Tolima, Valle del Cauca, Vichada), French Guyana, Guyana, Panama, Guatemala, Paraguay, and Surinam.

Etymology. The name "*brunneus*" means dark brown. It is the main color of worker, queen, and male.

Natural History. D. brunneus is mainly restricted to arid environments of the Andean region of Colombia, at elevations above 1000 m. Some lowland populations live in dry forests of western Colombia (Valle del Cauca, 400–500 m), savannas in eastern plains (Vichada, 240 m), and in Colombian Amazon basin (Amazonas, 200–300 m).

Like *Dorymyrmex biconis, D. brunneus* is well adapted to anthropic environments. Most of the specimens studied here have been collected in areas transformed by humans, mainly in open areas with low vegetation (stubble), coffee plantations (shade coffee culture), wooded areas for cattle grazing, and urban areas.

5.4.3. Comments. D. brunneus is one of the most variable species of *Dorymyrmex* with the widest distributional range. This species was found from Panama (Canal Zone) up to the central part of Argentina. This species is the most frequently

collected in Brazil, from sea level to more than 2000 m. As suggested by the variability observed in specimens from Figures 7(a)–7(d), *D. brunneus* may be a species complex. More detailed studies are needed based on series of specimens collected from same nest and different localities from all over its distributional range.

The shape of the mesosomal profile was one of the most frequently characters used to separate species of *Dorymyrmex* but is almost unusable to identify *D. brunneus*. Local populations of this species have strong differences in the profile of mesosoma (Figures 7(b) and 7(d)) and scape length. There are morphological variations among Colombian populations which seem to be stable: only workers with broad head (CI: 90–107) have a strong mesonotal depression making a sort of tubercle at the middle of mesonotum (different from *D. biconis*), with a deep and wide metanotal groove. Besides, workers have a median ocellus and, sometimes, two tiny lateral ocelli (populations from Boyacá, Colombia). The shape of queen head in frontal view (Figure 6(b)), mandibles with only three teeth in male and the number of closed cells in forewings have proved to be useful to separate *D. brunneus* from other *Dorymyrmex* species.

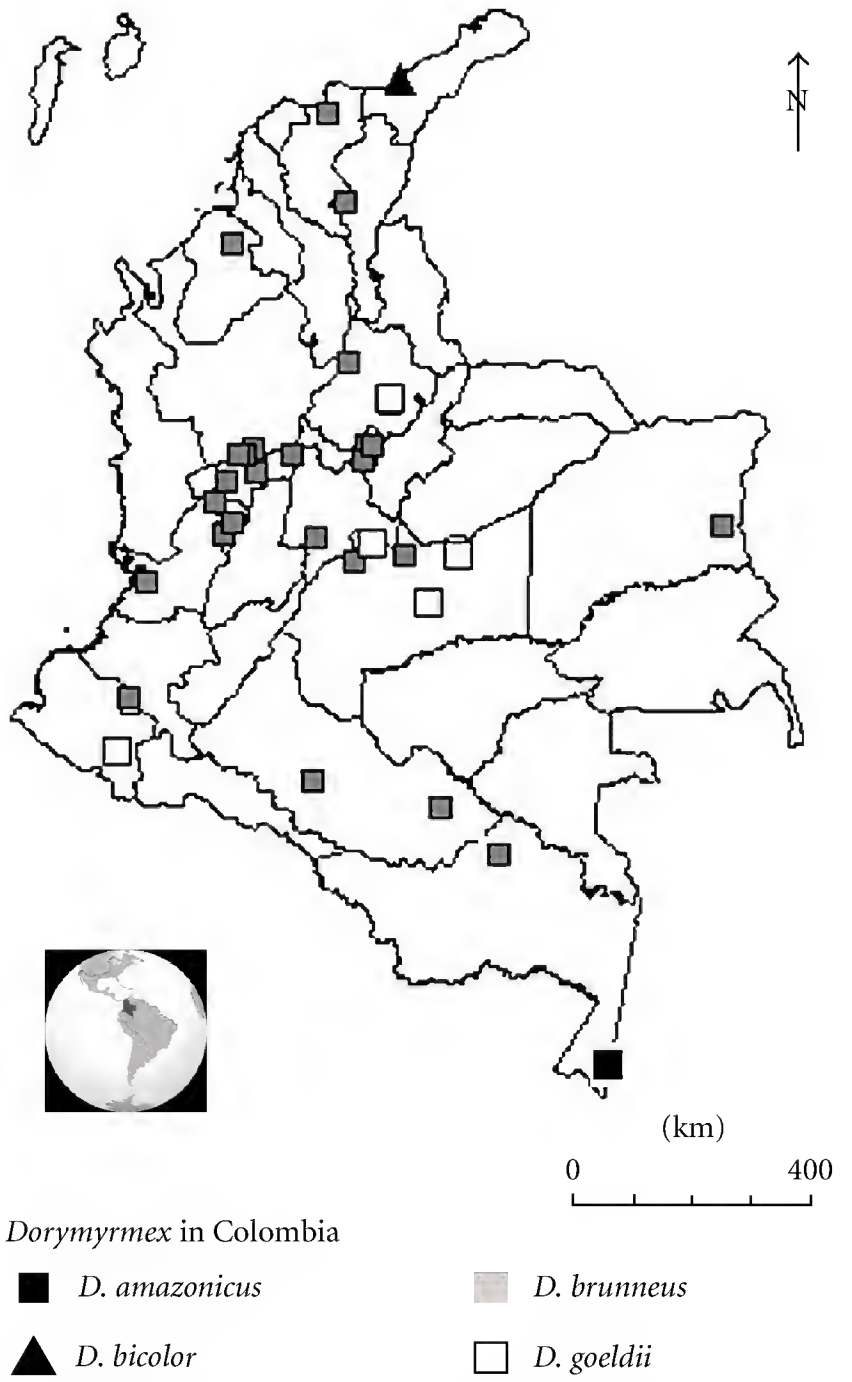


FIGURE 13: Geographic distribution of *Dorymyrmex amazonicus* n. sp., *Dorymyrmex bicolor*, *Dorymyrmex brunneus*, and *Dorymyrmex goeldii* in Colombia.

5.5. *Dorymyrmex goeldii* Forel, 1904 [38] *(Figures 8(a)–8(c); 13)*

Dorymyrmex Göldii Forel, 1904: 41 [38].

Dorymyrmex (Ammomyrma) goeldii Forel: Kusnezov, 1952: 429 [22].

Conomyrma goeldii (Forel): Kempf, 1972: 79 [10].

Dorymyrmex goeldii Forel: Shattuck, 1992: 85 [15]; Bolton et al. 2006 (catalog) [46].

5.5.1. Diagnosis

Worker. Concolours, light brown. Head longer than wide, with the posterior margin of head strongly convex, scape long (SI: more than 115), long mesosoma, in profile.

Queen and Male. Unknown.

5.5.2. Descriptions

Worker

Measurements. (n = 30): HL: 0.70–0.86; HW: 0.55–0.72; EL: 0.20–0.26; EW: 0.10–0.18; SL: 0.85–1.00; WL: 1.05–1.36; CI: 77–84; SI: 116–160; REL: 29–32; OI: 50–69; TLI: 147–163.

Concolours, light brown. Sculpture reticulated, widely spaced. Pubescence whitish and dense. Pilosity brownish. *Head* (Figure 8(a)): psammophore triangular with short setae, the hairs in the top line close to the foramen magnum and do not reach the oral cavity. Uppermost setae of psammophore close to the lower margin of foramen magnum. Scape surpassing occipital margin by more than twice its apical width. Posterior margin of head slightly straight. *Mesosoma* (Figures 8(b) and 8(c)): 0–2 pronotal erected setae; propodeal tubercle round and short tubercle, directed dorsally; declivitous face of propodeum straight to feebly convex. *Metasoma*: low petiolar scale.

Queen and Male. Unknown.

Examined Material. COLOMBIA: Meta: Puerto López, 6w (IAvH); PNN Sierra de la Macarena, Caño Curía, Sendero Cachicaos, 29w (IAvH); same locality, 29w (IAvH); same locality, 25w (IAvH); Cumaral, 5w (LACM). Nariño: R.N. La Planada, 1w (IAvH). Santander: Socorro, Vereda Alto de Reinas, Finca San Luis, 1w (IAvH).

Additional Examined Material. Outside COLOMBIA: BOLIVIA: Ignacio de Velasco, 3w (MZSP). BRAZIL: MT: Rondonópolis, 11w (MZSP). PE: Araripina, 9w (MZSP). PI: 5 km E Oeiras, Faz. Talhada, 10w (MZSP); 10 km N. Corrente, Faz. Maracujá, 2w (MZSP); Canto do Buriti, 2w (MZSP). SP: Agudos, 9w (MZSP); same locality, 11w (IFML).

Geographic Distribution. Bolivia, Brazil, and Colombia (Meta, Nariño and Santander). Figure 13 shows distribution of *D. goeldii* in Colombia.

Etymology. Named in honor of Emilio Goeldi.

Natural History. Geographical distribution of *D. goeldii* has a strong disjunction in Colombia: some populations are found in the Andean region (Deptartment of Santander and Nariño) at high altitude, between 1700 a 1900 m; other colonies, where *D. goeldii* is more common, prefer open areas of an isolated chain of mountains in the La Macarena National Park; in this area, specimens were collected at 493 m.

5.5.3. Comments. Characters given in the diagnosis are enough to differentiate this species from all other *Dorymyrmex* found in Colombia.

5.6. Dorymyrmex insanus (Buckley, 1866) [39] *(Figures 9(a)-9(b); 10(c)-10(d); 14)*

Formica insana Buckley, 1866: 165 [39].

Dorymyrmex insanus: McCook, 1880: 185-186 [53].

Dorymyrmex pyramicus (as senior synonymy of *D. insanus*): Mayr, 1886: 433 [1]; Emery, 1895: 331 [54]; W. M. Wheeler, 1902: 6-7, [55]; W. M. Wheeler, 1906: 342 [36]; Creighton, 1950: 346–349 [56].

Dorymyrmex pyramicus v. *insana*: Santschi, 1920: 381 [57] (revived from synonymy as variety of *D. pyramicus*).

Dorymyrmex (*Conomyrma*) *pyramicus*: Smith, 1951: 837 [20]; Gregg, 1963: 432–434 [58] (in part).

Conomyrma insana: Snelling, 1973 [25] (in part), Johnson, 1989: 185 [42] (Senior synonym of *D. medeis* and *D. reginicula*).

Dorymyrmex insanus (Buckley): Shattuck, 1992: 85 [15]; Shattuck, 1994: 84 [45]; Snelling, 1995: [4]; Bolton et al. 2006 [46].

5.6.1. Diagnosis

Worker. Medium brown to dark. Head longer than wide, lateral sides parallel and slightly convex. Posterior margin of head with a weak median emargination. Pronotum with 0–2 erect setae. Pro-mesonotum slightly convex with a weak subangle behind, forming a feeble tubercle in the same line of propodeum in profile.

Queen. Maximum cephalic width at the level of compound eyes, weakly narrow behind.

Male. Dark brown to black. Maximum head width after level of eyes. Posterior margin of head feebly concave in the middle; only three teeth present on the masticatory margin of the mandible. Forewing with no discoidal nor cubital cells.

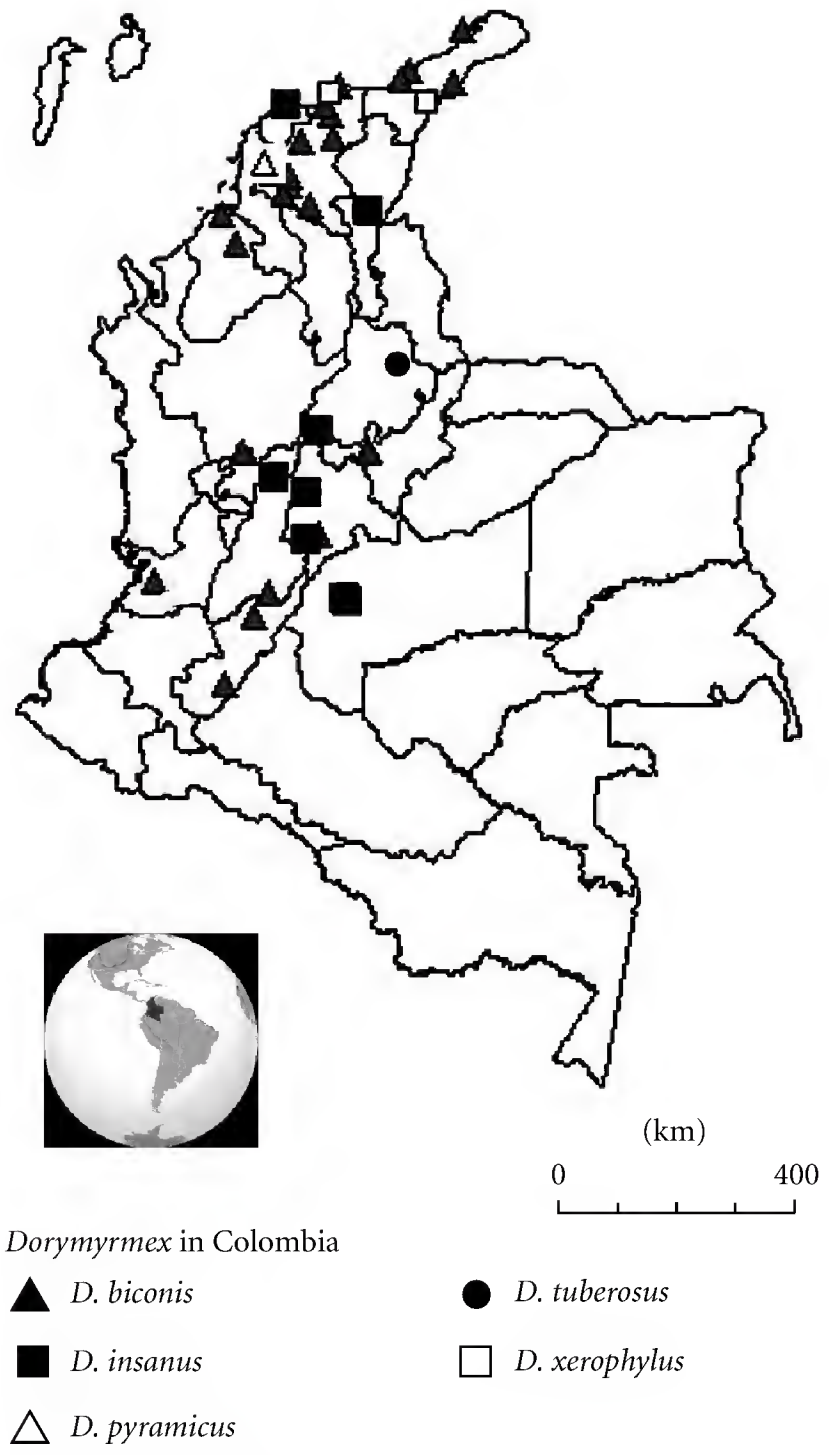


FIGURE 14: Geographic distribution of *Dorymyrmex biconis*, *Dorymyrmex insanus*, *Dorymyrmex pyramicus*, *Dorymyrmex tuberosus* n. sp., and *Dorymyrmex xerophylus* n. sp. in Colombia.

5.6.2. Descriptions

Worker

Measurements. (n = 55): HL: 0.78–1.16; HW: 0.70–1.00; EL: 0.22–0.57; EW: 0.15–0.22; SL: 0.80–1.12; WL: 1.08–1.44; CI: 79–96; SI: 74–119; REL: 25–71; OI: 26–82; TLI: 125–147.

Head, mesosoma, and gaster medium brown to dark; some specimens are almost black. Head and dorsal part of mesosoma covered with a dense and whitish pubescence but lighter than in *D. brunneus*. *Head* (Figure 9(a)): subquadrate, convex laterally. Compound eyes placed in first cephalic third. Scape surpassing the posterior margin of head by 1/3 of its length. Posterior margin of head concave medially. Psammophore with short setae, disposed in a double file, forming a semicircle; the hairs in the top line are disposed far from the foramen magnum and do not reach the oral cavity. *Mesosoma* (Figure 9(b)): profile interrupted in the middle, promesonotal profile higher than apical summit of propodeal tubercle. Mesonotal sclerite, in profile, forming an angle defining two faces, one dorsal and one posterior but not forming a real tubercle. In profile, the dorsal face

of propodeum is feebly sinuate. *Metasoma*: petiolar scale directed dorsally. No ventral petiolar process.

Queen. Well described by Snelling [4].

Male (First Description)

Measurements. (n = 4): HL: 0.55–0.6; HW: 0.58–0.63; EL: 0.25–0.28; EW: 0.15; SL: 0.25–0.28; WL: 1.08–1.23.

Body dark brown, mandibles yellowish brown except masticatory margin which is reddish brown. Whitish pubescence covering all the body. *Head* (Figure 10(c)): square with round occipital corner; posterior margin of head feebly concave in the middle. Mandible falcate, with subparallel inner and outer sides, with only three teeth: apical tooth three times longer than the preapical one; there is no well differentiated angle between masticatory and basal margins; basal margin completely devoid of tooth or denticles. Posterior margin of clypeus wide, reaching torulus; anterior margin of clypeus convex. Scape long (> or = to EL) surpassing posterior margin of compound eye; pedicel as long as each flagellomere. Compound eye large, exceeding the lateral margin of head. Hyaline ocelli well developed; lateral ocelli placed close to the posterior cephalic margin. *Mesosoma* (Figure 10(d)): pronotum projected forwards as an elbow; parapsidal furrows strongly divergent, reaching the middle part of pronotum. Mesonotum twice longer than wide in lateral view. Anepisternum and katepisternum completely divided by a mesopleural suture. Profile of propodeum continuous, dorsal and declivitous faces not well defined. Forewing with no discoidal nor cubital cell. Hindwing with 0–3 closed cells. Hamuli with 12 hooks. *Metasoma*: petiole low; scale apically rounded in lateral view; ventral petiolar process short. Pygostyle short, stout and covered with white, erect setae. Stout paramere, differentiated from volsella by a sulcus; digitus curved surpassing the volsellae in length, no cuspis present; aedeagus ventrally serrate.

Examined Material. COLOMBIA: Atlántico: Barranquilla, Km. 6 vía Puerto Colombia, 5w (CEUM). Boyacá: Puerto Boyacá, Puerto Romero, Vereda La Fiebre, Finca El Golfo, 1w (ICN). Cesar: Chiriguaná, 14w (CEUM). Cundinamarca: Fusagasugá, Rio Cuja, [1w (ICN-022530), 1w (ICN-022467), 1w (ICN 022468), 1w (ICN-022534), 1w (ICN-022529), 1w (ICN-022533), 1w (ICN-022536), 1w (ICN-022531), 2w (ICN-CORD78)]; Villeta, Buenos Aires, [1w (ICN-022538), 1w (ICN-022537)]. Meta: Vereda el Cocuy, 3°19′7.68″N 73°54′21.96″W 467 m, 23 Apr 1978, [1w (ICN-022542), 1w (ICN-022543)]; San Juan de Arama, Vereda Monserrate, PNN Sierra de la Macarena, 1w (ICN-F86M159); Meta, loc. not recorded, 2w (LACM). Tolima: Municipio Fresno, Vereda Colombia, Finca Las Perlas, 1w (IAvH).

Additional Examined Material. (Outside of COLOMBIA): USA: Arizona: Cochise Co., Portal, 33w (LACM); same locality, 2w, 2m, 2q (LACM); 4 mi. N. Sedona, Oak Co., 2w (LACM). California: Glamis, Riverside Co., 9w (LACM); 3.5 mi NW Glamis, Algodones Dunes, Imperial Co., 5w; La Jolla, San Diego Co., 4w (LACM). Kansas: Anthony City Lake, Harper Co., 5w, 1m, 1q (LACM).

Geographic Distribution. Colombia (Boyacá, Cesar, Cundinamarca, Meta and Tolima), Costa Rica, El Salvador, Honduras, Panama, USA (Arizona, California and Kansas).

Etymology. Insanus means mad, probably because of the crazy movement of foraging workers on the ground.

Natural History. In Colombia, *Dorymyrmex insanus* is commonly found in lowlands. This species nests close to the sea level (75 m.) in anthropic sub-xerophytic deciduous forests and in temporal cultivated areas. Some populations are restricted to arid and open areas of central Colombia, between 400 and 700 m, where small herbs cover the ground.

5.6.3. Comments. Taxonomical limits of this species are here clearly defined. In the past, the names of *D. pyramicus* and *D. insanus* were used as synonyms, in a sort of confusion, considering both species as one with a wide distributional range (from Texas, USA, to Argentina). Snelling [4] was the first to recognize this mistake and to propose a clear description of *D. insanus*, designating a neotype worker (Figures 9(a) and 9(b)) and neoparatypes. According to him, *D. insanus* can be found from Central Texas to Kansas and westward to Northern California. In the same paper, Snelling [4] says that the southern limit of its range is unclear in part due to inadequate collecting. With new material examined from Central and South America, we can confirm the presence of *D. insanus* in Central America and in the north part of South America. For more information, see comments of *D. pyramicus* below. Another interesting data is that *D. insanus* was considered as a vulnerable species by de IUNC Red List as fitting the "D2" criteria of the vulnerable (VU) category in the "1994 Categories & Criteria," meaning the population has an acute restriction in its area of occupancy (typically less than 100 km^2) or in the number of locations (typically less than five). With the distributional data provided here, this species can be considered to exceed the above criteria and, therefore, should be removed from the list of endangered species.

5.7. Dorymyrmex pyramicus Roger, 1863 [40] *(Figures 9(c)-9(d); 10(e)-10(f); 14)*

Prenolepis pyramica Roger, 1863: 160 [40]. Description of worker.

Dorymyrmex pyramicus (Roger): Mayr, 1870 : 947 [59]; Mayr, 1870b: 394 [60].

Dorymyrmex pyramicus: Emery, 1888: 362 [3]. Description of male.

Dorymyrmex (*Conomyrma*) *pyramicus* (Roger): Forel, 1913: 350 [11].

Dorymyrmex pyramicus (Roger): Gallardo, 1916: 54 [41] (w, q, m redescribed).

Dorymyrmex (Conomyrma) pyramicus (Roger): Santschi, 1925: 244 [61].

Dorymyrmex (Conomyrma) pyramicus G. C. Wheeler & J. Wheeler, 1951: 83 [62] (description of larvae).

Conomyrma (Conomyrma) pyramica (Roger): Kusnezov, 1952: 430 [22]; Snelling, 1973: 4 [25]; Goñi et al. 1984: 366 [63] (karyotype).

Dorymyrmex pyramicus (Roger): Shattuck, 1992: 85 [15]; Shattuck, 1994: 85 [45]; Bolton et al. 2006 [46] (catalog).

5.7.1. Diagnosis

Worker. Promesonotal profile continuous, strongly convex. Head, mesosoma, and legs reddish-yellow with gaster dark brown to black. Psammophore reaches the posterior margin of hypostoma.

Queen. Head subquadrate, maximum width at level of compound eyes; scape surpassing the posterior margin of head by no more than its maximum width; mandibles feebly striated with four teeth and two denticles on the masticatory margin; posterior margin of head straight. Forewing with only one large cubital cell.

Male. Head wider than long; posterior margin of head medially concave; scape long, surpassing the level of compound eyes, pygostyle poorly developed.

5.7.2. Descriptions

Worker

Measurements. (n = 10): HL: 0.78–0.88; HW: 0.73–0.78; EL 0.20–0.25; EW: 0.1–0.13; SL: 0.78–0.85; WL: 1.18–1.20; CI: 89–94; SI: 97–100; REL: 26–29; OI: 50–52; TLI: 137–152.

Head, mesosoma, and petiole concolorous reddish yellow; gaster always darker than the rest of the body, frequently dark brown to black. Whitish and sparse pubescence covering all body tagma. *Head* (Figure 9(c)): posterior margin of head feebly emarginated medially. Psammophore with short hairs forming a triangle; the hairs in the top line are dispose near to the foramen magnum and do not reach the oral cavity. Upper seta line of psammophore close to anterior margin of foramen magnum. *Mesosoma* (Figure 9(d)): pronotum with two subdecumbent short setae. Promesonotal profile strongly convex. Mesonotum not angulated, as in *D. insanus*. Propodeal tubercle well developed and directed dorsally. Declivitous face of propodeum, straight. *Metasoma*: petiolar scale pointing dorsally.

Queen

Measurements. (n = 2): HL: 1.15–1.18; HW: 1.18–1.2; EL: 0.33–0.4; EW: 0.13–0.15; IOD: 0.73; SL: 0.93–0.95; WL: 1.93–1.95; CI: 102-103; SI: 80-81.

Color and pubescence as in worker. *Head*: subquadrate; scape surpassing the posterior margin of head by no more than its maximum width; mandibles feebly striated, four teeth and two denticles on the masticatory margin; posterior margin of head straight; external margin of compound eye included in head surface in frontal view; ocelli hyaline, close to the posterior margin of head. *Mesosoma*: parapsidal furrows not well developed but parallels, axilla not divided medially. Anepisternum incompletely separated from katepisternum by a short suture. Forewing with only one close cubital cell; radial cell open. *Metasoma*: petiolar scale low, stout, and rounded apically.

Male

Measurements. (n = 2): HL: 0.6–0.64; HW: 0.7–0.75; EL: 0.2–0.26; EW: 0.13–0.14; SL: 0.34; WL: 1.48–1.50.

Body color similar to worker and queen. *Head* (Figure 10(e)): subquadrate, wider than long; lateral side of clypeus feebly projected forward; mandibles thin, with four teeth, apical tooth more than twice longer than the others; scape long, surpassing posterior margin of compound eyes. *Mesosoma* (Figure 10(f)): parapsidal furrows present and parallel, axilla not divided medially; forewing with one close radial cell and no cubital nor discoidal cell. Hindwing with two basal cells. *Metasoma*: petiole stout and low, directed dorsally, ventral process round, feebly developed. Pygostyle poorly developed; gonystylus stout covered with few erect setae; digitus short and no cuspis. Aedeagus with serrate ventral border.

Examined Material. COLOMBIA: Bolivar, Zambrano, 1w (IaVH), no more data available.

Additional Examined Material. Outside Colombia: ARGENTINA: La Rioja: Guayapa, 17w (IFML). Salta: PN El Rey, 2w (IFML); Campo Quijano, 19w (IFML). Tucumán: Salinas, 12w (IFML); Villa Nougués, 123w, 37q, 21m (IFML); Tucumán, 2w (MZSP). BRAZIL: Bahia: Rodelas, 2w (CEPEC); Domingos, Ilhéus, 1w (CEPEC); Simões Filho, 1w (CEPEC); Planalto, 1w (CEPEC). ES: Cda. Barra, 1w (CEPEC); REG-Linh, 1w (CEPEC); Itaúnas, Cord. De Antonia. 1w (MZSP); Rio de Janeiro: Macaé, 2w (MZSP); MT: Rondonopolis, 1w (MZSP); Tres Lagoas, 4w (MZSP); RS: Tramandaí, 49w (MZSP); Porto Alegre, 2w (MZSP). SC: Florianópolis, Praia da Joaquina, 1w (MZSP). CUBA: Guavia Cave, 3w (LACM). GUATEMALA: Escuintla, 20w (USNM); PARAGUAY: Central, Areguá, 3w (IFML); URUGUAY: Carmelo, 2w (LACM).

Geographic Distribution. Central and South America. Cuba, Guatemala, Colombia (literature records), Brazil (Bahía, Espírito Santo, Mato Grosso, Rio de Janeiro, Rio Grande and Santa Catarina States), Uruguay, Paraguay, Argentina (La Rioja, Salta, and Tucumán Provinces).

Etymology. The name of *pyramicus* refers to the typical tubercle or cone on propodeum present in all species of *Dorymyrmex*, giving to the propodeal angle an appearance of pyramid. Several *Dorymyrmex* species are known as "pyramid ants."

5.7.3. Comments. This species was described by Roger [40] as *Prenolepis pyramica* from one worker collected in Bahia, Brazil, and transferred to *Dorymyrmex* by Mayr [59]. Unfortunately, Wheeler [55] erroneously stated that *Formica insana* Buckley [39] (*Dorymyrmex insanus*) was an "undoubtedly synonym" of *D. pyramicus*. Originally, *Formica insana* was described from Texas and southern states of the United States. Workers of *D. insanus* are concolorous black to dark brown, as Snelling [25] says (see Figures 8(a) and 8(b)), differing from workers of *D. pyramicus* that are typically bicolored, as we describe above. Nevertheless, this mistake persisted, authors having considered *D. pyramicus* as a species with a very large distribution, from the south part of the United States throughout the Caribbean area to the north of Argentina. We only found one worker of *D. pyramicus* in Colombia, but there are bibliographic records that confirm its presence in this country [35]. Apparently, *D. insanus* and *D. pyramicus* are only sympatric in Central America (Cuba and Guatemala) and in the north part of South America (Colombia, Venezuela, and Northern Brazil). Beside color, *D. pyramicus* and *D. insanus* can be differentiated by the shape of head in full-face view and the shape of the promesonotal profile (continuous in *D. pyramicus*, interrupted at its end in *D. insanus*).

5.8. Dorymyrmex tuberosus Cuezzo & Guerrero n. sp. *(Figures 11(a)–11(c); 14)*

5.8.1. Diagnosis

Worker. Dark brown; whitish pubescence in all tagma; scape long; posterior margin of head concave in the middle; mesonotal profile interrupted by a short but distinct tubercle, besides a thin tubercle directed dorsally between dorsal and declivitous faces of propodeum.

5.8.2. Description

Worker

Measurements. Holotype (paratype): HL: 0.88 (0.93); HW: 0.80 (0.88); EL: 0.24 (0.25); EW: 0.20 (0.23); SL: 1.04 (0.98); COD: 0.18 (0.2); WL: 1.18 (1.20); CI: 90 (95); SI: 105 (106); REL: 27 (28); OI: 83 (90); TLI: 134 (131).

Concolorous, dark brown with the lateral corners of the clypeus reddish brown. Whitish and dense pubescence covering the all body. *Head* (Figure 10(a)): longer than wide. Compound eye placed in the middle of the lateral part of cephalic capsule, not surpassing the lateral margins. Psammophore with short hairs disposed in a triangle; the hairs on the top line are close to the foramen magnum and not reach the oral cavity. Scape long, surpassing the posterior margin of head. Mandibles strongly striate, with five teeth and at least two denticles along the masticatory margin and numerous denticles along the basal margin. Posterior margin of head with a feeble medial emargination. *Mesosoma* (Figures 11(b) and 11(c)): in profile with two tubercles, one in the posterior end of the mesonotum and one between the dorsal and the declivitous faces of the propodeum. *Metasoma*: petiolar scale directed dorsally, thin apically.

Queen and Male. Unknown.

Examined Material. Type series.

Geographic Distribution. COLOMBIA: (Bolivar: San Juan Nepomuceno, Santander: Bucaramanga, type localities).

Etymology. The name *tuberosus* refers to the presence of two tubercles on the dorsal face of both mesonotum and propodeum.

Natural History. Known only from museum collections. Specimens of Santander were collected in the campus of the Industrial University of Santander and those deposited in LACM have a label saying: "ex-Manihot," probably referred to be collected in a cultivate place. According to this data, *D. tuberosus* prefers, as several species of *Dorymyrmex*, disturbed habitats.

Examined Material. Type series.

5.8.3. Comments. Two well-developed tubercles on the mesosoma, along with whitish pubescence, and general dark color can be useful to differentiate *D. tuberosus* from the other species of *Dorymyrmex* found in Colombia. This species could be confused with *D. brunneus* by color but differs by the following characters: shape of the head, slightly wider after compound eyes and always with an emargination in the middle of posterior margin. Pro-mesosomal profile always at level or higher than the apex of propodeal cone. In contrary to *D. brunneus, D. tuberosus* has well-developed tubercles on the mesonotum.

5.9. Dorymyrmex xerophylus Cuezzo & Guerrero n. sp. (Figures 12(a)–12(c); 14)

5.9.1. Diagnosis

Worker. Small ants, TLI: <117. Head oval in full-face view, lateral margins parallel, and posterior margin strongly convex. Compound eyes not surpassing the sides of cephalic capsule. Propodeal tubercle short, stout, and lower than mesonotum. Pubescence dense and golden.

5.9.2. Description

Worker

Measurements. Holotype (Paratype): HL: 0.60 (0.62). HW: 0.44 (0.46). EL: 0.18 (0.18). EW: 0.12 (0.12). SL: 0.52 (0.54). WL: 0.70 (070). CI: 73 (74). SI: 87 (87). REL: 41 (39). OI: 67 (67). TLI: 117 (113).

Concolorous, light brown; only tergites 2 and 4 of gaster, darker. *Head* (Figure 11(a)): longer than wide. Mandibles feebly striate (only seen at more than 100x), with 4 teeth and 2 denticles. Compound eye well-developed in the first part of head in full-face view. Psammophore with only few hairs disposed in the central part of ventral cephalic face, not reaching the oral cavity; those hairs are equidistant between

the foramen magnum and the oral cavity. Scapes short (SI = 87). *Mesosoma* (Figures 12(b) and 12(c)): dorsal face of pronotum with two erect hairs lengthless than the greatest width of the antennal scape. Mesonotum straight in profile, lower than pronotum, only interrupted in its posterior end, forming a declivitous face continuous with propodeum. *Metasoma*: petiolar scale wide, thin and rounded apically.

Queen And Male. Unknown.

Examined Material. Type series.

Geographic Distribution. COLOMBIA (La Guajira and Magdalena, type localities).

Etymology. The specific name is in apposition, refers to the extremely arid environments where *D. xerophylus* usually nests.

Natural History. Ants of Magdalena were collected with sausage baits, between 10:00 and 11:00 a.m. Apparently, *D. xerophylus* prefers, like other *Dorymyrmex*, open areas of dry forests in lowlands. Specimens were found in dry forest of Sierra Nevada de Santa Marta, dominated by Poaceae. This habitat is subjected to occasional human disturbances resulting from logging. Ants collected in La Guajira live in restored areas of opencast coal mines, abandoned 10 years ago.

5.9.3. Comments. *D. xerophylus* is close to *D. goeldii* but differ by size, pubescence, and color. In Colombia, only this two species have the posterior margin of head strongly convex and head more than twice longer than wide (CI: 73-74 and 77–40 for *D. xerophylus* and *D. goeldii*, resp.).

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Research Article

Ant Community Structure (Hymenoptera: Formicidae) in Two Neighborhoods with Different Urban Profiles in the City of São Paulo, Brazil

Alcione Piva and Ana Eugênia de Carvalho Campos

Unidade Laboratorial de Referência em Pragas Urbanas, Instituto Biológico, Avenida Conselheiro Rodrigues Alves, 1252 São Paulo, SP, Brazil

Correspondence should be addressed to Ana Eugênia de Carvalho Campos, anaefari@biologico.sp.gov.br

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Some ant species are highly abundant in cities, may form huge unicolonial populations with thousands of individuals able to displace native fauna, and impoverish ecological relationships in urban environments. In this work, we study the ant community in two neighborhoods with different urban profiles, one recently populated and another from the 1900s in the city of São Paulo, Brazil. Two hundred and ninety houses were sampled with baits for ant collections. Results show that the recent urbanized neighborhood with greater disturbance favors opportunistic and dominant species to colonize it, like *Tapinoma melanocephalum*. We also made a temporal analysis in the ancient neighborhood, collecting ants after ten years from a first survey. *T. melanocephalum* has a broader range than ten years ago, displaced other ant species, but confronts with *Pheidole megacephala* that was not found in the recent urbanized neighborhood.

1. Introduction

Some ant species promote severe problems in Brazil, especially exotic ones with invading status, which were accidentally introduced in the country and may be found with huge populations, displacing native ant fauna, other arthropod species, and even vertebrates [1]. Among the most common invading species found in Brazilian cities, *Pheidole megacephala* (Fabricius, 1783), *Tapinoma melanocephalum* (Fabricius, 1793), *Nylanderia fulva* (Mayr, 1862), and *Paratrechina longicornis* (Latreille, 1802) [2–5] are highlighted.

Around the world, recordings on *P. megacephala* point this species as one of the most dominant and aggressive tramp ant species. In 1908, the big-headed ant invaded a Caribbean island, and surveys show that this is the only ant species found in that environment [6]. The same data were found in two, from five islands surveyed in Florida [7]. In Australia, *P. megacephala* displaced dominant Dolichoderinae species, Camponotini, and other *Pheidole* species in forested areas [8]. The big-headed ant biomass in areas where it dominates is 18 times greater than the native ant species biomass where it does not occur [9]. In a study conducted in urban areas from the Brazilian Cerrado, *P. megacephala* predominated in areas with low conservation efforts [5].

Tapinoma melanocephalum originated from the Indo-Pacific region is one of the ant species with broader distribution [10]. As in other parts of the world, it was the most abundant species in a neighborhood in the city of São Paulo [3], in a hospital in the Central-Eastern Brazil [11], in residences in Ilhéus [2], Uberlândia [4], and Mogi das Cruzes [12].

But not only are these common worldwide tramp ants found in Brazilian cities. Others, native or exotic, are also household invaders. *Brachymyrmex* sp., *Solenopsis saevissima* (F. Smith, 1855), *S. invicta* Buren, 1972, *Monomorium* spp.,

Crematogaster sp., *Cyphomyrmex* sp., *Cardiocondyla* sp., *Linepithema humile* (Mayr, 1868), *Dorymyrmex* sp., *Camponotus* spp., and *Pachycondyla* sp. [2, 3, 13, 14].

Studies have focused on the biology of invading species and found out that they share some common characteristics like multiple queens, polidomic nests with a huge cooperation net among the nests, without any aggressive behavior [15], and also their clear association with disturbed environments [16]. On the other hand, one might understand which are the patterns and characteristics in the urban environment to shelter invading ant species and use this knowledge to promote satisfactory management and control. In this way, Clarke et al. [17] examined soil characteristics and type of vegetation in urban parks in San Francisco, USA, and concluded that soil moisture positively affects ant species richness, and percent of natural area that is forested was negatively related. Forys and Allen [18] established a positive correlation between exotic and native ant species in Florida. The authors stated that the same biotic and abiotic characteristics may favor both species. But they highlighted that an increase on urban sprawl leads to an increase on the number of exotic ant species, once they are known to displace native ant fauna.

In New York, a study revealed that besides introduced species that are usually favored by disturbance, this process must be much more complex than it is known [19]. In this survey, *Tetramorium caespitum* (L.), a generalist exotic species that nests in cracks on sidewalks, was the most collected ant, but in most streets, it occurred with other ant species, exotic or native to the USA. This same ant species was also collected in a survey conducted in the surroundings of Montreal close to a forested area [20].

For this purpose, two neighborhoods were surveyed, and ant presence was associated to recent or ancient urbanization and other abiotic factors furnished by man.

2. Material and Methods

2.1. Surveyed Neighborhoods. Ant collections were made in households in two neighborhoods in the city of São Paulo: Vila Mariana and Itaquera.

Itaquera is located in the Eastern side of the city of São Paulo 20 Km apart from downtown. Its 55.32 km^2 have low- and medium-density residential areas, few apartment buildings, an environmental protection area with a rainforest remaining with 867.60 hectares, and an industrial and an agriculture area. Besides the first residences date from the 1900s, the urbanization process initiated 35 years ago and is still growing.

Vila Mariana is 26.87 km^2, 5 Km from downtown, with median- and high-density residential blocks, with many apartment buildings and commercial areas. There are no industrial, agricultural, or environmental protection areas. Urbanization process started in the 1900s, and most houses are from the 1950s. Today, some houses are being substituted by modern apartment buildings, but still ancient houses are found, and most of them are well preserved.

Ant collections were performed in Vila Mariana in two periods: from December 1998 to October 1999 and ten years later, from December 2009 to January 2011. In Itaquera, collections were made from December 2009 to January 2011.

Two hundred and ninety houses were sampled: 132 in Vila Mariana in the period 1998/1999, 79 in Vila Mariana (2009–2011), and 79 in Itaquera (2009–2011).

Households were randomly chosen. We could repeat the ant collection in the same 25 houses in Vila Mariana in 2009–2011 after 10 years. The other 54 houses were surveyed for the first time in the recent years. Baits based on dehydrated liver, pineapple cake, and honey were set in 7 cm length straws which were placed in the houses, 15 baits in each house and three in each room: living room, bedroom, kitchen, bathroom, and outdoors (peridomiciliar area). In addition, manual collections were also performed using brushes and a bottle-type vacuum. The baits were left for 24 hours, and collected ants were taken to the laboratory for identification and counting. In addition to the collections, it was also filled in a form with data relating to the environment as house conservation, cleanness, number of children, and presence of pets.

2.2. Statistical Analysis. We used descriptive statistics with graphics and tables. Categorical and numerical variables for neighborhood characterization were obtained from a form filled in for each ant collection (Table 1). We computed percentage values for categorical analysis (number of floors and children, pet and garden presence) and the mean number for numerical variables (number of rooms per house, number of residents and house age).

Association analysis was calculated for the five most collected ant species in the three samples. For that purpose, the Pearson's chi-square test was used or the Fisher's exact test (for expected values less than 5).

For numerical variables, the Mann-Whitney test was used to compare two categories, and the Kruskal-Wallis Test was used to compare three or more categories due to the absence of normal distribution from the variables [21]. The level of 5% of significance was used ($P < 0.05$). The SAS system for Windows (statistical analysis system), version 8.02, was used for statistical analysis [22].

Relative frequency was calculated to analyze the most frequent ant species indoors and outdoors in each neighborhood, in each period of collection.

Shannon diversity index, equitability index, and Simpson's Index were calculated with the BioDap software [23] in order to record ant community in the neighborhoods.

3. Results

3.1. Ant Community. It was collected 7.249 ant specimens in the 132 collections in Vila Mariana in 1998/1999, 6.477 specimens in the 79 collections in Vila Mariana in 2009–2011, and 12.054 specimens in the 79 collections in Itaquera in 2009–2011 (Table 2).

In Itaquera, 33 ant species were found, most native to Brazil, but 4 were exotic ones. In Vila Mariana, we collected 23 ant species in 1998/1999, 3 were exotic, and from 2009–2011 we collected 25 ant species and 4 were exotic.

Table 1: Data from surveyed households in Vila Mariana and Itaquera neighborhoods in the city of São Paulo, Brazil.

Control number	() Itaquera		() Vila Mariana	Date: / /
Address				
Contact			() Single-story house	() Two-story house
Number of rooms ()	Number of residents ()		Children: () Yes	() No
House age:			Garden: () Yes	() No
			Pets: () Yes	() No
Conservation condition	() Very good	() Good	() Bad	() Very bad
Cleanness condition	() Very good	() Good	() Bad	() Very bad

Tapinoma melanocephalum was the most abundant species in the three collections (VM 1998/1999, VM 2009–2011, and ITA 2009–2011).

In Vila Mariana, ten years later, it was found an increase on *T. melanocephalum* frequency and a decrease on the other ant species frequency as *N. fulva, Brachymyrmex* sp. 1, and *Solenopsis* sp., which are all native to Brazil. *L. humile*, also native to Brazil and found in 1998/1999, was not found in the surveys from 2009 to 2011. This is a species that could have had success in dominating the neighborhood. But the exotic big-headed ant, *P. megacephala,* expanded its foraging range ten years later. Besides its high frequency in Vila Mariana, *P. megacephala* was not found in Itaquera.

Ten years ago, *N. fulva* was the most frequent species, present in more than 40% of the sampled houses in Vila Mariana. Its occurrences were even indoors and outdoors. In 1998/1999, *P. longicornis* was also found outdoors with *N. fulva.* Our last survey from 2009 to 2011 showed that *P. longicornis*' frequency increased almost 3 times (Table 2), but its occurrence was limited to the peridomiciliar area. As *N. fulva, P. longicornis* is also an invasive ant species and can be displacing the native *N. fulva.* But the association analysis of *N. fulva* was negative with *T. melanocephalum* and *P. megacephala* (Figure 1) in the survey from 1998 to 1999. Thus, *N. fulva* may not succeed in colonizing Vila Mariana all these years, once it competed with three other invasive ants. As we calculated the association only among the five most frequent species, *P. longicornis* did not enter in 1998/1999 analysis.

From 2009 to 2011, few associations could be established among the ant species, but *P. longicornis* frequency increased and showed negative association with *P. megacephala* (Figure 2).

T. melanocephalum was mainly collected indoors (living rooms, bedrooms, kitchens, and bathrooms). When we compared samples from *T. melanocephalum* in 1998/1999 and ten years later, its relative frequency increased from 18% to 53% inside houses. *P. megacephala* indoor frequency was the same ten years later, but it expanded its foraging areas outdoors from 2009 to 2011. *Brachymyrmex* sp. 1 and *Solenopsis* sp. frequencies decreased in the outdoors in the last samples (Figure 3).

We analyzed the same 25 houses surveyed in 1998/1999 and 2009–2011, and data do not show significant differences from the 79 surveyed houses analyzed together (Figure 4).

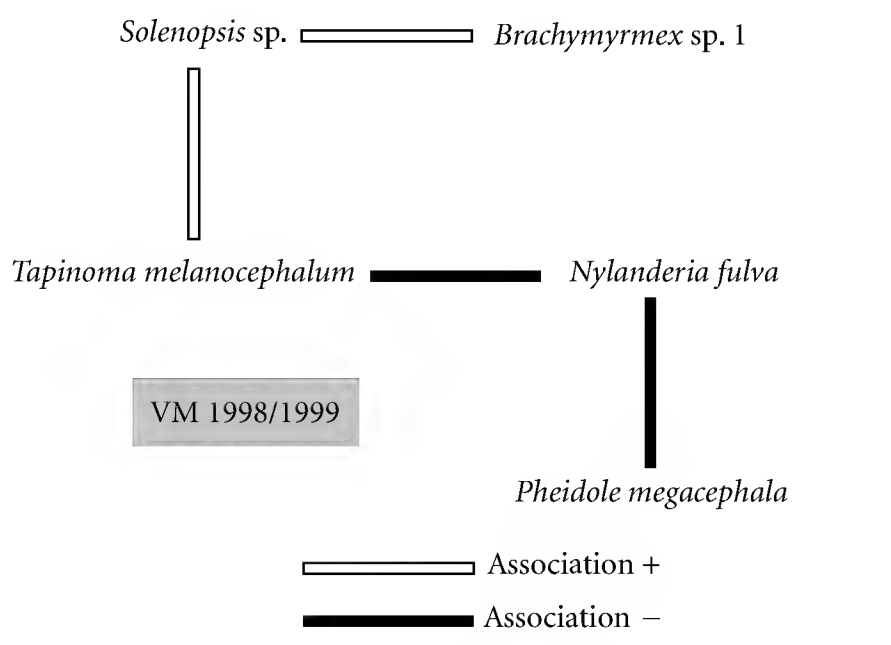

Figure 1: Community structure of house-infesting ants in Vila Mariana, São Paulo, from 1998 to 1999.

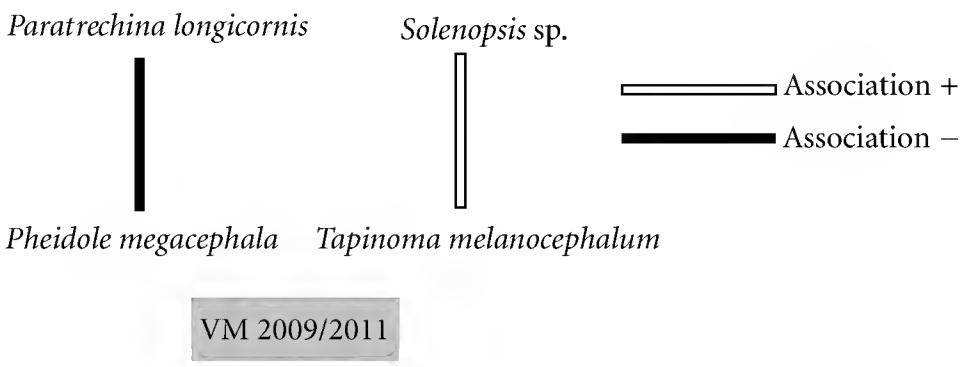

Figure 2: Community structure of house-infesting ants in Vila Mariana, São Paulo, from 2009 to 2011.

T. melanocephalum and *P. megacephala* are the most frequent species, and *N. fulva* decreased its frequency in ten years (Figure 4).

The number of ant species per house varied from 1 to 10 (Figure 5). The number of houses with only one ant species increased in the survey from 2009 to 2011 in Vila Mariana, due to *T. melanocephalum* increase.

In the opposite, Itaquera shows more houses with more ant species, compared to Vila Mariana.

The diversity indexes revealed that Vila Mariana has a more diverse and balanced ant community compared to Itaquera. The temporal analysis showed a decrease in the ant diversity in Vila Mariana (Table 3).

Table 2: Ant species richness and relative frequency in Vila Mariana (VM 1998/1999 and VM 2009–2011) and Itaquera (ITA 2009–2011), in the city of São Paulo, Brazil.

Taxon	Relative frequency (%) VM 1998/1999	Relative frequency (%) VM 2009–2011	Relative frequency (%) ITA 2009–2011
Acromyrmex sp. 1	—	—	3.80
Brachymyrmex sp. 1	36.36	17.72	8.86
Brachymyrmex sp. 2	—	6.33	—
Brachymyrmex sp. 3	—	1.27	—
Camponotus (*Tanaemyrmex*) sp. 1	6.82	1.27	1.27
Camponotus crassus (Mayr, 1862)	—	1.27	7.59
Camponotus rufipes (Fabricius, 1775)	—	—	6.33
Camponotus sp. 1	1.52	—	—
Camponotus sp. 2	—	2.53	—
Cardiocondyla sp. 1	15.15	—	1.27
Crematogaster sp.	1.52	—	1.27
Cyphomyrmex sp. 1	0.76	1.27	3.80
Dorymyrmex sp. 1	0.76	—	3.80
Ectatomma sp. 1	—	1.27	5.06
Nesomyrmex sp. 1	5.30	2.53	16.46
Linepithema humile (Mayr, 1868)	10.61	—	—
Linepithema sp. 1	—	—	3.80
Linepithema sp. 2	—	—	1,27
Monomorium floricola Jerdon, 1851	—	6.33	5.06
Monomorium pharaonis (Linnaeus, 1758)	—	—	2,53
Monomorium sp.	6.06	—	—
Nylanderia fulva (Mayr, 1862)	40.15	5.06	17.72
Octostruma sp. 1	—	—	1.27
Pachycondyla sp. 1	0.76	2.53	3.80
Pachycondyla sp. 2	—	—	1.27
Paratrechina longicornis (Latreille, 1802)	6.06	17.72	22.78
Pheidole dimidiata Emery, 1894	7.58	2.53	1.27
Pheidole fallax Mayr, 1870	3.79	—	1,27
Pheidole megacephala (Fabricius, 1783)	24.24	37.97	—
Pheidole risii Forel, 1892	0.76	1.27	1.27
Pheidole sp. 1	—	7,59	2,53
Pheidole sp. 2	—	—	1.27
Pheidole sp. 3	—	—	3.80
Pheidole sp. 4	—	7.59	2.53
Pheidole sp. 5	—	—	1.27
Pheidole sp. 6	0.76	1.27	—
Pheidole sp. 7	0.76	—	—
Pheidole nubila Emery, 1906	0.76	—	6.33
Hypoponera sp. 1	—	1.27	—
Pseudomyrmex termitarius (Smith, 1855)	—	1.27	1.27
Solenopsis sp.	23.48	7.59	25.32
Solenopsis (Diplorhoptrum) sp. 1	12.12	3.80	2.53
Tapinoma melanocephalum (Fabricius, 1793)	19.70	56.96	79.75
Wasmannia auropunctata (Roger, 1863)	—	3.80	—

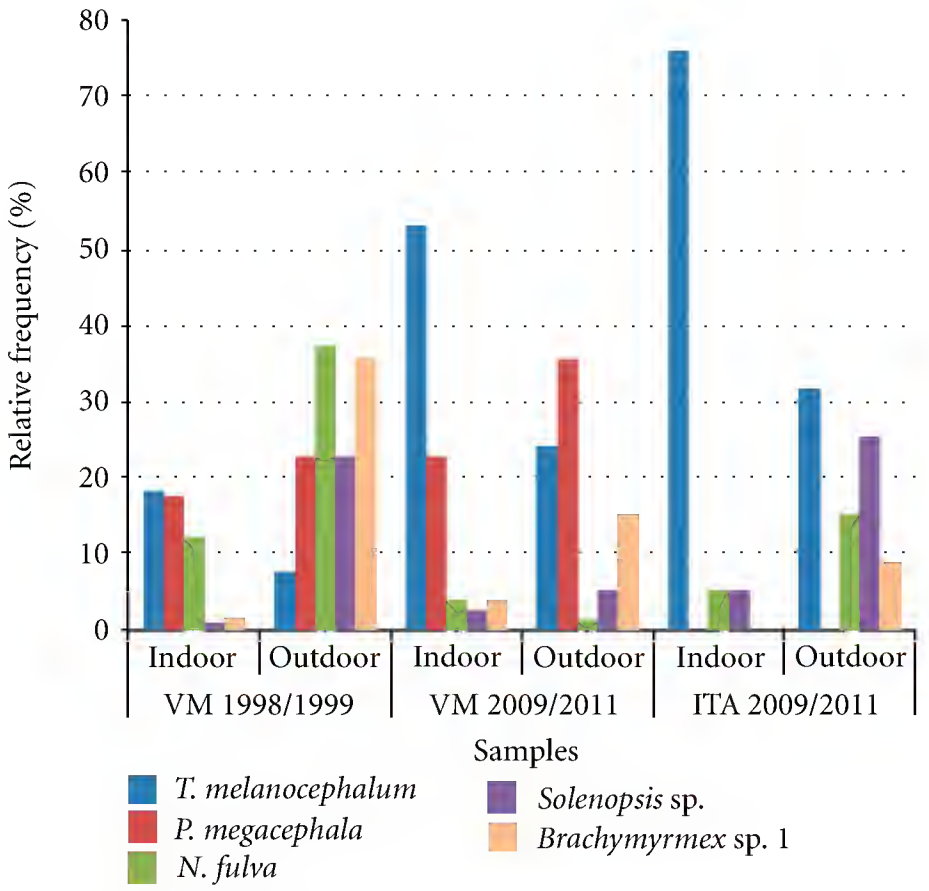

FIGURE 3: Relative frequencies of the most common sampled ant species in Vila Mariana from 1998 to 1999 (VM 1998/1999), 2009 to 2011 (VM 2009–2011) and Itaquera, from 2009 to 2011 (ITA 2009/2011), in the city of São Paulo.

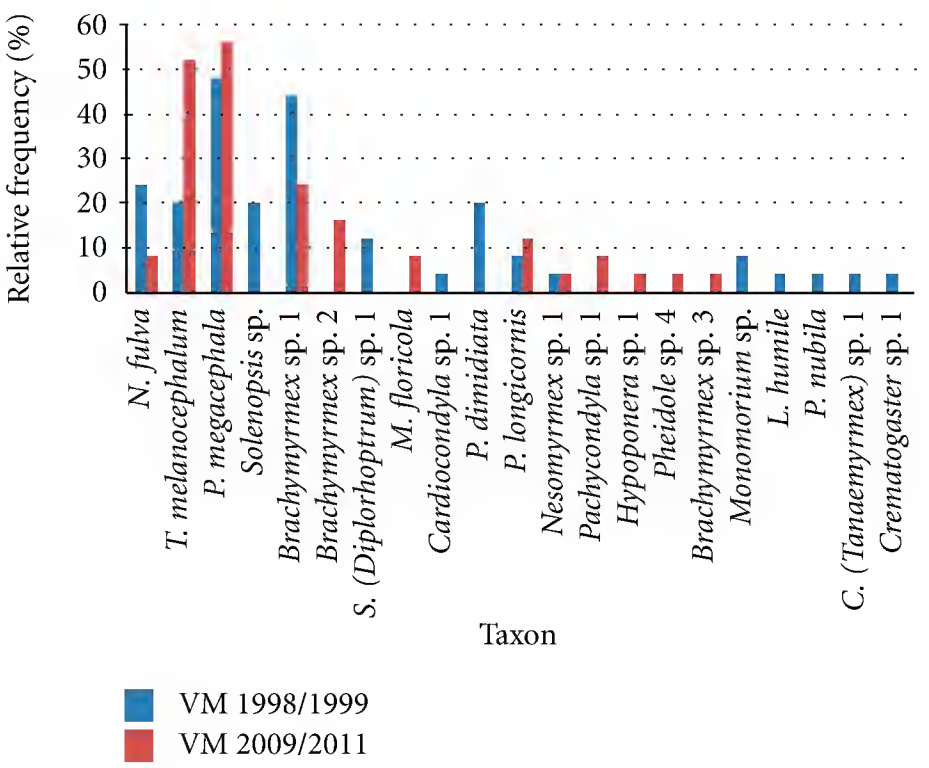

FIGURE 4: Relative frequency of ant species collected in the same 25 houses in 1998/1999 and 2009–2011, in Vila Mariana (VM), São Paulo, Brazil.

3.2. Neighborhood Profiles. Itaquera is a neighborhood with small- and single-story houses, recently constructed, with more children and few areas of gardens and backyards. The worst conserved and with less cleanness houses were found there. In Vila Mariana, houses are bigger, more ancient than in Itaquera, with a large number of two-story houses, and with more gardens and backyards (Table 4).

3.3. Comparative Analysis between Ant Samples and Environment Characteristics. The Mann-Whitney and Kruskal-Wallis tests showed that more ant species were found in

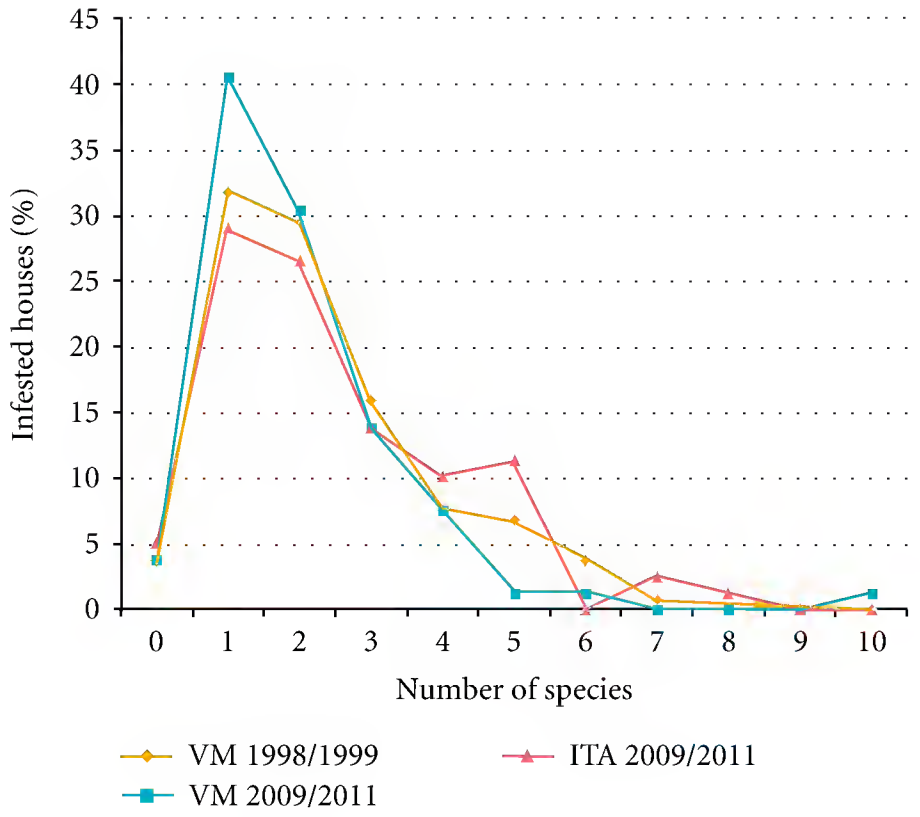

FIGURE 5: Number of ant species collected per house in Vila Mariana (VM), 1998/1999 and 2009–2011, and Itaquera (ITA) in the city of São Paulo, Brazil.

TABLE 3: Species richness, Shannon-Wiener diversity index (H′), equitability (E), and Simpson's diversity index (D) in Vila Mariana and Itaquera, in the city of São Paulo.

	Vila Mariana (1998/1999)	Vila Mariana (2009–2011)	Itaquera (2009–2011)
Species richness	24	25	33
H′	1.87	1.42	0.96
E	0.60	0.44	0.27
D	0.207	0.381	0.648

TABLE 4: Characteristics of houses sampled in Vila Mariana and Itaquera, in the city of São Paulo, Brazil.

Characteristic	Vila Mariana (1998/1999)	Vila Mariana (2009–2011)	Itaquera (2009–2011)
One-story houses (%)	11.4	15.2	74.7
Two-story houses (%)	88.6	84.8	25.3
Mean number of rooms per house	9.4	8.1	4.8
Mean of residents per house	3.4	3.4	3.7
Houses with children (%)	20.8	16.4	43.5
Mean house age (years)	44.8	49.9	25.1
Houses with gardens (%)	85.6	72.1	34.1
Houses with pets (%)	47.3	50.6	60.7

single-story houses ($P = 0.007$), mainly in their outdoors, and in both neighborhoods, in both periods of collections. *T. melanocephalum* was more frequent in houses with children ($P = 0.001$). On the other hand, *P. megacephala* was more

frequent in houses without children ($P = 0.007$). Yet, according to the Mann-Whitney test, the highest frequencies of ant specimens outdoors ($P < 0.001$), as the number of ant species ($P = 0.002$), are higher in external areas in houses with gardens and backyards. *P. megacephala* is mainly found in houses with gardens and backyards ($P < 0.001$). Houses without pets showed the highest species richness even indoors ($P = 0.042$), and outdoors ($P = 0.016$), the worst conserved houses showed species richness ($P = 0.042$) and pour cleanness houses showed higher ant abundance ($P = 0.047$).

4. Discussion

Tapinoma melanocephalum was the most common species in Vila Mariana in both studied periods and in Itaquera. Other surveys in urban areas also pointed to this same species [4, 14, 24] as one of the most common ants in urban environments. *T. melanocephalum* is distributed throughout the world [10] and recruits a huge number of workers when a food resource is found [13], explaining its high abundance in our samples. As in São Paulo, in a survey conducted in New York in 2010, an exotic species was the most collected [19].

After ten years, it expanded its range in Vila Mariana as did *P. megacephala* and *P. longicornis,* contributing to the displacement of *N. fulva*, *Brachymyrmex* sp. 1, and *Solenopsis* sp. populations. *P. megacephala* is an aggressive and dominant ant species known to displace native ant fauna where it is introduced [7–9]. *P. longicornis* was pointed by Forys and Allen [18] as the most common ant species in the region of Lower Florida Keys, and it was positively associated with the increase of urban development.

In a survey conducted in 1994 in a banana crop in southeastern Brazil, Fowler et al. [25] reported negative association between *T. melanocephalum* and *P. longicornis*. From 80 surveyed banana farms, 31 showed *P. longicornis* and 18 had *T. melanocephalum* presence, and in none of them both species were collected together. In our study, *P. longicornis* association was not analyzed with other ant species in Vila Mariana in 1998/1999 once its frequency and abundance were too low. But frequency data compared in both collected periods in Vila Mariana showed that this species is expanding its range. When association analysis was calculated to the most recent ant collections in Vila Mariana, *P. longicornis* only showed negative association with *P. megacephala*. This is explained by the fact that *P. longicornis* was not collected inside households, only outdoors where we also found *P. megacephala*. As *T. melanocephalum* occurrence is more frequent indoors than outdoors, they did not establish any ecological association.

In the central-eastern Brazil, surveys showed *P. megacephala* in ancient neighborhoods, with intense commercial activities and without expressive green areas [4], environments similar to Vila Mariana. Pacheco and Vasconcelos [5] also found this species in commercial areas and poorly preserved. In fact, commercial areas were also reported in a survey conducted by Menke et al. [26] with several invading ant species, as we have found in Vila Mariana. Therefore, it cannot be affirmed that this species prefers such urban environments. The establishment and dominance of invading species are related to several biotic and abiotic factors that must be better studied.

For *T. melanocephalum* to establish its nests, cleanness and resident habitats seem not to be relevant, but they can be decisive to their high frequency and abundance. In Itaquera, around 43% of the total sampled houses showed regular or very bad cleanness, and almost 25% were poorly preserved. Such conditions favor food offering and shelter [27] in order to promote suitable conditions for ant colonies development. Therefore, the best condition observed in Itaquera to be a good environment for *T. melanocephalum* was the absence of other ant species, as dominant as the ghost ant is, like *P. megacephala*.

Children presence may favor *T. melanocephalum* establishment, once data were similar in Vila Mariana after one decade and when the neighborhoods were separately analyzed. Children may offer more food on several rooms of the house favoring this opportunistic ant species.

A decrease on the ant diversity in Vila Mariana after 10 years was found when we calculated the Shannon-Wiever diversity index, but even though it is higher than in Itaquera. The low ant diversity in Itaquera must be attributed to *T. melanocephalum* abundance. Simpson's index corroborates this statement, once its value was higher in Itaquera, pointing to the dominance of one ant species.

An interesting data showed by the comparative analysis using the Mann-Whitney test between the collections from 2009 to 2011 in the two different neighborhoods is that *T. melanocephalum* was found in the peridomiciliar area in Itaquera and Vila Mariana equally. Peridomiciliar in these neighborhoods was different, with more gardens and backyards in Vila Mariana. But *T. melanocephalum* occurrence outdoors was lower than indoors, in both neighborhoods, probably due to the competition with other ant species and to abiotic factors such as temperature and moisture. In a survey conducted in 2009 by Wetterer [10], he reports that the ghost ant is restricted to indoors once the species can live wherever man is. He also emphasizes that it is found in tropical and subtropical zones in latitudes higher than 30°. MacGown and Hill [28] confirm this statement and comment that, besides, it is found in the United States and in some states in Canada; the occurrences are in greenhouses and other warm places, strengthening that temperature influences its distribution, but it can be solved when occupies human dwellings with controlled temperature. In our work, *T. melanocephalum* was found mainly in the indoors even though there were differences among houses and human habits in both neighborhoods. Menke et al. [26] also highlight the role of cities as a refuge for generalist species that show more tolerance to dryer and hotter environments.

Differences in the environment outdoors are not significant for *T. melanocephalum* once this is not the most favorable environment for its establishment.

Itaquera has a recent urbanization process; new households, streets, and avenues have been continually built as other landscape transformation caused by such processes, what may favor opportunistic ant species with flexible adjustments to such disturbances, like *T. melanocephalum*.

Many authors discuss this fact like Tschinkel [16] who suggested that *S. invicta* whose native environment is seasonally flooded and is a species that easily adapts to highly disturbed environments, even in different continents. When native ant populations were compared with invasive/exotic ant species in recent and ancient urban areas, the exotic species succeed in establishing in recent urbanized areas [29], while the native species remained in the ancient urban areas. Native and exotic species distribution in a city is still a not very well-explained question although the preference from exotic species for disturbed environments is known. Studies suggest that there is a series of relationships between biotic and abiotic factors that makes this question so complex [18, 19].

This explains the species richness found in Itaquera compared to Vila Mariana and the high dominance by *T. melanocephalum* in the former neighborhood, a still unknown ant species but intimately linked to the São Paulo citizens.

Yet, the number of houses with several ant species in Itaquera is related to the green area that surrounds the neighborhood and many species that are not common to urban areas like *Acromyrmex* sp., *C. crassus, C. rufipes, Cyphomyrmex* sp., *Linepithema* spp., *Octostruma* sp., and 9 *Pheidole* species. The bad conservation of houses in Itaquera also may contribute to the establishment of ants inside and in the peridomiciliar areas of the houses.

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Research Article

Contribution of Cytogenetics to the Debate on the Paraphyly of *Pachycondyla* spp. (Hymenoptera, Formicidae, Ponerinae)

Cléa dos Santos Ferreira Mariano,[1,2] Silvia das Graças Pompolo,[3] Janisete Gomes Silva,[1] and Jacques Hubert Charles Delabie[2,4]

[1] *Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Km 16 Rodovia Ilhéus-Itabuna, 45650-000 Ilhéus, BA, Brazil*
[2] *Laboratório de Mirmecologia, Centro de Pesquisas do Cacau (CEPEC/CEPLAC), Caixa Postal 7, 45600-000 Itabuna, BA, Brazil*
[3] *Laboratório de Citogenética de Insetos, Departamento de Biologia Geral, Universidade Federal de Viçosa, 35570-000 Viçosa, MG, Brazil*
[4] *Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Km 16 rodovia Ilhéus-Itabuna, 45650-000 Ilhéus, BA, Brazil*

Correspondence should be addressed to Cléa dos Santos Ferreira Mariano, camponotu@hotmail.com

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We present evidence of the paraphyly of the ant genus *Pachycondyla* resulting from our cytogenetic studies on 29 populations in 18 species from Brazil and French Guyana. It is likely that karyotypes with a large number of chromosomes and comprising mostly small acrocentric chromosomes in species within the *Pachycondyla stricto sensu* group resulted from a succession of centric fission events. On the other hand, karyotypes with a small chromosome number comprising mostly metacentric chromosomes are also interpreted as little derived and tend to undergo centric fission. The karyotypes of the group *Neoponera* are more heterogeneous and probably undergo successive cycles of rearrangements tending to increase the chromosome number by centric fission. The *apicalis* and *verenae* complexes form two probable sister groups that evolved independently due to centric fissions (*verenae*) and pericentric inversions (*apicalis*). Our results reveal the karyotype diversity in the genus and reinforce the hypothesis on the paraphyly of *Pachycondyla*.

1. Introduction

Among the Ponerinae, the genus *Pachycondyla* (Ponerinae, Ponerini) is one of the most ancient known genera of ants and still extant. A fossil species, *Pachycondyla rebekkae* Rust and Andersen, was found in calcareous rocks from the early Tertiary (±55 million years) in formations in northwest Denmark [1]. The current distribution of this genus (*Pachycondyla sensu* Brown, in Bolton, [2]) is pantropical with 197 valid species [3, 4]. A recent review of the New World species of *Pachycondyla* reports 92 species alone in the Neotropics and characterizes 18 complexes of species based on morphological characters [4].

According to Kempf [5], *Pachycondyla* comprised only 10 species in the Neotropical region, whereas other Neotropical taxa currently included in this group [3, 4] were distributed within the genera *Neoponera, Mesoponera, Pachycondyla, Termitopone,* and *Trachymesopus* in his catalogue. This classification was maintained until Bolton [2] proposed a synonymization based on arguments already discussed by Brown [6]. According to Schmidt's conclusions [7], and recently commented by Ward [8] who called it the "*Pachycondyla* problem," the group of ants currently denominated "genus *Pachycondyla*" is paraphyletic. Taking into account only the Neotropical taxa, this taxon would comprise six species groups (according to Schmidt's classification) not necessarily related.

Cytogenetic studies on insects not only can significantly contribute to the understanding of morphological characteristics but also can shed some light on taxonomic and

evolutionary aspects, as, for instance, on groups of species in sympatry [9] competing for the same resources or cryptic species complexes [10, 11]. In the order Hymenoptera, cytotaxonomy has been used by Baldanza et al. [12], Hoshiba and Imai [13], Gokhman [14], and Gokhman and Kuznetsova [15] as a character for taxonomic and evolutionary studies. The determination of a karyotype and the occasional observation of the occurrence of chromosome rearrangements are especially important to make inferences regarding evolutionary or speciation processes. Since gene expression is regulated at least partially by the location of neighboring genes, chromosome alterations can result in phenotype alterations [16] and drive speciation processes. Thus, the understanding of karyotype evolution is valuable for evolutionary, phylogenetic, and taxonomic studies [17, 18] and can be used as a tool to evaluate species diversity.

Regarding Formicidae, Lorite and Palomeque [19] report more than 750 morphospecies with known chromosome number, which is still a relatively small number considering the diversity of this family estimated to be about 21.000 species [20]. In the Ponerinae, cytogenetic studies have been published for 95 morphospecies in 12 genera with chromosome numbers ranging between $2n = 8$ and $2n = 120$ [19], which is a considerable variation when compared to the remaining ant families, except for Myrmeciinae. It is noteworthy that karyotype variation among populations of the same species is frequent in the genera *Myrmecia* [21] and *Pachycondyla* [22–25]. Among the Ponerinae, *Pachycondyla* has been the most studied genus (40 morphospecies) and also the one with the highest variation in chromosome number $2n = 12 - 104$ [19]. Several hypotheses have been tested to understand karyotype evolution in ants including the fusion, fission, and modal hypotheses summarized by Imai et al. ([26], see also [19, 27]). In 1988, Imai and collaborators proposed the Minimum Interaction Theory that states that the chromosome interactions in the interphasic nucleus are responsible for changes in the karyotypes [28, 29]. The same research group [21, 27] developed the karyographic method as a tool that allows to visually explain karyotype evolutionary processes based on metaphase rearrangements. Although this method has not been much used [18, 21, 30–32], it is the only way to compare a large set of karyotype data and make inferences about the studied groups, except for comparative studies using molecular cytogenetic techniques [33, 34].

According to Lorite and Palomeque [19], the chromosome groups reported in Formicidae suggest the occurrence of different patterns of karyotype evolution in different taxonomic groups. Aiming at contributing to the knowledge of Neotropical poneromorphs, our research group has been developing interdisciplinary studies on different species of the subfamily Ponerinae. In this study, we investigated a series of Neotropical taxa within the genus *Pachycondyla sensu* Brown (Table 1) whose monophyly has been questioned by some authors [7, 23]. We also discussed hypotheses regarding the evolution of lineages that comprise this taxon, which is so important for the conservation of forest biomes in the Neotropical region [4].

2. Material and Methods

Colonies of *Pachycondyla* spp. were collected in 13 localities (Table 2) in several states in Brazil and in French Guyana in areas of the Atlantic rainforest, cocoa plantations, Caatinga, and the Amazonian rainforest between 2000 and 2010. In order to make comparisons feasible, we used original and published information as shown in Table 2.

Species identification was carried out following the review by MacKay and MacKay [4] and the species complexes proposed by them. However, aiming at comparing the studied taxa, we also refer to the previous classification by Kempf [5], to the synonymization of different genera of Ponerinae under *Pachycondyla* by Brown *in* Bolton [2] and to a recent generic reclassification proposed by Schmidt [7] but still not fully formalized (Table 1).

Mitotic metaphases were obtained from cerebral ganglia and male gonads treated with 0.005% colchicine for 20–40 minutes and the chromosomes were stained with Giemsa 2% according to Imai et al. [29]. The images were captured using Image-Pro Discovery version 4.5 software under a clear field microscope. Metaphases of some the taxa studied were used to exemplify chromosome patterns.

Our analyses were based on chromosome number and morphology. Unpublished information or available in literature [19, 35–37, Mariano et al., unpublished information] on chromosome number and their structure in the Ponerinae subfamily and for *Pachycondyla sensu* Brown is used for comparison. Chromosomes were classified according to Imai's terminology [38]. The karyotypes studied were grouped and compared mainly based on Schmidt's classification [7]. Inferences on karyotype evolution in groups within *Pachycondyla sensu* Brown were carried out based on karyographs following Imai and Crozier [27] and Imai et al. [21]. Such an analysis allows for the discussion of the direction of karyotype evolution at the taxonomic group level (Figures 1 and 6).

3. Results

A graphic comparison of the karyotype diversity among Neotropical species of Ponerinae and the taxa within the genus *Pachycondyla sensu* Brown is shown in a histogram (Figure 2). Species within the genus *Pachycondyla sensu* Brown and the Neotropical species in the same genus were discriminated from the remaining genera belonging to the subfamily Ponerinae. We found an ample chromosome variation, which had already been observed in Ponerinae, showing the extreme karyotype heterogeneity within this subfamily, especially when compared with the remaining subfamilies of Formicidae, except for the Australian Myrmeciinae [19].

The Neotropical taxa within the genus *Pachycondyla sensu* Brown studied and their respective classification according to Kempf [5], Bolton [2], Schmidt [7], and MacKay and MacKay [4] are listed in Table 1. Our results comprise four groups similar to Schmidt's proposal [7], and among these groups, *Neoponera* was the largest in this study and also the group with the most variable chromosome

Table 1: List of species considered here, generic classification according to Kempf, 1972 [5]; Brown [6] *in* Bolton, 1995 [2]; Schmidt's (2009) proposition for genera names; *Pachycondyla* species complex according to MacKay and MacKay's [4] and taxonomic unit names used in this study and based on ecological, cytogenetic, and morphological evidences.

Pachycondyla species	Genera according to Kempf, 1972 [5]	Genus according to Brown *in* Bolton, 1995 [2]	Schmidt's (2009) genera name proposition	MacKay and MacKay's [4] *Pachycondyla* species complex	Name of the taxonomic unit used in this study
Pachycondyla apicalis (Latreille, 1802)	*Neoponera*	*Pachycondyla*	*Neoponera*	*apicalis*	*Neoponera-apicalis*
Pachycondyla arhuaca (Forel, 1901)	*Mesoponera*	*Pachycondyla*	*Pachycondyla (Incertae Sedis)*	*arhuaca*	—
Pachycondyla carinulata (Roger, 1861)	*Neoponera*	*Pachycondyla*	*Neoponera*	*crenata*	*Neoponera-crenata*
Pachycondyla concava (Mackay and Mackay, 2010)	—	*Pachycondyla*	—	*emiliae*	*Neoponera-emiliae*
Pachycondyla constricta (Mayr, 1884)	*Mesoponera*	*Pachycondyla*	*Mayaponera*	*constricta*	—
Pachycondyla crassinoda (Latreille, 1802)	*Pachycondyla*	*Pachycondyla*	*Pachycondyla*	*crassinoda*	*Pachycondyla*
Pachycondyla crenata (F Smith, 1858)	*Neoponera*	*Pachycondyla*	*Neoponera*	*crenata*	*Neoponera-crenata*
Pachycondyla curvinodis (Forel, 1899)	*Neoponera*	*Pachycondyla*	*Neoponera*	*foetida*	*Neoponera-foetida*
Pachycondyla gilberti (Kempf, 1960)	*Trachymesopus*	*Pachycondyla*	*Pseudoponera*	*stigma*	*Pseudoponera*
Pachycondyla goeldii (Forel, 1912)	*Neoponera*	*Pachycondyla*	*Neoponera*	*crenata*	*Neoponera-crenata*
Pachycondyla harpax (Fabricius, 1804)	*Pachycondyla*	*Pachycondyla*	*Pachycondyla*	*crassinoda*	*Pachycondyla*
Pachycondyla impressa (Roger, 1861)	*Pachycondyla*	*Pachycondyla*	*Pachycondyla*	*crassinoda*	*Pachycondyla*
Pachycondyla inversa (F Smith, 1858)	*Neoponera*	*Pachycondyla*	*Neoponera*	*foetida*	*Neoponera-foetida*
Pachycondyla marginata (Roger, 1861)	*Termitopone*	*Pachycondyla*	*Neoponera*	*laevigata*	*Neoponera*
Pachycondyla metanotalis (Luederwaldt, 1918)	*Pachycondyla*	*Pachycondyla*	*Neoponera*	*emiliae*	*Pachycondyla*
Pachycondyla moesta (Mayr, 1870)	*Neoponera*	*Pachycondyla*	*Neoponera*	*crenata*	*Neoponera-crenata*
Pachycondyla stigma (Fabricius, 1804)	*Trachymesopus*	*Pachycondyla*	*Pseudoponera*	*stigma*	*Pseudoponera*
Pachycondyla striata (Smith, 1858)	*Pachycondyla*	*Pachycondyla*	*Pachycondyla*	*crassinoda*	*Pachycondyla*
Pachycondyla succedanea (Roger, 1863)	*Trachymesopus*	*Pachycondyla*	*Pseudoponera*	*stigma*	*Pseudoponera*
Pachycondyla unidentata Mayr, 1862	*Neoponera*	*Pachycondyla*	*Neoponera*	*crenata*	*Neoponera-crenata*
Pachycondyla venusta (Forel, 1912)	*Neoponera*	*Pachycondyla*	—	*emiliae*	*Neoponera-emiliae*
Pachycondyla verenae (Forel, 1922)	*Neoponera*	*Pachycondyla*	*Neoponera*	*apicalis*	*Neoponera-verenae*
Pachycondyla villosa (Fabricius, 1804)	*Neoponera*	*Pachycondyla*	*Neoponera*	*foetida*	*Neoponera-foetida*

Table 2: Chromosome number and karyotypes of 29 Neotropical *Pachycondyla* populations/species. FG: French Guyana, others: Brazil: BA: state of Bahia, MG: state of Minas Gerais, SP: state of São Paulo. *Referred to as *P.* gp. *venusta* in the original publication.

Species	2*n*	Karyotype	Locality/coordinates	Reference
P. apicalis	2*n* = 36	28M + 8A	Ilhéus-BA; 14°45′S 39°13′W	[25]
P. apicalis	2*n* = 40	30M + 10A	Belmonte-BA; 16°05′S 39°12′W	[25]
P. apicalis	2*n* = 68	48M + 20A	Uruçuça-BA; 14°34′S 39°16′W	[25]
P. arhuaca	2*n* = 36	36A	FG: Chutes Voltaire 05°27′N 54°03′W	this study
*P. concava**	2*n* = 54	6M + 48A	Itororó-BA; 15°7′S 40°5′W	[35]
P. carinulata	2*n* = 24	4M + 20A	Ilhéus-BA; 14°45′S 39°13′W	[35]
P. constricta	2*n* = 30	30A	Ilhéus-BA; 14°45′S 39°13′W	[35]
P. crassinoda	2*n* = 62	22M + 40A	Ilhéus-BA; 14°45′S 39°13′W	[14]
P. crenata	2*n* = 26	2M + 24A	Viçosa-MG; 20°45′S 45°52′W	[23]
P. curvinodis	2*n* = 26	4M + 22A	Ilhéus-BA; 14°45′S 39°13′W	[35]
P. curvinodis	2*n* = 28	22M + 6A	Una-BA; 15°16′S 39°05′W	[35]
P. gilberti	2*n* = 12	10M + 2A	Arataca-BA; 15°15′S 39°24′W	this study
P. goeldii	2*n* = 24	24A	FG: Petit Saut; 05°20′N 53°41′W	[35]
P. harpax	2*n* = 96	12M + 84A	Ilhéus-BA; 14°45′S 39°13′W	[24]
P. impressa	2*n* = 94	8M + 86A	Ibicuí-BA; 14°53′S 40°02′W	this study
P. inversa	2*n* = 30	20M + 10A	Ilhéus-BA; 14°45′S 39°13′W	[35]
P. marginata	2*n* = 46	28M + 18A	Viçosa-MG; 20°45′S 45°52′W	[35]
P. moesta	2*n* = 26	26A	Viçosa-MG; 20°45′S 45°52′W	[23]
P. metanotalis	2*n* = 70	16M + 54A	Camacã-BA; 15°23′S 39°33′W	this study
P. stigma	2*n* = 12	12M	Porto Seguro-BA; 16°23′S 39°10′W	this study
P. striata	2*n* = 104	4M +100A	Camacã-BA; 15°23′S 39°33′W	[24]
P. succedanea	2*n* = 14	14M	FG: Chutes Voltaire 05°27′N 54°03′W	this study
P. unidentata	2*n* = 12	12M	Ilhéus-BA; 14°45′S 39°13′W	[35]
P. venusta	2*n* = 48	26M + 22A	Viçosa-MG; 20°45′S 45°52′W	[35]
P. verenae	2*n* = 42	30M + 12A	Ilhéus-BA; 14°45′S 39°13′W	[25]
P. verenae	2*n* = 62	14M + 48A	Ilhéus-BA; 14°45′S 39°13′W	[25]
P. verenae	2*n* = 58 − 60	14M + 44A	Viçosa-MG; 20°45′S 45°52′W	[25]
P. verenae	2*n* = 64	12M + 52A	Rio Claro-SP; 22°23′S 47°32′W	[25]
P. villosa	2*n* = 34	12M +22A	Ilhéus-BA; 14°45′S 39°13′W	[35]

number and karyotypes (Table 2). We present information on taxa within nine of the 18 species complexes defined by MacKay and MacKay [4] (Table 2). When comparing these classifications, there is unanimity among the authors solely on the *Pachycondyla stricto sensu* group. There is a certain consensus regarding the group *Neoponera* according to Kempf's catalogue [5] and Schmidt's proposal [7] (Table 1). Although Schmidt [7, page 197] has placed *Pachycondyla metanotalis* Luederwaldt, 1918 in his clade *Neoponera*, we followed Kempf's classification [5] for the aforementioned species since Schmidt's proposal is not backed by any new data, for the fact that *P. metanotalis* is a soil-dwelling species as most species in the clade *Pachycondyla* [5, 7], for morphological criteria not detailed herein, and because its karyotype is much closer to other species in the *Pachycondyla stricto sensu* group than to the *Neoponera* in this study.

A total of 29 populations was studied (Table 2), and several different populations were sampled for some taxa, therefore each line in this table represents one of these such populations as they can have different karyotypes with distinct characteristics. The chromosomes are classified [38] according to if they are acrocentric (A) or metacentric (M). The chromosome complements found are extremely variable showing from a few metacentric chromosomes ($2n = 12$) of large size (*Pachycondyla unidentata* Mayr, 1862) to a large number of minute acrocentric chromosomes ($2n = 104$ in *Pachycondyla striata* (Smith, 1858) (Table 2) and confirming the tendency shown in Figure 1: karyotypes with a few chromosomes have large chromosomes whereas karyotypes with a large number of chromosomes have small chromosomes (see examples in Figure 3).

The simple observation of chromosome morphology reveals great similarity among karyotypes of *Pachycondyla crassinoda* (Latreille, 1802), *Pachycondyla harpax* (Fabricius, 1804), *Pachycondyla impressa* Roger, 1861, *P. metanotalis,* and *P. striata* (group *Pachycondyla sensu stricto*) and also in the karyotypes of *Pachycondyla gilberti* (Kempf, 1960), *Pachycondyla succedanea* (Roger, 1863), and *Pachycondyla*

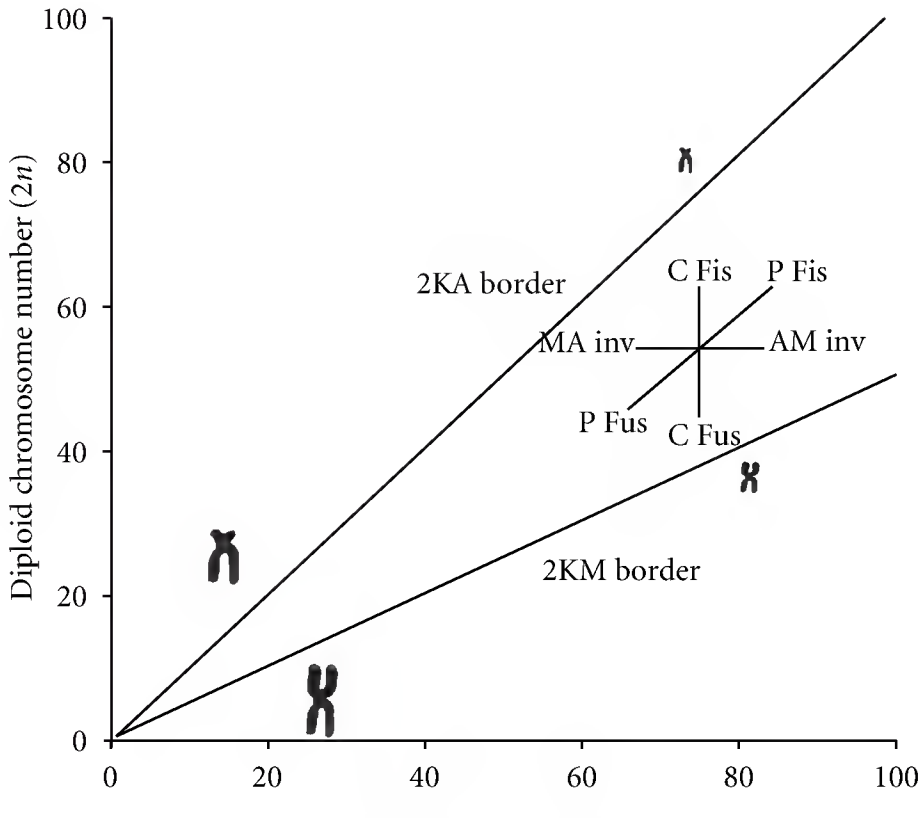

FIGURE 1: Karyograph adapted from Imai et al. [21] for ants. Since the genome is approximately constant for the whole Formicidae family, a proportional smaller chromosome size corresponds to the increase in the chromosome number. 2KA border: upper limit to the number of acrocentric chromosomes in diploid cells; 2KM border: lower limit to the number of metacentric chromosomes in diploid cells. C Fis: centric fission, C Fus: centric fusion, P Fis: pericentric fission, P Fus: pericentric fusion, AM inv: acrocentric-metacentric inversion, MA inv: metacentric-acrocentric inversion.

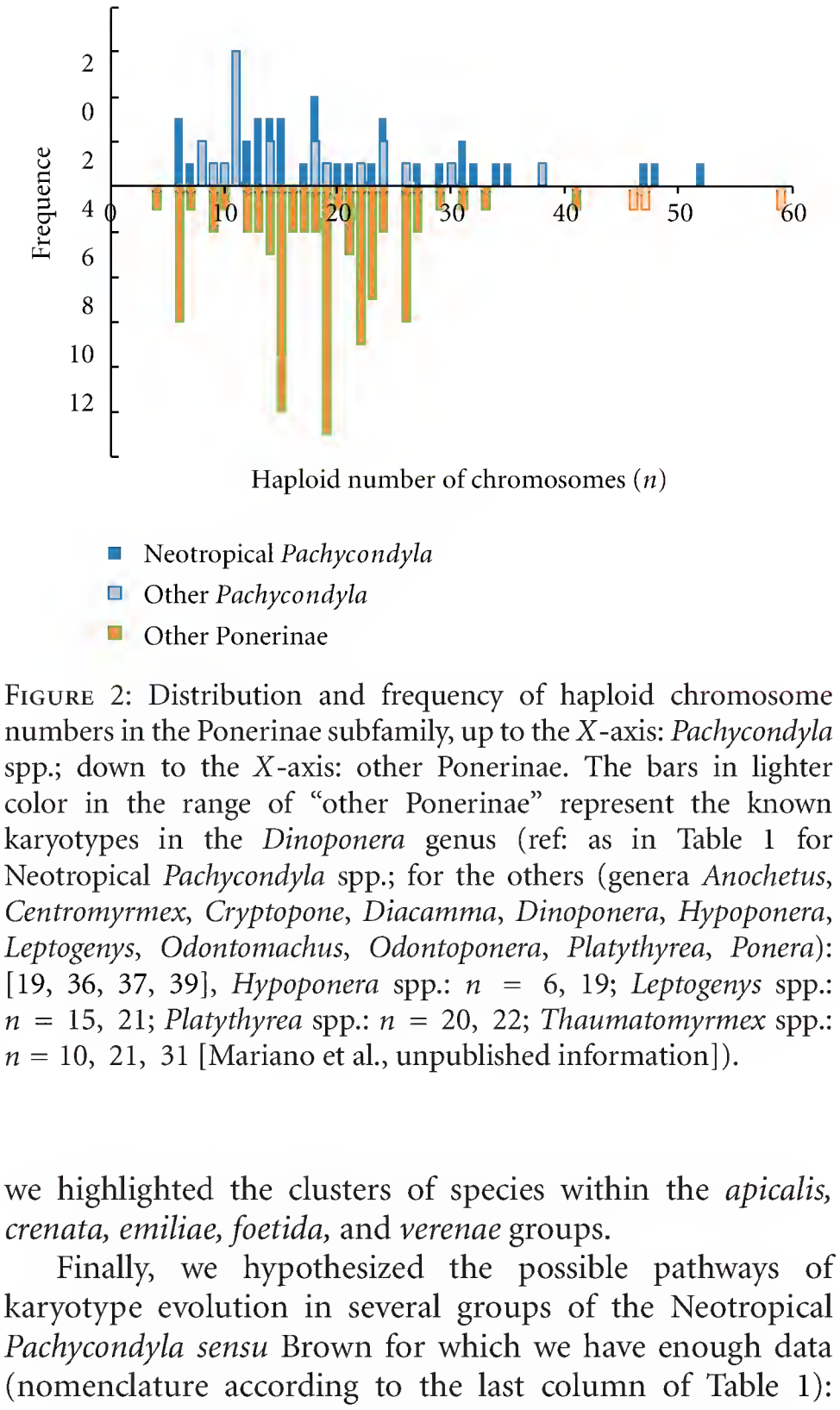

FIGURE 2: Distribution and frequency of haploid chromosome numbers in the Ponerinae subfamily, up to the *X*-axis: *Pachycondyla* spp.; down to the *X*-axis: other Ponerinae. The bars in lighter color in the range of "other Ponerinae" represent the known karyotypes in the *Dinoponera* genus (ref: as in Table 1 for Neotropical *Pachycondyla* spp.; for the others (genera *Anochetus*, *Centromyrmex*, *Cryptopone*, *Diacamma*, *Dinoponera*, *Hypoponera*, *Leptogenys*, *Odontomachus*, *Odontoponera*, *Platythyrea*, *Ponera*): [19, 36, 37, 39], *Hypoponera* spp.: $n = 6, 19$; *Leptogenys* spp.: $n = 15, 21$; *Platythyrea* spp.: $n = 20, 22$; *Thaumatomyrmex* spp.: $n = 10, 21, 31$ [Mariano et al., unpublished information]).

stigma (Fabricius, 1804) (group *Pseudoponera*), which coincidentally also form such groups according to Kempf [40], Schmidt [7], and MacKay and MacKay [4] (Table 2).

The study of the several clusters of *Pachycondyla sensu* Brown using the karyograph method (Figure 4) shows the clustering of species within the *Pachycondyla sensu stricto* group, all with a large number of acrocentric chromosomes, of species of *Pseudoponera*, with predominately metacentric chromosomes, and the great variation found in the karyotypes of species classified within the group *Neoponera*. The point distribution suggests that the most frequent rearrangements in these karyotypes were centric fissions and pericentric inversions (A-M type), and these rearrangements favor an increase in the number of chromosomes. Except for an isolated point on the right close to the 2KM limit in Figures 4 and 5 (which represents the population of *Pachycondyla apicalis* (Latreille, 1802) from Uruçuca), the karyotypes with larger numbers of chromosomes also tend to have mostly acrocentric chromosomes.

Some species have the same chromosome number but their morphology can be quite variable as a result of the aforementioned rearrangements. Six species have karyotypes that comprise only one morphological type of chromosome; in three of these species the karyotype is comprised of acrocentric chromosomes exclusively, and the other three species have karyotypes with only metacentric chromosomes (Table 2, Figures 4 and 5). In the karyograph, which shows taxa within the group *Neoponera* (11 species, 16 karyotypes, Figure 5), we highlighted the clusters of species within the *apicalis, crenata, emiliae, foetida,* and *verenae* groups.

Finally, we hypothesized the possible pathways of karyotype evolution in several groups of the Neotropical *Pachycondyla sensu* Brown for which we have enough data (nomenclature according to the last column of Table 1): *Neoponera apicalis*, *Neoponera crenata, Neoponera foetida*, *Neoponera verenae*, *Pseudoponera* and *Pachycondyla sensu stricto*. The representation (Figure 6) follows the model suggested by Imai and Crozier [27] developed for the interpretation of mammal karyotype evolution and is based on a hypothesis of karyotype variation essentially driven by fission.

4. Discussion

We can observe groups associated to the taxonomic position of species (Table 1) and some coincided with Schmidt's proposal [7], which splits *Pachycondyla* into 13 clades, with *Mayaponera* and *Neoponera* (both endemic), *Pseudoponera,* and *Pachycondyla* for the Neotropical Region.

Contrary to what has been reported for genera such as *Atta, Acromyrmex,* and *Pheidole,* in which the species already studied have a constant or not so variable karyotype [19], the karyotype groupings are extremely variable in species of *Pachycondyla* as well as in some distinct populations of the nominal species. Chromosome morphology is also variable, and it is noteworthy that, in most karyotypes with large chromosome numbers ($n > 11$, according to

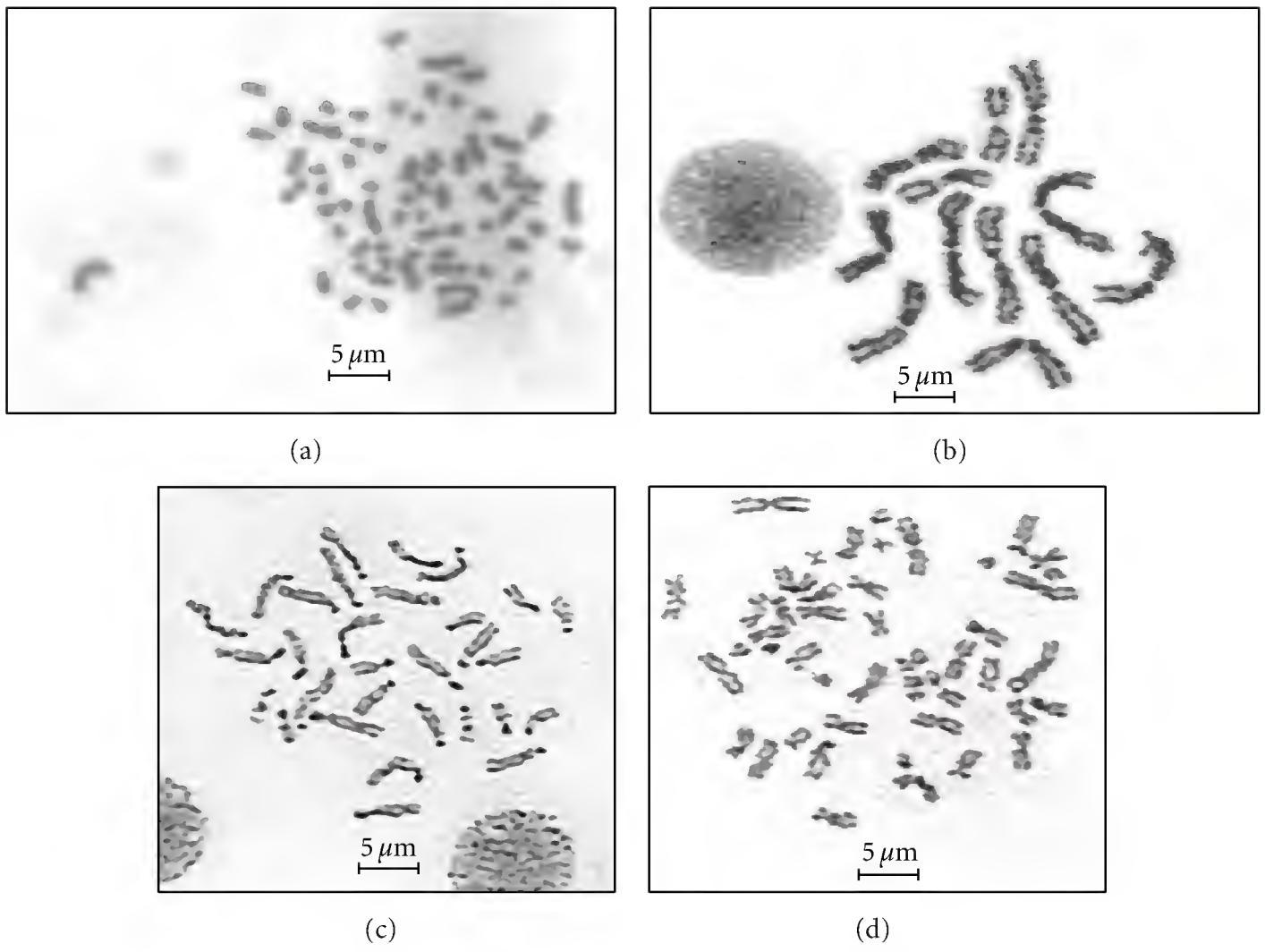

FIGURE 3: Metaphasic plates representing the different chromosome patterns found in *Pachycondyla* species. (a) *Pachycondyla impressa*, $2n = 70$, karyotype with a high number of chromosomes, mostly acrocentric. (b) *Pachycondyla unidentata*, $2n = 12$, karyotype with a low number of chromosomes, comprised exclusively by type M chromosomes of large size. (c) *Pachycondyla arhuaca*, $2n = 36$, Karyotype comprised exclusively by type A chromosomes. (d) *Pachycondyla venusta*, $2n = 54$, Karyotype comprised by types A and M chromosomes, a pattern found in many species and very common in the *Neoponera* group.

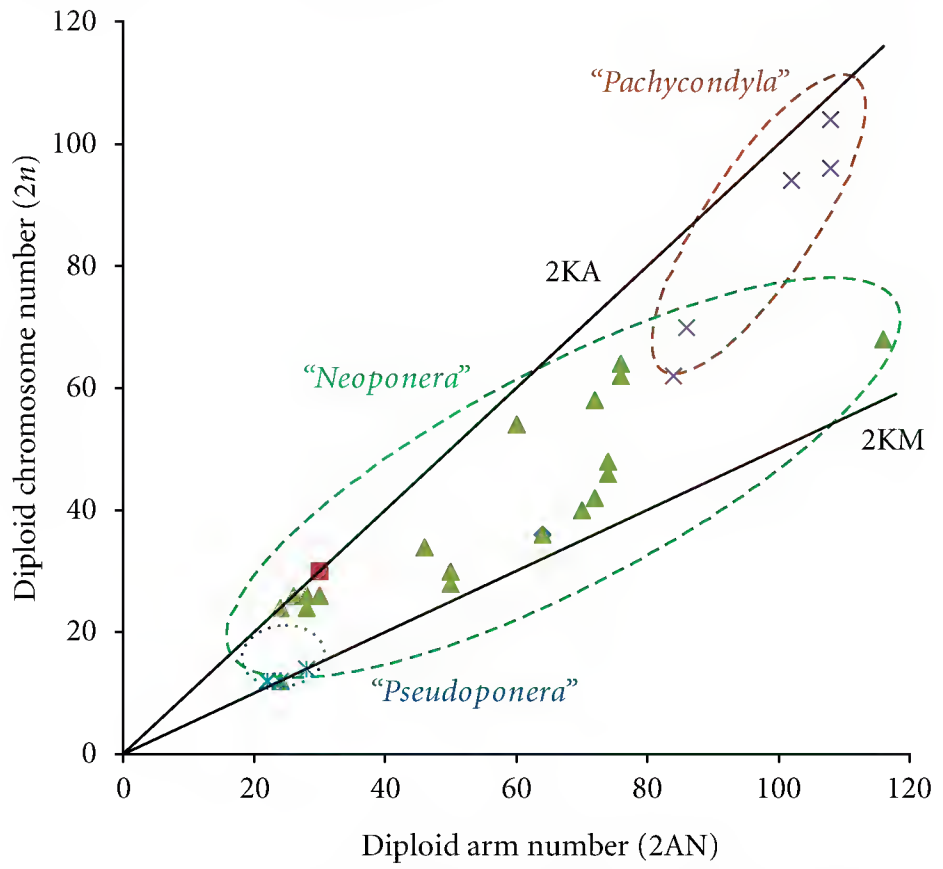

FIGURE 4: Karyograph of the Neotropical *Pachycondyla sensu* Brown. The ellipses circle the more representative species groups of our sampling according Schmidt [7] (see Table 1); two isolated points (square, diamond) represent single taxa not incorporated in a group (*P. arhuaca*, *P. constricta*).

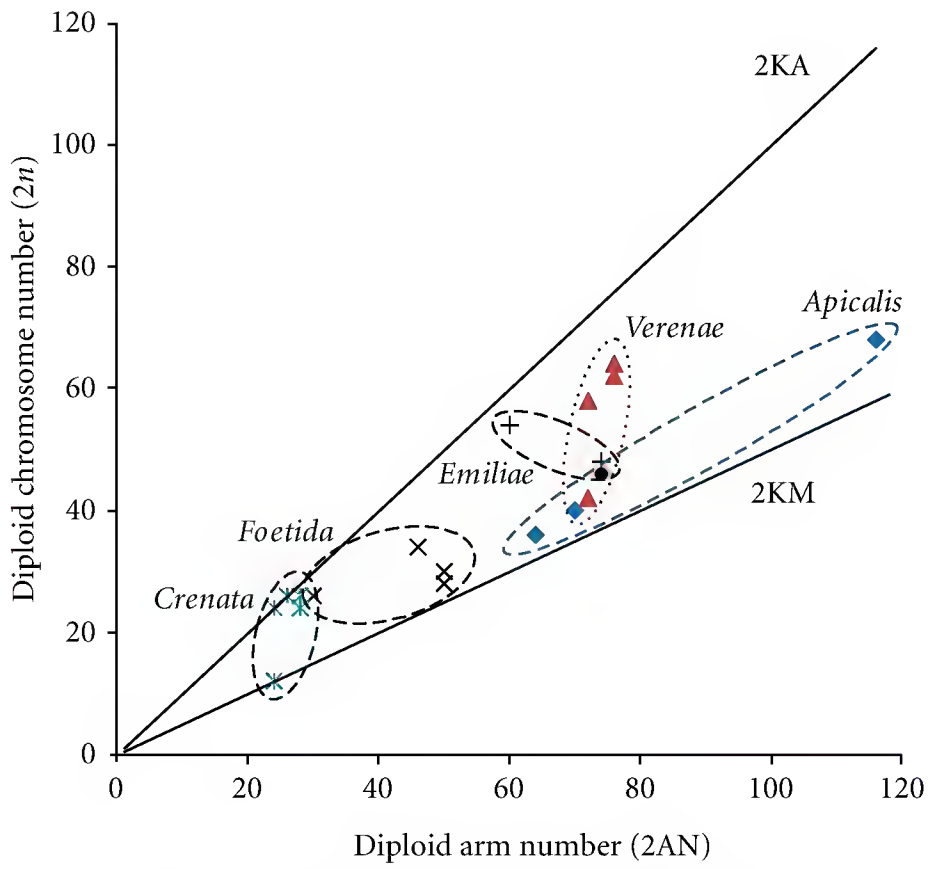

FIGURE 5: Karyograph of the Neotropical *Neoponera* according the Schmidt's proposal. The ellipses circle the more representative species groups (last column of Table 1); an isolated black circle represents a single species (*P. marginata*) not incorporated in a group.

the criteria of Imai et al., [41]), the chromosomes are submetacentric and acrocentric, which allows us to infer that fission and pericentric inversions (A-M ou M-A) are the most frequent chromosome rearrangements in the evolution of these karyotypes. These rearrangements can be either responsible or coadjuvant in speciation processes, especially in the complexes of cryptic species sampled in this study (*apicalis, verenae,* and *foetida* groups).

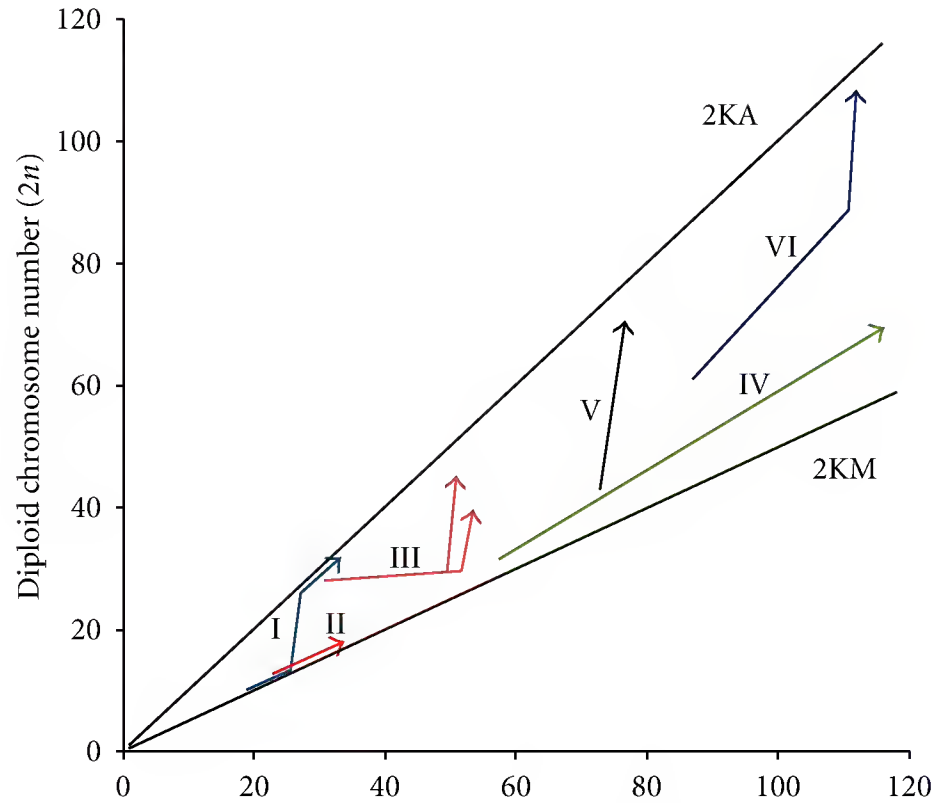

FIGURE 6: Possible pathways of karyotype evolution in several groups of Neotropical *Pachycondyla sensu* Brown. The Roman numbers correspond to the more representative species groups studied here (nomenclature according the last column of Table 1): I: *Neoponera-crenata*; II: *Pseudoponera*; III: *Neoponera-foetida*; IV: *Neoponera-apicalis*; V: *Neoponera-verenae*; VI: *Pachycondyla sensu stricto*. This schematic representation follows the suggested model of Imai and Crozier [27] for karyotypical evolution in mammals and is based on a hypothesis of a karyotype variation essentially driven by fission.

According to MacKay and MacKay [4], even though *Pachycondyla sensu* Brown is morphologically heterogeneous, the morphological characters were not consistent enough to justify splitting the group into distinct genera. However, similarly to Schmidt's [7], our results question the monophyly of *Pachycondyla* in its current acceptation. Thus, our results suggest the cooccurrence of multiple genera once there are totally independent patterns of karyotype evolution that strongly converge with Schmidt's conclusions [7]. We found groups with distinct patterns of karyotype evolution thus organized.

(a) Karyotypes with a large number of chromosomes and comprising mostly small acrocentric chromosomes in species within the *Pachycondyla stricto sensu* group (*P. crassinoda, P. harpax, P. impressa, P, metanotalis,* and *P. striata*) which most likely resulted from a succession of centric fission events (Figures 3(a), 6). These karyotypes follow the same pattern of those found in the three species of *Dinoponera* with available cytogenetic information [42], which is the sister genus of the clade *Pachycondyla* according to Schmidt [7].

(b) Karyotypes with a small chromosome number ($n \leq 11$ according to criteria in Imai et al. [41]) and comprising mostly metacentric chromosomes correspond to the pattern found in species within the group *Pseudoponera* (*P. cauta, P. gilbertii*, and *P. stigma*) and can be interpreted as little derived karyotype patterns which tend to undergo centric fission (Figure 3(b)).

(c) The karyotypes of *Neoponera* exemplify the karyotype evolution according to the model proposed by Imai et al. [21]: the karyotypes undergo successive cycles of rearrangements tending to increase the chromosome number by centric fission. The species included in *Neoponera* are considered the most diverse morphologically and behaviorally among the ponerine [7] and this diversity translates into the variety of karyotypes (Figure 3(c)).

(d) The case of populations within the taxa *P. apicalis* and *P. verenae* studied herein exemplifies an interesting evolutionary model based on biogeography. The two forms coexist along their range, which comprises practically only tropical and subtropical terrestrial environments in the Neotropical Region [25, 43]. A more refined analysis of the morphological criteria suggests that each nominal taxon is a complex of cryptic species of allopatric distribution [25], which is corroborated by the cytogenetic study: the *apicalis* and *verenae* complexes form two probable sister groups that probably evolved independently due mainly to centric fissions (*verenae*) and pericentric inversions (*apicalis*) (Figures 5 and 6).

All these examples adequately illustrate the karyotype heterogeneity in *Pachycondyla* and reinforce the argument of the cooccurrence of several genera, at least in the Neotropical region. The cytogenetic studies indicate groupings that do not seem to have recent ancestry and also strongly suggest the paraphyly of the "*Pachycondyla* problem," according to Ward [8], as each group follows a distinct evolutionary pattern (Figure 4).

Some of these patterns are not exclusive of the species represented herein; they have been reported in known ant karyotypes such as in species of the Australian genus *Myrmecia* [21] and corroborate the occurrence of different evolutionary patterns in insects.

The diversity of karyotypes found in the known species of *Pachycondyla* in the Neotropics is supported by the antiquity of this group of ants and reinforces a tendency observed in karyotypes of Formicidae: the increase and diversification of chromosome number and morphology in a basal subfamily such as Ponerinae contrasting with the low variation and relative stability in some genera of more derived subfamilies such as Dolichoderinae, Formicinae, and Myrmicinae [22, 44]. A similar phenomenon was observed in the Australian Myrmeciinae [21, 26], but the idea formerly well accepted that these ants are basal and ancestral is no longer supported by recent molecular phylogenies [45]. This situation leads to the very intriguing question of what is shared by the Myrmeciinae and Ponerinae to be so variable with respect to their karyotypes whereas karyotypes seem to be rather uniform in related subfamilies?

Among the ants, it is noteworthy the occurrence of cryptic species complexes and sibling species: morphologically indistinguishable species recently diverged (sibling-species) or that maintain strongly convergent characters

(cryptic species), and not separable using the traditional methods of identification [10, 11], in which characters such as behavior, chemical signature, and karyotype composition act as mechanisms of reproductive isolation (in Neotropical *Pachycondyla*, see, for instance, Lucas et al. [46]). Such a phenomenon has been reported for ants and many other organisms, and there are likely to be different speciation processes as there are multiple species concepts [47]. Thus, the use of different criteria for the description of species (alpha level taxonomy) is justified, and these criteria have been tested using the integrated taxonomy approach, which consists of using complementary areas such as molecular genetics, ecology, behavior, cytogenetics, and chemistry among others [48]. This approach strengthens the necessity of interdisciplinary studies and emphasizes the importance of multiple tools for taxonomic studies, a consensus among several authors [48, 49]. Therefore, besides confirming the validity of a species recognized by other methods, cytogenetics can contribute to the study of the origin and definition of species limits, as well as to the understanding of the evolution of organisms [49]. We hope our study will shed some light on the classification of the genus *Pachycondyla*, which still needs further disentangling.

Acknowledgments

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Review Article

Positive-Strand RNA Viruses Infecting the Red Imported Fire Ant, *Solenopsis invicta*

Steven M. Valles

USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

Correspondence should be addressed to Steven M. Valles, steven.valles@ars.usda.gov

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The imported fire ants, *Solenopsis invicta* and *S. richteri* were introduced into the USA between 1918 and 1945. Since that time, they have expanded their USA range to include some 138 million hectares. Their introduction has had significant economic consequences with costs associated with damage and control efforts estimated at 6 billion dollars annually in the USA. The general consensus of entomologists and myrmecologists is that permanent, sustainable control of these ants in the USA will likely depend on self-sustaining biological control agents. A metagenomics approach successfully resulted in discovery of three viruses infecting *S. invicta*. *Solenopsis invicta virus 1* (SINV-1), SINV-2, and SINV-3 are all positive, single-stranded RNA viruses and represent the first viral discoveries in any ant species. Molecular characterization, host relationships, and potential development and use of SINV-1, SINV-2, and SINV-3 as biopesticides are discussed.

1. Introduction

The black imported fire ant (*Solenopsis richteri*) and red imported fire ant (*S. invicta*) were thought to have been introduced into the United States in 1918 [1] and sometime between 1933 and 1945 [2], respectively. *S. invicta* has clearly emerged as the most successful of the two ant species, largely displacing and relegating *S. richteri* to a roughly contiguous area in eastern Mississippi, western Alabama, and western Tennessee [2]. In contrast, since its introduction, *S. invicta* has expanded its range to infest more than 138 million hectares (Figure 1) from Virginia, south to Florida, and west to California [3]. Although both of these fire ant species are invasive, *S. invicta* is by far the most successful and considered the major pest species in the USA. Thus, in the strictest sense, the term "imported fire ants" (in the USA) refers to both *S. invicta* and *S. richteri*. However, in reality, efforts to study, understand, and control imported fire ants are focused nearly completely on *S. invicta*.

Introduction of these ants into the USA has had significant economic consequences. Damage attributed to *S. invicta* is quite diverse, including, physical damage to agricultural commodities, livestock, and equipment, infrastructure (e.g., roads and electrical equipment), negatively impacting biological diversity, and even human health [4]. Costs associated with damage and control efforts are estimated to cost 6 billion dollars annually in the USA [5]. Although a number of highly effective insecticides are available to control *S. invicta* and *S. richteri*, they must be used regularly to provide sustained control. If insecticide use is discontinued, fire ant populations invariably re-inhabit these previously treated areas. In addition, because fire ants are so ubiquitous within the infested region, insecticide-based control is impractical, from both environmental and economic standpoints.

A number of comparative ecological studies have demonstrated that *S. invicta* nest density, nest volume, and population density compared with other ant species in the community are significantly greater in the USA compared with South America where the ant is native [6, 7]. These differences have been attributed to a lack of natural enemies in the USA as a result of a bottleneck event at the time of introduction [7]. The enemy release hypothesis [8] states that introduced species arrive without their complement of natural enemies, and release from these organisms confers

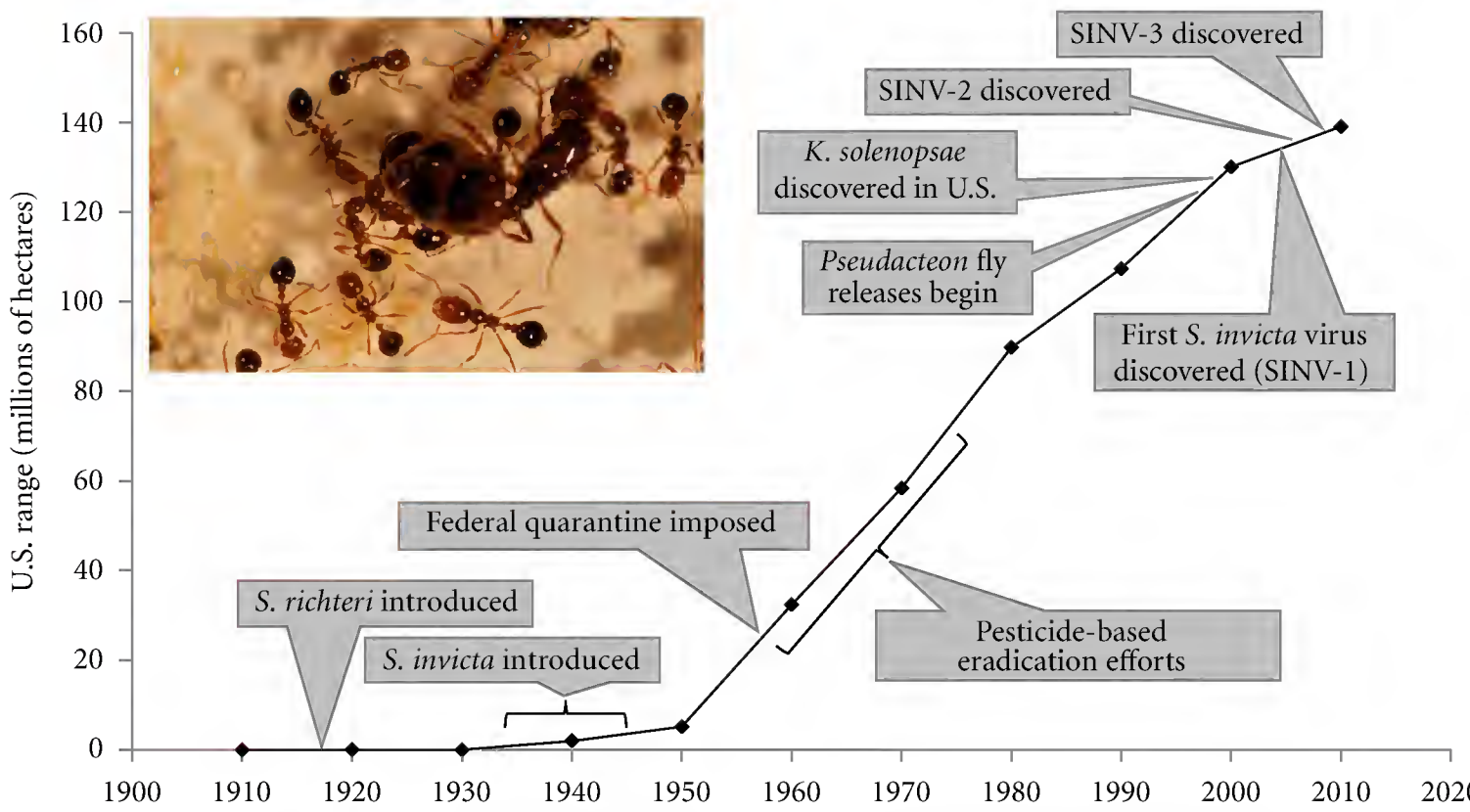

Figure 1: Seminal events during the invasion of *Solenopsis invicta* and *S. richteri* in relation to their USA quarantined range. Since the introduction of *S. invicta* and *S. richteri* into the USA, efforts to contain and eradicate the ants have been attempted [2, 4]. The USA federal government emplaced a quarantine in May 1958 to limit the rate of range expansion of the ants. This quarantine is still enforced by the Animal and Plant Health Inspection Service (APHIS) today and prohibits movement of soil-containing products (e.g., sod, nursery stock, sand, etc.) or soil-moving equipment from a quarantined to a non-quarantined area unless first treated in an APHIS-specified manner to kill fire ants. The range values correspond to areas quarantined and do not relate changing population densities in the USA. Rather, the graph illustrates the expanding geographic range of these ants. Eradication efforts were attempted from 1957 through 1978 using several organochlorine insecticides (heptachlor, dieldrin, and mirex). Research efforts to discover, develop, and release pathogens and parasites as control agents in the USA resulted in identification and/or release of a microsporidian pathogen (*Kneallhazia solenopsae*) [18], viruses [24–26], and *Pseudacteon* parasites [28]. Inset: *S. invicta* queen surrounded by workers and brood. Quarantine data were provided by APHIS [3] and the figure was adapted from Lofgren [29].

superior performance and attainment of higher densities in the introduced region [9]. The *S. invicta* introduction into the USA exemplifies this hypothesis; at least 30 fire ant natural enemies have been identified in South America, but nearly all of these are absent among USA populations [7, 10–14]. Indeed, this premise has served as the impetus for research based on discovery, development, and use of pathogens and parasites because permanent, sustainable control of *S. invicta* across its USA range will likely depend on self-sustaining biological control agents as part of an integrated management strategy.

A limited number of pathogens and parasites of *S. invicta* have been detected or intentionally released in the USA. Currently, 2 species of endoparasitic fungi [15, 16], a microsporidian obligate parasite [17, 18], a neogregarine parasite [19, 20], a strepsipteran parasite [21], phorid flies in the genus *Pseudacteon* [22, 23], and 3 RNA viruses [24–26] comprise the known self-sustaining, biological control agents found in North American *S. invicta*. Discovery and exploitation of additional biological control agents, from either South or North American populations, could aid the control and suppression of fire ants and remain a key research topic for a number of academic and government laboratories [10]. Indeed, the number of natural enemies found in recently introduced *S. invicta* populations in Australia and China are even fewer than in the USA [27].

2. Virus Discovery

Although viruses can be important biological control agents against pest insect populations [30], until recently, no viruses had been shown to infect *S. invicta*. Indeed, no virus had been reported in any species of the Formicidae before the discovery of the fire ant virus, SINV-1. Extensive searches for pathogens, including viruses, of *S. invicta* have been conducted in the introduced (USA) and native (South America) ranges using traditional methods (identification of unhealthy ants followed by microscopic examination or simply examination of large numbers of healthy fire ant colonies) [11–14]. However, with the exception of "virus-like particles" observed in an unidentified species of *Solenopsis* from Brazil [31], no viruses had been described by these methods. Further complicating discovery of pathogens in fire ants by traditional methods is their fastidious nature [32]; sick or dying colony members are promptly removed from the nest precluding detection.

In an effort to identify virus infections of *S. invicta*, a metagenomics approach was employed [33]. The primary intention of this analysis was to utilize homologous gene identity to facilitate discovery of viruses infecting *S. invicta* that could potentially be used in pest management. A non-normalized gene expression library was created from a monogyne colony of *S. invicta* and a relatively small number (2,304) of clones

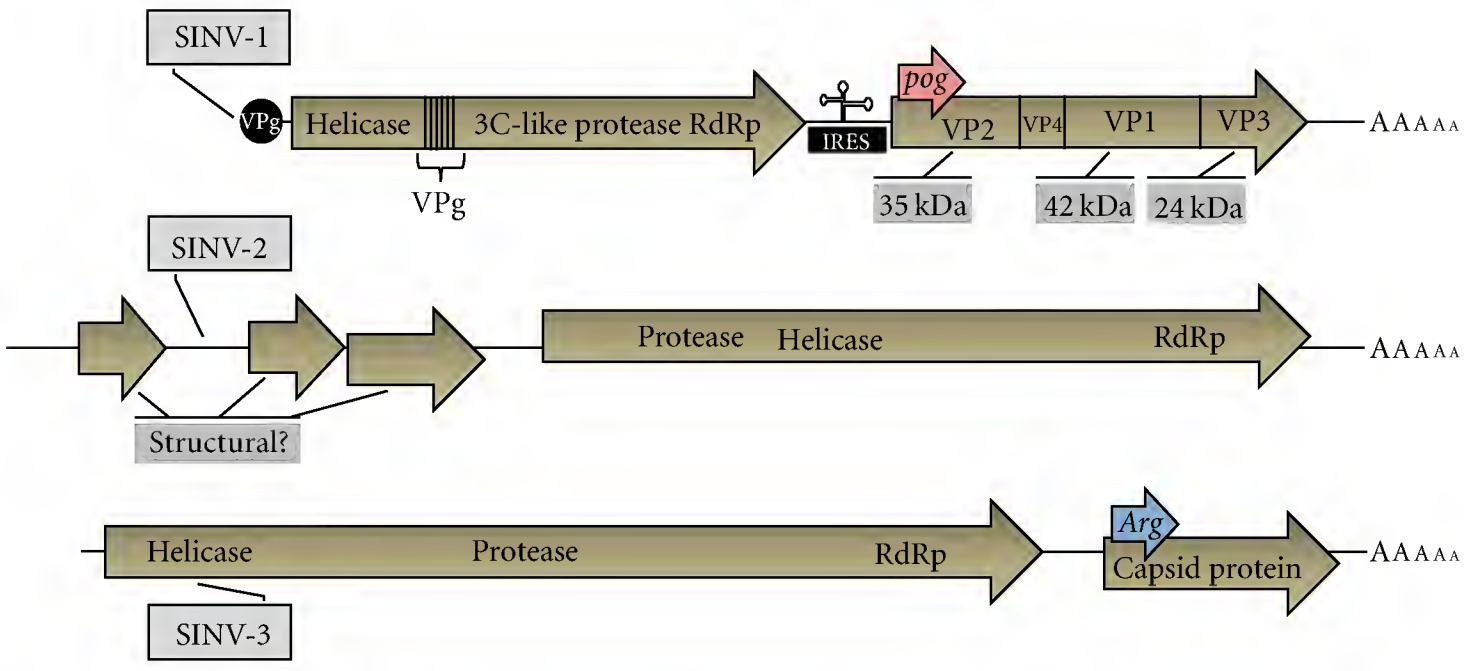

FIGURE 2: Comparative genome architecture of SINV-1, SINV-2, and SINV-3. Orientation of each genome is 5′ to 3′ (left to right). Relative positions of non-structural and structural proteins are indicated within each open reading frame (rectangles). ORFs are illustrated in different vertical positions to show their in-frame, comparative relationships. The SINV-1 genome is monopartite, dicistronic, and possesses 6 copies of the viral protein genome (VPg) peptide, an intergenic IRES, a predicted overlapping gene (*pog*) within ORF 2, and well-characterized capsid proteins (VPs) of known mass. The SINV-2 genome is monopartite with non-structural proteins encoded by the 3′-proximal ORF. The 3 small ORFs at the 5′-proximal end of the genome are presumed capsid proteins. The SINV-3 genome is monopartite with non-structural proteins encoded by ORF 1 (5′-proximal) and capsid proteins by ORF 2 (3′-proximal). An overlapping ORF (*Arg*) was identified within ORF 2 of a virus isolate from infected *S. invicta* ants from Argentina.

were sequenced. After assembly, 1,054 unique sequences were yielded and deposited into the GenBank database (accession numbers EH412746 through EH413799). Six sequences exhibited significant identity with RNA viruses. Subsequent analysis of these expressed sequence tags led to the discovery of three, positive, single-stranded RNA viruses, *Solenopsis invicta virus 1* (SINV-1), SINV-2, and SINV-3 [24–26].

3. *Solenopsis invicta* Virus 1

3.1. Genome Characterization. SINV-1, the first virus discovered in *S. invicta*, is the best characterized of the three currently described fire ant viruses [25]. Acquisition of the SINV-1 genome sequence was completed by a series of 5′ and 3′ rapid amplification of cDNA ends (RACE) reactions using expressed sequence tags identified from an expression library as anchor templates. The A/T rich genome is composed of 8,026 nucleotides excluding the poly(A) tail found on the 3′ end (Genbank accession AY634314). Analysis of the genome (Figure 2) revealed 2 large open reading frames (ORFs) in the sense orientation (within frame) with an untranslated region (UTR) at each end and between the two ORFs. BLAST analysis [34] of ORFs 1 (5′-proximal) and 2 (3′-proximal) revealed identity to nonstructural and structural proteins, respectively, from positive, single-stranded RNA viruses. ORF 1 was found to exhibit a characteristic helicase, protease, and RNA-dependent RNA polymerase (RdRp) cassette ascribed to viruses in the *Picornavirales* [35] and ORF 2 the structural, or viral capsid, proteins. No large ORFs were found in the inverse orientation suggesting that the SINV-1 genome is a positive, single-stranded RNA virus. The 5′, 3′, and intergenic UTRs were comprised of 27, 223, and 204 nucleotides, respectively.

ORF 1 commenced at the first start AUG codon present at nucleotide position 28 and ended at the UAA stop codon at nucleotide 4,218 which encoded a predicted product of 1,397 amino acids with a molecular mass of 160,327 Da. ORF 1 conspicuously lacks a region thought to suppress host antiviral responses at the N-terminus—a characteristic exhibited by other dicistroviruses [36–38]. Thus, Nakashima and Shibuya [39] have suggested that SINV-1 lacks approximately 1,500 nucleotides at the 5′ end of the genome. However, no empirical evidence for this suggestion has been reported. Sequence similarity analyses of ORF 1 identified domains consistent with a helicase, protease, and RdRp (Figure 2) [25]. Nakashima and Shibuya [39] identified the putative viral protein genome (VPg) sequence and location in SINV-1 ORF 1. The VPg is a peptide covalently linked at the 5′ terminus of picornavirus genomes and serves as a primer for viral RNA genome replication [40]. Six copies of the heterologous 18 amino acid VPg peptide were identified between the helicase and 3C-like protease of SINV-1 ORF 1 (Figure 2), the most for any dicistrovirus [39]. Multiple VPg copies are thought to facilitate multiplication of dicistroviruses because fewer translation cycles of the non-structural polyprotein (ORF 1 for SINV-1) are necessary for viral replication to occur compared with the intergenic internal ribosome entry site-mediated production of the structural polyprotein (ORF 2). The 2C/3A and 3C/3D cleavage site positions were predicted within ORF 1 of SINV-1 [41].

ORF 2 was originally reported [25] to commence at nucleotide position 4,390 (canonical AUG start codon), however, it was later revealed empirically to actually start at codon GCU (genome position 4423–4425) encoding an alanine [42]. ORF 2 initiation at this noncanonical codon is a consistent characteristic of dicistroviruses [43] and its presence and location were predicted to occur in SINV-1 [44]

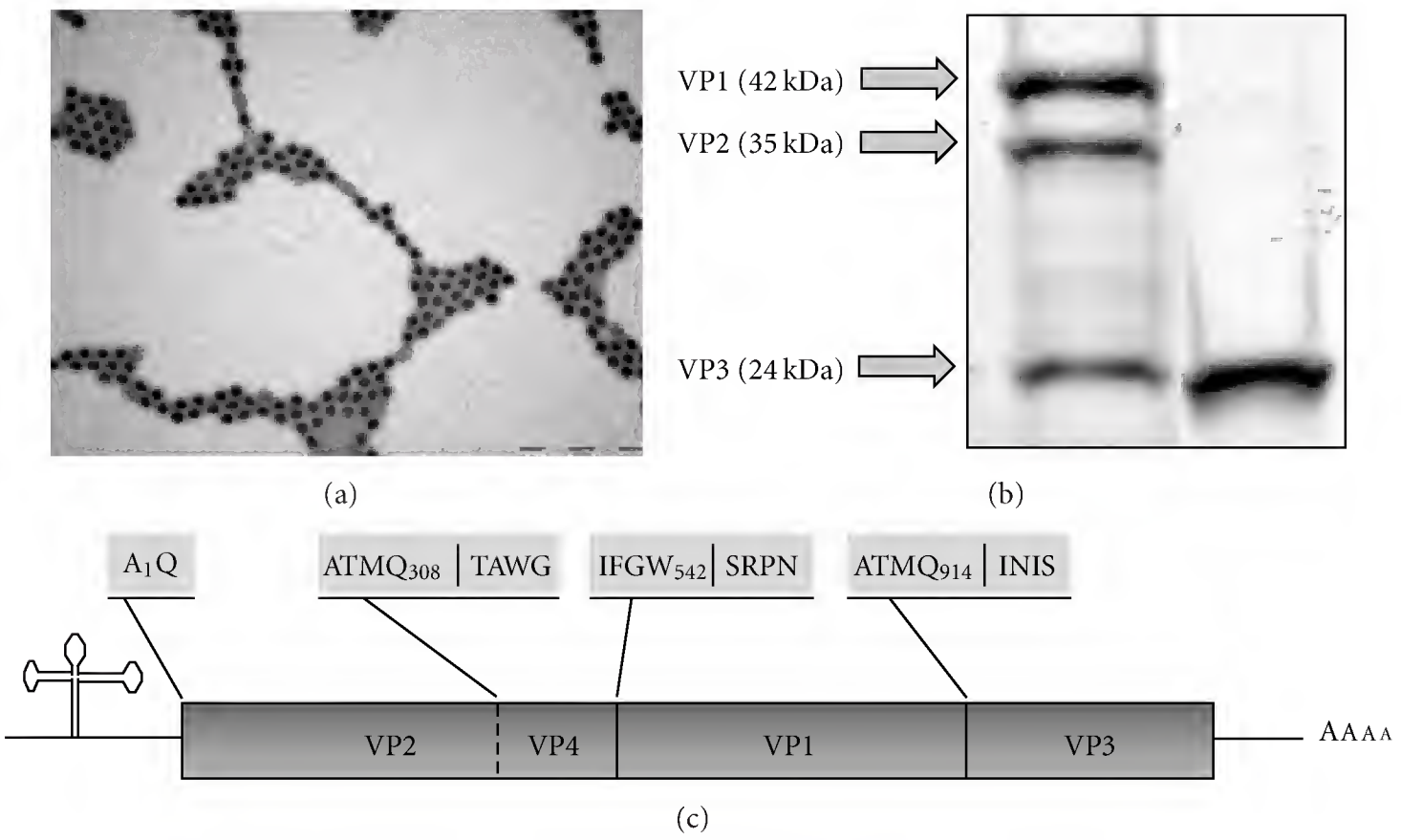

FIGURE 3: (a) Electron micrograph of purified SINV-1 particles. Scale bar represents 200 nm. (b) SINV-1 capsid proteins separated by SDS-PAGE (lane 1) and probed with polyclonal antibodies developed toward a portion of the predicted capsid protein, VP3 (lane 2). (c) Illustration of the intergenic region, IRES, and ORF 2 of SINV-1. Scissile bonds of each capsid protein of ORF 2 as determined by N-terminal sequence analysis of VP1, VP2, and VP3. Cleavage positions (subscript numeral) and amino acid residues about the cleavage site (vertical line) are illustrated.

before being empirically determined [42]. Thus, ORF 2 is comprised of 1,126 amino acids with a molecular mass of 126,434 Da. SDS-PAGE analysis of purified SINV-1 particles yielded 3 major and one minor protein band (Figure 3). The capsid proteins were labeled VP1, VP2, VP3, and VP4 based on mass and N-terminal sequence analysis of each of these proteins identified their respective positions within ORF 2 (Figures 2 and 3). Western analysis conducted with polyclonal antibodies developed from a peptide synthesized from the predicted amino acid sequence of VP3 (SRGGYRYKFFADDN) confirmed its location and synthesis from ORF 2 (Figure 3). The empirically determined and predicted molecular mass of VP0 (VP2 + VP4) (60.6 kDa), VP1 (41.8 kDa), and VP3 (24.0 kDa) were in agreement [42]. The positional organization of the capsid proteins of SINV-1 ORF 2 did not follow the pattern exhibited by most known dicistroviruses when based on mass (i.e., NH_2-VP2-VP4-VP3-VP1). Although VP0 (VP2 + VP4) was at the N-terminus of ORF 2, and VP1 and VP3 were downstream of VP0, VP1 was found between VP0 and VP3. This organization (NH_2-VP2-VP4-VP1-VP3) was also reported for deformed wing virus (DWV), an iflavirus [45]; SINV-1 and DWV both possess an unusually large VP1. The scissile bonds for VP0/VP1 and VP1/VP3 were located at amino acid positions 542/543 and 914/915, respectively. Amino acid residues at these junctions were consistent with other dicistroviruses and unclassified picorna-like insect-infecting viruses [46]. Amino acid residues G_{541}, S_{543}, and P_{545} at the VP4/VP1 cleavage site and Q_{914} at the VP1/VP3 cleavage site were conserved. These sites exhibited highest identities with Kashmir bee virus (KBV), Acute bee paralysis virus (ABPV), and Israeli acute paralysis virus (IAPV), all of which infect honey bees [47, 48].

A third overlapping ORF of unknown function has been identified at the 5′ end of SINV-1 ORF 2 in the +1 reading frame (Figure 2) [49]. The gene has been provisionally named *predicted overlapping gene* (*pog*). Protein motif searches of *pog* revealed weak relationships precluding assignment of a potential function. Interestingly, all hymenopteran-infecting dicistroviruses in the *Aparavirus* genus (KBV, ABPV, SINV-1 and IAPV) feature the *pog* gene. However, neither a transcript nor protein encoded by *pog* has been detected.

The 5′ and intergenic UTRs of dicistroviruses characteristically contain IRES regions that direct translation independent of a 7-methyl guanosine cap [50, 51]. SINV-1 has been shown to possess a Type II intergenic IRES based on sequence, structure, and homology within the dicistroviruses [44, 52]. Hertz and Thompson [53] have demonstrated that the SINV-1 IGR IRES is translation competent in yeast and mammalian cells.

Although positive, single-stranded RNA viruses, like SINV-1, do not synthesize a DNA template during any portion of their life cycle, portions of some positive, single-stranded RNA virus genomes (including a dicistrovirus) have been reported to be integrated into their host genomes. Interestingly, these integration events apparently afforded protection to the host from infection by the corresponding virus [54–56]. Because SINV-1 may be exploited as a microbial control agent, it was important to determine whether integration of a portion of the virus genome occurred in the host. A series of oligonucleotide primer pairs covering the entire

genome of SINV-1 were used to probe the genome of its host for integrated fragments of the viral genome [57]. Among 32 *S. invicta* genomic DNA samples collected from Argentina and the USA, no SINV-1 genome integration was detected.

3.2. Host Specificity and Prevalence. SINV-1 has been shown to infect *S. invicta* in the USA and Argentina [58–61]. Monogyne and polygyne *S. invicta* colonies [62] serve as hosts for SINV-1 [63]. However, SINV-1 infections appear to be more prevalent among polygyne *S. invicta* colonies [64]. *S. geminata*, *S. richteri*, the *S. invicta*/*S. richteri* hybrid, the *S. geminata*/*S. xyloni* hybrid (SMV unpublished) and *S. carolinensis* were also found to be infected with SINV-1 [58]. The infections in *Solenopsis* species other than *S. invicta* appear to be limited to areas in which *S. invicta* is sympatric and well established. SINV-1 was not detected in *S. xyloni* nor was it detected in *S. geminata* from southern Mexico (where *S. invicta* is not found currently), Hawaii, or Australia (SMV unpublished). Although still developing, these data suggest that *S. invicta* is the primary host of SINV-1 with other species in the *Solenopsis* genus serving as hosts occasionally (acquired from sympatric, SINV-1-infected *S. invicta*).

SINV-1 was distributed widely among *S. invicta* populations throughout the USA and Argentina [58, 60] with intercolony infection rates ranging from <10% [58] to >90% [63]. SINV-1 was detected in fire ants collected from all USA states examined with the exception of New Mexico. Although some dicistroviruses, like Cricket paralysis virus (CrPV), exhibit extremely wide host ranges, others, like Drosophila C virus (DCV), exhibit a genus-limited host range as observed for SINV-1 [50, 51, 65].

A strong relationship between temperature and SINV-1 colony prevalence was reported in two separate studies [58, 63]. Thus, time of collection (as it relates to temperature) must be considered when evaluating comparative prevalence data for SINV-1. This temperature dependency may be the result of more efficient IGR IRES activity. Hertz and Thompson [53] have shown recently that the SINV-1 IGR IRES exhibits increased activity at higher temperatures (3 to 5-fold). Further, the temperature-dependent enhanced activity resided in the ribosome binding domain of the IRES [53, 66]. So, the seasonally observed prevalence of SINV-1 appears directly related to the ability of the virus to replicate more efficiently at higher temperatures and not necessarily influenced by the behavior of the ant host.

Multiple genotypes of SINV-1 have been identified [25, 67, 68], and genomic diversity has been attributed to a high mutation rate characteristic of positive, single-stranded RNA viruses [69, 70]. Phylogenetic analysis of nucleotide sequences from the structural protein regions of the SINV-1 genome indicated divergence between isolates infecting North American and South American *S. invicta* [58] suggesting a prolonged duration of separation on the two continents. The analysis also indicated that North American SINV-1 had diverged more recently compared with those from Argentina. Indeed, a more extensive examination of the conserved RdRp region of the SINV-1 genome from *Solenopsis* hosts across the USA and northern Argentina revealed clustering of Argentinean sequences, distinct from the USA sequences [59]. Thus, SINV-1 in North America likely arrived with one of the founding introductions of *S. invicta* from South America. This conclusion is supported by the lack of infection among other *Solenopsis* species (*S. geminata* and *S. xyloni*) in areas devoid (Mexico and Hawaii) or with incipient infestations of *S. invicta* (New Mexico, California, and Australia). SINV-1 infection of *S. geminata* and *S. carolinensis* (in Florida and Northern Mexico/Southern Texas) may have originated from introduced *S. invicta* or *S. richteri*.

SINV-1 was capable of being detected retrospectively in alcohol-stored arthropods for at least 2 years facilitating host specificity evaluations [71]. Pitfall collections of 1,523 ants from 16 genera (excluding *Solenopsis*) tested negative for SINV-1 from areas in Florida where SINV-1 was present in the *S. invicta* community [58]. Likewise, 282 other arthropods in four classes and ten families within the Hexapoda were negative for SINV-1. Even *Pseudacteon* parasitoids that complete development within *S. invicta* do not serve as hosts of SINV-1 [72]. Thus, SINV-1 appears limited to the *Solenopsis* genus with *S. invicta* likely the primary host.

3.3. Stage and Tissue Tropism, Transmission. Real-time PCR was employed to determine the presence of SINV-1 in tissues, individual ants, and among colonies of *S. invicta* by quantifying the genome of the virus [73, 74]. Initial experiments examined groups of tissues collectively to pinpoint the location of the virus infection. In workers, the abdomen contained the highest proportion of SINV-1; virus was also detected in the head and thorax of worker ants, but at very low rates (Figure 4). Among the remaining abdominal tissue groups, SINV-1 was detected occasionally in worker Malpighian tubules, the poison sac, hindgut and crop, but the greatest concentration of SINV-1 was in the midgut. Larval infection was also largely limited to the alimentary canal (Figure 4). SINV-1 specificity for the midgut of *S. invicta* is consistent with a number of other insect-infecting positive, single-stranded RNA viruses. Ingestion and the alimentary canal feature prominently in dicistrovirus infection acquisition and transmission processes [75]. Indeed, among the 14 described dicistroviruses, 12 exhibit a tissue tropism toward some part of the alimentary canal of their hosts [76]. Also, the gut contents of many hosts have been shown to contain high numbers of viral particles (e.g., Himetobi P virus (HiPV) [77], SINV-1 [73], DCV [78], and Triatoma virus (TrV) [79, 80]).

Electron microscopy of worker and larval gut homogenates revealed the presence of spherical virus particles with a diameter of 30–35 nm, consistent with SINV-1. The molecular and microscopic data suggest that SINV-1 replicates in gut epithelial cells of *S. invicta* and infectious viral particles are shed into the gut lumen [76, 81]. From there, the particles may be passed to nestmates by trophallaxis or substrate contamination by defecation [81]. Large quantities of SINV-1 detected in the gut contents of *S. invicta* larvae [73] suggest that this stage facilitates intra-colony dissemination of SINV-1. Late-instar *S. invicta* larvae digest all solid food for the colony which is redistributed in liquid form to nestmates

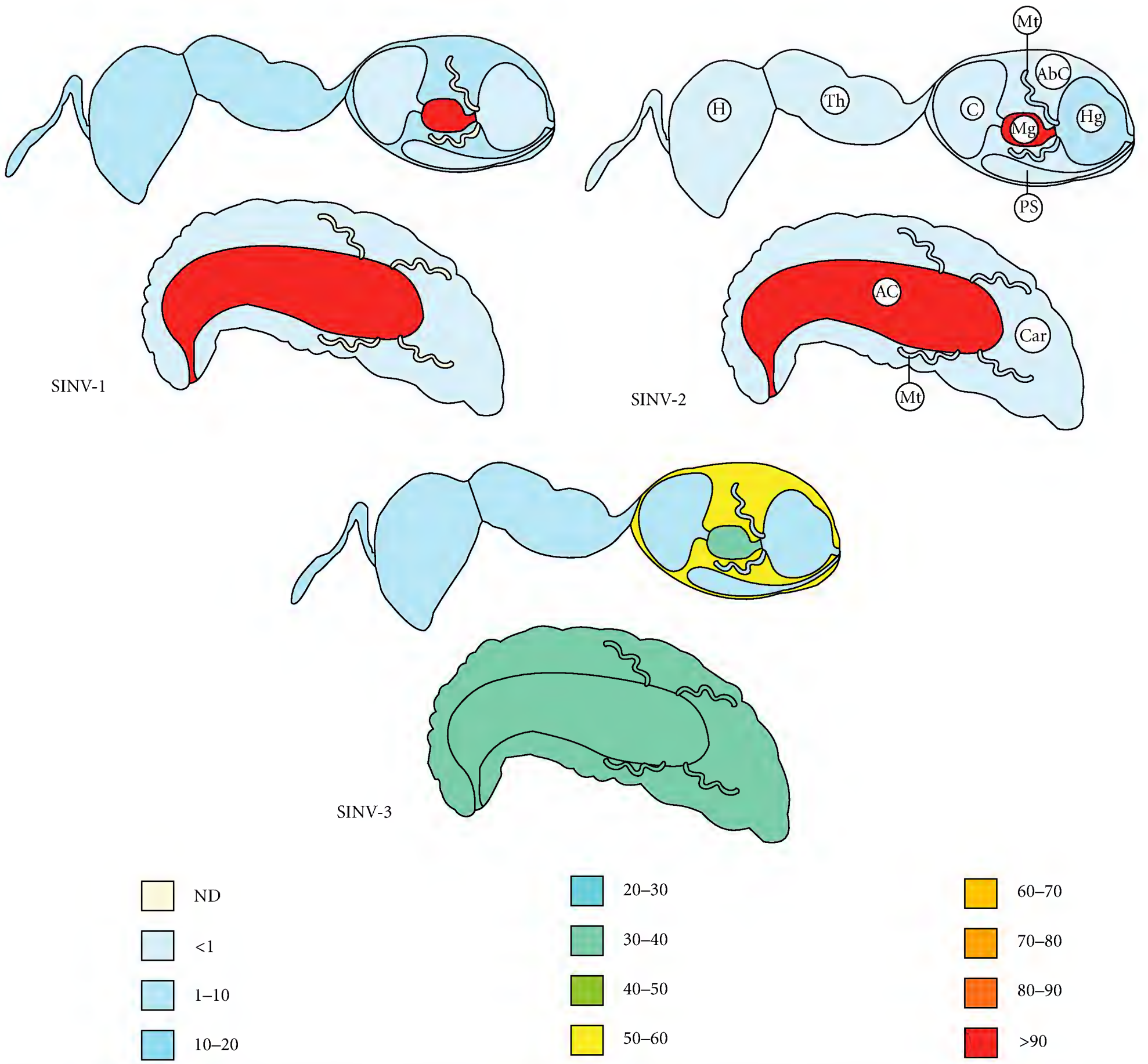

Figure 4: Tissue tropism of SINV-1, SINV-2, and SINV-3 among larval and worker *S. invicta* ants. Virus was quantified by real-time PCR. Color key reflects the proportion of the total for each virus detected in each tissue. (ND = not detected). Tissue key is identical for all viruses, H: head; Th: thorax; C: crop; Mg: midgut; Hg: hindgut; PS: poison sac; Mt: Malpighian tubules; AbC: abdominal carcass, AC: alimentary canal; Car: carcass.

[83]. Thus, larvae not only appear to serve as reservoirs for SINV-1, but also conduits for SINV-1colonial dissemination (Figure 5).

Evidence for the importance of the alimentary canal in the horizontal transmission of dicistroviruses is further illustrated by the presence of virus particles in the excreta of infected hosts. The excreta serves as an important source of viral inoculum for Plautia stali intestine virus (PSIV) [84], Black queen cell virus (BQCV), Acute bee paralysis virus (ABPV), [85] Kashmir bee virus (KBV) [86], HiPV [87], and TrV [80]. The fecal-oral route of infection has even been shown to play a prominent role in the infection process of many of the *Picornaviridae* in mammals [88].

SINV-1 was detected in all developmental stages of *S. invicta* including eggs and queens indicating vertical transmission of the virus [25, 73, 74]. Larval and worker ants generally exhibited the highest viral loads reaching levels of 10^8 to 10^9 per individual [74]. The SINV-1 titer was generally similar between larvae and workers collected from the same colony. A positive relationship was observed between the SINV-1 titer in individual ants and intracolony SINV-1 prevalence; colonies with higher intracolony

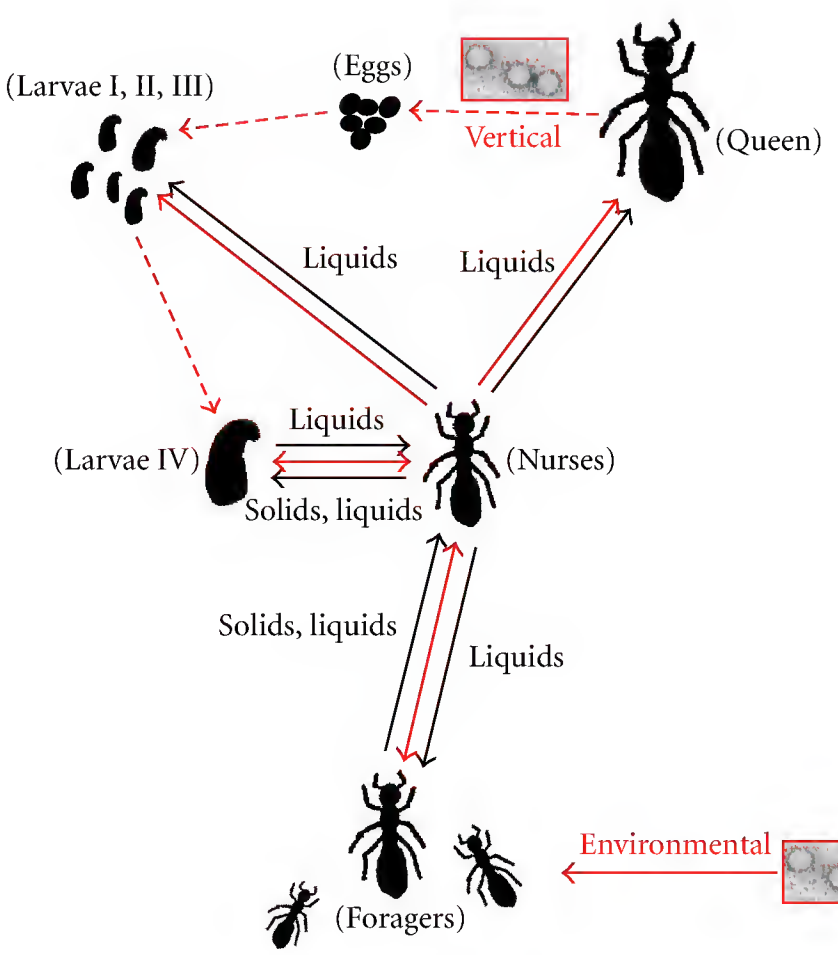

FIGURE 5: Routes proposed for SINV-1 (and possibly SINV-2 and SINV-3) colony acquisition and dissemination [81]. SINV-1 exhibits a tissue tropism toward the midgut, and large quantities of virus are detected in the midgut contents of adults and larvae of infected colonies. Thus, colony dissemination of SINV-1 appears to coincide with the food distribution route for the colony. Black arrows illustrate the flow of food throughout the colony and red arrows the corresponding movement of SINV-1. Two modes of virus acquisition are likely environmental (from outside sources entering the colony by way of foragers) and vertical (from infected queens). The vertical route of distribution is illustrated by broken red lines. The food flow diagram was adapted from Vinson and Sorensen [82].

SINV-1 prevalence exhibited higher SINV-1 titers among individuals [73].

3.4. Colony Effects. Initially, SINV-1 was associated with larval mortality in laboratory *S. invicta* colonies but no observable symptoms were detected among field populations [25]. A number of factors, including undetected pathogens and the inability to produce pure SINV-1 have limited the ability to directly infect colonies with SINV-1 and measure the impact of the infection on individual ants and colonies. SINV-1 appears to conform to the paradigm of many arthropod-infecting positive, single-stranded RNA viruses. Specifically, the virus persists as a chronic, asymptomatic infection that does not cause any overt signs or symptoms. However, under certain circumstances (e.g., environmental stress) the virus replicates rapidly causing overt symptoms and even death [89–91].

A recent series of experiments examined the ability of SINV-1-infected and -uninfected *S. invicta* ants and colonies to compete with native ant species *Pheidole fervens* and *Monomorium chinense* [92]. SINV-1-infected *S. invicta* were more quickly eliminated by *M. chinense* than healthy *S. invicta*. Direct confrontation tests confirmed these results; *M. chinense* killed significantly more *S. invicta* minors from SINV-1-infected colonies compared with healthy colonies. Against *P. fervens*, SINV-1-infected *S. invicta* required significantly more time to eliminate competing *P. fervens* colonies compared with healthy *S. invicta*. The study revealed that SINV-1 infection weakened the competitive ability of *S. invicta* and made them more susceptible to elimination by some species of sympatric ants, like *M. chinense*.

4. *Solenopsis invicta* Virus 2

SINV-2 is the second virus discovered that infects the red imported fire ant, *S. invicta* [26]. This virus possesses a genome structure that is unique and differs considerably from currently described positive, single-stranded RNA viruses. As with SINV-1, the SINV-2 genome was constructed by compiling sequences from successive 5′ and 3′ RACE reactions using an expressed sequence tag (Genbank accession EH413675) as the initial anchor template (Figure 2). The genome (Genbank accession EF428566) was monopartite, 11,303 nucleotides in length, polyadenylated at the 3′ end, A/U rich (27.9% A, 28.9% U, 20.1% C, 23.1% G), and encoded 4 major ORFs (comprised of ≥100 codons) in the sense orientation (Figure 2). Untranslated regions were present on the 5′ (nts 1–301) and 3′ ends (nts 10917–11303) of the genome. Intergenic regions were also indicated between ORFs 1 and 2 (nts 1079–1828), and ORFs 3 and 4 (nts 3793–4454). ORFs 2 and 3 overlap a stop and start codon, respectively, and are not, therefore, interrupted by an intergenic region. ORF 3 was in the first reading frame, ORFs 1 and 2 were in the second reading frame, and ORF 4 was in the third reading frame. ORFs 1 through 4 encoded predicted proteins of 29,413; 31,160; 43,224; 246,845 Da, respectively. Blastp analysis [34] of SINV-2 ORF 4 identified regions with significant identity to RdRp, helicase, and protease conserved domains from positive, single-stranded RNA viruses [35] (Figure 2). Blastp analysis of ORFs 1, 2, and 3 yielded poor identity (expectation scores greater than 1) to corresponding capsid proteins from positive, single-stranded RNA viruses.

The monopartite, multiple ORF-encoding genome structure of SINV-2 is unique. Regions of the polyprotein encoded by SINV-2 ORF 4 exhibited identity with RdRp and helicase domains characteristic of positive, single-stranded RNA viruses. However, only a partial domain for a protease was recognized near the amino end of the ORF 4 polyprotein (amino acid residues 330 to 410). A similarly unique genome structure was reported for the Nora virus, an unclassified virus that persistently infects *Drosophila melanogaster* [93]. The Nora virus genome is also monopartite and encodes 4 major ORFs. In addition to genome structural similarities, Nora virus and SINV-2 contained truncated protease domains [93]. Amino acids thought to form the catalytic triad of the protease (H, E, C) and the consensus GxCG sequence motif were absent in all ORFs of SINV-2 [94, 95]. Additional differences between Nora virus and SINV-2 included positional relationships of the nonstructural proteins and relative ORF positions.

S. invicta colonies infected with SINV-2 did not exhibit any discernable symptoms in the field or consistently when reared in the laboratory. Occasionally, infected laboratory colonies exhibited brood die-off. Whether SINV-2 was responsible for this pathology was undetermined. The negative strand of the SINV-2 genome was detected in larvae and adults of *S. invicta* indicating that the virus was replicating [96].

SINV-2 host specificity evaluations have not been conducted. All developmental stages of *S. invicta* have been shown to be infected with SINV-2, including the queen and eggs suggesting vertical transmission of the virus [97]. Larvae and workers generally exhibited the highest viral load. Horizontal transmission of SINV-2 to uninfected *S. invicta* colonies was accomplished by feeding a homogenate of SINV-2-infected ants [97]. Tissue specificity of SINV-2 closely reflected that of SINV-1. The midgut of workers and alimentary canal of larvae possessed the highest quantities of SINV-2 (Figure 4).

5. *Solenopsis invicta* Virus 3

SINV-3 is the most recent virus to be discovered from *S. invicta* using the metagenomics approach [24, 33] and it also possesses features consistent with placement within the order *Picornavirales* [35]. As with SINV-1 and -2, the genome of SINV-3 was constructed by compiling sequences from successive 5′ and 3′ RACE reactions using an EST sequence (Genbank accession EH413252) as anchor template. SINV-3 possesses a genome that is 10,386 nucleotides in length, excluding the poly(A) tail present on the 3′ end (Genbank accession FJ528584). Also consistent with SINV-1 and -2, the SINV-3 genome was A/U rich (70.9% A/U; 29.1% G/C). It encodes 2 large ORFs in the sense orientation with a UTR at each end and between the two ORFs (Figure 2). The 5′ proximal ORF (ORF 1) began at nucleotide position 92 and ended at a UGA stop codon at nucleotide 7,834 yielding a predicted polyprotein of 299,095 Da (2,580 amino acids). The 3′ proximal ORF (ORF 2), commenced at nucleotide position 8,308, terminated at nucleotide position 10,263 and encoded a predicted protein of 73,186 Da (651 amino acids). No large ORFs were found in the inverse orientation. The 5′, 3′, and intergenic UTRs were comprised of 91, 123, and 473 nucleotides, respectively. Blastp analysis [34] of the polyprotein encoded by ORF 1 identified conserved domains for RdRp, protease, and helicase (Figure 2). Blastp analysis of the ORF 2 polyprotein did not yield any sequences with significant identity.

For comparison, the genome of an Argentinean isolate of SINV-3 (SINV-3^{ArgSF}) obtained from the Santa Fe region of Argentina was sequenced in entirety [98]. Argentina is thought to be the region from which the USA *S. invicta* population originated [99]. Excluding the poly(A) tail, the genome length of SINV-3^{ArgSF} (Genbank accession GU 017972) was identical to the North American isolate (referred to as SINV-3). The SINV-3^{ArgSF} genome possessed 3 major ORFs in the sense orientation; SINV-3 possessed only two ORFs [24]. Both isolates exhibited identical start and stop codon positions for ORFs 1 and 2. Blastp analysis of the translated ORF 1 of SINV-3^{ArgSF} recognized conserved domains for helicase, protease, and RdRp, and their corresponding positions were identical to those reported for SINV-3. ORF 3, unique to the SINV-3^{ArgSF} genome (Figure 2), was located at nucleotide positions 8,351 through 8,827 and overlapped ORF 2. ORF 3 yielded a predicted protein sequence comprised of 158 amino acids with a molecular mass of 18.8 kDa. Blastp analysis of the translated amino acid sequence of ORF 3 revealed no significant similarity in the Genbank database.

The two SINV-3 isolates exhibited 96.2% nucleotide sequence identity across the entire genome [98]. The 5′, 3′ and intergenic UTRs of the genomes exhibited 100, 99.2, and 92.6% identities, respectively. The amino acid sequences of ORFs 1 and 2 exhibited 99.0 and 96.6% identities, respectively, indicating that the nucleotide differences between isolates were largely synonymous. Indeed, the proportion of amino acid residues that were similar in ORFs 1 and 2 were even higher (99.6% and 98.2%, resp.).

Tracking changes in pathogen genomes (including viruses) can be a useful and indirect method of providing information about their hosts [59, 100] and have been employed to construct demographic histories of host populations [101]. Unlike SINV-1 [59], comparison of the genome sequences of SINV-3 isolates indicated that no significant directional selection has occurred despite separation of the host populations geographically (Argentina/United States) and temporally (approximately 70 years). Thus, SINV-3 may have been a relatively recent introduction into the North American *S. invicta* population. SINV-1, -2, and -3 may aid a number of phylogenetic-based studies and reveal information about movement and establishment of the *S. invicta* host population.

SINV-3 infects all developmental stages of *S. invicta*, including the queen and eggs suggesting that vertical transmission occurs. Unlike SINV-1 and SINV-2, SINV-3 exhibits a broad tissue tropism. SINV-3 was detected in all tissues of *S. invicta* queens, workers and larvae examined (Figure 4). Thus, the SINV-3 infection appears to be systemic. This systemic characteristic appears to coincide with the association between SINV-3 infection and significant mortality among *S. invicta* laboratory colonies [24]. Signs of infection included large midden piles of ants, brood mortality, and colony collapse. Dead, dried brood found on the midden piles exhibit a crystallized appearance. Some workers may remain alive for considerable periods after the initial die-off, and occasionally, if the queen survived, colonies will rebound exhibiting normal brood production.

Although SINV-1, SINV-2, and SINV-3 are all positive, single-strand RNA viruses infecting *S. invicta*, they exhibit differences in their genome organizations; SINV-1 and SINV-3 encode 2 ORFs, while SINV-2 encodes 4 ORFs. However, the most important difference between SINV-1, SINV-2, and SINV-3 is pathogenicity. SINV-1 and -2 appear to cause chronic, asymptomatic infections that may result in mortality under certain stressful conditions, as reported in honeybees [90]. Although SINV-1 and SINV-2 were regularly transmitted to healthy colonies of *S. invicta* ants by feeding,

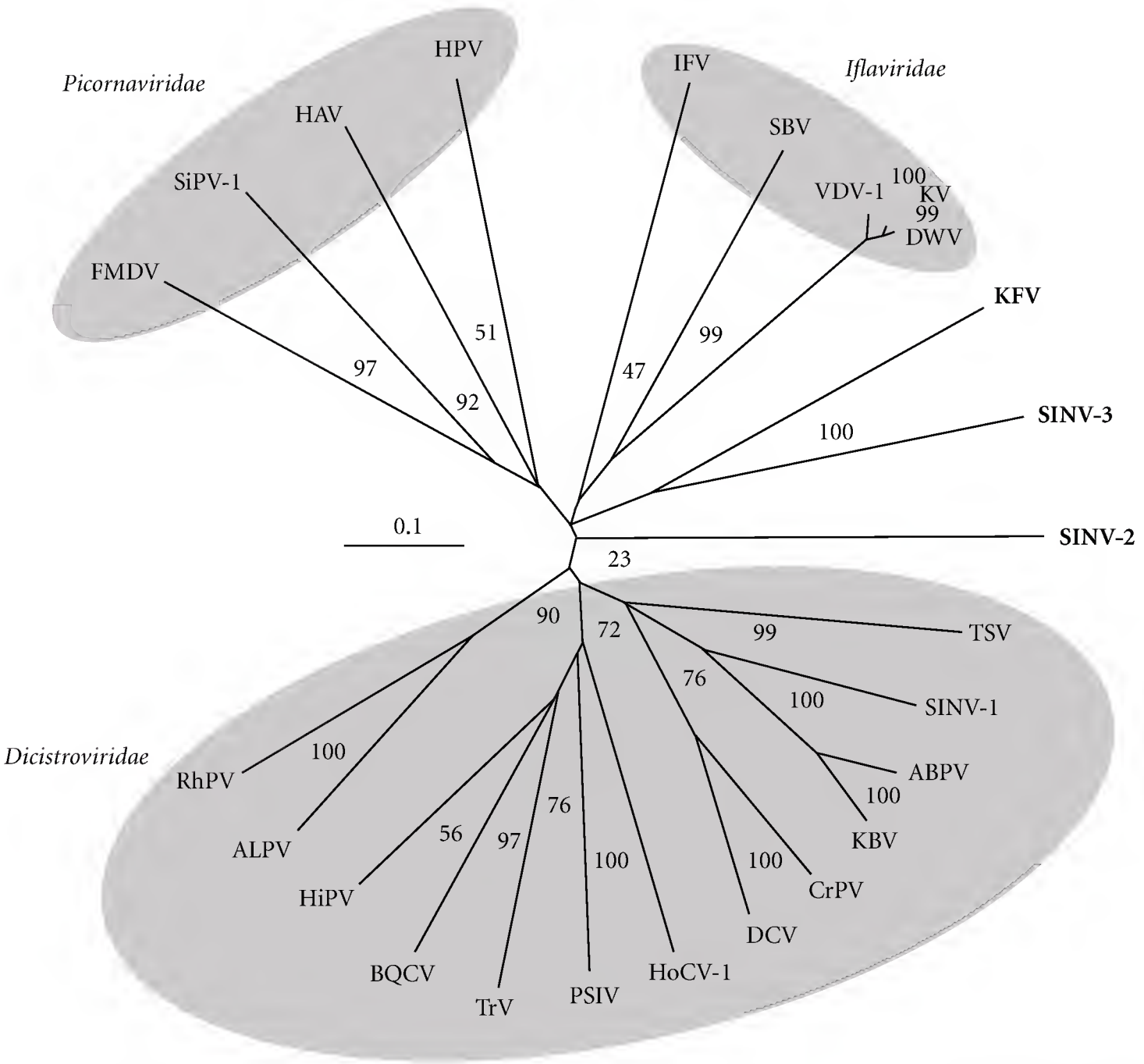

FIGURE 6: Phylogenetic analysis of the conserved amino acid sequences containing domains I to VIII of the putative RdRp from 13 dicistroviruses, 5 iflaviruses, 4 picornaviruses, and 3 unassigned viruses (bold). Virus abbreviation, Genbank accession number of the virus sequence, and amino acid residues of aligned sequences in a specific ORF (5′ proximal ORF of dicistroviruses, otherwise an ORF number is specified) include the following. Aphid lethal paralysis virus (ALPV) [AF536531], 1661–1955; Black queen cell virus (BQCV) [NP620564], 1317–1585; Cricket paralysis virus (CrPV) [NP647481], 1423–1697; Drosophila C virus (DCV) [AF014388], 1415–1693; Himetobi P virus (HiPV) [AB017037], 1441–1710; Plautia stali intestine virus (PSIV) [NP620555], 1465–1739; Rhopalosiphum padi virus (RhPV) [AF022937], 1625–1916; Triatoma virus (TrV) [AF178440], 1446–1716; Acute bee paralysis virus (ABPV) [AAG13118], 1566–1837; Homalodisca coagulata virus-1 (HoCV-1) [DQ288865], 1446–1716; Kashmir bee virus (KBV) [AY275710], 1594–1864; Solenopsis invicta virus-1 (SINV-1) [AY634314], 1052–1327; Taura syndrome virus (TSV) [AF277675], 1770–2036; Infectious flacherie virus (IFV) [AB000906], 2618–2888; Sacbrood virus (SBV) [NC002066], 2522–2790; Deformed wing virus (DWV) [AJ489744], 2556–2826; Kakugo virus (KV) [AB070959], 2556–2826; Varroa destructor virus 1 (VDV-1) [AY251269], 2556–2826; Foot-and-mouth disease virus (FMDV) [AF308157], 2011–2264; Hepatitis A virus (HAV) [NC001489], 1904–2161; Human parechovirus (HPV) [AJ005695], 1871–2117; Simian picornavirus 1 (SiPV-1) [AY064708], 2119–2368; Solenopsis invicta virus 2 (SINV-2) ORF 4 [ABQ01575], 1814–2081; Kelp fly virus (KFV) [YP415507], 3015–3272; Solenopsis invicta virus 3 (SINV-3) ORF 1, 1848–2107.

mortality among recipient colonies was an occasional event [25]. On the other hand, SINV-3 was associated consistently with ant mortality and a correspondingly high SINV-3 titer ($>10^9$ viral particles in a single dead ant carcass). Furthermore, SINV-3 was detected systemically—unlike SINV-1 and SINV-2 which were largely limited to the gut [73, 74, 97].

SINV-3 is readily transmitted to healthy colonies in the laboratory by exposure to homogenates of SINV-3-infected ants and by simply being confined in areas containing SINV-3-infected colonies (SMV unpublished). SINV-3-containing fire ant body parts become friable and airborne, contaminating surrounding areas. Disinfection of contaminated areas is extremely difficult. However, hypochlorite solution is an effective means of disinfection.

6. *S. invicta* Virus Phylogenetic Analysis

Phylogenetic analysis of the conserved amino acid sequences containing domains I to VIII of the RdRp from representative dicistroviruses, iflaviruses, picornaviruses, and unassigned positive, single-stranded RNA viruses revealed a phenogram with SINV-1 clearly part of the *Dicistroviridae*, SINV-2 forming its own unique clade, and SINV-3 and Kelp fly virus (KFV) comprising a unique group (Figure 6). SINV-1 has

been placed formally into the *Dicistroviridae* family [102] and the recently proposed *Aparavirus* genus (pending approval from the International Committee for the Taxonomy of Viruses). Bootstrap values between the major clusters and SINV-2 were relatively low indicating an uncertain common ancestor for this virus. This independent placement of SINV-2 is further supported by phylogenetic results for the helicase region of ORF 1 [26]. SINV-3 is also unique, but exhibits a relationship with KFV. Both of these viruses exhibited a small virion size (27.3 ± 1.3 nm diameter) with apparent surface projections [103] and a high buoyant density (1.39 ± 0.02 g/mL). They also appear to possess only 2 major capsid proteins (VP1 and VP2) as opposed to 3 or 4 which is typical of the *Iflaviridae* and *Dicistroviridae*. The capsid proteins of KFV and SINV-3 exhibited poor comparative sequence identity (<10%).

7. Potential as Control Agents

SINV-1, -2, and -3 represent the only known viruses infecting any ant (Hymenoptera: Formicidae) species. As stated, the intention of virus discovery in *S. invicta* was to utilize viruses as novel control agents against this ant pest. Development and use of positive, single-stranded RNA viruses as insect control agents has been proposed [104, 105] and successfully demonstrated for a number of insect pests. CrPV was evaluated against the olive fruit fly, *Dacus oleae*, and shown to cause up to 80% mortality [106]. CrPV was also reported to be an effective control agent for adult Mediterranean fruit flies, *Ceratitis capitata* [107]. High rates of mortality were observed in laboratory and field tests of RNA viruses against *Epicerura pergrisea* and *Latoia viridissima* in Côte d'Ivoire [108, 109]. Unfortunately, a major limitation of the use of RNA viruses in insect control is large-scale production. This problem can be ameliorated when virus growth is supported by a cell line. However, insect host cell lines supporting viral production are available for only a handful of viruses. Indeed, the absence of a fire ant cell line has hampered investigation and development of the *Solenopsis invicta* viruses as microbial control agents. However, alternative methods of virus production have been demonstrated.

Production of infectious RNA transcripts [110–112] and *in vitro* baculovirus-driven expression of insect-infecting positive, single-stranded RNA viruses have been reported [113]. These methods facilitate study of virus biology and provide a means for their large-scale production. Development of a SINV-3 construct and subsequent *in vitro* expression of SINV-3 has been underway in our laboratory for the last year. Successful transcription of the SINV-3 genome has been accomplished, but production of encapsidated SINV-3 genome has not been observed (SMV unpublished). Indeed, transcript production and translation have proven extremely limited in this system. Because SINV-3 is associated with significant mortality among *S. invicta* colonies (reminiscent of colony collapse disorder of honeybees), our research is focused on studying this virus, including production, host specificity, efficacy, dose responses, mechanisms of action, and development as a biopesticide. Although SINV-1 and SINV-2 appear to cause chronic, asymptomatic infections, they might find utility as control agents once their biology is more fully understood. Sodium alginate microencapsulated formulations of SINV-1 have been demonstrated to successfully transmit virus to uninfected colonies [114]. SINV-1 and -2 may also be exploited in unique ways, for example, as delivery vehicles for toxins or RNA interfering molecules after modification. Only through additional research to characterize the biology of these viruses will their full potential as control agents be realized.

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Research Article

Annual and Seasonal Changes in the Structure of Litter-Dwelling Ant Assemblages (Hymenoptera: Formicidae) in Atlantic Semideciduous Forests

Flávio Siqueira de Castro,[1,2] Alexandre Bahia Gontijo,[1,2] Paulo de Tarso Amorim Castro,[3] and Sérvio Pontes Ribeiro[1,2]

[1] *Laboratory of Evolutionary Ecology of Canopy Insects and Natural Succession, Department of Biodiversity, Evolution and Environment, Federal University of Ouro Preto, 35400-000 Ouro Preto, MG, Brazil*
[2] *Post-Graduate Program in Ecology of Tropical Biomes, Department of Biodiversity, Evolution and Environment, Federal University of Ouro Preto, 35400-000 Ouro Preto, MG, Brazil*
[3] *Departament of Geology, Federal University of Ouro Preto, EM/ Universidade Federal de Ouro Preto, 35400-000 Ouro Preto, MG, Brazil*

Correspondence should be addressed to Flávio Siqueira de Castro, fsiqueiradecastro@gmail.com and Sérvio Pontes Ribeiro, spribeiro@iceb.ufop.br

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We surveyed ant fauna in the leaf litter in an Atlantic Semideciduous forest in the State Park of Rio Doce (PERD). The work aimed to produce basic information about habitat effects on diversity, as well as about how the ant fauna in a such buffered forest habitat, as the litter layer, could respond the climate variation in a short and long term. We sampled two years in two distinct forest physiognomies, which respond to different geomorphologic backgrounds, in dry and rainy seasons. Species composition, richness and abundance of these forests were distinct. However, both forests hosted similar numbers of rare and specialized, habitat demanding species, thus suggesting both are similarly well preserved, despite distinct physiognomies. However, the lower and more open forest was, more susceptible to dry season effects, showing a steeper decline in species numbers in such season, but similar numbers in the wet seasons. The pattern varied between years, which corroborates the hypothesis of a strongly variable community in response to subtle climatic variation among years. The present results are baselines for future long term monitoring projects, and could support protocols for early warnings of global climatic changes effects on biodiversity.

1. Introduction

Species richness and composition respond to different habitat variables and abiotic factors that influence climate, seasonality, humidity, topography, and lithology [1–4]. The construction of the concept of "habitat components" is based on the interactions between abiotic and biotic variables, which result in the parameters on which the niches of species evolve [5]. One habitat component which is hardly studied is its temporal variance, due to the fact that it is highly unpredictable [6].

Forest litter is a crucial habitat compartment for mineral cycling, humidity retention, and, greatly, to biodiversity maintenance [7, 8]. Conversely, the insect fauna that dominates the litter is a fundamental factor for its transformation. In a whole tropical forest, ants and termites are the most important animals in relation to biomass and relative abundance. Ants are found in virtually all strata of forests, playing a key role in structuring ecological communities in tropical ecosystems [9]. They are responsible for processes of soil mineralization due to its extensive bioturbation activity [10], promoting changes in physical environments [7], and, consequently, a vast movement of nutrients [8, 11]. Furthermore, an ant assemblage responds positively to natural succession [12–14], causing feedback responses, such as plant species dispersal and seed collecting [15–17]. They

are also responsible for important predation rates on tropical forests (e.g., army ants) [11].

For ant assemblages, the negative effects of low temperature [18], intense rainfalls (daily and cumulative), plus the positive effects of high relative humidity of the understorey and forest ground, influence directly the foraging and nesting [19]. The effect of intense rainfall may occur due to interference in the communication process between individuals, by literally washing down the worker's chemical trails, or by flooding areas with soils less susceptible to drainage [20]. Thus, these factors affect many phenological activities in the colonies [20, 21] and are crucial parameters in structuring ground-dwelling ant assemblages in tropical forests [18–20]. Additionally, it may have confounding positive effects with the rainfall, such as increasing humidity or increasing the litter volume [22], thus resulting in a difficulty to evaluate the real effects on the ants assemblages.

Therefore, the way how seasons and years (namely general weather conditions) should affect ant species parameters along time must be highly variable and unpredictable. Campos et al. [23] have shown that arboreal ant assemblage in an Atlantic semideciduous forest, in the State Park of Rio Doce, responded as strongly to host trees as to time passing, and more significantly than to seasonality. Further, the authors observed that changes in ant fauna was not affected by the host plant habitat specificities, such as being in a forest artificial border, within the forest, or in a natural lake ashore. In other words, ant species composition and relative density may respond to more subtle components of the environment.

In the present study, we aimed to evaluate the effects of habitats and temporal variation on the litter-dwelling ant species richness, abundance, and composition in this same semideciduous Atlantic forest. In order to investigate the hypothesis that temporal variation may have stronger effect than habitat specificities, we sampled in two contrasting forest physiognomies, in two different geomorphologic backgrounds.

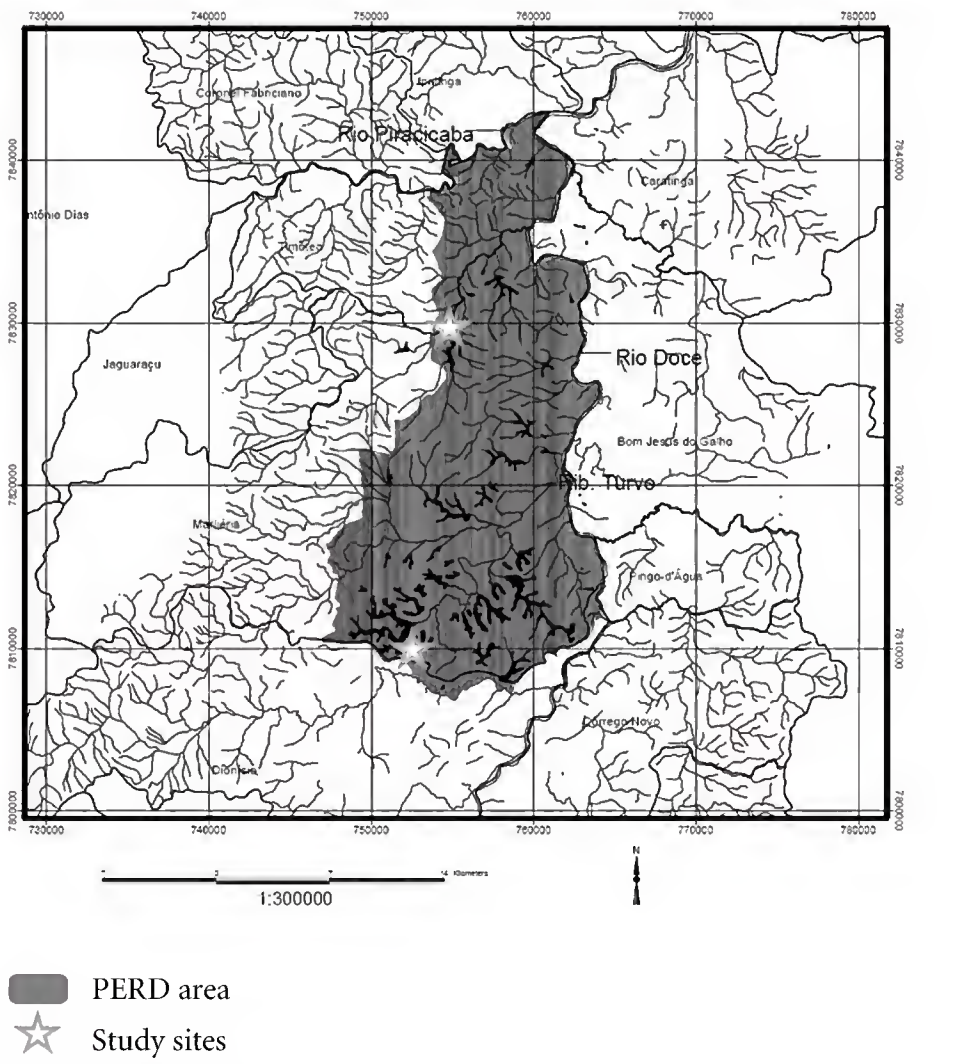

Figure 1: Map showing the location of the PERD and the study sites in relation to the surrounding region. Star at the north showing the location of Macuco's IMA (TM) and at the south showing the Gambazinho's IMA (LG) (Source: [22].)

2. Materials and Methods

2.1. Study Sites. Samples were taken in the State Park of Rio Doce (PERD-IEF), Marliéria, Minas Gerais. This is approximately 36,000 ha of protected forests and lakes, comprising part of the municipalities of Timóteo, Marliéria, and Dionísio—between the parallels 19°48′18″–19°29′24″S and meridians 42°38′30″–42°28′18″W. The park is bound in the East by the Doce River and Piracicaba River to the North [24]. The vegetation is characterized as lower montane Atlantic semideciduous forest, with a percentage of deciduous trees between 20 and 50% [25, 26]. The forest varies greatly in physiognomy and soil conditions from north to south. Also, the park preserves the largest natural lake system in the Atlantic rainforest domain (10% of its area) that resulted from geological movements around the old Doce river and affluents during the Pleistocene [27].

The climate is tropical humid mesothermal [28]. The wet season occurs from October to March and the dry season from April to September [29]. Samples were taken in August (dry season) and November (rainy season) in the years of 2005 and 2006, in two different areas: the Lagoa do Gambazinho's IMA (Integrated Monitoring Array) (hereafter LG) (southern PERD) and the Trilha Macuco ou Juquita's IMA (hereafter TM) (northern PERD) (Figure 1).

The LG is composed by a secondary, edaphically constrained low forest vegetation (10–15 m high) [30] in an area with irregular topography, varying from hills to lowlands, with permanent and temporary swamps [31]. In the TM, there is a predominance of high and medium forest in the lowlands and medium forests in the slopes and crests, with little topographic variation, but the presence of alternating hills and lowlands. The whole area of the TM is in an old alluvial terrace, the paleochannel of the Belem River (tributary of Doce River), while LG has a distinct geological unit, and this entire area lies on a unit called litostatigragphic Mantiqueira Complex [31].

2.2. Sampling Design. The study areas are two permanent plots of 100 ha (IMA) produced during the Rio Doce TEAM Project, a long-term project coordinated by the Conservation International through the TEAM (Tropical Ecology, Assessment, and Monitoring) Initiative network [32]. The samples used in this work are part of Rio Doce TEAM Project—Ant Protocol [33]. The chosen areas are permanent plots set to attend several projects, and transects were easily set in a full random design due to the existence of open narrow research paths. Eight transects were sampled per season in both areas,

Table 1: Number of hits (records) and overall frequency (%) of ant species per IMA, LG, and TM.

Species	2005				2006				Frequency (%)
	LG		TM		LG		TM		
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	
Solenopsis (*Diplorhoptrum*) sp1	27	26	23	27	34	36	3	26	63.13
Pyramica denticulata (Mayr, 1887)	21	16	1	15	27	22	19	19	43.75
Brachymyrmex australis Forel, 1901	12	22	12	28	4	22	13	18	40.94
Strumigenys elongata Roger,1863	13	9	6	12	21	13	16	18	33.75
Hypoponera trigona (Mayr, 1887)	16	1	8	9	13	15	24	19	32.81
Pheidole diligens Smith F., 1858	1	7	11	15	11	21	15	24	32.81
Odontomachus meinerti Forel, 1905	12	11	8	19	19	7	15	12	32.19
*Solenopsis (Diplorhoptrum)*sp5	29	7	24	6	13	3	11	4	30.31
Hypoponera distinguenda Emery, 1890	13	12	1	11	18	19	12	7	29.06
Pheidole cf. *flavens* Roger, 1863	5	14	3	1	18	16	16	9	25.63
Crematogaster longispina Emery,1890	1	4	13	5	17	5	17	8	21.88
Wasmannia auropunctata (Roger,1863)	9	6	6	4	26	1	11	1	20.00
Strumigenys cf. *silvestrii* Emery, 1906		13	11	11	2		9	16	19.38
Pheidole cf. *dimidiata* (Emery, 1894)	1	6	2	1	6	17	5	18	17.50
Pheidole midas Wilson, 2003	7	11	2	5	1	9	9	11	17.19
Carebarella sp1	5	2	2	8	11	4	14	8	16.88
Sericomyrmex cf. *bondari* Borgmeier, 1937	14	5	1	9	5	12	6	1	16.56
Apterostigma gp. p*ilosum* Mayr, 1865	6	8		1	11	4	13	3	14.38
Solenopsis (Diplorhoptrum) sp2	4	7	5	13	3		6	8	14.38
Brachymyrmex longicornis Forel, 1907		1	13	9			9	12	13.75
Pachycondyla gp. *harpax* (Fabricius,1804)	9	4	6		2	5	3	3	10.00
Solenopsis sp4	1		1	11			7	11	9.69
Pheidole cf. *minutula* Mayr, 1878	1		5	8	4		1	11	9.38
Brachymyrmex sp3	15	1			8	5			9.06
Pyramica crassicornis (Mayr,1887)	6	5	1	3		4	1	9	9.06
Cyphomyrmex tranversus Emery, 1894	1	1	1		4	5	7	8	8.44
Octostruma iheringi (Emery,1888)	2	1	1	1	2	7	5	7	8.13
Crematogaster (Orthocrema) sp6	3		7	4	5	2	1	3	7.81
Hylomyrma reitteri (Mayr,1887)	3	5		2	3	9		3	7.81
Solenopsis cf. *terricola* Menozzi, 1931	4	5		3	2	3	3	5	7.81
Octostruma rugifera (Mayr, 1887)		4		5		5		8	6.88
Hypoponera sp6	3	5	1	1	1	9		1	6.56
Pyramica eggersi (Emery,1890)		1			1	11	5	2	6.25
Crematogaster nigropilosa Mayr, 1887	6	7		5		1		1	6.25
Mycocepurus smithii Forel, 1893	1	3	1	3	3	2	2	4	5.94
Carebara panamensis (Wheeler, 1925)	2	2			1	4	4	5	5.63
Rogeria besucheti Kluger, 1994	2	1			4	2	4	5	5.63
Discothyrea sexarticulata Borgmeier,1954	1	2		5			4	5	5.31
Megalomyrmex modestus Emery, 1896	2	3	1	3			2	6	5.31
Crematogaster limata Smithi F., 1858	3	4		6		1	1	1	5.00
Paratrechina sp4	1			1	7	2	4		4.69
Ectatomma permagnum Forel, 1908	3	1	1	3	2		3	1	4.38
Brachymyrmex heeri Forel, 1874	6	5	1	1					4.06
Octostruma cf. *balzani* (Emery, 1894)		2		4		6	1		4.06
Carebara urichi (Wheeler, 1922)	1	1		2	1	1	1	5	3.75

Table 1: Continued.

Species	2005				2006				Frequency (%)
	LG		TM		LG		TM		
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	
Dolichoderus imitator Emery, 1894	4		4		3		1		3.75
Acromyrmex subterraneus brunneus Forel, 1912	2	2	2	1	1		3		3.44
Camponotus (Tanaemyrmex) punctulatus andigenus Emery, 1903		1	3		3	1	2	1	3.44
Hylomyrma sp2		2		1	1	3		4	3.44
Pyramica sp5	1	5				5			3.44
Strumigenys perparva (Brown, 1958)	1		1		1	1	5	1	3.13
Megalomyrmex drifti Kempf, 1961	3	4	1	1			1		3.13
Pheidole sp9						4		6	3.13
Brachymyrmex sp5	3	6							2.81
Ectatomma tuberculatum (Olivier,1792)	2		1	1	3		2		2.81
Pachycondyla venusta Forel, 1912	1		2	1	1	1		3	2.81
Pyramica alberti (Forel, 1893)				3			6		2.81
Pyramica brevicornis (Mann, 1922)		1	1	2	3	1	1		2.81
Brachymyrmex cf. *pictus* Mayr, 1887	2		1		1		4		2.50
Ectatomma suzanae Almeida, 1986	3			1			3	1	2.50
Hypoponera foreli (Mayr, 1887)	3	1	1		1		2		2.50
Hypoponera cf. *punctatissima* (Roger 1859)				2			6		2.50
Prionopelta antillana Forel, 1909	1			1	2		2	2	2.50
Pyramica subedentata (Mayr, 1887)	2			1	1		2	2	2.50
Trachymyrmex cornetzi Forel, 1912	1		1		1	3	1	1	2.50
Hypoponera sp8		3	2		2				2.19
Odontomachus haematodus (Linnaeus,1758)	1		3		1		1	1	2.19
Wasmannia cf. *sigmoidae* (Mayr, 1884)							7		2.19
Acanthognathus ocellatus Mayr, 1887		1	1	1				3	1.88
Brachymyrmex sp7	5				1				1.88
Myrmicocrypta squamosa Smith F., 1860		1		2	2	1			1.88
Pachycondyla gilberti Kempf, 1960		1			1	1	1	2	1.88
Pheidole sp13		3		2		1			1.88
Pyramica maynei (Forel, 1916)		3				2		1	1.88
Solenopsis sp16			1	3	2				1.88
Strumigenys precava (Brown, 1954)	1	2					2	1	1.88
Acropyga goeldii Forel, 1893		1						4	1.56
Apterostigma sp5					3		2		1.56
Brachymyrmex sp4	4					1			1.56
Camponotus (Tanaemyrmex) melanoticus Emery, 1894	1	1			1	2			1.56
Megalomyrmex goeldii Forel, 1912		5							1.56
Pheidole sp12	5								1.56
Solenopsis sp6	2	3							1.56
Linepithema pulex Wild, 2007	1		1	1	1			1	1.56
Wasmannia sp3	1	1	2						1.25
Cryptomyrmex longinodus Férnandez & Brandão, 2003		2				2			1.25
Amblyopone lurilabes Lattke, 1991				1		2		1	1.25
Crematogaster acuta (Fabricius,1804)		4							1.25

Table 1: Continued.

Species	2005				2006				Frequency (%)
	LG		TM		LG		TM		
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	
Gnamptogenys porcata (Emery,1896)		1					1	2	1.25
Paratrechina steinheili (Forel, 1893)		1	3						1.25
Pheidole sp14	1			1				2	1.25
Pheidole sp16			1					3	1.25
Wasmannia rochai Forel, 1912			1	1		1		1	1.25
Amblyopone cf. *armigera* Mayr, 1897	1						1	1	0.94
Brachymyrmex sp8					1	2			0.94
Camponotus (Mymocladoecus) latangulus Roger, 1863		2		1					0.94
Camponotus (Myrmophaenus) novogranadensis Mayr, 1870	1	1		1					0.94
Crematogaster sp3	2					1			0.94
Cyphomyrmex peltatus (Kempf, 1966)		1					2		0.94
Eurhopalothrix prox. *bruchi* Santischi, 1922		1		1			1		0.94
Gnamptogenys gracilis (Santischi, 1929)	1							2	0.94
Octostruma sp4				1		1		1	0.94
Paratrechina sp6					1		2		0.94
Solenopsis sp14				2				1	0.94
Trachymyrmex prox. *relictus* Borgmeier, 1934			1		1		1		0.94
Azteca cf. *alfari* Emery, 1893	1	1							0.63
Camponotus femoratus (Fabricius,1804)		1						1	0.63
Cephalotes pusillus (Klug,1824)	1		1						0.63
Crematogaster curvispinosa Mayr, 1862	2								0.63
Hylomyrma sp3	1						1		0.63
Hypoponera sp12	1	1							0.63
Megalomyrmex silvestrii Wheeler W.M., 1909				1		1			0.63
Mycocepurus goeldii Forel, 1893	2								0.63
Myrmelachista (Hincksidris) sp2			1		1				0.63
Pachycondyla verenae (Forel, 1922)	1					1			0.63
Pheidole sp4					1			1	0.63
Pseudomyrmex tenius (Fabricius, 1804)			2						0.63
Rogeria microma Kempf, 1961	2								0.63
Solenopsis sp3						2			0.63
Stegomyrmex olindae Feitosa, Brandão & Diniz, 2008							2		0.63
Wasmannia cf. *lutzi* Forel, 1908	2								0.63
Acanthognathus prox. *rudis* Brown & Kempf, 1969		1							0.31
Acropyga smithii Forel, 1893						1			0.31
Anochetus diegensis Forel, 1912				1					0.31
Brachymyrmex sp9					1				0.31
Camponotus (Tanaemyrmex) balzani Emery, 1894		1							0.31
Camponotus (Myrmoclaedocus) bidens Mayr, 1870			1						0.31
Camponotus (Mymocladoecus) crassus (Mayr, 1862)			1						0.31
Camponotus (Mymocladoecus) rectangularis Emery, 1890	1								0.31

Table 1: Continued.

Species	2005				2006				Frequency (%)
	LG		TM		LG		TM		
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	
Camponotus (Myrmobrachys) trapezoideus Mayr, 1870		1							0.31
Carebara pilosa Férnandez, 2004								1	0.31
Cephalotes maculatus (Smith F.,1876)	1								0.31
Cerapachys splendens Borgmeier, 1957		1							0.31
Crematogaster sp8								1	0.31
Dolichoderus lutosus Smith F., 1858				1					0.31
Eciton burchelli (Westwood, 1842)		1							0.31
Gnamptogenys sp3					1				0.31
Heteroponera angulata Borgmeier, 1959		1							0.31
Hypoponera sp10					1				0.31
Hypoponera sp13		1							0.31
Hypoponera sp17							1		0.31
Labidus coecus (Latreille, 1802)		1							0.31
Linepithema iniquum (Mayr, 1870)			1						0.31
Myrmelachista sp1	1								0.31
Myrmelachista sp3							1		0.31
Neivamyrmex sp1						1			0.31
Nesomyrmex spininoidis Mayr, 1887	1								0.31
Nesomyrmex wilda Smith M.R., 1943			1						0.31
Pachycondyla ferruginea Smithi F., 1858		1							0.31
Pachycondyla villosa inversa Smith F., 1858							1		0.31
Pheidole fallax Mayr, 1870							1		0.31
Pheidole sp17					1				0.31
Pseudomyrmex gp. *pallidus* Smith F., 1855			1						0.31
Pyramica appretiata (Borgmeier, 1954)		1							0.31
Rogeria scobinata (Kluger, 1994)		1							0.31
Solenopsis (Euophthalma) globularia Smith F., 1858				1					0.31
Solenopsis sp15								1	0.31
Strumigenys sublonga Brown, 1958							1		0.31
Strumigenys schmalzi Emery, 1905		1							0.31
Wasmannia villosa Emery, 1894					1				0.31

thus 16 per year, with 10 samples of 1 m^2 litter per transect, using the apparatus of Mini-Winkler [34], equidistant 10 m one from another, summing up 320 samples on two years. Each transect had its exact position previously sorted using random numbers and a plotted map of the transect, thus assuring a fully random sampling design.

Ants were taken to the lab, sorted, and identified to genera. Species confirmations were achieved in collaboration with the Myrmecological laboratory of CEPLAC, Bahia. The collection is saved in both CEPLAC and in the collection of the Laboratory of Evolutionary Ecology of Canopy Insects, in DEBIO/ICEB/UFOP.

2.3. Data Analysis. In order to evaluate the effect of accumulation of species in each sampling unit and for all observed data, we made species accumulation curves (Coleman method), which devise the expected richness for random subsamples of the data set grouped [35, 36]. Calculations were made using the computer program EstimateS version 8 [35]. The Coleman curve is essentially the same to a rarefaction curve and more efficient computationally [37, 38]. The Abundance-based Coverage Estimator (ACE) was used as estimator of species richness [35, 39], because the coefficient of variation (CV) was larger (CV = 0.519) than abundance distribution. When the CV was larger than CV > 0.5, Chao [39] and Colwell [35] recommend Chao 1 and ACE as the best estimates for abundance-based richness.

A nonsmetric multidimensional scale analysis (NMDS) was used to demonstrate overall differences in species composition between the two areas. The ordination was carried

out for the data on species presence and absence in each plot, using the Jaccard index. We used analysis of similarities (ANOSIM) [40] to test for differences in species composition between areas. In order to investigate patterns of similarity between the ant communities in both areas, we used the relative differences between R-value of the ANOSIM test [41]. These analyses were performed using the software PAST [42].

Factorial analysis of variance (ANOVA) models [43], with Poisson distribution of data (which is automatically log-transformed in the model in order to best fit the distribution) [44], were used to analyse the results. The statistical package GLZ-Generalized Linear/Nonlinear Models (Statsoft Statistica 7.0 software) was used when generating analyses of the frequency of occurrence of ants (number of records) and the total number of species for each sample (transect) in different areas, seasons, and between years. Wald's test was used to verify the true parameter value based on the sample estimate, assuming that the value of $P < 0.05$ is significant. The measures of relative abundance (frequency of species per transect) were based on the number of occurrences of species per point (each 1 m^2 of the transect), summing up 10 possible occurrences of each species per transect, or 40 per season/IMA.

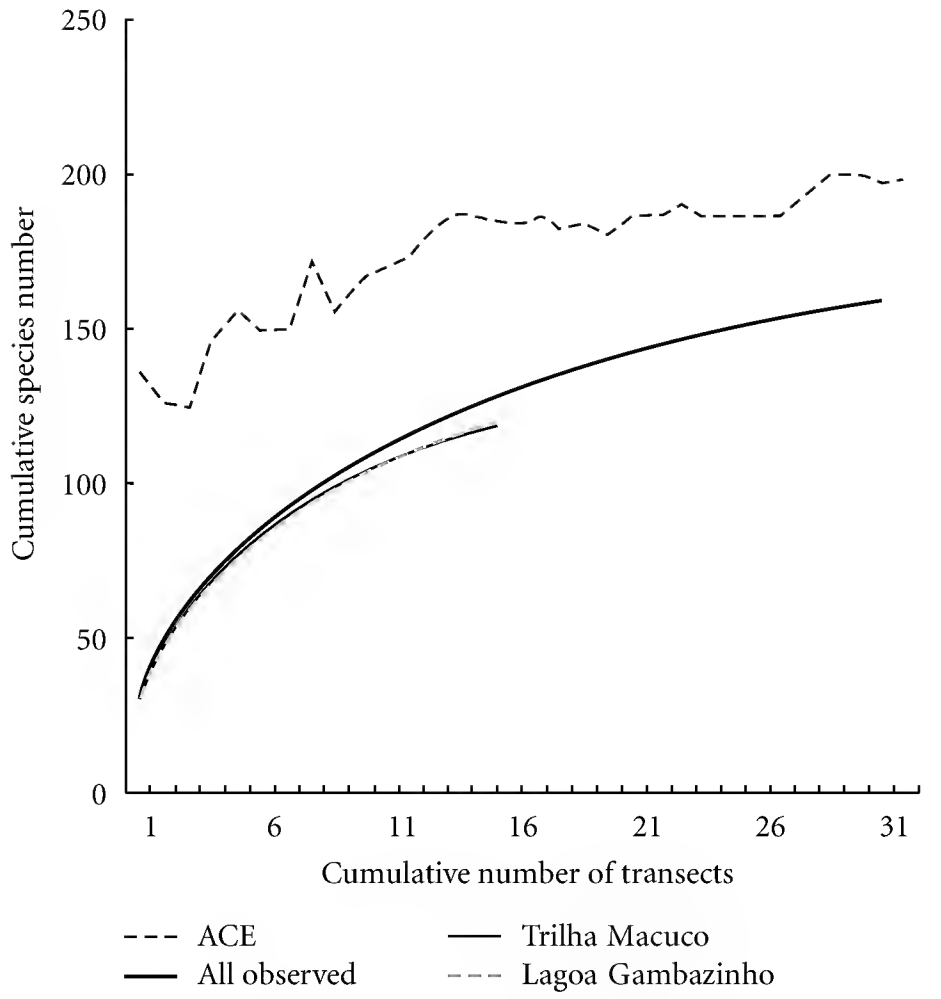

FIGURE 2: Accumulation curve of species richness (obtained by Coleman's method) for all sampling (solid black line), for Lagoa Gambazinho (dashed grey line), and for Trilha Macuco (thin black line). The overall species richness for both IMAs was estimated with Chao 2.

3. Results

In total 2851 individuals, 48 genera and 160 morphospecies were identified and recorded, belonging to 11 subfamilies: Amblyoponinae, Cerapachyinae, Dolichoderinae, Ecitoninae, Ectatomminae, Formicinae, Heteroponerinae, Myrmicinae, Ponerinae, Proceratiinae, and Pseudomyrmicinae. Most of species and genera found belong to the subfamily Myrmicinae, followed by Formicinae and Ponerinae. Only four of the 11 subfamilies of ants were not common to the two areas: Cerapachyinae, Ecitoninae, and Heteroponerinae were found only in the TM, while Pseudomyrmicinae was found only in LG.

The number of genera occurrences was very similar between the two sites. LG showed 40 genera, being four habitat-specific genera: *Cephalotes*, *Myrmelachista*, *Pseudomyrmex*, and *Stegomyrmex*. TM had 44 genera, eight habitat-specific genera: *Acropyga*, *Anochetus*, *Cerapachys*, *Cryptomyrmex*, *Eciton*, *Heteroponera*, *Labidus*, and *Neivamyrmex* (Table 1). In both areas, we found typical litter-forest genera (*Stegomyrmex* in LG, *Cerapachys* and *Cryptomyrmex* in TM), typical forest species or species only recently described, for example, *Wasmannia villosa* and *Stegomyrmex olindae* in LG, and the typical soil ant *Cerapachys splendens* and *Cryptomyrmex longinodus* in TM. New species for science, being in process of description, were also found, as *Hylomyrma* sp2 (MZUSP).

Species richness did not reach stabilized at the end of the sampling, even after combining all samples ($n = 32$; Figure 2). The total species richness of the ant community per transect (1 ha) was estimated to be around 200 species, and rare-faction curve was reached to be around 160 species (Coleman's method) (Figure 2). For both IMA, species richness was similar (rarefied species richness for 122 occurrences Coleman method = 4.22 ± 1.63 for LG and 4.27 ± 1.53 for TM, Figure 2). However, differences in species composition were detected among areas, as revealed by NMDS (ANOSIM, $R = 0.23$, $P < 0.001$; Figure 3).

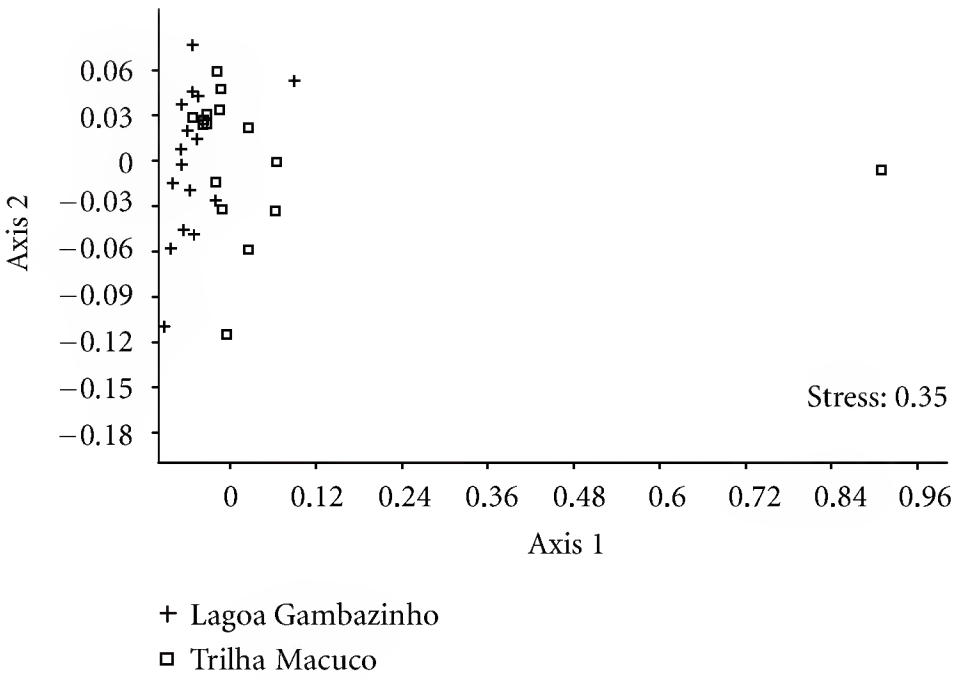

FIGURE 3: NonMetric Dimensional Scale (NMDS) ordination of species composition of the ant community in both IMA as sampled by Mini-Winkler.

Regardless seasonal and yearly variations, species richness was very similar between both areas (Factorial ANOVA, Wald $X^2(1) = 1.06$, $P = 0.30$). The LG had 126 species, 36 of these habitat-specific species, and the TM showed 124 species and 35 habitat-specific species (Figure 4 and

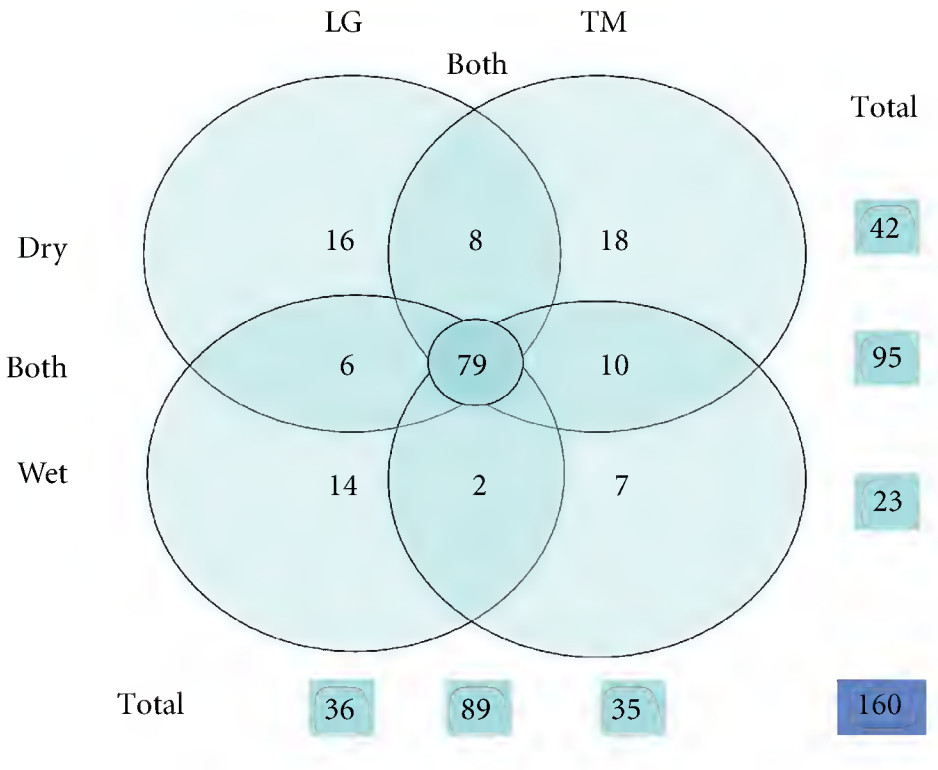

FIGURE 4: Diagram representing the number and distribution of ant species in both IMA (Lagoa Gambazinho and Trilha Macuco) and in two seasons (dry and wet). Separation criteria were unique to each of the IMA and weather stations (large circles), collected in both IMA (intersections of top and bottom), collected in two seasons (intersections of left and right), and collected in two IMA and in two seasons or in at least one weather station (central circle).

Table 1). However, the species richness found were quite different between the dry period (42 species) and the rainy season (24 species), and this difference was due to a strong reduction of species in LG, the low and more open forest, during the dry periods (Figure 5(a)). It is worth to notice that there were more species in common between the areas during the dry season than in rainy season. Species such *Azteca* cf. *alfari*, *Brachymyrmex* cf *pictus*, *B.* sp5, *Camponotus* (*Tanaemyrmex*) *balzani*, *Paratrechina steinheili*, *Hypoponera* sp12, *Solenopsis* sp5, and *Wasmannia* sp3 were found only in the dry season, while *Brachymyrmex* sp8 and *Pheidole* sp4 were found only in wet season (Figure 4 and Table 1). Species richness declined in the LG in the dry season, when compared to TM or to itself in the rainy season, but only for 2006 (Factorial ANOVA, Climatic Season∗Year, Wald $X^2(1) = 6.81$, $P = 0.009$; Figure 5(a)). The inconsistency of this decline in 2005 resulted in a lack of significant differences in species richness between areas (Factorial ANOVA, Wald $X^2(1) = 0.46$,$P = 0.50$; Factorial ANOVA, IMA∗Climatic Season∗Year, Wald $X^2(1) = 0.50$, $P = 0.48$) (Figure 5(a)).

A similar interaction effect between area and year effects (Factorial ANOVA, IMA∗Year, Wald $X^2(1) = 7.34$, $P = 0.006$) and the effects of season and year (Factorial ANOVA, Climatic Season∗Year, Wald $X^2(1) = 17.70$, $P = 0.00003$) defined the variance in ant abundance between the areas. Likewise species richness, the ant abundance declined strongly in the dry season of 2006 and only. In the present case, the strength of this interaction was perceptible in the three levels, thus reflecting in the mean numbers of ants between areas, greater in the TM, the tallest forest (Factorial

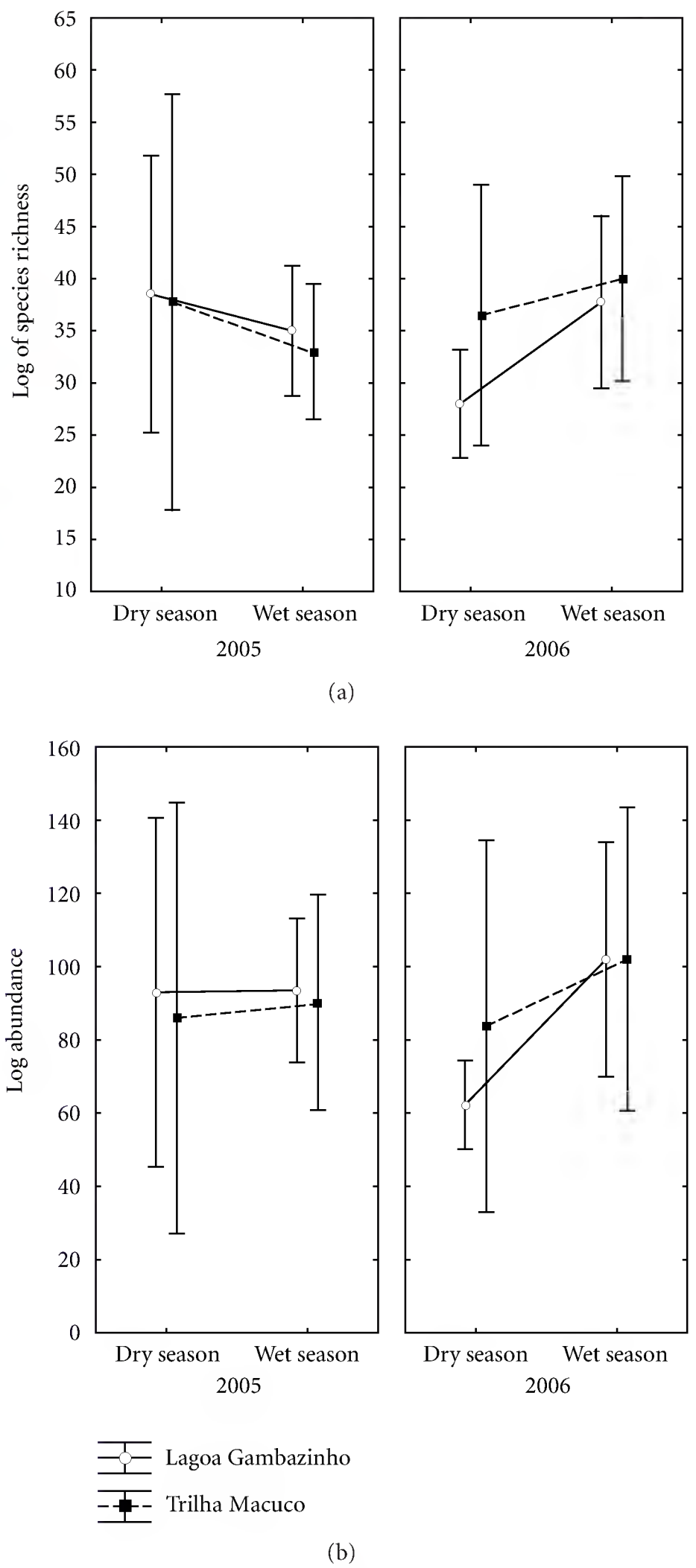

FIGURE 5: Species richness (a) and relative abundance (b) (number of occurrences on transects) of ant species in both IMA, in two seasons (dry and wet), and in two years (2005 and 2006).

ANOVA, IMA∗Climatic Season∗Year Wald, $X^2(1) = 5.02$, $P = 0.02$; Figure 5(b)).

The overall mean abundance was also very similar between areas (Factorial ANOVA, Wald $X^2(1) = 1.46$, $P = 0.23$). The 10 most abundant species throughout the sampling were *Solenopsis* sp1, followed by *Pyramica denticulata*, *Brachymyrmex australis*, *Hypoponera trigona*, *Strumigenys*

elongata, *Pheidole diligens*, *Odontomachus meinerti*, *H. distinguenda*, *S.* sp5, and *P.* cf. *flavens*. However, at LG, the species *B. australis* and *P. diligens*, were not among the most abundant species. In this area, besides the other eight previously mentioned, we found *Crematogaster longispina* and *Wasmannia auropunctata* amongst the most abundant. In the TM, *Solenopsis* sp5 was not as abundant as the others, while *P.* cf. *dimidiata* was found among the most abundant. Regarding rare species, the LG presented 36 species, 20 of these were habitat-specific species, while in TM were 33 rare species, and also 20 habitat-specific species (Table 1).

As observed for relative abundance, there was an increase in species richness in the wet season in both areas. However, despite low species richness in the dry season, species composition varied smoothly between years in both areas. The data of the rainy season in LG, for example, showed that the total number of species increased from 67 to 72 in a year to another, and the number of rare species (singletons) decreased from 26 to 21 species (38.8% to 29.2% of species). Values of relative abundance (number of occurrences per sampling event, or 4 transects and 40 points for either season/IMA) of intermediate species (species that were neither among the 10 most abundant or among singletons) varied greatly between years (Table 1), which was related to the variation in numbers of predatory ants species richness and abundance.

4. Discussion

The global ground-dwelling ant diversity in Rio Doce was remarkably high, as well as composed of rare and habitat-demanding species and genera (see below). The ant species richness in the forest litter was substantially higher than ants or general arthropods species in the canopy of these same forests [23, 45, 46]. In addition, it was comparably as high as in other tropical forests and sometimes comparable to the species richness found and wetter and closer equatorial forests [13, 19, 36, 38].

In spite of differences in physiognomy and geomorphology found in both areas, the overall mean values of species richness and abundance were not statistically different between these forest types, although there were substantial differences in relation to species composition. However, one could expect to sample more species, especially rare predators and cryptic specialists species in TM than in the LG, due to the tree heights, a better structured understorey, apparent constancy of conditions in the former compared to the latter. These conditions should allow high availability of resources and quality of sites for feeding and nesting areas [47, 48]. The TM pristine semideciduous forest was denser in trees, which also had larger basal area than the open, apparently secondary LG [31]. Among many other ecological implications, these traits imply in greater litter biomass in TM compared to LG.

In a close wet and tall forest, there might have less variation in abiotic conditions, such as local atmospheric humidity and temperature [19], and, sometimes, as is the case of PERD, this may be further buffered by a smooth and continuous topography [31]. On the other hand, ecological variables such as increased litter production in association with the high heterogeneity of the vegetation may also happen in association to close and tall forests [49], which results in high spatial complexity, allowing diversification of conditions, thus sheltering a large number of species of ants and other invertebrates in the litter and soil [31, 45, 48, 50–52]. Other studies in tropical forests have corroborated that differences of litter ants species diversity and distribution respond to habitats with low and high structural heterogeneity [53–55].

Hence, TM should have the best conditions for more habitat-demanding ant species than LG, unless the habitat conditions in the latter are rather natural too. The region around LG has indeed a more open canopy and lower tree heights than TM, but the cause for its physiognomic pattern is subject of debate. Although the area was impacted by a fire in 1967, there would have been time enough for full recover. Soares [31] and Ribeiro et al. [46] have discussed that a great deal of the observed differences in vegetation are natural rather than result of human past disturbance. Data suggest that physiognomic differences between the two studied areas are evolutionary rather than ecological, and both may sustain equivalent levels of heterogeneity. Indeed, the geomorphologic origins of both forests ought to have stronger effect on their productivity and then on their canopy traits that relate to understorey microclimate and heterogeneity. Hence, despite apparent vulnerability of LGs and its low resistance to the dry season extreme desiccation, both forests are equivalently rich and populated with ecologically demanding species. Such pattern only could happen if both places had long enough favourable conditions for species evolution.

The occurrence of rare species of ants in the LG, for example, *Stegomyrmex olindae*, so far found only in humid and mature forests [56], *Cryptomyrmex longinodus* (first occurrence in the southeastern Brazil; Fernández, personal communication), *Strumigenys sublonga* (cryptic species collected by first time in tropical semideciduous forest in Minas Gerais, Castro unpublished data), *Eurhopalothrix* prox. *bruchi*, and *Octostruma* species, support that this forest, whatever the human impacts suffered in the past, has several ant species typical of environments with greater habitat structural complexity and a well-preserved long evolutionary history. It is quite likely that edaphic-evolving condition found in the LG forest is the best explanation for the fact. This also may explain the fact that there is not any widely distributed tall and closed forest in southern PERD as a whole.

Seasonal effects are important regulators of ecological communities in tropical forests, especially plants and invertebrates [5]. Seasonal effects were more perceptible in 2006 than in 2005, concerning both species richness and abundance. According to PERD climatic station data and our microclimatic records, the 2005 had a dry season with little rainfall, mild air temperatures, and high mean relative humidity, rising up to 80% in the winter driest days. The year of 2006 was characterized by a dry season hotter than 2005, regarding air temperature in the understorey, with abrupt

changes in rainfall regimen, unlike 2005, even though we sampled exactly in the same time of the year. For instance, it rained practically every day in August 2006 (a usually very dry month), while in 2005 rainfall was recorded in only four days during the same month. In the rainy season, in relation to data of temperature and humidity, there was no significant dispatch from the expected and the understorey reached values at or near 100% relative humidity in both years. However, in November 2005, the accumulated rainfall index ranged from 312.5 to 330.61 mm (daily rain 89 ± 4 mm), while in 2006, it ranged from 48.43 to 29.99 mm (daily rain 17 ± 5 mm) [57].

These data suggest that in 2006, the rainy season started abnormally earlier (in August). In tropical forests, the cycle of ants colonies is synchronized with the seasonal rainfall and temperature [21], and, although the mechanism is not well known, in the more humid and hot (above 30°C and 50% of relative humidity), the faster is the development of ant colonies [20]. Nevertheless, the unpredictable start of rains may have a very negative effect in some habitats, by taking the colony not prepared for the change. On the other hand, a badly defined dry season in 2006, with subsequent early onset of the rainy season, may have provided a better partition of resources available for ant assemblage in both areas, especially preys, which could reflect the fast recovery and increasing abundance of predators in the wet season. Indeed, in 2005, some specialist predatory ants were rare or absent, as some species of the genus *Strumigenys* and *Pyramica*, known as specialist predators of Collembola, *Discothyrea sexarticulata*, a predator of spider eggs, and the generalist large predators as *Ectatomma* and *Pachycondyla* species, predators of insects and invertebrates with similar body size or also larger [58].

In conclusion, our results showed that contrasting forest types may have similar total ant species richness, as well as a similar amount of rare and ecologically specialized species. These similarities between these forests suggest that litter-dwelling species may have high resilience, related to the litter habitat conditions, to changes and disturbances in both ecological and evolutionary times.

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Review Article

Adult Diapause in Coleoptera

Ivo Hodek

Institute of Entomology, Biological Centre, Academy of Sciences, 37005 Ceske Budejovice, Czech Republic

Correspondence should be addressed to Ivo Hodek, hodek@entu.cas.cz

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Recent studies dealing with adult (reproductive) diapause in the Coleoptera are reviewed, as a kind of supplement to the classic compendia. In the first two sections, the general characteristics of adult diapause are described and principal terms explained. Original articles dealing with 19 species from nine coleopteran families (Coccinellidae, Chrysomelidae, Bruchidae, Curculionidae, Carabidae, Silphidae, Scolytidae, Scarabaeidae, and Endomychidae) are reviewed. Finally attempts are made at generalisations from the papers reviewed, and hypotheses on diapause evolution are inferred. A polyphenic character of diapause is a prominent feature in *C. septempunctata* and *L. decemlineata*, but has been found also in other Coleoptera and in insects generally and often generates voltinism heterogeneity within populations.

1. Introduction

Adult diapause is the most common form of diapause in Coleoptera. It occurs in about 90% of beetle species [1], belonging mostly to the families of Coccinellidae, Chrysomelidae, and Curculionidae, and partly also Carabidae (the so-called carabid "autumn breeders" diapause as larvae). Another insect order with a high incidence of species entering diapause in the adult stage, is the Heteroptera with about 70% species. The lowest incidence of adult diapause is among species in the orders Lepidoptera and Hymenoptera (about 5% each).

2. General Remarks on Adult/Reproductive Diapause

To save space in discussions of individual species and avoid repetition, we describe the common characteristics here. (For more details see [1, 2].)

Diapause is an adaptive arrestment of development that helps synchronize active stages with suitable environmental conditions and so increase survival potential during unfavourable periods of the year. Insects that diapause as adults, the larvae and the young adults, develop when the food resources are present. For the stressful period without food (often lasting many months) adults prepare in time by accumulating reserves (lipids, glycogen, proteins) and substances needed for resistance to future hazardous changes of environmental conditions. To begin early enough before the start of the dangerous period, diapause is induced by signals heralding the arrival of the unfavourable season; usually the cue is photoperiodic. Short (decreasing) day length serves as a signal of approaching winter and induces winter diapause (hibernation). In contrast, long-day photoperiods announce summer and induce estivation/summer diapause. Temperature and other environmental conditions act during the sensitive stage in concert with photoperiod in diapause induction or aversion (= prevention).

Such regulation is typical for facultative diapause that can, but need not, be entered in each generation. Quite often, however, the genetically fixed propensity is so strong that diapause, may be obligatory, is entered under any environmental conditions. Usually a population is not genetically homogeneous: both tendencies may be mixed, as we will see below, for example, in *Coccinella septempunctata* or *Leptinotarsa decemlineata*.

To terminate diapause, the insect has to go through diapause development, that is, horotelic processes of physiogenesis that often proceed best at temperatures in the range of 5–10°C, but cold is not always a prerequisite for the resumption of development [3]. In many cases, diapause may be terminated by tachytelic processes of termination that is

due to some environmental stimuli, such as a temperature increase or rainfall in case of summer diapause.

For quite a long period diapause research was focused only on hibernation/winter diapause, as the traditional view equated diapause with resistance to freezing. Estivation/summer diapause was long neglected, although it is rather common [4]. Quite often the terms hibernation versus estivation do not respond well to timing of seasons in the field: winter diapause may begin as early as in midsummer, as we will see below in several ladybird species.

Also, while hibernation lasts until spring, its diapause phase in temperate insect populations dwindles into postdiapause quiescence around the winter solstice. In this phase the morphological development of insects is arrested only directly by low temperature (or absence of food), and in spring (or by transfer to suitable laboratory conditions) morphological development is resumed.

The most conspicuous feature of adult diapause (often termed reproductive diapause) is the suppression of reproductive functions: maturing of ovaries and male accessory glands, and mostly also mating activity. Endocrinological regulatory pathways in adult diapause begin in the neurosecretory cells of brain whose axons terminate in corpora cardiaca. The pathway continues in another endocrinological organ, the corpora allata, where juvenile hormone is produced that regulates the activity of reproductive organs. In adults destined for diapause, food consumed is not used for maturation of gonads but for accumulating reserves. Thus the ovaries consist of threadlike ovarioles, so hidden in the much-enlarged fat body that they are sometimes difficult to find in dissection.

The above general traits were revealed in several coleopteran species that were studied in detail for many decades and published in series of papers, such as the studies on *Leptinotarsa decemlineata* and *Coccinella septempunctata.* The classic papers are not reviewed here as they are reviewed in the above-mentioned compendia [1, 2]. Here we focus on more recent findings, mostly those published in the last two decades. It may be warned that the basic paradigm of diapause, built in the previous century, had not yet been broken.

3. Coccinellidae

3.1. Coccinella septempunctata L. Among ladybirds, adult diapause has evidently been most studied in the originally Palaearctic species *Coccinella septempunctata*, the seven spot, that has in two recent decades invaded the Nearctic region and attracted attention of researchers there (e.g., [5]). Both in Europe and USA *C. septempunctata* has been found heterogeneous as to the induction of diapause (see Section 1).

In Bohemia (50°N, Western Czech Republic), the population in the autumn consists of two fractions. Although in some years aggregations of both sexes of dormant *C. septempunctata* may be found in their hibernation quarters in grass tussocks from early August onwards, one can also find actively feeding coccinellids on vegetation with aphids (often on different weeds, such as *Carduus* spp. and Daucaceae) for the whole of September and into early October [6]. The physiological condition of these two fractions was determined by dissection immediately after sampling and after rearing. Whereas the alimentary canal in the dormant beetles was empty of food and there were no traces of vitellinization in the ovaries, the digestive tract was full of food in more than half of the active adults sampled and 13–20% of females possessed vitellinized oocytes or even eggs. The difference between the dormant and active beetles became striking when they were reared for three weeks under long days, at 19–22.5°C with plentiful aphid food; the ovarioles of about 85–90% of dormant females remained without any vitellinization, while about 90% of the females collected on plants possessed vitellinized oocytes after rearing [6].

Dissections in mid-July of females collected outdoors a fortnight after adult emergence indicated a strong tendency to univoltinism: 84–93% of the females, entered diapause. The offspring of overwintered adults (F 1) also displayed a high incidence of diapause despite rearing under long-day conditions of 16L:8D or 18L:6D with a surplus of suitable prey. When such experiments were repeated in five years the incidence of diapause in F 1 fluctuated between 60 and 90%. A gradual decrease in diapause incidence across generations suggests selection against a propensity for obligatory diapause under long days [6].

A dominant effect of photoperiod and its modification by temperature was documented in samples from the selected lines. Under long days diapause was prevented in most females and 87–96% females reproduced in spite of low temperatures (18 or 18.5°C, resp.). Under short days of 12L:12D or 8L:16D, diapause incidence was high (85–94%) at low temperature (about 18°C), but low at high temperature: at temperatures alternating between 24-25°C (night) and 27-28°C (day) only 10% entered diapause.

In central Europe and the Paris region (France), the progress of diapause development in *C. septempunctata* was monitored by transfers of adults from the field to the laboratory at 25°C. Diapause was completed in December-January, whereupon it was replaced by postdiapause quiescence that lasted until spring when, under the influence of temperature increasing above around 12°C, the adults dispersed from hibernation sites to localities with aphids, where they fed and reproduced [6].

Whereas field observations indicate a univoltine cycle in central Greece, a tendency to multivoltinism was documented in *C. septempunctata* in this region, when four subsequent generations were reared in modified outdoor conditions [7]. The conditions were improved by shading of the rearing cages from direct insolation and continually providing surplus of suitable aphid prey.

3.2. Ceratomegilla undecimnotata (Schneider). Similar to *H. convergens*, the relative role of food and photoperiod in diapause regulation in *C. undecimnotata* is not yet clear enough, although the share of food/prey appears important.

The earlier studies on this Palaearctic species were undertaken in France and Czech Republic [6]. Detailed laboratory and field studies in central Greece [8] widened our knowledge of diapause in this species. The authors dissected

the females of *C. undecimnotata* that were sampled from the field in mid-June. About 40–50% of nonreproductive females were recorded in the plain, while most females (70–100% in different years) were at the same time immature in aggregations on mountain summits, where they remained until spring. This should indicate a univoltine cycle. When, however, the beetles were provided with a surplus of aphids outdoors (under shading, but natural photoperiod), five subsequent generations were produced. With about 30% of immature females in the first three generations, such diapause incidence was not far from the field records from the plain. These data indicate that *C. undecimnotata* populations from central Greece are heterogeneous as regards the induction of diapause. These findings are similar to *C. septempunctata* and *H. convergens*.

Samples from mountain tops were regularly transferred to laboratory (25°C, long day 16L : 8D, prey surplus). Females were activated under these conditions by tachytelic processes and laid eggs after a gradually shortened preoviposition period (92 d in July, 63 d in August, 20 d in September). This decrease in diapause intensity demonstrated the progress of diapause development by horotelic processes in the field.

All these results, similar to the French and Czech findings [6], indicate that *C. undecimnotata* is a long-day insect (long day is diapause preventing and not diapause inducing as assumed in the recent paper [8]). (The generic name *Hippodamia* used in [8] is not correct.)

3.3. Hippodamia convergens Guerin. In this common Nearctic aphidophagous ladybird, several possibilities of diapause induction were proposed by Hagen [9] for the plains of Northern California—and not much has been added later to his hypothesis. Originally, before the installation of irrigation systems, most individuals had an (obligatory ?) univoltine cycle (with a complex migration to mountains where enormous quantities of beetles aggregate for overwintering and are collected for biocontrol purposes). Later the Californian ladybird populations changed to multivoltine cycle, due to the high abundance of introduced aphids and are induced to diapause by photoperiod and temperature. However, diapause can still be nutritionally induced in a part of the population [9, 10]. In the upper coastal plain of South Carolina, diapause in *H. convergens* is terminated in December/January after the transfer to temperatures >15.5°C, despite short days 12L : 12D [11].

We need to know much more about the combined action of food and other factors in this species. Such studies have evidently begun with the analyses of the role of nutritional factors (nonaphid protein rich alternative food) in the arid conditions. In the Great Plains region in the central USA *H. convergens* is normally bivoltine, with obligate winter hibernation and facultative summer estivation which creates the possibility for additional generations when conditions permit. Various cases of nutritional regulation of reproductive diapause were analysed in females of *H. convergens* in these populations [12, 13]. The importance of drinking sap on sunflower in the summer months in West Kansas was examined. Sunflower petioles and pollen as well as lepidopteran eggs were provided to the beetles collected in early June. While these females did not oviposit in the absence of protein food, feeding on eggs of *Ephestia kuehniella* followed by pollen enabled 66% of the females to lay viable eggs at a low rate of 6.6 eggs/day. The females, transferred on 14 August to essential aphid food (*Schizaphis graminum*), laid six times more eggs.

These experiments stressed the adaptive role of the life cycle in *H. convergens* in that it enables survival during arid summer conditions when there is a shortage of the essential food, aphids. In the absence of protein-rich food, the 1st generation can enter diapause. Another tactic could be to wait in a state of lowered metabolism (but less lowered than in diapause) for the reappearance of essential aphid food, relying meanwhile on alternate foods. Then a switch to intensive egg laying can be quick, as was shown by a short oviposition delay of only 4 or 6–9 days on essential prey [12, 13].

3.4. Harmonia axyridis (Pallas). After the very early Russian studies from Asia (see [6]) only two Japanese papers were published, dealing with diapause of this coccinellid from east Asia—before its invasion to America and Europe. In Japan this bivoltine long-day insect hibernates in diapause [14, 15] and uses the polyol myo-inositol to increase its cold-hardiness [15].

After its arrival in Europe this invasive species was studied in South-Eastern France, Northern Italy, and Belgium and has become the most studied coccinellid. Facultative diapause of the multivoltine strain is induced by short-day photoperiod 12L : 12D at 23°C and lasted 1–3 months; eggs of *Ephestia kuehniella* were used as a suitable alternative food [16–19].

4. Chrysomelidae

4.1. Leptinotarsa decemlineata (Say). It was probably the first insect model for the detailed experimental study of adult diapause. Thanks to the intensive research by the team of Professor Jan de Wilde, Wageningen, The Netherlands, particularly the physiological/endocrinological aspects of diapause have been intensively investigated since the late 1950's [20]. These studies are reviewed in the important compendia [1, 2] and in the introduction of a paper by de Wilde's followers [21]. Research on the prolonged diapause in *L. decemlineata* and its dependence of soil types was focused by the team of Professor Raisa Ushatinskaya, Moscow, Russia [22].

The main facts on diapause regulation from the classic Dutch studies will be given here to make the reading of more recent studies below more easy. Diapause is induced by short-day photoperiod: 10L : 14D at 25°C have been used in Wageningen and 16L : 8D was the long-day photoperiod. Both larvae and adults are sensitive to induction [23]. 20–30% of beetles enter diapause under any photoperiod; thus a propensity to obligatory univoltinism is indicated, similar to the case of *C. septempunctata*, discussed above. Diapause development in *L. decemlineata* progressed well under any

of three temperatures, 4, 12, and also 25°C, that is, it does not need period of low temperatures for its completion (similar to quite a number of other insect species [3]). At 4°C mortality was high (15% after 3 mo, 50–70% after 6-7 mo), while it was <10% at 12 and 25°C. Diapause development was faster at 25 than 12°C, and 50% of females spontaneously emerged from soil after 14 and 21 wk, respectively. Sensitivity to photoperiod is retained during diapause development at least to February: at this sensitive period, diapause can be terminated by only three long days. The females lose photoperiodic response for at least 5 wk after the completion of diapause development, but the responsiveness is restored 3 wk after diapause in a part of the population (recurrent photoperiodic response, discussed in Section 12). However, photoperiodic sensitivity is never lost completely, even after the completion of diapause it affects the rate of vitellogenesis and ovarian maturation.

Because of the importance of the Colorado potato beetle as pest, the primary insect defoliator of solanaceous crops in North America and Eurasia [24], research continues on different aspects. Flight incidence and duration in relation to mating was recorded by flight mills [25]. Mating has a pronounced effect on flight activity decreasing it in females, evidently because migration and reproduction interfere with each other, and increasing it in males—they may thus mate with females from different localities.

Oviposition and burrowing behavior (as contrasting characteristics of nondiapause versus diapause, resp.) were compared in 1st generation females along a 5° latitudinal gradient, in six populations from North Dakota and Minnesota, USA, and Manitoba, Canada. Four locations were sampled in the Red River Valley region (between 49°49′N and 47°00′N) and two in east central Minnesota (45°20′N and 44°44′N). Different incidence of oviposition was recorded under long days in the RRV region (0-1%) and in ECM samples (9–15%). The authors conclude that *L. decemlineata* has the capability of becoming adapted to local environmental drivers, while retaining intrapopulation variability [26].

Some Colorado potato beetles enter prolonged (>1 yr) dormancy, an event quite common in adults dormant in the soil, such as some curculionids discussed below. This phenomenon was studied earlier in Russian populations and those from Western United States, where a very high incidence (22%) was recorded. M. J. Tauber and C. A. Tauber [24] studied its frequency in the Upstate New York in a 10-yr field study and recorded an average 2.04 (0–72)% in 12,607 beetles. They explain this relatively low incidence in North Eastern United States by the late arrival of *L. decemlineata* after the introduction of cultivated solanaceous crops. In Western United States, in contrast, the Colorado potato beetle commonly occurs on wild solanaceous host plants in drought-prone habitats.

Both the effect of age of potato foliage and temperature are important in the prediapause beetles [27]. The adults consumed older foliage at a faster rate, particularly at the higher temperature of 17°C (compared with 11.5°C) and consumed 45% higher weight of leaves. It is assumed that there is a fixed requirement of accumulated reserves to achieve prediapause satiation. If the food is less rich in needed substances, larger amounts have to be consumed.

In populations from Central Europe 70–80% of reproducing females develop under >15 h day length, while under <14 h day length all beetles enter diapause [28]. In experiments the photoperiod of 12L : 12D was used as short day, and 18L : 6D as long day. The index of food conversion was 5.4 under long days, but 7.2 and 11.9 under short days (at 20 and 25°C, resp.). Pupae were smaller under long days due to a greater loss of biomass during the prepupal stage that was almost twice as long as under short days.

The functional state of flight muscles was assessed by staining with commercially available (Sigma-Aldrich) tetrazolium salts; the color develops due to reduction of a colourless salt by mitochondrial enzymes [29].

The research on diapause of *L. decemlineata* continues also in the recent molecular biology age. In a study of gene expression patterns during the first 20 postemergence days in beetles programmed for diapause (at 8L : 16D, 24°C), that is, in prediapause phase, oxygen consumption was measured in this period. The respiration rate increased from 0.4 mL/g/h on day 1 to 1.1 mL/g/h on day 4, and after a plateau between days 4 and 7 the oxygen consumption decreased to 0.08 mL/g/h on day 15. The CO_2 production followed the same curve, with an additional conspicuous peak on day 7. Among the clones of genes isolated, elevated levels of expression of the glycine-rich transcripts (that function in structural support of insect cuticle) persisted for four days longer in diapause-programmed beetles, compared with nondiapause adults. The differentially regulated genes were downregulated between days 13 and 20, that is, at the end of prediapause when the metabolic rate was already much decreased [30].

The series of papers by Yocum and coauthors has continued by a recent one [31]. Prediapause and diapause phases of development are well marked by expression of genes in laboratory reared adults. However, it is much less clear in field collected adults, evidently due to the polyphenic character of diapause, mentioned earlier. The authors conclude that this property contributes to the status of *L. decemlineata* as a "superpest" of potatoes [31]. This characteristic is similar to that in *C. septempunctata*, where also the plastic character of adult diapause is obviously associated with the "success" of the species [32].

4.2. Colaphellus bowringi Baly. A complex analysis of diapause regulation was conducted by Professor Xue and coauthors in a series of recent papers. The cabbage beetle, *C. bowringi*, is a pest of cruciferous vegetables in mountain areas of Jiangxi Province, China. There are four generations per year, one in spring and three in autumn. The beetle estivates and hibernates as adult in the soil. A life-cycle polymorphism was reported by Xue and Zhang 20 yrs earlier (for an English summary of that paper published in Chinese, see [33]). Although the adults enter diapause at the same time, they differ much in diapause duration (several months–two yrs) and thus they expressed heterogeneous voltinism. Without regard to diapause induction and duration, the post-diapause beetles emerge from soil either between late

February and early April, or between mid-August and early October.

C. bowringi is a short-day species (i.e., long days induce diapause), but the photoperiodic response is strongly affected by temperature. High temperatures enhance the diapause-averting effects of short days and suppress the diapause-inducing effects of long days. Diapause incidence is 100% at <20°C at any photoperiod. Photoperiod plays a relatively small role in diapause induction; short days can prevent diapause only at temperatures above 20°C. The mechanisms ruling the complex seasonal life-cycle in *C. bowringi* are well explained by experimental results [33]. It is probably the first documented case of summer diapause induction by low temperature instead of high temperature. Diapause is entered by early-emerging individuals in April. The authors suggest that the photoperiodic and temperature controls of diapause induction have a different genetic basis.

Experiments on the effect of thermoperiods on diapause induction in *C. bowringi* showed again the importance of temperature, particularly during the photophase [34].

Other detailed experiments documented an important effect of host plants on diapause incidence in *C. bowringi* [35]. The highest incidence of diapause was caused by feeding on radish (*Raphanus sativus*) and the dark green variety of Chinese cabbage: the lowest incidence was obtained by feeding on the yellow-green variety of Chinese cabbage with thin leaves. Most adults entered diapause on mature and aged leaves. Diapause incidence was affected by host plants only within a certain range of photoperiods and temperatures; it was best manifested at 25°C and 13L : 11D. Regardless of host plants, all adults entered diapause at 20°C or at 16L : 8D, as indicated in the earlier papers.

There is no negative tradeoff between diapause duration and several parameters of performance in adults after diapause: the body weight, longevity, and fecundity of beetles with the longer diapause duration of 21 mo were higher than those with the shorter duration of 5, 11, and 17 mo [36].

Crossing a high diapause strain with a laboratory selected nondiapause strain showed that diapause capability is inherited in an incomplete dominant manner; maternal inheritance of diapause induction is stronger than paternal inheritance [37].

4.3. Zygogramma bicolorata Pallister. This chrysomelid was successfully introduced to Jammu and Kashmir, India for biological control of carrot weed, *Parthenium hysterophorus* L. Adults enter diapause from August to December with a peak in late November. They burrow into soil and are dormant about 1–3 cm bellow the surface. The incidence of burrowing adults increases with soil moisture and is higher in silty soil (47%) than in sandy soil (24%). Diapause is facultative as nondiapausing adults breed in winter under laboratory conditions. The beetles become active in March and, after having defoliated their host plants in an area, they disperse and need not be introduced to other areas. By treating the newly emerged beetles with human insulin (5 : g) the incidence of diapause was lowered and the fecundity increased [38].

In a population from Jabalpur, India, 64% of beetles entered diapause at 26°C and photoperiod was not important. Storage of females at 10°C for 6 mo did not lower their fecundity [39].

4.4. Plagiodera versicolora Laicharting. This is a species with facultative diapause that feeds on several species of willows. Experimental populations from the region of the river Ishikari (43°N, Hokkaido, Japan) had both univoltine and bivoltine life-cycles and were most abundant on *Salix sachalinensis* Fr.Smidt.

All females entered diapause at 10L : 14D, but a rather high incidence also was recorded at 16L : 8D (68% with a range of 40–100%) [40]. These are evidently results from rearing beetles on leaves of mixed quality, as only 10% diapause was reported in the 1st generation reared on 2–22 July on young leaves [41]. Diapause induced under short days of 10L : 14D at 22°C was terminated by long days of 16L : 8D at 22°C [42].

Later the effect of photoperiod and temperature (16L : 8D and 20°C in the laboratory) was experimentally isolated from the effect of seasonally changing quality of host-plant leaves [41]. While the abiotic laboratory conditions were kept constant, the leaves of *S. sachalinensis* were collected in the field and thus gradually more mature leaves were provided. The reproductive parameters declined in the 2nd and 3rd generations, in comparison with the 1st generation. Diapause incidence increased from 10 to 60%, the preoviposition period increased from about 9 to 16%, and the fecundity during the first 10 days of the egg laying period decreased from about 50 to 18 eggs per female. The authors thus documented the effect of host plant age and suggested that the combination of both day length and host-plant conditions cuing diapause is adaptive [41].

4.5. Galerucella calmariensis L. It was introduced to the United States for the biological control of purple loosestrife (*Lythrum salicaria* L.). The adults undergo a facultative reproductive diapause (the paper's abstract mentions obligatory diapause by mistake) during summer, autumn, and winter. Diapause can be averted by long days of 16L : 8D and induced by 8L : 16D. Adults are responsive to diapause-inducing photoperiods. The authors failed to isolate the cultures efficiently from insolation with white tissue tents as the natural photoperiod produced a seasonal effect; in early summer the ovaries matured better [43].

4.6. Crioceris sp. This undescribed chrysomelid species was studied in the Western Cape Province, South Africa (34°35′S) as a promising biocontrol agent of bridal creeper (*Asparagus asparagoides* (L.) W. Wight) with the intention to introduce it to Australia.

The majority of fully developed adults remain inside cocoons in soil for various periods of summer diapause. Field observations suggest that rainfall might be the cue for termination of diapause or dormancy. The effect of wetting was demonstrated in the laboratory. Only 29% ($n = 135$) adults emerged from dry cocoons at 20°C within 76 days.

This proportion was substantially increased by wetting, and even more by repeated wetting.

No research addressed the mechanism of diapause regulation by physical and biotic environmental factors, although larvae were reared successfully to pupation in soil at 15 and 20°C [44].

5. Bruchidae

5.1. Callosobruchus subinnotatus (PIC). It is a major pest of stored bambara groundnut, *Vigna subterranea* (L.) Verdcourt in sub-Saharan West Africa. Adult polymorphism was described in this bruchid, similar to that of some other species of the family, particularly *C. maculatus* (Fabricius), that was the model insect for a series of classic ecological studies by Professor Syunro Utida from Kyoto University, Japan, in the years 1954–1981. The terms for the two polyphenic forms, used in the earlier *C. maculatus* studies, were also used here although they do not seem very adequate: "active" and normal adults. While the normal adults have a high fecundity, low longevity, and lower tendency to dispersal, the "active" phase shows opposite qualities. Dissections reveal immature ovaries and male gonads, so we might consider this suspension of reproduction an adult diapause or at least a diapause-like phenomenon. Although high population density was suggested in several Utida's articles to be the factor responsible for the development of "active" form, no attempts have been made to address this influence in *C. subinnotatus* [45].

Another congeneric bruchid, *Callosobruchus rhodesianus* (Pic), suffering from strong competition by *C. maculatus* on cowpea, *Vigna unguiculata* (Walp.) in Togo, Africa, reproductive diapause was recorded [46] in a part of population.

5.2. Bruchidius dorsalis Fahraeus. This multivoltine seed-eater occurs in Central and Southern Japan. Females oviposit on seedpods of the Japanese honey locust, *Gleditsia japonica.* Newly matured seeds are available from August to autumn, but the females may use also dry, hardened seeds; thus host seeds can be utilized almost the whole year.

In contrast to most insect species, in warmer regions *B. dorsalis* enters diapause in different developmental stages: final larval instars and adults. Even nondiapausing early instars may overwinter [47]. In Sagamihara (35°34′N) 3 to 4 generations develop per year. Some autumnal adults produce the new generation before winter, while another part of the population overwinters before spring reproduction. Diapause is induced by short days and the first five days after adult emergence are sensitive to diapause-inducing factors.. Diapause incidence was higher and the critical photophase longer in cooler regions [48, 49].

6. Curculionidae

6.1. Curculio nucum (L.). This specialist of hazelnut trees has an obligate 2-yr cycle in France (45°46′N, 420 m a.s.l.) with one larval diapause in winter, pupation and ecdysis of adults (in soil) the next summer, and in the 2nd winter adult diapause. In spring the overwintered adults emerge from the soil in April and appear on trees from May to early July [50]. Early emergence from the soil enables females to oviposit in nuts before they fully harden. As they cannot penetrate the mature nut, they must oviposit before July.

Females lose about 8.5% of their weight during overwintering, but their lipid content does not decrease. Thus the authors suppose that females use lipids accumulated during the larval stage for egg production and obtain other nutrients from adult feeding. Evolutionary forces triggering the obligate 2-yr cycle are discussed [50].

6.2. Exapion ulicis (Forster). This univoltine species consumes the seed of gorse, *Ulex europaeus*, that has peak fruiting in spring and was introduced to some countries for its biological control. Adults lay eggs in spring into young green pods where the larvae develop adults feed on leaves and flowers of gorse and then diapause in autumn and winter. In winter the beetles stay on branches and are able to resist cold. In Brittany, France, the species was studied together with *E. lemovicinum* (Hoffmann), a species that overwinters in the larval stage, to understand its cold-hardiness. *E. ulicis* adults are freezing intolerant, but exhibit a low supercooling point of −17°C. The regulation of diapause induction and termination was not studied [51].

7. Carabidae

7.1. Nebria salina Fairmaire and Laboulbene. This species is common in unproductive habitats, such as sand dunes and upland grasslands. This short-day insect is an autumn breeder that enters a summer diapause. Females require at least two months of exposure to short-day photoperiods of <12L : 12D. Under long days of 18L : 6D the ovaries do not mature. Still shorter days of 6L : 18D stimulate better growth of ovaries. The males matured after two months irrespective of photoperiod.

In the field (Hamsterley, County Durham, UK) the main activity of *N. salina* was concentrated in September [52]. Thus, the life cycle of this species resembles the congeneric autumn breeder *Nebria brevicollis*, where diapause was studied in the 80's by Hengeveld and Loreau (quoted in Telfer and Butterfield [52]).

7.2. Carabus yaconinus. When the authors transferred this spring breeder from the field to laboratory experimental photoperiods, the beetles showed a long-day photoperiodic response in autumn and early winter. In the course of winter the response was gradually lost, so that in late April the ovaries of females matured both in short and long days [53]. However, in summer the photoperiodic response resumed again. Thus *C. yaconinus* appears to be another case of recurrent photoperiodic response that was revealed for the first time in a pentatomid bug *Aelia acuminata* [54] and recorded later also in *L. decemlineata* and *C. septempunctata* (see above).

8. Silphidae

8.1. Nicrophorus nepalensis. This subtropical short-day breeding carrion beetle that occurs in Taiwan (24°45′N, 745 m a.s.l.) is active mainly in early spring (February–May) and also in autumn (October-November). Reproduction is best promoted at 20°C and a photoperiod of 12.5L : 11.5D, but only in the presence of carrion, whereupon oviposition starts after 2 weeks. At a lower temperature of 15°C and 11L : 13D, maturation is slower, so that the oviposition begins after about 9 weeks. In contrast, longer days (14L : 10D) prevent oviposition at 25°C, but enable oviposition of 45–50% of females at 20°C. Summer diapause is an efficient adaptation as the development of offspring on carrion in summer would suffer from competition with more quickly developing dipteran larvae on quickly decomposing dead animals [55].

9. Scolytidae

9.1. Ips typographus L. The number of generations varies in different countries, similar to other insect species. Flight parameters were studied in young beetles from five populations: one in Denmark (56°51′N) and four in Sweden (between 57°40′N and 62°51′N). The flight propensity of beetles that emerged in the period 34–80 days was tested and those flying less than 100 s ("non-fliers") were found by dissection to be in diapause with undeveloped ovaries and large fat reserves. The frequency of such beetles increased with increasing latitude from 35 to 70%. While "fliers" migrate to find a breeding site, diapausing "non-fliers" often overwinter on the ground beneath the brood tree [56].

In a Central European population, diapause was induced by <16L : 8D at 20°C and the critical photoperiod (50% diapause incidence) was 14.7L : 9.3D. Temperature of >23°C prevented diapause even at 12L : 12D. Neither gonads nor flight muscles matured in diapausing adults. Overwintering adults, shown to be in diapause by their response to photoperiod, reproduced at the long-day photoperiod of 18L : 6D but not at 12L : 12D when transferred in October from the forest to laboratory 20°C [57].

10. Scarabaeidae

10.1. Dasylepida ishigakiensis Niijima and Kinoshita. This "white grub" is a serious pest of sugarcane on Okinawa Islands, Japan. It is the only scarabaeid beetle in which the regulation of adult diapause has been studied. Both larvae and adults of this subtropical beetle undergo diapause in a semivoltine (2-yr) life cycle. Adults emerge from pupae in the soil and stay there for about two months. This delayed emergence from the soil cannot be related to synchronization with food, because the adults have degenerated mouthparts and do not feed. The beetles begin to leave the soil in late autumn when cooler temperatures are favorable for mating. Sexual maturation in the laboratory is suppressed at temperatures of 25–30°C, but proceeds well at 15–20°C. Photoperiod does not seem to act in diapause regulation [58].

11. Endomychidae

11.1. Stenotarsus subtilis (=rotundus) Arrow. S. subtilis provides a case of tropical diapause that was studied in Panama [59, 60]. This beetle forms large aggregations for a long dormancy, comprising 6 months of the wet season and 4 months of the dry season. Breeding sites, food, and diapause-inducing factors all remain unknown. Experiments with beetles collected from aggregations revealed the role of environmental factors in diapause development. Although in Panama (9°N) the difference between the longest and shortest day is only 1 h, increasing day length from March onward stimulates weak development of corpora allata, primary oocytes, and flight muscles that had remained resorbed for about 6 months. Mating and dispersal coincide with the onset of rains in late April. Two-month exposures to contrasting humidities revealed that higher humidity also stimulates development of the aforementioned organs [59, 60].

12. Concluding Remarks

It is almost impossible to make general conclusions from the recent data given above on the diapause of beetles. There are at least two obstacles: (1) diapause is a seasonal adaptation (see the Introduction) and it apparently evolved independently in individual species under specific environmental selective pressures that ultimately result in a genetic basis. Diapause of individual species/populations is thus intrinsically diversified—and inherently resists generalization. (2) The conditions for reasonable comparisons are further aggravated by diverse research protocols that have been employed by individual researchers either arbitrarily or under various technical constraints. Even in the case of currently studied species (that often are economically important), such as some chrysomelids, curculionids, or coccinellids, research analyses have often remained incomplete. This is still even more true for fragmentary studies some of which are included here only to show the wide range of records.

In spite of these difficulties, we may try to deduce some general features (that may apply to other insect orders as well). No particular break of the classic paradigms was made in the recent papers. They rather were further corroborated and extended. The most general, and the most studied, is the signaling function of photoperiod, often modified by effects of temperature and food that announces seasonal transitions with astronomical precision. In particular populations, photoperiodic response is always adapted to geographic latitude, as has been shown above best in *L. decemlineata* populations originating from locations separated by about 5° latitude. In some common and widely studied species, such as the Nearctic coccinellid *H. convergens*, the evidence of the photoperiodic regulation has still remained rather scarce.

An effect of food quality implicated in several of the discussed species. The difference of old versus young leaves is well documented in *L. decemlineata* and *C. bowringi*; the effect of alternative food (e.g., pollen) versus essential aphid species has been studied in detail in *C. septempunctata* [6]

and *H. convergens* [12, 13]. In the ladybird *Ceratomegilla undecimnotata* (Schneider) the physiological age of host plant can even act through the aphid prey, as reported in the 70's see [61] (see also) [6].

The effect of population density has remained rather neglected in the beetles discussed here. The exceptions are early articles on the bruchid *C. maculatus*, where the effect was recorded long ago by Professor Utida, and the observations on *C. subinnotatus.*

As mentioned above, diapause adaptations are very plastic in response to selection. Similar to changes in photoperiod, beetles can adapt quite quickly to environmental changes associated with changes in food supply. Thus, for example, introduction of irrigation in arid areas led to the establishment of prey in two coccinellids, *H. convergens* in California and *Chilocorus bipustulatus* L. in Israel, and thereafter to changes in their life cycles [6].

It is worthwhile to speculate about a hierarchy of individual factors governing diapause regulation. The basic driver is usually photoperiodic response to the precise annual astronomical repetition of day length. We may assume that less rigid reactions to less predictable environmental changes in food availability and quality, and other factors such as temperature, humidity, and population density, can be superimposed. The archetypal nutritive factor is "prepared to enter the game" in the case of unpredictable events affecting prey abundance—and this is facilitated by phenotypic plasticity.

Polyphenic character of diapause is a very important feature in *C. septempunctata* and *L. decemlineata*, but it has been found also in other Coleoptera and in insects generally: populations are heterogeneous as to voltinism tendencies. For populations with mixed uni- and polyvoltine tendencies we might envisage a scenario which combines plasticity with resilience. One aspect of the life-cycle strategies is the "safety" ("insurance") factor of the univoltine trait which is permanently perpetuated in the gene pool and maintained (i.e., not selected out) despite the frequent momentary occurrence of conditions favourable for the production of an additional generation, since these transitory conditions are unreliable in the long run. However, polygenes facilitate population responses to changes in the environment. If there is a promising improvement they may "open the gate" for intermittent multivoltine development, that may be more or less appropriate to capitalize on transitory environmental improvement. The system remains resilient because the univoltine trait is maintained quite intensively. This scenario is adequate for *C. septempunctata* and perhaps also *L. decemlineata* living in temperate regions/climate. In different climatic areas, the regulation of voltinism can differ.

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Review Article

Biological Control of *Solenopsis* Fire Ants by *Pseudacteon* Parasitoids: Theory and Practice

Lloyd W. Morrison

Department of Biology, Missouri State University, 901 S. National Avenue, Springfield, MO 65897, USA

Correspondence should be addressed to Lloyd W. Morrison, lloydmorrison@missouristate.edu

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Pseudacteon parasitoids are potential biocontrol agents of invasive *Solenopsis* fire ants. *Pseudacteon* species that parasitize the invasive *S. invicta* Buren and *S. richteri* Forel have been introduced to, and naturally dispersed across, the southeastern USA, although there is no evidence yet that *Solenopsis* host ant populations have decreased. The ability of introduced *Pseudacteon* species to regulate *Solenopsis* populations will depend upon the relative importance of top-down effects in the recipient communities. In this paper, I examine the characteristics of the *Pseudacteon/Solenopsis* parasitoid/host system and evaluate the extent to which research findings are consistent with top-down control. Laboratory and field experiments evaluating *Solenopsis* population regulation have been equivocal, and overall the available evidence provides little support for strong top-down effects in this system. Competitive exclusion may occur among introduced *Pseudacteon* species, and future efforts at biological control are likely to be more efficacious if they focus on other types of natural enemies.

1. Introduction

Many species of *Pseudacteon* (Diptera: Phoridae) are parasitoids of *Solenopsis* (Hymenoptera: Formicidae) fire ants. Several species of *Solenopsis* fire ants are invasive pests and others have the potential to be [1]. High densities of the invasive *S. invicta* Buren in North America are usually attributed to an escape from natural enemies [2]. Much recent research has focused on the potential use of *Pseudacteon* parasitoids as classical biological control agents to regulate *Solenopsis* fire ant populations, particularly *S. invicta* and *S. richteri* Forel in North America. Two South American *Pseudacteon* species—*P. tricuspis* Borgmeier and *P. curvatus* Borgmeier—have been released at multiple locations and dispersed naturally across the southeastern USA. It is estimated that *P. tricuspis* now occurs in 65%, while *P. curvatus* may occur in as much as 90% of the invasive *S. invicta/S. richteri* range [3]. Two other species—*P. litoralis* Borgmeier and *P. obtusus* Borgmeier—have been established in localized areas, *P. cultellatus* Borgmeier has been recently released in Florida, and releases of additional species are planned [3, 4]. In addition to the direct effect of mortality, *Pseudacteon* phorids may have indirect effects on their *Solenopsis* hosts, affecting their behavior and potentially putting the host species at a relative disadvantage with competing ants [5].

There have been many studies conducted on various aspects of the *Pseudacteon/Solenopsis* parasitoid/host system, and the literature is in need of an objective, critical review. There is great interest in whether the introduction of *Pseudacteon* phorids can regulate invasive fire ant populations, and if so, to what degree. I conducted original research on this system for a decade, but have more recently pursued other avenues of study. The success or failure (perceived or actual) of this biological control program has no bearing on my obtaining funding, promotion, or tenure. Thus, I am in a good position to conduct a knowledgeable, yet detached review. I do not attempt to review all the *Pseudacteon/Solenopsis* literature, but focus on the potential of introduced *Pseudacteon* parasitoids from South America to regulate population densities of host *Solenopsis* ants in their invasive range in North America, through both direct and indirect effects.

2. The Species

2.1. The Host. The genus *Solenopsis* contains about 185 described species worldwide; the ~20 *Solenopsis* species known

as "fire ants" are all native to the new world [1]. Three of these fire ants are invasive pests: *S. invicta*, the most notorious, is native to South America but has invaded North America, the Caribbean, Australia, Taiwan, and China [6]. *S. richteri*, also native to South America, has invaded the southeastern USA, where it has hybridized with *S. invicta* [7]. *S. geminata* (Fabricius), whose natural range spans southern North America to northern South America, has been found at numerous low latitude sites around the globe [7]. The vast majority of *Solenopsis* fire ant research has focused on these three species. All three are characterized by polymorphic workers, which live between 2 and 8 months, depending on worker size and temperature [8]. The massive fire ant literature has been summarized by Taber [7] and synthesized by Tschinkel [1].

One important aspect of *Solenopsis* biology that is relevant here is the seasonal cycle of population abundance. In the southeastern USA, *S. invicta* reaches peak abundances in midwinter, and the lowest worker numbers occurring in midsummer are only about half that of winter highs [9]. *S. invicta* above ground foraging activity is highest in summer, however, and lowest in winter when soil temperatures are too cold to forage [10, 11]. Thus, the availability of hosts to *Pseudacteon* parasitoids is greatest in the summer months, even though *S. invicta* absolute densities are near their lowest.

Beyond the seasonal oscillations in abundance, *Solenopsis* population size may vary with other factors. The *Solenopsis* species in question are disturbed habitat specialists [1, 12]. Disturbances come in all degrees, however, and across a broad scale either too much or too little disturbance may result in lower fire ant abundances [13]. Disturbance regimes undoubtedly vary temporally and result in variability in *Solenopsis* populations. Climatic events (i.e., droughts, floods, and unusually cold weather) may also affect *Solenopsis* abundances [14, 15]. Fluctuations in abundance due to a variable disturbance regime or such climatic events could either amplify or dampen the inherent seasonal oscillations.

Finally, *Solenopsis* fire ants have the propensity to rapidly increase in abundance. After removing all *S. invicta* from experimental plots in Florida, for example, *S. invicta* recolonized the plots and in only two years reached abundances similar to control plots [16]. It is against this background of wide and potentially variable fluctuations in host population size, in addition to a strong potential for colony growth, that the regulatory effect of *Pseudacteon* parasitoids must be evaluated.

2.2. The Parasitoid. Although a number of taxonomic issues remained unresolved, over 20 *Pseudacteon* species are known to parasitize *Solenopsis saevissima* complex fire ants in South America [17]. Similarly, more than 20 *Pseudacteon* species parasitize *Solenopsis geminata* complex fire ants from North America to northern South America [18]. The basic biology and natural history of *Pseudacteon* phorids that parasitize *Solenopsis* fire ants have been summarized by Porter [19] and Morrison [20]. The life cycle of *Pseudacteon*, in brief, is as follows. A female *Pseudacteon* hovers near *Solenopsis* worker ants and inserts eggs into the thorax of hosts in aerial attacks with a specialized ovipositor. Three larval instars—the second of which migrates to the head—precede pupation. At pupariation, the worker is killed by the parasitoid consuming all the tissue inside the head capsule, which is then used as a pupal case. Development from egg to adult takes from 5 to 12 weeks, depending upon temperature and the *Pseudacteon* species. *Pseudacteon* are solitary parasitoids, with only one larva able to complete development in each host. Each female *Pseudacteon*, however, may produce >200 eggs [21].

3. Theory

Whether or not *Pseudacteon* parasitoids control or regulate population densities of *Solenopsis* fire ants can be thought of as a function of the relative importance of top-down versus bottom-up effects in the communities in question. In food web terminology, bottom-up effects occur when the abundance of a resource affects the population of the consumer of that resource. The higher the abundance of resources at lower trophic levels, the higher the abundance or diversity that can be obtained at higher trophic levels. Top-down effects, on the other hand, occur when the population density of a consumer affects the abundance of its resource. Top-down control refers to the situation where the abundance or diversity of lower trophic levels is dependent on effects from consumers at higher trophic levels [22]. There has been much discussion in the literature over the relative importance of top-down versus bottom-up effects in arthropod communities, including the seasonal and spatial variability in such effects [23–29].

In a community with strong top-down effects, *Solenopsis* populations would be regulated by *Pseudacteon* parasitoids (or other predators or parasites). In contrast, in a community with strong bottom-up effects, *Solenopsis* populations would ultimately be regulated by the food resources available to them, and simply support *Pseudacteon* populations but not be controlled by them. Larger *Solenopsis* populations could support larger *Pseudacteon* populations. Obviously, biological control of host ants requires a system with relatively strong (and consistent) top-down effects.

Interspecific competition has traditionally been viewed as the primary mechanism organizing ant communities and limiting ant populations [30]. It has been suggested, however, that top-down processes such as parasitism may also play an important role in some ant communities [31]. Here, I make no attempt to evaluate the importance of parasitoids to ant communities in general, but rather to determine which characteristics of the *Pseudacteon*/*Solenopsis* parasitoid/host system are consistent with top-down control. I refer to the prevalence of such top-down effects as "strong control," in reference to the goal of regulating invasive *Solenopsis* populations.

The relative importance of top-down effects can be illustrated by comparing two scenarios: in the first scenario—"strong control"—top-down effects prevail. This scenario is characterized by (1) a diversity of *Pseudacteon* species that exert a broad range of parasitism pressure on host *Solenopsis* ants, (2) consistently high abundance and activity of *Pseudacteon*, (3) high rates of mortality resulting from parasitism, (4) a lack of refuge for, or ability to behaviorally adapt in,

host ants, and (5) shifting of the outcome of interspecific interactions with competing ants. The second scenario, which I term "weak control", is characterized by (1) low diversities of *Pseudacteon* species, (2) low or fluctuating abundance or activity of *Pseudacteon*, (3) low rates of mortality resulting from parasitism, (4) the presence of a refuge or the ability to behaviorally adapt by host ants, and (5) little or no effect on the outcome of interspecific interactions with competing ants.

These two scenarios more appropriately represent the ends of a continuum rather than two mutually exclusive states (or because multiple characteristics are involved, the margins of a multidimensional space). Moreover, the characteristics of each scenario are largely independent of each other. Evaluation of recent research results relative to these scenarios allows for a greater understanding of the degree to which *Pseudacteon* parasitoids may control or regulate population densities of *Solenopsis* fire ants.

Given the high background population fluctuations of host *Solenopsis,* the potential effects of *Pseudacteon* as described in the two scenarios above are illustrated as a conceptual model in Figure 1. In the strong control scenario (Figure 1(a)), broad parasitism pressure (direct and indirect) depresses fire ant populations consistently over time, resulting in peaks and troughs of fire ant population cycles that are lower than without the parasitoids. The mean *Solenopsis* abundance over time is also lower.

In the weak control scenario (Figure 1(b)), parasitism pressure is weak and may only affect host ant populations seasonally, or is otherwise greatly limited in intensity. *Pseudacteon* populations also fluctuate, reaching their highest abundances and thus exerting peak parasitism pressure in the fall, due to greater host availability in the summer (because of higher above-ground foraging activity by the ants). Because of low rates of mortality due to parasitism and the ability of ants to adapt behaviorally and lessen the indirect effects, colony fitness is only slightly affected, and this decrease comes at a time when overall colony size is peaking. Because of the overall cyclical nature of *Solenopsis* abundance and the added stochastic effects of disturbance and climate (depicted in this figure as irregular seasonal oscillations), the impacts of *Pseudacteon* may be relatively small. Over the long term, such effects may be washed out by the greater population variability due to other factors. Under optimum disturbance intensity and climatic conditions, host ants may regain previous population peaks. In this scenario, the long-term average *Solenopsis* abundance or range of fluctuations may change relatively little due to *Pseudacteon*.

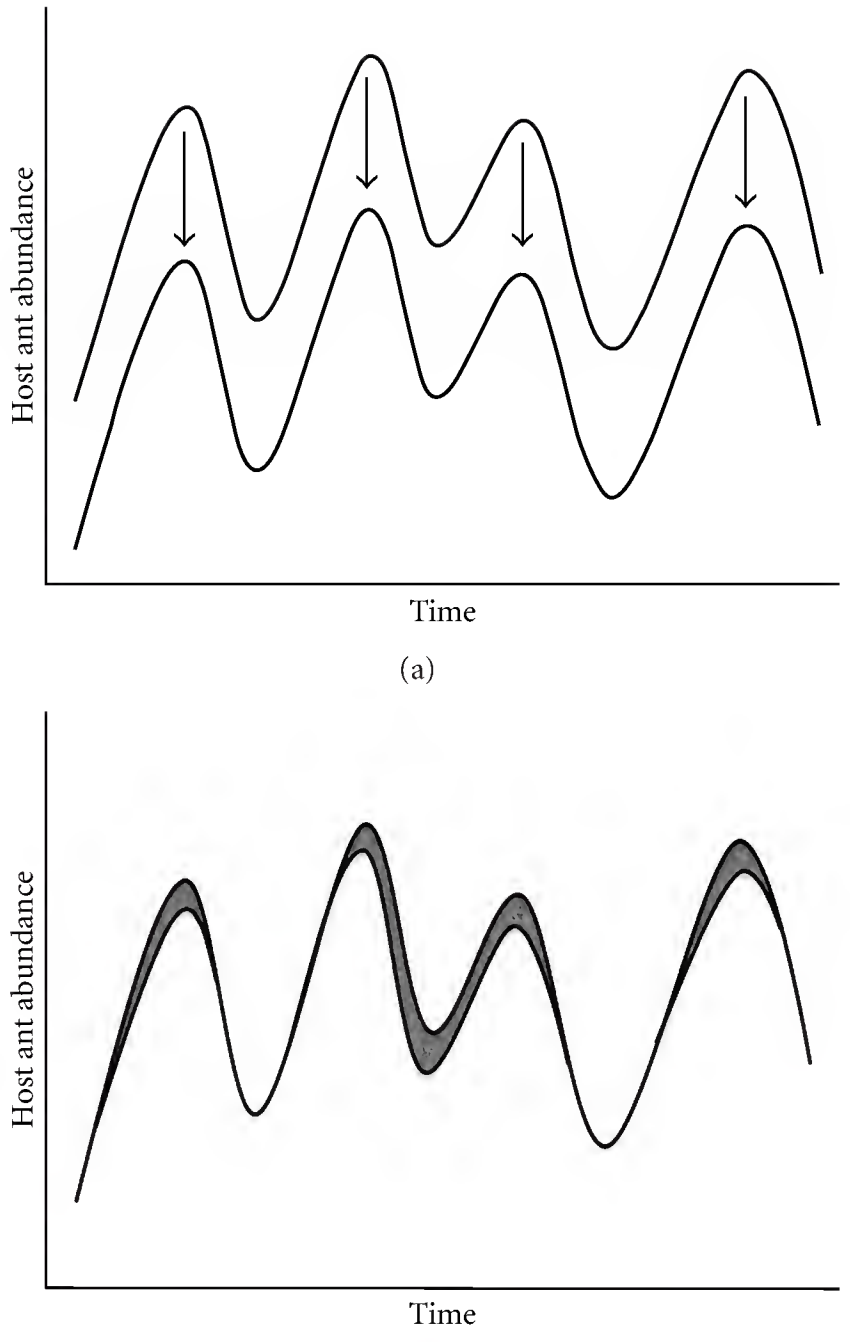

FIGURE 1: Two hypothetical scenarios of the potential effects of introduced *Pseudacteon* parasitoids on *Solenopsis* fire ant populations. (a) Strong control: parasitism pressure depresses fire ant populations consistently over time, resulting in peaks and troughs of fire ant population cycles that are lower than without the parasitoids. (b) Weak control: parasitism pressure primarily affects host ant populations seasonally, or to an otherwise relatively small extent, and ants rebound under optimum growth conditions. (Shaded areas indicate the amount of decrease due to the parasitoids.)

4. The Evidence

4.1. Diversity of Pseudacteon Species. In most locations that have been studied, multiple *Pseudacteon* species have been found. This is true for *Solenopsis saevissima* complex fire ants in South America and *Solenopsis geminata* complex fire ants in North America [17, 32–35]. Host ants are partitioned among *Pseudacteon* species along several axes, including size of worker [36–38], host location preferences [33, 39], and time of day [35–40]. Thus multiple *Pseudacteon* species attack a greater size range of workers, engaged in a wider diversity of activities, over a longer period of time than a single *Pseudacteon* species would.

Although multiple *Pseudacteon* species may cooccur at a site, most species are usually relatively rare. This is also true both in North America [5, 34] and South America [32, 33, 35, 41–43]. The niche segregation observed is likely the result of competition among *Pseudacteon* species for hosts. There is now evidence that introduced South American *Pseudacteon* species are competitively displacing each other in North America [4, 44, 45], and a reanalyses of abundance data from South America suggests competitive exclusion exists there as well [44].

4.2. Abundance and Activity of Pseudacteon Species. Adult *Pseudacteon* live for only a few days [46], and reveal relatively great variability in abundance over time [34, 41, 47–49]. In

tropical and subtropical climates, *Pseudacteon* species are active year round, although relative abundance varies seasonally [32, 35, 41, 49]. *Pseudacteon* species are active from dawn to dusk, but diel variation exists among species [35]. In more temperate zones, flight activity of *Pseudacteon* is limited by cool air temperatures and adults may not be active in the winter months [34].

Introduced *P. tricuspis* populations in the southeastern USA reveal cyclical patterns of seasonal abundance, with peaks in the fall and troughs in the spring [48, 50, 51]. *Solenopsis* above ground foraging activity, and thus host availability is highest in summer. Thus *P. tricuspis* densities track host ant availability (with some lag time to be expected based on the 5–12-week-long development cycle) and greater availability of hosts (i.e., resources) is correlated with higher parasitoid (i.e., consumer) populations. Abundance patterns of *P. curvatus*, however, may differ, at least in some areas [S. D. Porter, unpublished data].

4.3. Mortality due to Parasitism. Parasitism rates (i.e., the percentage of *Solenopsis* workers in a colony infected with a *Pseudacteon* egg or larva at a given point in time) have been reported to be very low in a number of studies. In Texas, *S. geminata*—hosts of native North American *Pseudacteon* species—had parasitism rates of <3% [36]. Parasitism rates of *Solenopsis invicta* in its native range in Argentina by native South American *Pseudacteon* species were similarly <3% [35]. Parasitism rates of *Solenopsis invicta* in its exotic range in Florida from introduced *Pseudacteon* are generally ≤2% [48, 52, S. D. Porter, unpublished data]. These observed rates are an order of magnitude below the lowest parasitism rates associated with successful biological control programs (>30%) [53, 54]. These rates for successful biocontrol for other types of parasites, however, are based on the direct effect of mortality only.

Workers that are parasitized by *Pseudacteon* are not a random assortment from the colony, but rather represent primarily older ants with a shorter life expectancy. Fire ants exhibit a division of labor, in which the particular task engaged in by a worker depends on the age and size of that worker. In general, younger workers engage in relatively safe activities within the central colony, whereas older workers are found near the periphery of the nest, and the oldest workers engage in the most dangerous activity: foraging [55]. Mortality rates of foragers may be as high as 5% *per day* [1]. Because of the high mortality rates associated with foraging activities in ants in general, foragers have been described as a "disposable caste" [56].

Pseudacteon phorids attack host workers involved in foraging, interspecific interactions, and colony defense—all relatively dangerous activities. Thus most of the workers parasitized are engaged in high-risk activities and near the end of their natural lives, and colony fitness is affected to a lesser degree than if workers were parasitized at random with respect to age.

4.4. Host Ant Refuges and Behavioral Adaptations. A primary focus for research into the indirect effects of *Pseudacteon* has been the reduction of foraging in host ants in the presence of these parasitoids. A number of studies involving different *Pseudacteon*/*Solenopsis* combinations in both North and South America have revealed that in the presence of these parasitoids, worker behavior changed and foraging at rich food resources dramatically diminished [5, 57–61]. The size of foraging *Solenopsis* workers has also been observed to decrease in the presence of *Pseudacteon* [52–64]. These studies, however, have almost exclusively focused on short-term effects of phorids at rich food resources, characterized by the recruitment of many workers in the absence of any shelter or refuge for the host workers and during ideal conditions for *Pseudacteon* flight activity.

Yet foraging in these *Solenopsis* species occurs under a great variety of conditions. In other words, these ants are characterized by a relatively broad foraging niche (*sensu*, [65]). They may forage by day or night [5, 66], as long as the soil temperature is at least 15°C [10, 11]. *Solenopsis* fire ants are omnivorous, with a very catholic diet [67, 68]. They excavate elaborate underground tunnel systems [69], and some unknown proportion of their food may be derived from underground sources (i.e., plant roots or root homopterans) [67]. The range of food items and types varies greatly; they may retrieve small items individually, but many workers may recruit to larger or long-lasting resources.

In contrast, *Pseudacteon* flies are not active after dark. Their aerial mode of attack makes it impossible to attack workers in underground tunnels (worker ants will quickly kill *Pseudacteon* flies if they can catch them). Additionally, *Pseudacteon* are unlikely to affect foraging of items that can be retrieved individually, although this has not been carefully studied. Some *Pseudacteon* species may not even affect foraging of relatively rich food resources if these are uncontested. Introduced *P. tricuspis* in North America, for example, were not attracted to workers foraging at such resources, unless involved in interspecific interactions with other ants [39]. *P. tricuspis* is attracted to the alarm pheromones and venom alkaloids that are typically released in such interactions [70, 71].

Pseudacteon native to North America are not active when air temperatures drop below 20°C [34], although some species in South America are active down to 14°C [41]. Thus there may be a narrow temperature zone in which it is warm enough for above-ground *Solenopsis* foraging, but not for *Pseudacteon* activity, although this appears to vary geographically and by species. Thus, *Pseudacteon* phorids will affect some unknown fraction of the overall broad spatiotemporal foraging niche of *Solenopsis*.

Furthermore, although the reduction in food obtained from such rich food resources may appear very large in the short term, in the longer term *Solenopsis* ants may be able to adapt behaviorally and still obtain much of the resource in question, if not removed by competitors. Field studies have shown that although forager numbers may decrease, *Solenopsis* workers do not usually completely abandon food resources in the presence of *Pseudacteon*, but some workers remain behind to guard the resource [34, 47, 59]. Foraging has even been observed to rebound to earlier levels after a depression by *Pseudacteon* [33]. Workers have been observed tunneling

beneath a rich food source and covering it with dirt and debris [5, 47].

Although most laboratory studies have constrained foraging to occur only in the presence of *Pseudacteon*, the design of one laboratory study allowed a colony of *S. invicta* to forage simultaneously in one arena with *P. tricuspis* and in a second arena without the parasitoid. Although less food was retrieved from the arena with *P. tricuspis*, the sum of food obtained from both foraging arenas was not different from that obtained in control trials without parasitoids [61]. Thus the colonies were able to compensate for decreased food retrieval caused by *P. tricuspis* harassment by simultaneously increasing food consumption when food was available elsewhere.

4.5. Effects on Interspecific Interactions. *Pseudacteon* species were never expected to have large direct effects of mortality on *Solenopsis* ants, and the expectation of this parasitoid's ability to regulate host ant populations was based primarily on the indirect effect on host behavior. These short-term behavioral effects would mean relatively little to colony fitness if they merely represented a delay in obtaining food resources. If, however, other ant species are able to secure a competitive advantage due to *Solenopsis*' parasitoid evasion behavior and are able to obtain relatively more food resources, then *Pseudacteon* could play an important role in mediating overall ant community dynamics and species abundance relationships.

Orr et al. [60], working in South America, observed *S. invicta* to lose food resources to competing ants in the presence of *Pseudacteon* phorids. Other studies, however, have found that host *Solenopsis* workers under *Pseudacteon* attack often did not lose control of the resource to competing ants. Studies of *Pseudacteon* parasitoids specific to *S. geminata* in Texas revealed that the presence of the parasitoids had no effect on the outcome of interspecific interactions involving *S. geminata* [5, 47]. A comparative study in Brazil revealed that *S. invicta*-specific *Pseudacteon* had no effect on the outcome of interspecific competition between *S. invicta* and other ants at two of three sites [72]. Thus, based on the available evidence, the ability of parasitoids to affect the outcome of interference competition among ant species appears to be too weak to be scientifically documented in many communities.

5. Long-Term Experiments

5.1. Laboratory Experiments. It has proven difficult to provide evidence for the success (or failure) of introduced *Pseudacteon* species in regulating host *Solenopsis* ants at the population level. Most evidence for a *Pseudacteon* effect, as discussed above, comes from short-term behavioral studies (minutes to hours in duration). In a relatively long-term lab study (28 days) incorporating both *Pseudacteon* phorids and a competing ant, *Forelius pruinosus* (Roger), Mottern et al. [73] reported a reduction in foraging in S. *invicta* due to *Pseudacteon* harassment, but no change in the colony growth rate of *S. invicta*. It is possible that the duration of this experiment was too short, or the method used to measure colony growth (i.e., photographing brood piles) was not precise enough. The most likely explanation, however, is that ants were allowed to forage beyond the period that *Pseudacteon* attacked, and *Solenopsis* was able to compensate by increasing food retrieval when *Pseudacteon* were not active. Mottern et al. [73] state that such conditions would be "representative of those found in nature." No change in the growth rate of the competing ant was observed either, although this was not surprising because *F. pruinosus* never entered the communal foraging chamber.

In a longer lab study (50 days), Mehdiabadi and Gilbert [74] documented that the reduction of foraging in *S. invicta* due to *Pseudacteon* harassment did, as expected, eventually result in reduced colony fitness. In that study, the presence of *P. tricuspis* reduced the abundance of middle-sized workers, but not small-or large-sized workers. Worker ants, however, were always constrained to forage in small trays for limited periods in the presence of *P. tricuspis*, without any refuge or potential to adapt their foraging behavior. It is noteworthy that a combination of *P. tricuspis* phorids and a competing ant—this time *Forelius mccooki* (McCook)—had no greater effect on colony fitness than the competing ant alone [74]. As in the Mottern et al. [73] study, no increase was observed in the reproductive output of the competing ant [75]. (Interestingly, Mottern et al. [73] criticized the statistical analysis of Mehdiabadi and Gilbert [74], claiming that no significant differences existed for any of their treatments!)

Thus, the ambiguous results of these laboratory experiments provide little empirical support for the idea that *Pseudacteon* phorids could mediate competitive interactions that would ultimately lead to a decrease in *Solenopsis* populations, while allowing for a relative increase in competing ant populations. The general problem with such laboratory experiments is that the design can greatly influence the outcome. Given the complexity of fire ant foraging and the multitude of interactions with other species, any community-level laboratory experiment is destined to be an oversimplification of the natural world with limited inference.

5.2. Field Experiments. Field experiments, while more realistic, have their own limitations, in this case primarily logistical. Fire ant populations are undoubtedly affected by many factors, and while many of these variables can be controlled for in the laboratory, attempting to isolate the effect of one factor in the context of a broad field experiment is very difficult. Moreover, introduced *Pseudacteon* species spread naturally at a rapid rate; *P. tricuspis* dispersed at rates of up to 30 km/year for the first few years after establishment in north Florida [76], and at rates of up to 57 km/year over the following four years [77]. After three and a half years, *P. curvatus* had dispersed even farther in Florida than *P. tricuspis* did over the same period after initial release [78]. In Texas, small satellite populations of *P. tricuspis* have been found tens of km beyond the main expansion front; this jump dispersal was probably assisted by the prevailing winds [79].

Thus it is difficult to have true control sites that are not colonized within the time course of an experiment. Control sites would have to be placed so far away that there could be systematic differences in environmental variables between the treatment and control sites. This is almost a moot point,

as by now the vast majority of the invasive range of *Solenopsis* in the southeastern USA is estimated to have been colonized by at least one introduced *Pseudacteon* species [3]. Because of the tiny size of *Pseudacteon*, it is impractical to attempt to construct enclosures or exclosures, as these would include or exclude almost all other species (except microscopic ones).

The only published field experiment—including control plots (albeit 2 counties away) and spanning relatively large spatiotemporal dimensions—failed to find any measurable effect of introduced *P. tricuspis* on *S. invicta* in Florida [50]. The study was ended after 3 years when *P. tricuspis* dispersed to control plots. Relatively large variabilities were observed in fire ant activity and abundance, however, in both treatment and control plots. Thus the effects of this parasitoid would have had to be relatively large (perhaps reducing host ants by as much as 30%) to be detectable [50].

Studies are underway to gauge impact by comparing fire ant abundances before and after the introduction of *Pseudacteon*, in the absence of any control sites [3]. Such comparisons could be misleading, however, and should be interpreted with extreme caution. Fire ant abundances can and do change in response to many factors. *S. invicta* abundance, for example, was found to decrease by almost an order of magnitude over 12 years at a Central Texas site [80]. *Pseudacteon* species had not become established in Central Texas at the time, although other natural enemies of fire ants were present [80]. Additionally, numerous pathogens of *Solenopsis* fire ants are now known to be present in North America, and many have relatively high infection rates [52, 81, 82]. Finally, broad scale trends (i.e., climate cycles or directional change) may affect fire ant abundances independently of parasitoids.

6. Synthesis

6.1. Summary of the Evidence in Relation to Theory. Multiple *Pseudacteon* species frequently cooccur, although usually only one or a few species are very abundant. Overall, the *Pseudacteon* assemblage present at a given location may reach relatively high abundances at times, although populations fluctuate, and in the case of the introduced *P. tricuspis* in Florida, in synchrony with host availability. Parasitism rates are usually very low, and most workers parasitized are probably near the end of their natural lives, so this direct effect of parasitism may be almost negligible. Host ants may engage in much of their foraging in the absence of *Pseudacteon* flies and have the ability to adapt their behavior in the presence of this parasitoid, so that in the long term, the overall reduction in resource retrieval is likely much less than that suggested in short-term observations. Finally, *Solenopsis* species often do not lose control of rich food resources to competing ants in the presence of *Pseudacteon*.

Thus the available evidence suggests that any impacts of *Pseudacteon* phorids on host ant populations are generally small, especially when measured over the relatively large population variability of *Solenopsis* fire ants. Moreover, given the ability of *Solenopsis* to rapidly increase in population size under ideal conditions, any depression in *Solenopsis* populations by *Pseudacteon* phorids could be ephemeral if parasitism pressure is not consistent. Thus, the effects are probably much closer to the "weak control" scenario described above, although such effects could vary geographically and temporally. Experimental assessments of the impact of introduced *Pseudacteon* species have been few, and the results equivocal, although certainly no large impacts have been documented with any scientific rigor. Unfortunately, due to the constraints described above, we may never have a reliable, precise estimate for the effect of *Pseudacteon* parasitoids on *Solenopsis* fire ant populations in nature.

Thus the available evidence provides little support for strong top-down effects in this system. The accumulated data reveal that introduced *P. tricuspis* in North America are positively correlated with *S. invicta* availability, both temporally [48, 50, 51] and spatially [51]. These findings are not inconsistent with the hypothesis that larger *Solenopsis* fire ant populations simply support higher abundances of *Pseudacteon* parasitoids, and that *Solenopsis* populations are primarily regulated by other factors.

6.2. Host Specificity and Knowledge Gaps. Although *Pseudacteon* phorids may have relatively small effects on fire ant populations, they possess two very desirable qualities of a biocontrol agent. They have been documented to be highly host specific, in a battery of tests conducted: (1) in the field in South America [83, 84], (2) in the lab prior to the release of South American *Pseudacteon* species to North America [85–88], and (3) in the field in North America after the establishment of introduced populations [89, 90]. Thus, *Pseudacteon* phorids appear to be safe (i.e., no documented adverse effects on any other species), which is the most important quality of any biocontrol agent. Additionally, once established, *Pseudacteon* species will persist as a permanent component of communities in which host *Solenopsis* are found, and naturally disperse to others.

Several aspects of *Pseudacteon*/*Solenopsis* interactions need more study. Some *Pseudacteon* are attracted to mound disturbances [33, 39], although the potential effects of *Pseudacteon* on mound disturbances are less clear than those of interruption of foraging. The presence of *Pseudacteon* may delay the mound rebuilding process, but only for a matter of hours, until *Pseudacteon* activity ceases with darkness. The mound is important in regulating brood temperature and thus development [91], and movement of brood out of the mound due to disturbance and delayed reconstruction could adversely impact colony fitness, although such an effect seems ephemeral and thus relatively small. The frequency of natural mound disturbance events is an important variable in assessing this impact, and this disturbance regime no doubt varies geographically.

Morrison and King [39] found that numerous *P. tricuspis* flies were attracted to *S. invicta* mound disturbances when nonnestmate *S. invicta* were added, resulting in interspecific interactions, but on average fewer than one parasitoid was attracted in the absence of such interactions. Thus, most mound disturbances in the absence of interspecific interactions would probably be little affected by *P. tricuspis*, although other *Pseudacteon* species may differ in this aspect of their behavior. *Pseudacteon* are also attracted to *Solenopsis* mating flights [92]. Although *Solenopsis* reproductives are

not suitable hosts [19], workers are very active on the mound surface before the reproductives take flight [93], and the presence of *Pseudacteon* could dampen this activity, potentially disrupting the mating flight. Large numbers of workers may be vulnerable to parasitism during mating flights or mound reconstruction, and such workers may be younger than workers engaged in foraging, thus representing a potential greater loss to overall colony fitness. Finally, *Pseudacteon* are carriers of pathogens that infect *Solenopsis* [94], although actual vectoring of diseases among colonies has not been demonstrated.

6.3. Implications and the Bigger Picture. Efforts are underway to introduce additional South American *Pseudacteon* species to North America. The question is how many additional species should be introduced? Because of the relatively high degree of niche partitioning observed in *Pseudacteon* species in South America, and the coexistence of multiple species at a site, the traditional wisdom has been that multiple *Pseudacteon* species will coexist at North American release sites, and that more *Pseudacteon* species will exert greater parasitism pressure on host *Solenopsis* ants. The species abundance patterns of *Pseudacteon* in both North and South America, and the recent, relatively unexpected finding of competitive exclusion among introduced *Pseudacteon* species, however, suggest only one or two introduced species may be abundant at any given location. Thus the introduction of additional *Pseudacteon* species may simply reduce the abundance of already-established species, without substantially increasing the density of the overall *Pseudacteon* assemblage.

An argument can be made that multiple *Pseudacteon* species are necessary because of the diversity in invasive *Solenopsis* populations in North America. Both *S. invicta* and *S. richteri* (and a hybrid) are present, and colonies may be either polygyne (i.e., multiple queen) or monogyne (i.e., single queen) [1]. There have been attempts to "match" or find the best combination of South American *Pseudacteon* species or biotypes with North American *Solenopsis* populations [95]. Ultimately, given the speed of dispersal of this parasitoid, after introductions of multiple *Pseudacteon* species to multiple locations in the *Solenopsis* invasive range, the flies may eventually sort themselves out. Not all *Pseudacteon* species may disperse at the same rate as *P. tricuspis* and *P. curvatus*, however. *P. litoralis* has spread much more slowly from a single release site in Alabama [4]. Although *Pseudacteon* species appear to have no detrimental effects, the time and effort involved in evaluating, rearing, and releasing these flies might be better spent evaluating other types of natural enemies. At this point, it seems likely that the marginal contribution of each additional *Pseudacteon* species released will be diminished as more species are added.

In South America, a diversity of parasites, pathogens, predators, and competitors affect *S. invicta* [1, 96]. Thus, introduction of a single type of natural enemy is unlikely to result in outsized reductions of invasive *Solenopsis* populations. It is more likely that regulation is incremental, and that each type of introduced natural enemy may have a relatively small effect, yet one that is cumulative so that overall control becomes greater with the addition of more types of natural enemies. Thus, the continued search, evaluation, and introduction of other safe (i.e., host-specific) natural enemies of *Solenopsis* fire ants may eventually lead to measurable levels of fire ant population regulation.

Given the general lack of evidence for strong top-down control in this system, it is possible that other ants represent the greatest natural enemies of *Solenopsis*. The South American ant fauna contains more species that are strong competitors of *S. invicta* than does the North American fauna [72, 97, 98]. Many of these ant species would have their own deleterious impacts to recipient biotas, perhaps even greater than *Solenopsis* fire ants, and introductions of such natural enemies are not seriously contemplated. If competition from other ants is the primary reason that *S. invicta* is less abundant in South America, it follows that introductions of all possible other types of natural enemies will not result in a decrease of North American *S. invicta* or *S. richteri* populations to South American levels.

The conventional wisdom of *Pseudacteon* biological control is that the presence of these parasitoids may shift the competitive balance away from *Solenopsis* fire ants to native ant species, allowing for a relative increase in abundance of the native species at the expense of the invasive *Solenopsis*. Recent work on the effects of disturbance, however, challenges the conventional wisdom that *Solenopsis* fire ants are strong competitors that have displaced native ants primarily due to a competitive asymmetry. King and Tschinkel [99] obtained experimental evidence suggesting that native ants are first displaced as a result of habitat disturbance, and then *Solenopsis* fire ants—which are disturbed habitat specialists—move into the disturbed areas. Experimentally removing *S. invicta* from disturbed areas did not result in an increase of native species [100]. In this study, conducted in forest habitat in Florida, "disturbance" resulted in the simplification of habitat structure to a type that was more similar to the habitat where *S. invicta* is native (i.e., open areas with high insolation). Thus, at least in some areas, disrupting the behavior of *Solenopsis* fire ants or even reducing their abundances may have limited effects on native ant diversity, in the absence of restoring disturbed or simplified habitats.

Habitat type or disturbance alone, however, cannot adequately explain the high abundances of invasive *Solenopsis* fire ants in North America. *Solenopsis* species were found to be more abundant at the same type of disturbed (i.e., roadside) sites in North America relative to South America [2]. Thus the abundance of *Solenopsis* fire ants in an area is likely the result of a number of factors (and interactions of factors), including habitat type, degree of habitat disturbance, and the presence of natural enemies. Ultimately, efforts to reduce invasive fire ant densities would probably benefit by taking a broad perspective and include attempts at habitat restoration in addition to the introduction of an array of natural enemies.

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Review Article

Temperature-Driven Models for Insect Development and Vital Thermal Requirements

Petros Damos and Matilda Savopoulou-Soultani

Laboratory of Applied Zoology and Parasitology, Department of Plant Protection, Faculty of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Correspondence should be addressed to Petros Damos, damos@agro.auth.gr

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Since 1730 when Reaumut introduced the concept of heat units, many methods of calculating thermal physiological time heat have been used to simulate the phenology of poikilothermic organisms in biological and agricultural sciences. Most of these models are grounded on the concept of the "*law of total effective temperatures*", which abstracts the temperature responses of a particular species, in which a specific amount of thermal units should be accumulated above a temperature threshold, to complete a certain developmental event. However, the above temperature summation rule is valid within the species-specific temperature range of development and therefore several empirical linear and nonlinear regression models, including the derivation of the biophysical models as well, have been proposed to define these critical temperatures for development. Additionally, several statistical measures based on ordinary least squares instead of likelihoods, have been also proposed for parameter estimation and model comparison. Given the importance of predicting distribution of insects, for insect ecology and pest management, this article reviews representative temperature-driven models, heat accumulation systems and statistical model evaluation criteria, in an attempt to describe continuous and progressive improvement of the physiological time concept in current entomological science and to infer the ecological consequences for insect spatiotemporal arrangements.

1. Introduction

Climate has a profound effect on the distribution and abundance of invertebrates such as insects, and the mathematical description of the climatic influence on insect development has been of considerable interest among entomologists. Additionally, as temperature exerts great influence among the climate variables, by directly affecting insect phenology and distribution, most of the models that describe insect development are temperature driven [1–5].

This first effort for a formal description of the relation between temperature and developmental rate was taken by botanists, to model the effect of temperature on plant growth and development [6–10]. However, similar modeling procedures extended to most of the poikilothermic organisms, including insects as well [1–3]. To date, the earliest experiment that related the velocity of insect development and heat, was made by Bonnet (1779) [11] on the study of the reproduction rate of *Aphis evonymi*, F. [12], while the major assumption and principles that have been brought out by these earlier works, constituted the basis for all future research. Nevertheless, since then, several theoretical and experimental works have been carried out and current progress in entomology, mathematics and computation offers new means in describing the relation of temperature to insect development [13–20].

Thus, although simple predictive models have been developed during the last century, the development and broader availability of personal computers in the 70s and 80s resulted in the rapid development of computer-based models to predict responses of insects in relation to climate [21, 22].

Insects are adapted to particular temperature ranges and temperature is often the most detrimental environmental factor influencing their populations and distribution. In general, within optimum ranges of development and as environmental temperature decreases, their rates of development

slow and cease at the lowest (base) temperature, while as temperature rises, developmental rates increase up to an optimum temperature, above which they again decrease and eventually cease at their temperature maximum [4, 5, 15, 23, 24].

It is proposed that this effect of temperature on poikilothermic organism functioning is related to the effect on enzymatic activities. For instance, the conformation of enzymes is the essential step in the enzymatic reaction and this conformation depends on temperature [22, 25, 26].

One common approach to model temperature effects on insect development is to convert the duration of development to their reciprocals. This simple transformation is used to reveal the relationship type, as it will be shown later close to linear, between temperature and rate of development and permits the determination of two vital parameters of development namely, the thermal constant (K) and the base or lower temperature of development ($T_{\min}$). The thermal constant is expressed as the number of degree-days (in °C) and provides an alternative measure of the physiological time required for the completion of a process or a particular developmental event [4, 5, 21, 27].

Attempts to quantify the influence of temperature on insect development rates, growth, and fecundity have been carried out by several studies for species of economic significance [16, 27–32]. Entomologists have strong interest on this kind of relationships, since they are prerequisite to predicting timing and phenology of insect life cycle events and to initiating management actions [33–35], while application of temperature driven models are also essential in epidemiology modeling, development of effective vector control programmes [36] and prediction of biological invasions [37, 38]. From an agronomical standpoint, empirical models are often used to predict specific population events and provide means for precisely applied control methods, reducing costs as well as insecticide use [39, 40]. Furthermore, the determination of insect-specific vital thermal requirements provides evidence to infer on observed geographical distributions and predict future dynamics [8, 41].

The current review highlights the importance of the relationship between insect development and its vital thermal requirements and outlines important constraint and challenges regarded to model selection and applicability in pest management and insect ecology. Within our aims, building on preview reviews, was to provide a simple account for applied entomologists and field ecologists by avoiding complex and technical details. Furthermore, efforts are also made to present a short example of the linear model and to propose a simple three parameter non-linear equation for modeling temperature effects on insect developmental rates.

The rest of this article is structured as follows. The first section describes and explains the concept of the *law of total effective temperatures* and how it is related to the linear models of insect development. A paradigm of the *x-intercept* method is presented in defining lower developmental threshold for *Grapholitha molesta* (Lepidoptera: Gelechiidae). This threshold is vital in applying phenology models in field, and to our knowledge estimated for first time in a laboratory trial. The next section summarises the most common nonlinear regression models, including the derivation of the biophysical ones, which have been proposed by researchers in order to estimate cardinal temperatures of insect development. Additionally, among the given functions, a new 3-parameter equation is proposed and its general shape is also presented. Section 3 lists principal statistics that are used for parameter estimation in regression analysis and criteria for model selection among candidate equations. Section 4 briefly outlines the major heat accumulation systems for estimating species-specific heat energy in field during the growth season. Finally, there is extensive discussion regarding constraints and challenges of the models for pest management while efforts have been made to discuss how the estimated insects vital thermal requirement are related to the species environmental adaptation and field distribution.

2. Mathematical Models and Insect Development

Mathematical models represent a language for formalizing the knowledge on live systems obtained after experimental observation and hypothesis testing. An empirical model, if successful, determines result and cause and can be further used to describe the behavior of the system under different conditions [39, 40, 42].

Since temperature is considered as the most critical factor affecting insect development, numerous efforts have been made by researchers to propose models to describe such relations either in laboratory or field [6, 16, 22, 28, 29, 39, 40, 43–45]. Moreover, several of these models have been constructed in the view to be applicable for pest management [1, 21, 23, 27, 39, 42, 43, 46–48].

The term model emphasises some qualitative and quantitative characteristics of the process, which are actually abstracted, idealized, and described mathematically rather than the system itself.

Most of these approaches are based on the empirical detection of relationships and the construction of relative models that in brief capture all information about the response variable in relation to temperature. It should be noted that the presented temperature relationships can be judged as deterministic or empirical, by the sense that they consist of descriptions in which processes are not known, but where relations are established. However, all regression procedures that are followed, for parameter estimation, are purely probabilistic.

In applied entomology, empirical approaches are often used in the construction of developmental models. In general, the procedures include the delimitation of all the factors that affect development to the most limiting one, which is further chosen (i.e., temperature), in order to reveal empirical dependence of the developmental variable upon the limiting factor. A function which describes the data with higher accuracy is plugged to this relation, and its prediction power is further evaluated by using new datasets.

2.1. The Law of Total Effective Temperatures and the Linear Model. All poikilothermic organisms are related to a species-specific thermal constant that corresponds to time units that

must be accumulated to complete a particular developmental event. The above principle forms the basis for all modeling approaches that have been developed since the first introduction of the heat units concept by Reaumut on 1730 and the following initiation of the temperature summation rule [20, 49]. This rule, which was first proposed by Candolle [6] and characterized the development of all poikilothermic species, is referred to as the *law of total effective temperatures* and consists of the first effort in modeling temperature-dependent developmental rates instead of developmental times [7, 31, 50].

The model is characterized by universality, since development of all species is addressed by a thermal constant which corresponds to the accumulated degree-days that are needed to complete a particular developmental stage. This principle is further related to most other cumulative degree-day approaches.

According to the *law of total effective temperatures*, it is possible to estimate the emergence and number of generations for a given duration, of the organism of interest, according to the following fundamental equation:

$$K = D(T - T_0), \tag{1}$$

where K is the species (or stage-specific) thermal constant of the poikilothermic organism, T temperature, and T_0 developmental zero temperature. This thermal constant provides a measure of the physiological time required for the completion of a developmental process and is measured in degree-days (DD).

One popular method of estimating the above parameters is to use a linearizing transformation of the above function by calculating the rate of development $y = 1/D$ for the day variable resulting to the following equation [44]:

$$\frac{1}{D} = -\frac{T_0}{K} + \frac{1}{K}T. \tag{2}$$

Equation (2) is often referred to as the linear degree-day model or as the *x-intercept* method [24, 51], which is simply derived after growth rate fitting to a simple linear equation and then extrapolated to zero:

$$y = a + bT. \tag{3}$$

The lower theoretical temperature threshold (i.e., base temperature) is derived from the linear function as $T_b = -a/b$ whereas 1/slope is again the average duration in degree days or thermal constant K.

Equation (3) simply means that the thermal constant is a product of time and the degrees of temperature above the threshold temperature.

2.2. Lower Developmental Threshold for Grapholitha Molesta (Lepidoptera: Gelechiidae). Figure 1, for instance, describes a typical temperature effect on the developmental time of the pupae of *G. molesta* as well as the respective linear relationship between temperature and developmental rate according to (3). To reveal the above relations, larvae were reared in the laboratory at the Aristotle University of Thessaloniki

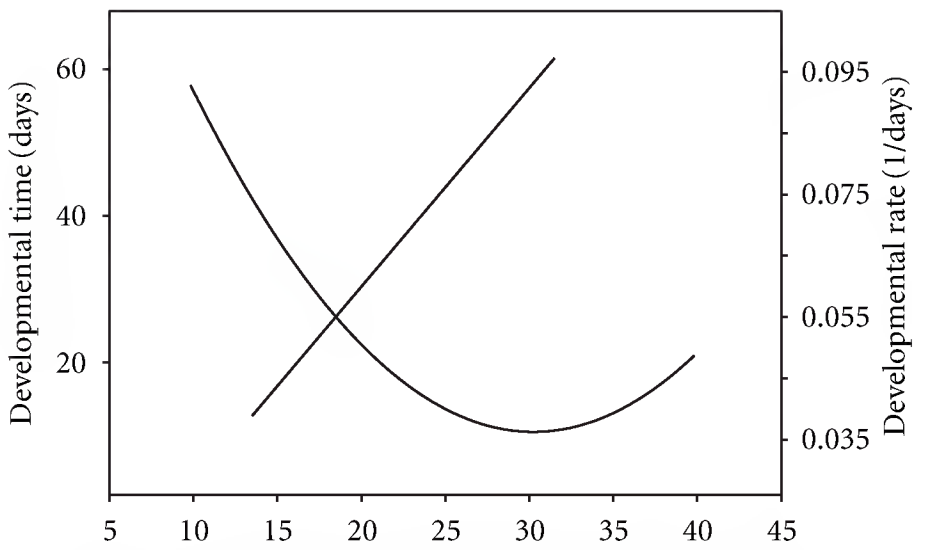

FIGURE 1: Typical response and temperature effect on the developmental time ($y = 115.5 - 6.9x + 0.1134x^2$) of an insect (i.e., pupal stage of *G. molesta*) and respective linear relationship between temperature and developmental rate according to the linear model ($y = 0.041x - 0.0412$, $T_{\min} = 10°\text{C}$).

and respective pupae were incubated at different constant temperatures at constant laboratory conditions (15, 20, 25, and 30°C, and 65 ± 5% R.H., 16:8 h L : D).

The need for inverse regression, as also displayed in the above paradigm, arises most often when the observed variable (developmental time) is the result of the major factorial cause variable (temperature) which is not subjected to error. Thus, in order to measure the predicted variable with negligible error and avoid bias, such kind of "physical problems" should be treated as inverse even if causality is not known or not considered [21, 27, 39, 52, 53].

However, if the dependent variable is measured with negligible error (relative to error in the measurement of the factorial variable), or is much smaller than that of the response variable, the direct prediction will involve bias, unless the two variables are perfectly correlated [53]. Therefore, regressions in which both variables are subjected to error have been also proposed [12] and are applied to insect temperature-dependent development to improve prediction precision [21, 27]:

$$DT = K + T_b D, \tag{4}$$

where D is development time (days) and T is temperature.

One of the major advantages of this equation, as in the case of the *x-intercept* method, is simplicity and the existence of biological interpretation over the estimated parameters: thermal constant and lower temperature threshold. Its added value, however, is increased precision in parameter estimation and the detection of outliers that reside on the non linear response curve and should be eliminated by the regression [44].

2.3. Nonlinear Regression Models. Although in practice the linear models are quite adequate over a range of favourable temperatures, they proved unsecure in predicting development in extreme conditions and temperatures in which the relationship becomes non linear [21, 27, 48, 55, 57]. Hence, ideally one should know the response of the organism

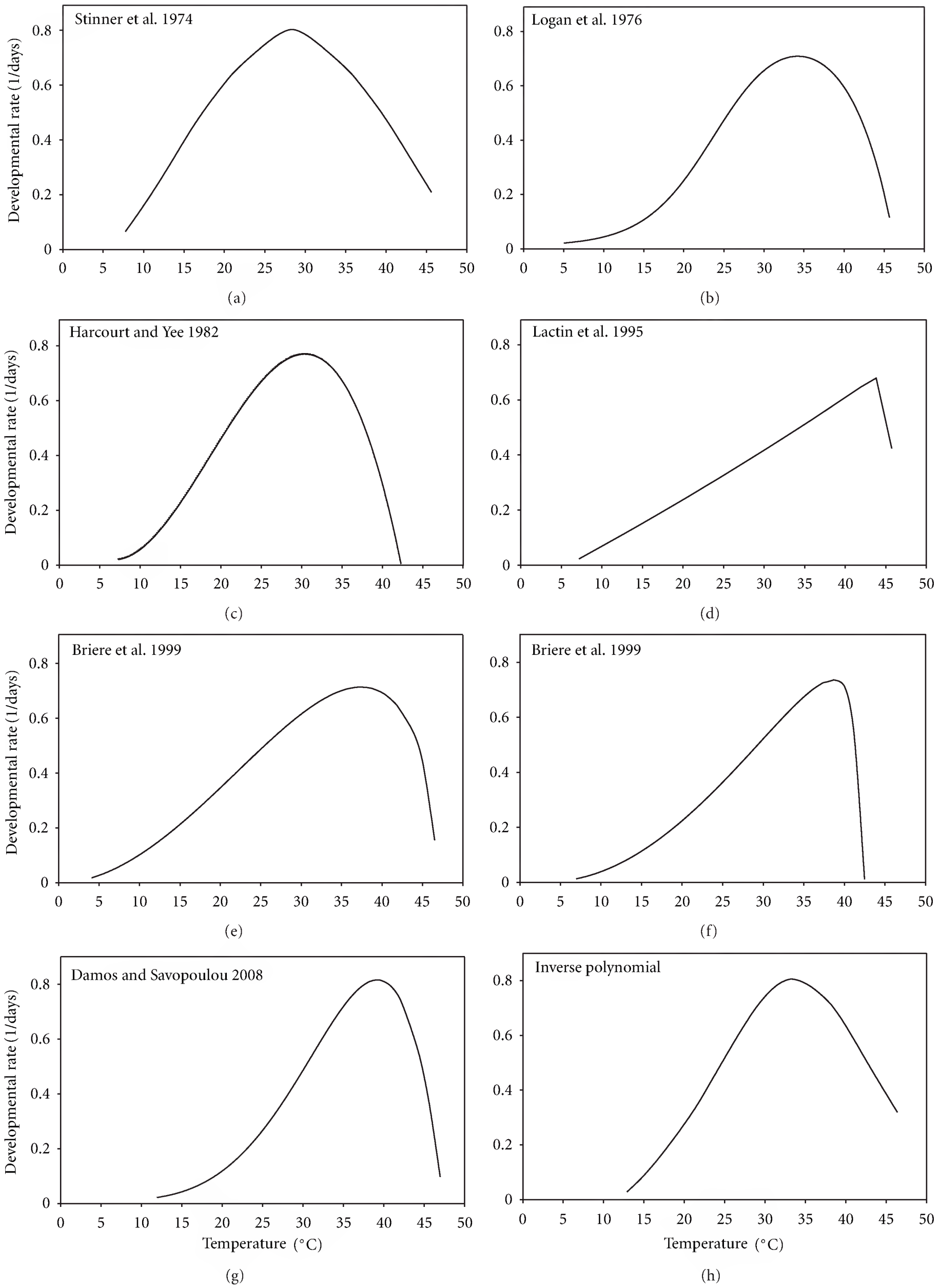

Figure 2: Typical relationships between temperature and insect developmental rates according to several representative non-linear models.

Table 1: Some representative regression models that have been created for the description of temperature-dependent development of insects and related arthropods.

Non-linear model	Equation	Description	Reference
$1/D = c/(1 + e^{(a+b\cdot T)}), \quad \text{if } T \le T_{\text{opt}}$ $1/D = c/(1 + e^{[(a+b\cdot(2\cdot T_{\text{opt}} - T)]}), \quad \text{if } T > T_{\text{opt}}$	(1)	"Stinner" (non-linear)	Stinner et al. 1974 [54]
$1/D = \psi \cdot [1/(1 + k \cdot e^{-\rho\cdot T}) \cdot e^{-((T_{\max}-T)/\Delta)}]$	(2)	"Logan 10"	Logan et al. 1976 [55]
$1/D = a \cdot T^3 + b \cdot T^2 + c \cdot T + d$	(3)	"3rd-order polynomial" (non-linear)	Harcourt and Yee 1982 [56]
$1/D = e^{\rho\cdot T} - e^{(\rho\cdot T_{\max} - (T_{\max} - T/\Delta))} + \lambda$	(4)	"Lactin" (non-linear)	Lactin et al. 1995 [57]
$1/D = a \cdot T \cdot (T - T_{\min}) \cdot (\sqrt{T_{\max} - T})$	(5)	"Briere 1" (non-linear)	Briere et al. 1999 [29]
$1/D = a \cdot T \cdot (T - T_{\min}) \cdot (\sqrt{T_{\max} - T})^{(1/m)}$	(6)	"Briere 2" (non-linear)	Briere et al. 1999 [29]
$1/D = \rho \cdot (a - T/10) \cdot (T/10)^{\beta}$	(7)	"Simplified beta type" (non-linear)	Damos and Savopoulou-Soultani 2008 [27]
$1/D = a/(1 + bT + cT^2)$	(8)	"Inverse second-order polynomial 1"	This study

over the entire range of temperatures to compute accurately developmental rates over all temperature range.

Several non linear models have been proposed to describe developmental rate response curves over the full range of temperatures, aimed either to build general insect phenology models, or to be used as forecasting tools for pest management [4, 5, 20, 21, 27, 29, 31, 34, 45, 50, 57–60]. Although the procedure can be easily generated using several different softwares, one important limitation is that the optimization procedure is performed only for the dependent variable and assumes that the residual errors of the independent variable are negligible.

Table 1 presents some of the most common non-linear models that have been developed to describe insect development rates over the whole range of temperature. Figure 2 depicts typical temperature response curves according to some common non-linear equations that are presented in Table 1. The models have been abstracted by the respective references and are additionally generated for representative selected empirical data.

Typically, and according to all models, there is no growth below the lower temperature threshold, while developmental rate increases and reaches a maximum at optimal temperature and declines rapidly approaching zero at the higher temperature threshold that is often considered as lethal temperature.

2.4. Biophysical Models. Biophysical models predict the behavior of insect developmental rate in physical terms. Since "*temperature rate biophysical models*" are representations of temperature-depended development and based on the primitive rules of temperature dependence of reaction rates narrowed by biophysics, they are differentiated to all the other non-linear models.

The conformation of enzymes is the essential step in the enzymatic reaction and this conformation depends on temperature. Because poikilothermic development can be considered as a macroscopic revelation of enzyme reactions, in which temperature exerts a catalytic effect at a molecular level, these equations have been applied in modeling microorganism growth and in describing temperature-dependent development of arthropods.

Traditionally, such kinds of relations are based on the empirical equations of *Van't Hoff's law* [7], Arrhenius [46], and Eyring [50, 60–62]; and these relationships provided the principal foundation of later works.

Van't Hoff, based upon the experimental results of the botanist and pharmacist Pfeffer (who first measured osmotic pressure in 1877), concluded that the osmotic pressure π of a sugar solution in relation to its volume is constant and directly related to the absolute temperature T:

$$\pi = kT, \tag{5}$$

where k is a constant of analogy. Furthermore, by applying the ideal gas state equation to describe the osmotic pressure, as in the case of ideal gas, results in

$$\pi = RT\Sigma c_i, \tag{6}$$

where R is the universal gas constant, T is the absolute temperature, and c_i is the molar concentration of solute i. Interpretation of (5) and (6) simple states that the rate of chemical reactions increases between two- and threefold for each 10°C rise in temperature. This conclusion, according to *Van't Hoff's law,* that an increase in temperature will cause an increase in the rate of an endothermic reaction had a huge impact in chemistry, biochemistry, and physiology.

The Arrhenius equation relates the chemical reaction rate constant to temperature T (in Kelvins or degrees Rankin) and the activation energy of the reaction E_α as follows:

$$k = k_0 e^{-E_a/RT}, \tag{7}$$

where K_0 is the rate coefficient, E_a the activation energy, R the universal gas constant, and T absolute temperature. According to the Eyring function [61] any biochemical reaction rate (without prior enzyme activation) increases exponentially while in the equation parameterized by Schoolfield et al. [60] the reaction rate $r(T)$ is given as a modification of a reference reaction rate to a respective reference temperature:

$$r(T) = \rho \frac{T}{T_{\text{ref}}} e^{[H_\alpha/R\ (1/T_{\text{ref}} - 1/T)]}. \tag{8}$$

In (8), ρ is considered as *1/time* (reference rate) and H_α corresponds to the temperature sensitivity coefficient

(or activation enthalpy in J/mol) and R is the universal gas constant (8.314 J K^{-1} mol^{-1}). The above equation can be applied to any intended temperature sensitive rates including developmental rates as well.

However, when dealing with biological rates, exponential increase is observable on a limited range and not throughout all temperature regimes. Sharp and DeMichele [63] considered activation process of the two extreme temperatures as independent and proposed a modification of the Arrhenius equation. This result to an equation having two components in the denominator, each for the description of the reversible inactivation of the rate-controlling enzyme considering both low and high temperatures and including "linearity" at middle temperatures:

$$r(T)=\left[\frac{T\cdot\exp\left[\left(\Phi-\Delta H_A^{\neq}/T\right)/R\right]}{1+\exp[(\Delta S_L-\Delta H_L/T)/R]+\exp[(\Delta S_H-\Delta H_H/T)/R]}\right], \tag{9}$$

where $r(T)$ is the mean developmental rate at temperature T (1/time), T is the temperature in K, R is the universal gas constant (1.987 cal deg^{-1} mol^{-1}), while the other parameters are associated with the rate-controlling enzyme reaction: ΔH_A is the activation enthalpy of the enzyme reaction while ΔH_H is the change in enthalpy associated with high-temperature inactivation of the enzyme (cal mol^{-1}), ΔS_L is the change in entropy associated with low-temperature inactivation of the enzyme (cal deg^{-1} mol^{-1}), and Φ is a conversion factor having no thermodynamic meaning.

Figure 3 gives the biophysical model (9) for representative datasets as well as the respective Arrhenius plot. The biological interpretation of the above function has analogies to those of the Arrhenius function in which the dominator represents the fraction of rate-controlling enzyme that is in the active state. Derivation of the above mathematical function as well as the basic assumptions and modifications of the original formula are covered in details in [60, 63].

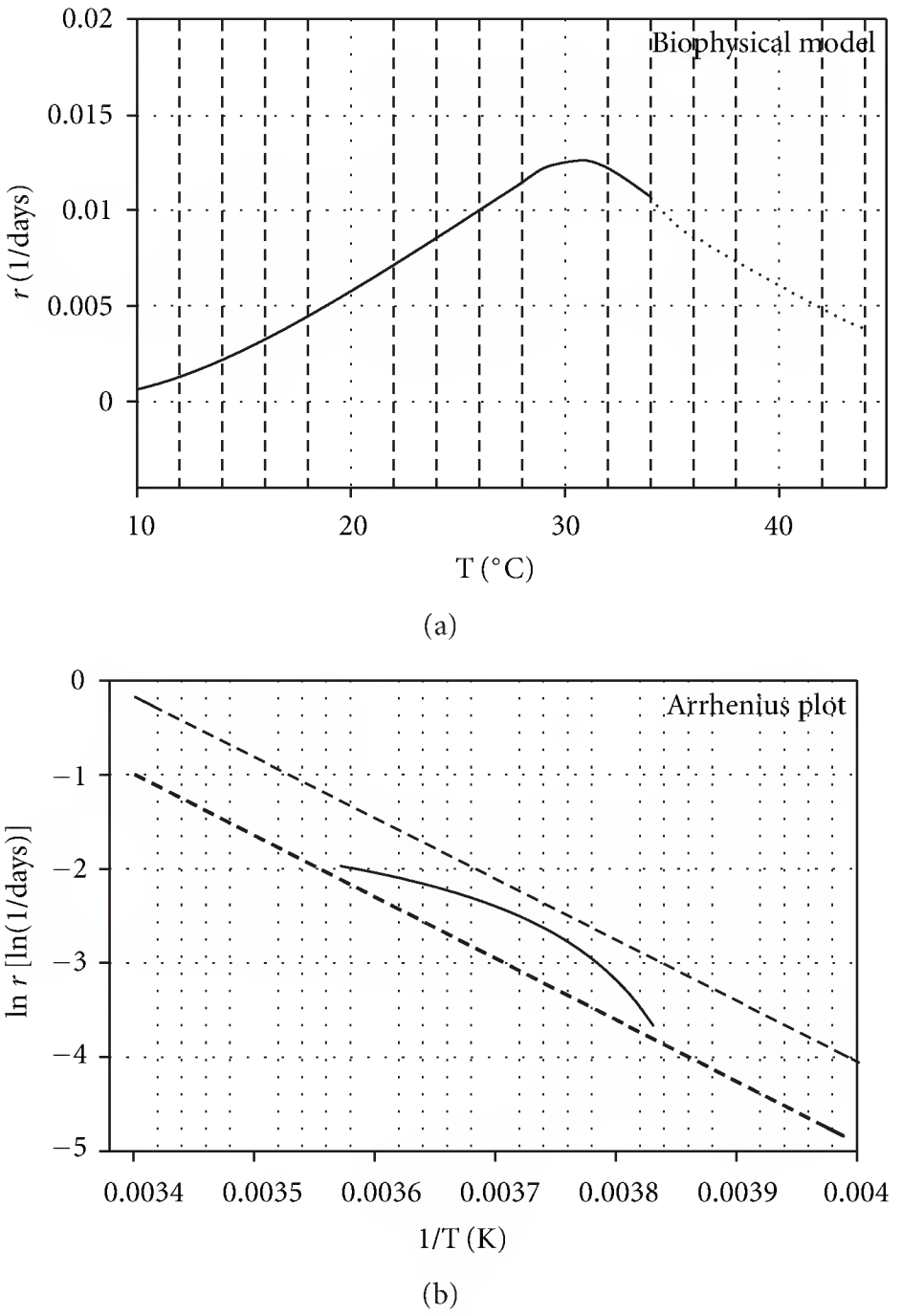

Figure 3: Curve shape of the biophysical model of sharp and DeMichelle [63] as modified by Schoolfield et al. [20] (a) and the respective Arrhenius plot (b).

3. Statistics for Parameter Estimation and Model Comparison

3.1. Parameter Estimation. Numerous procedures have been developed for parameter estimation and inference in regression analysis.

Campbell et al., 1974 [43, 64], provide statistics for the Standard error (SE) of the lower developmental threshold ($T_{\min}$) and the thermal constant K for the linear model based on "principal-manually" derived statistics:

$$\mathrm{SE}_{T_{\min}}=\frac{\bar{r}}{b}\sqrt{\frac{s^2}{N\cdot\bar{r}^2}+\left[\frac{\mathrm{SE}_b}{b}\right]^2}, \tag{10}$$

where s^2 is the residual mean square of r, $\bar{r}$ is the sample mean, and N is the sample. Additionally, the size of the SE_K for the thermal constant K for the linear model having slope b is, respectively [64],

$$\mathrm{SE}_K=\frac{\mathrm{SE}_b}{b^2}. \tag{11}$$

However, several other procedures are also proposed for parameter estimation and relative statistics. The most common are the maximum likelihood (ML) and the ordinary least square (OLS) estimation, and they are used for both linear and non linear models [65].

Point and interval estimation using ML relies on distributional assumptions (here a specific probability function for error dispersion must be specified), in contrast to OLS point estimates, which generally do not require hidebound distributional assumptions, are unbiased, and have minimum variance.

The OLS minimise the sum of square residuals of the regression function of interest. Additionally, most statistical packages of parameter estimation are based on the Levenberg-Marquardt algorithm (LMA) which provides a numerical-iterative solution of curve fitting over a space of parameters of the function.

The Marquardt algorithm [66] is a least squares method based on successive iterations for parameter optimization. Thus, if $(x_i,\ y_i)$ is the given set of n empirical observation pairs of the independent (temperature) and dependent (developmental times) variables, the algorithm optimizes the

parameters p of the model curve $f(x, p)$ so that the sum of the squares of the deviations is minimum:

$$g(p) = \sum_{i=1}^{n} [y_i - f(x_i, p)]^2 \quad (12)$$

The method is that the analyst has to provide an initial starting guess for final parameter estimation. This is an important constrain of the method and especially in curves with multiple minima the initial guess must already to be closed to the final solution. Furthermore, problems can arise in the case of observational data (i.e., time series) in which covariates can exist between observed and response variables.

The methods described above for calculating standard error and confidence intervals for a parameter relay on the assumption that the statistic of interest is assumed to be normal distributed. Thus, there is no need whatsoever for bootstrapping in regression analysis if the OLS assumptions are met. However, in the case of estimating population values in the absence of any information (i.e., variables in which sampling distributions and variances are unknown due to limited data), or in the case in which the variable is the final result of several observations (as in the case of life table statistics), parameter estimation and standard errors can be based on resampling methods such as the Bootstrap and/or the Jackknife method, or even based on Bayesian inference estimation.

For more details on resampling the reader should consider the references cited [67, 68].

3.2. Model Comparison. Since several regression models are available it is convenient to provide criteria or goodness of fit tests for model comparison. For instance, a common question that applied entomologists are facing is how to compare two different models for a given species and/or how to compare two different species with a given model.

Generally, several criteria have been proposed to evaluate model performance including the root mean square error (RMSE), the Pearson x^2, the deviance (G^2) statistics, regular and adjusted to the parameter numbers regression coefficients, and information criteria such as the *Akaikes* and *Bayes-Schwarz* information criteria [21, 27, 39, 69].

The idea behind most of these criteria is to measure the "range" of which the predicted values of a given model match the observed and can be applied in evaluating prediction capability for a particular dataset (i.e., one species-several models). Some of them are described in brief.

The Pearson x^2 statistic is based on observed (O) and expected fitted or predicted (e) observations and has similarities to the Root Mean Square Error [27, 65]:

$$x^2 = \sum_{i=1}^{n} \frac{(o - e)^2}{e} = \sum_{i=1}^{n} \frac{(y_i - n\hat{\pi}_i)^2}{n\hat{\pi}_i(1 - \hat{\pi}_i)} \quad (13)$$

Where y_i is the observed value of Y, $\hat{\pi}_i$ is the predicted or fitted value of x_i and n is the number of observations. Additionally, based on the same concept a "prediction capability" index d can be addressed to be used to compare candidate models and rank them according to the degree to which the predictions are error-free:

$$d = 1 - \frac{\left[\sum (P_i - O_i)^2\right]}{\sum \left[\left(\left|P_i - \overline{O_i}\right|\right) + \left(\left|O_i - \overline{O_i}\right|\right)\right]^2}, \quad (14)$$

where $\overline{O}_i$ is the average of the observed values [27, 70].

For a comparison of only two models, an efficacy ratio can be calculated as follows [27, 70]:

$$E_{1,2} = \frac{\mathrm{MSE}_1}{\mathrm{MSE}_2}. \quad (15)$$

Where the respective to the models efficacy ratio E is based on the mean square errors (MSE) and can be used as evaluation index [70]. Values close to 1 indicating very low differences between the selected models in predicting a particular dataset [21, 27].

Considering that there are cases in which different datasets (i.e., two different species) are described with a particular model and cases in which there is model selection among equations that differ on the number of parameters, model performance comparisons can be made according to the adjusted coefficient of determination (Adj $\cdot r^2$) and on the Akaike's information criteria [71].

The Adj $\cdot r^2$ is a modification of r^2 that adjust for the number of explanatory terms in a model. Unlike r^2, Adj $\cdot r^2$ increases only if an additional new term improves the model more than would be expected by change [21, 39]. The Adj $\cdot r^2$ is defined as

$$\mathrm{Adj} \cdot r^2 = 1 - \frac{\mathrm{RSS}/(n - (\vartheta + 1))}{\mathrm{SS}/(n - 1)}. \quad (16)$$

Akaike's information criterion (AIC) developed and proposed by Akaike in 1974 [39] is

$$\mathrm{AIC} = n \cdot [\ln(\mathrm{RSS})] - [n - 2 \cdot (\theta + 1)] - n \cdot \ln(n) \quad (17)$$

and the Bayesian-Schwartz information criterion (BIC or SIC) was proposed on 1978 and is [39]

$$\mathrm{BIC} = n \cdot [\ln(\mathrm{RSS})] + (\theta + 1) \cdot \ln(n) - n \cdot \ln(n), \quad (18)$$

where RSS is the residual sum of squares and SS total sum of squares, θ number of parameters and n observation number. These criteria permit to infer on how the different number of parameters add to the explanatory power of the candidate model.

4. Physiological Time and Heat Unit's Accumulation Systems

Considering the above models in defining cardinal temperatures of development in the laboratory, as well as the respective for each stage and species thermal constants, the interest is to apply this knowledge in order to make field predictions of temperature effects on insect phenology in time and space, according to the physiological time and related heat accumulation systems [50, 72–75].

Often referred to also as thermal time, the progress of the development of an organism is viewed as a biological clock that measures time units. Thus, although physiological time accelerates or slows according to prevailing temperatures, the time units to complete a particular developmental event in field should be the same as defined in the laboratory and equals the species specific thermal constant.

Thus, since the *law of effective temperatures* suggest that the completion of a given stage in development requires an accumulation of a definite amount of heat energy, similar approaches can be followed in which effective accumulated temperatures are estimated by the respective heat energy in field during the growth season.

According to this approach the amount of age or development accumulated from time 0 to t, and for discrete time intervals is

$$\Delta\alpha = \sum f\ [T(t)]\Delta t, \qquad [T(t)] > 0. \tag{19}$$

According to this function the species integrate temperature effects according to some function, f, peculiar to their species. This function, $f[T(t)]$, can be either linear or non-linear. If $f[T(t)]$ is assumed to be linear, then the developmental rate is proportional to temperatures above threshold (as defined according to the *x-intercept* method and apart from the linearity check of the rate-temperature curve), on the other hand, several non linear relations exist such as the logistic curve. However, in order to be effective, heat summation takes into account only the active temperatures within the species-specific range of development [24, 51].

Several methods have been proposed in calculating degree days accumulated in field, as well as related software. However, for the sake of brevity, in this review, the following three widely applied methods the average method, the modified average method, and the modified sine wave method, are briefly discussed.

4.1. Average Method. According to the average method developed by Baskerville and Emin [14], which is the simplest one, the number of daily degree-days is calculated by subtracting the base temperature from the average daily temperature as follows:

$$DD = \left[\frac{\min T + \max T}{2}\right] - T_{\min}. \tag{20}$$

Among the disadvantages of the above approach is that it does not take into account those daily minimum temperatures that can fall below the species lower temperature thresholds. This situation is very common in spring and results in bias and underestimation of degree-days accumulated by the insect since not all hourly temperatures during a day are above the threshold level. Thus, during this short period, development proceeds but is not taken into account by the proposed heat accumulation system.

4.2. Modified Average Method. In order to avoid the above-mentioned disadvantage it is convenient to modify the first component of (20) by substituting minimum temperature with lower temperature threshold, thereby approximating closer reality by calculating the daily temperature accumulation that corresponds to the interval between maximum temperature and that which is higher than the lower threshold of the species, or

$$DD = \left[\frac{T_{\min} + \max T}{2}\right] - T_{\min}. \tag{21}$$

This approach will result in a higher number of degree-days by taking into account development during the short periods in which temperature is slightly above the lower developmental threshold.

4.3. Modified Sine Wave Method. In principle mathematical relationships for this technique were given by Baskerville and Emin [14], Allen, and Watanabe [2]. Arnold [24, 51, 76] showed that the area under the temperature curve, the amplitude of which has been adjusted to the daily maximum and minimum temperatures for a given day, can be approximated according to sine curve.

Thus, according to the modified sine wave method, proposed by Allen [51], a trigonometric sine function is being used to describe this kind of daily temperature fluctuations. Based on the same principle as previously stated, heat accumulations during a day correspond to the area above the species lower temperature threshold. It is also noteworthy to state that this method leads to similar results as the modified average method in the case where minimum temperature is higher than the base temperature.

All these methods that are briefly described are based on the principle that the specimen is accumulating climate temperatures that are limited within its thresholds. Heat units are expressed as accumulated degree-days that correspond to a 24-hours daily interval that is limited between minimum and maximum temperature range and the predetermined species-specific thresholds.

5. Discussion

Among the scopes of this article was the description of representative models that have been proposed to model insect temperature dependent development either in the laboratory or field. However, a tremendous amount of prior work has been done in the field of insect temperature modelling since the first defined principles and the reader should consider the work of Ludwig [18], Uvarov [49], Powsner [19], Wigglesworth [26], Laudien [25] and Wagner et al. 1984 [20] for additional information.

Nevertheless, among the purposes of this review was to popularise prior studies. Several statistical criteria for model comparison are also gathered in order to integrate and familiarise most current approaches and tools for modelling the effect of temperature on insect development. This is an essential step to be made in order to draw inference upon the species ecology, spatiotemporal arrangement, and abundance.

According to selected linear and non linear models, that are presented in brief, developmental responses can

be summarized in terms of the three critical, or cardinal, temperatures of development. In addition, since calculation of physiological time by temperature-driven field models is related to the area summated by the chosen heat-accumulation system, the definition of these temperatures is a prerequisite for accurate phenology prediction. Thus, apart from the ecological concerns, the importance of finding a mathematical/statistical model which describes and then simulates the phenology of individuals under field conditions is a prior constraint for further successful timing of pest management practices in field.

Depending on their parameters, the presented models can be judged more or less complex and several algorithms for least squares estimation have been proposed for nonlinear parameters [66, 77]. By incorporating several more factors-parameters on the equations, the authors search to gain higher accuracy on data description. However, complexity does not assure more accuracy in all cases. Prior comparative approaches should be followed to choose among most appropriate models that are available. To put forward, since most model shapes are quite similar, comparative differences of model performances can be only indicated by detailed statistical measures [39].

Hence, not all models display the same fit behaviour when carefully observed while very few provide a detailed biological interpretation of the estimated parameters. For instance, the advantage of the models proposed by Logan and Lactin over the other equations is due to the fact that they incorporate parameters that have direct biological interpretation and this is a major asset. In addition, the models proposed by Sharpe and DeMichele [63] and Schoolfield et al. [60], based on enzyme kinetic reactions, display a radical departure from those based on empirical fits to data. Nevertheless, it is common that temperature affects not only the rate of chemical reactions, but also induces conformational changes in biological systems [49].

Moreover, one disadvantage of complexity in models is that it strongly influences parameters estimation [39]. For example, although most of the polynomial models do not have any biological interpretation, probably the most important advantage they have is that parameter estimation can be easily done [56].

One other characteristic, among the presented models, is that not all of them are able to make predictions that are matched over, the experimentally derived, observed values. Unfortunately, there are instances in which optimum and upper threshold temperature predictions are quite overestimated when compared to real data [21, 27]. For instance the lower temperature threshold for *G. molesta*, as estimated in the current laboratory trial, slightly deviates from that estimated by prior field studies [47]. Nevertheless, differences in respect to insect stage can also exist so it is important to model all development of *G. molesta* for safer interpretations. Thus, a good fit for a respective model has no utility if it predicts temperature thresholds that have no biological meaning. Such false predictions can result in bias on the estimation of cardinal temperatures. In most cases overestimation of optimum and maximum temperature thresholds is the result of skewed curve, although coefficients of determination are quite high but can be the result of a good data fit on the intermediate temperature range. In other words, a good fit is not always a guarantee for biologically significant model performance and a reliable and accurate data description over all temperature range [21, 27, 44].

On the other hand, not all models can predict lower temperature thresholds, since there is no intersection with the temperature axis, when rate of development is zero [27], while in some cases cardinal temperatures are derived graphically and not numerically. In addition, the assumption of a base temperature close to 0°C, in the cases in which the curve approximates origin may seem unreasonable, considering that it is well accepted that lower temperature thresholds for most arthropods are well above 0°C, usually around 6–10°C, or higher. This is also displayed for the dataset used to model *G. molesta* in the current study. Thus, the most currently used non-linear temperature models describe only part of the whole picture of insect temperature-dependent development. The equation of Logan et al. [55], as modified by Lactin et al. [57], due to the constant factor that intersects with the temperature axis, as well as the equation proposed by Hilbert and Logan [16], proposes a lower threshold as well, although proved rigid in describing particular datasets [27].

The above reasons, as well as the species and stage-specific plasticity on temperature responses, give important reasons that should be taken into account to choose among several available formulas. These trends have been pointed out by several researchers and are probably the major cause that resulted to the development of plethora of non-linear models in the literature [27, 31, 55–57, 78, 79].

Another important constraint is that most of these models are directly related to temperature and do not take into account other climatic variables. For insects in particular, temperature is probably the most critical abiotic factor that influences their developmental rates and their life cycles, although other factors such as photoperiod, humidity, and nutrition should not be excluded, as well as crowding or density and competition [13, 40, 44].

Furthermore, in most cases it is virtually impossible to measure the temperature that an insect experiences in its original microenvironment. For example, most plant-feeding insects display a species-specific behavior in relation to their host (i.e., crawling inside of shoots or barks at the larval stage) while others exert some control over their body temperature through their behavior (i.e., they rest at shadowed and cool places when temperature is high) [21, 40].

Considering that the existence of alternating temperatures is more probable in reality [80], there are cases in which models displayed considerable inaccuracy in predicting insect development and phenology under field conditions [21, 39, 42, 44, 58, 81, 82, 82, 83].

Hence there is no perfect model, but we rely on the available ones that best describe our datasets, under certain conditions, and even though most models are oversimplifications, they are acceptable for empirical predictions in some defined ranges and instances.

Thus, if the model is proved reliable after seedily experimental evaluation, heat accumulations of a phenological event that occurs in field should reflect that which have been estimated by the model and thereby provide means of accurate timing of pesticides and initiation of pest management tactics. Therefore, it is not risky to claim that temperature has a prominent role in insect biology and by understanding the temperature effects on insect development we are able to describe and predict the distribution and abundance of insect species in any locality [83–85].

From an ecological standpoint, insect vital thermal requirements, as described in this article (i.e., thermal constant and temperature thresholds) provide ecologically and practically useful information [34, 66, 86]. For instance, as the thermal constant differs among genera, species or even stages, their study reveals various aspects of temperature adaptation and in particular the adaptation of each to its environment. On the other hand, species specific thermal requirements can also be used as indicators of the distribution and abundance of insect populations [32].

The effect of a climatic factor, such as temperature for instance, sets the tolerance limits for a species, and this has been acknowledged by earlier studies (i.e., Shelfold, 1913: The Law of Tolerance). Later studies [13, 87, 87, 88, 88] discuss how the species-specific "environmental boundaries" are determined by the ultimate tolerance factor (i.e., temperature) which may further restrict geographic distribution [8, 37, 41, 89].

Moreover, is it though for species whose geographical distributions ultimately are determined by temperature, global warming should result in spatial range shift [33]. Thus, the speculations on the effects of climatic change on the spatial dynamics of insect species have been quite general and populations are expected to extend their ranges to higher latitudes and elevations [37, 38, 90–94].

However, contrasting results concerning future projecting of species distribution have been also reported [90, 95], and one cannot exclude a progressive temperature selection of individuals that are adapted to the new temperature environment and especially for species with high reproductive potential [96–98] and host alternatives. Furthermore, the rate of temperature change affects species acclimation potential which further results in different conclusions regarding the responses of the species to acclimation [38, 99] and that thermal tolerances of many organisms to be proportional to the magnitude of temperature variation they experience.

Since genetic variation and potential response to selection should be positively correlated with population size, species with restricted ranges, or smaller populations, are predicted to have reduced capacity to adapt to environmental change [96, 97, 100]. On the other hand, it is more likely that temperature alteration can affect the reproductive potential of a species (i.e., abundance) and its life cycle, since additional generations or/and outbreaks are possible during the growth season [101] when not limited by photoperiod [48].

For a particular species, there is an inverse relation between the thermal constant and the lower developmental threshold and it is suggested that this trade modifies the fitness of the species and finally influences the outcome of competition between related species and their distributions [85, 88, 102–104]. Moreover, tropical species and warm-adapted species tend to have higher values on their lower temperature thresholds when compared to cold-adapted species that had greater *DD* requirements and much lower temperature ranges [85, 88, 102, 104].

Based on such linear relationships, between thermal constants and lower temperature thresholds, for several cold-blood species, it is suggested that there is an inverse relationship between lower temperature thresholds and the thermal constant associated with latitude and/or habitat that adapts each species to its thermal environment [85, 103]. Thermal constant and respective *DD* requirements are also based on the particular morphology and size of the species. For example, size at maturity is a function of the rate and duration of growth, and large size at maturity implies a long generation time and a correspondingly large *DD* requirements [17, 102, 105].

Hence, insect thermal requirements have a strong physiological and ecological interpretation since they modify species-specific ecological strategy which is adapted to a particular thermal environment [26, 49, 74, 84, 104, 106].

Thus, any model which provides biologically important parameters is useful in modeling population dynamics under several temperature regime alterations. In addition, by incorporating more factors in the equations, climate-driven models have the potential to describe the general ecological behaviour, abundance, distribution, and outbreaks of insects on a regional or even global scale, with important practical applications.

Finally, future research must be carried out in the direction of insect thermal adaptation in order to assess the species reproduction potential and related evolutionary properties as they respond to short- and long-term temperature alterations. The development of more sophisticated models, such as demographic system models and ecological niche models, that incorporate species-specific vital thermal requirements as well, is also an urgent necessity to improve and complete all current models. Thus models that are based on weather and other factors can more realistically estimate the spatiotemporal population evolution and invasive potential of native and nonindigenous species in new areas.

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Research Article

A New Lycid Genus from the Dominican Amber (Insecta, Coleoptera, Lycidae, Leptolycinae, Leptolycini)

Sergey V. Kazantsev

Coleoptera Division, Insect Centre, 13-326 Donetskaya Street, Moscow 109651, Russia

Correspondence should be addressed to Sergey V. Kazantsev, kazantss@yahoo.com

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A new fossil genus, *Electropteron* **gen.n.**, and a new species, *E. avus* **sp.n.**, are described from the Dominican Amber. *Electropteron avus* **gen.n., sp.n.**, appears to be related to some of the extant Great Antillean lycids and is the first fossil taxon from the subfamily Leptolycinae.

1. Introduction

No taxa of the family Lycidae have so far been known from the ambers of the New World. All previously described amber lycids come from the Baltic Amber and all belong to the subfamily Erotinae [1–3], although Klebs [4] signaled, also from the Baltic Amber, a representative of *Lygistopterus* Mulsant, 1838 (Calochrominae).

The first Dominican Amber lycid, a well preserved and clearly observable male specimen, turned out to represent a new genus and a new species, apparently very close to some of the recent Leptolycinae from Hispaniola and Puerto Rico. Its gender is easily defined by the characteristic structure of the terminal abdominal segments, with the elongate, pointed at apex ultimate sternite enveloped laterally by a tergite. The description of the new taxon is given below.

2. Description

2.1. Electropteron ***gen.n.***

2.1.1. Type Species: Electropteron Avus ***sp.n.***

Description. ***Adult male.*** Alate, slender, elongate (Figure 1(a)). Head subquadrate, slightly narrowed behind eyes. Fastigium right-angled. Eyes relatively small, spherical. Maxillary palps slender, with ultimate palpomere pointed distally. Gula prominent. Antennal prominence conspicuous, antennal sockets approximate. Antenna 11-segmented, moderately long, slightly widening distally; antennomeres 4–11 flattened, with slightly uneven edges, antennomeres 2 and 3 short, transverse, subequal in length (Figure 2(a)); pubescence on antennomeres 4–11 relatively short and erect (Figures 1(a) and 1(b)).

Pronotum small, ca. 6 times shorter than elytra, transverse, trapezoidal, with obscure median impression in posterior third; posterior angles produced laterally (Figure 1(a)). Prosternum short, V-shaped (Figures 1(b), 2(b)). Thoracic spiracles small, not projecting beyond coxae. Mesoventrite transverse, short. Mesonotum with rather prominent, elongate scutellum (Figure 1(a)). Elytra long, narrowing, and dehiscent distally, covering abdomen, except genital capsule, with two noticeable primary costae (presumably, costae 2 and 4); interstices irregularly areolate; short and erect elytral pubescence uniform (Figure 1(a)). Metaventrite transverse, with acute posterior angles; discrimen complete, attaining to mesosternum.

Pro- and mesocoxae elongate; metacoxae approximate; angle between metacoxae ca. 90°. Legs slender; trochanters elongate, but considerably shorter than femurs, cylindrical, connected to femora distally; femurs and tibiae flattened, tibiae straight, widened distally; tarsomeres 1–4 narrow, without plantar pads; all claws simple. Ultimate sternite and tergite elongate, pointed at apex (Figures 1(a), 1(b), and 2(c)).

Female. Unknown.

Figure 1: General view of *Electropteron avus* **gen.n., sp.n.**, holotype male: (a) dorsally; (b) same, ventrally.

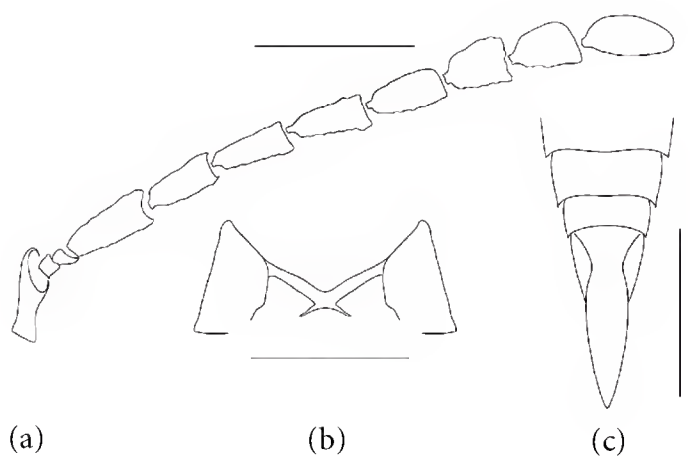

Figure 2: Details of *Electropteron avus* **gen.n., sp.n.**, holotype male: (a) antenna; (b) prosternum; (c) apex of abdomen, ventrally. Scale: 0.5 mm.

Etymology. The name of the genus is derived from "electron" and "pteron," the Greek for "amber" and "wing." Gender neuter.

Diagnosis. Electropteron **gen.n.** appears to be related to the extant genus *Tainopteron* Kazantsev, 2009, from Puerto Rico [5], but is distinguishable by the flattened and distally slightly widening antennomeres 4–11 (Figure 2(a)), less transverse pronotum and more elongate elytra completely covering the folded wings (Figure 1(a)). The new genus is different from *Leptolycus* Leng et Mutchler, 1922, another Greater Antillean extant endemic [5], by the flattened antennomeres 4–11, their short pubescence, transverse pronotum with explanate sides, and short V-shaped prosternum (Figures 1(a), 1(b), and 2(b)). On the other hand, *Electropteron* **gen.n.** is somewhat similar to *Ceratoprion* Gorham, 1884, distributed in the highlands of Central America and the Ands south to Ecuador, differing by the nonserrate antennomeres 4–11 and their erect pubescence and by the absence of the median longitudinal pronotal carina.

2.2. Electropteron avus ***sp.n.*** *Figures 1(a)–2(c)*

2.2.1. Material. Holotype, Male, specimen no. 09155/2198988208, Dominican Amber, Oligocene (Insect Centre, Moscow).

Description. ***Male.*** Dark brown to black; antennomere 11, pronotum, scutellum, elytra proximally, at scutellar level, meso- and metaventrites, genital capsule, coxae, trochanters, and femurs yellowish.

Head with deep impression behind antennal prominence. Eyes small, interocular dorsal distance over 2 times greater than eye radius. Antennae attaining to elytral middle, with antennomere 3 subequal in length to pedicel (antennomere 2) and 5.5 times shorter than antennomere 4 (Figures 1(a)–2(a)).

Pronotum transverse, ca. 1.5 times as wide as long, slightly narrowing anteriorly, with almost straight anterior margin, noticeable anterior, and small acute posterior angles. Scutellum parallel-sided and medially emarginate at apex (Figure 1(a)).

Elytra elongate, 3.3 times as long as wide at humeri, narrowing distally, dehiscent in distal two fifths, with two primary costae reaching their apices and costa 1 noticeable in proximal fourth (Figure 1(a)).

Legs are relatively short, tibiae subequal in length to femurs (Figures 1(a), 1(b)).

Length (from anterior head margin to end of abdomen): 3.3 mm. Width (humerally): 0.7 mm.

Female. Unknown.

Etymology. The name of the new species is derived from "avus," the Latin noun for "grandfather," alluding to its hypothetic ancestry to some of the extant Greater Antillean lycids.

Diagnosis. Electropteron avus **sp.n.**, the only known representative of the genus, is easily distinguishable from the described extant lycids, as well as from the Baltic Amber lycid taxa, by the generic characters.

3. Discussion

Electropteron avus **gen.n., sp.n.**, which is evidently close to some of the recent Leptolycini from Hispaniola and Puerto Rico, is tentatively attributed to the same tribe, although the tribe itself with the unusually wide range of morphologies of its members [5–7] may well prove to represent several independent lineages. The tribe Leptolycini is confined to Central America, Greater Antilles, and mostly northern part of South America. It is one of those enigmatic groups of net-winged beetles where females are not known and the pupa phase is presumably absent in female development [8]. The discovery of a representative of this group in the ca. 30-million-year-old Dominican Amber, actually in the area of the current distribution of its close relatives, gives further clues for the reconstruction of the history and phylogenetics of the family.

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Review Article

Ants as Indicators in Brazil: A Review with Suggestions to Improve the Use of Ants in Environmental Monitoring Programs

Carla R. Ribas,[1] Renata B. F. Campos,[2] Fernando A. Schmidt,[3] and Ricardo R. C. Solar[3]

[1] *Laboratory of Ant Ecology, Sector of Ecology, Department of Biology, Federal University of Lavras, 37200-000 Lavras, MG, Brazil*
[2] *Institute of Education of Divinópolis, Educational Foundation of Divinópolis, 35501-170 Divinópolis, MG, Brazil*
[3] *Post-Graduate Program in Entomology, Department of Entomology, Federal University of Viçosa, 36570-000, Viçosa, MG, Brazil*

Correspondence should be addressed to Carla R. Ribas, crribas@gmail.com

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We describe the use of ants as indicators in Brazil, based on a critical review of published articles. The analysis of fifty-eight papers, encompassing a range of almost 25 years, indicates an increased number of studies using ants as indicators in the last decade. Among the parameters analyzed in the papers, species composition is the most suitable to evaluate the effect of the disturbance on ant communities. The use of other metrics that consider the specificity and fidelity (e.g., IndVal index) of ant species to a level or state of disturbance is also highly desirable. We discuss several alternative ways of overcoming many of the drawbacks related to the robustness of the results and to reduce the financial, logistic, and time costs involved with the use of ants as indicators in monitoring programs. By doing so, we expect to encourage new research on ants as bioindicators as well as to summarize current knowledge, facilitating further research.

1. Introduction

Intensive exploitation of natural resources and the resulting impacts on pristine habitats have led to calls from the scientific community and the general public to measure or monitor the level of these environmental impacts [1–3]. Bioindicators are a useful way to evaluate such impacts, since changes in their population dynamics or community parameters can indicate an environmental state more easily, quickly, and safely and with lower financial and labour inputs than direct measurements [4–6].

McGeoch [7] divided the general use of the term bioindication into three categories according to the three main applications: (i) environmental indicators: used to detect or monitor changes in the environmental state, (ii) ecological indicators: used to demonstrate the impact of an environmental stress on the biota or monitor longer-term stress-induced changes in the biota, and (iii) biodiversity indicators: used to identify the diversity of a taxa in a specified area or to monitor changes in biodiversity.

Therefore, there are several characteristics that an indicator species must have, the most notable being ease of measurement, sensitivity to environmental stress, and predictable responses to environmental stress [4, 8]. The use of certain species or groups of species as indicators of successful rehabilitation practices or for environmental monitoring has been recommended in recent years (e.g., [5, 6, 9]).

Ants have been used as a powerful tool in several ecological studies [10, 11]. This group has useful characteristics for successful indication and monitoring of environmental impacts, including widespread distribution, high abundance, importance in ecosystem functioning, ease of sampling, and relatively well-known taxonomy and ecology [12].

Thus, ants have been used as indicators of several environmental impacts, such as fire, deforestation and logging, agricultural intensification, mining, and urbanization [13, 14]. The first study suggesting the use of ants as indicators was in the early 1980s [15], and the use of ants as indicators is now widespread in Australia (e.g., [16–19]) and is becoming

a major focus of myrmecological research worldwide (e.g., [20–25]).

Although ants are a simple, cheap, and powerful indicator of environmental impacts and rehabilitation (e.g., in Australia [17]), in Brazil, a country which harbours enormous diversity and complexity of habitats, the standard use of ants as indicators is still relatively new and should be evaluated in greater detail (see [26, 27]). According to Philpott et al. [14] and Gardner [6], a critical need is the selection of ant species that are affected by distinct types of disturbance in different regions, in order to guarantee their usefulness as good indicators.

Therefore, as we described above, given the international use of ants as indicators, several studies have investigated the use of ants as indicators in Brazil. In order to describe the background of bioindication with ants in Brazil, we carried out a critical review of several studies concerned directly or indirectly with the use of ants as indicators.

Using the three categories proposed by McGeoch [7], environmental, ecological, or biodiversity indicators, we describe the historical development of ants as indicators and evaluate the implications of these studies. Additionally, we highlight ways of overcoming the major challenges to the widespread use of ants as tools in environmental monitoring programs.

2. Methods

We searched for papers regarding ants as indicators, restricting our search to those carried out in Brazil. To encompass a broad time range of papers, we used the following keywords in Portuguese and English, respectively: "formiga," "ant," "indicador," "bioindicador," "indicator," "bioindicator," "Brasil," "Brazil," and the combination of the words cited above in the Scielo and in the ISI Web of Knowledge websites. We also used papers from our personal archives, gathered under several keywords.

In all papers, we accessed the following information: the language, the general idea of the paper (i.e., descriptive, a general survey, a test of correlations or hypotheses), if the paper specifically analyzed ants as indicators; the aims; the ant sampling methodology, the parameters of ant fauna which were analyzed (i.e., diversity, composition, population dynamics), the environmental parameters which were observed, the results which were obtained, and the main conclusion reached in the study.

We define the paper as specifically analyzing ants as indicators if it explicitly declared this intention in the aim or the introduction (Explicit indication papers). However, if this criteria was not clear but the article still analyzed ants as indicators, we defined these as "Implicit indication papers." Papers in which the major aim was not the use of ants as bioindicators, but which presented results that could Potentially enable the use of ants as indicators, were considered as "Potential indication papers." Finally, papers that did not meet any of the above criteria, that is, did not mention in any way the use of ants as indicators, or with results that could not be used to evaluate ants as indicators, were considered as "Indirect bioindication papers."

The disturbances or aims investigated in the papers were split into the categories "Agriculture," "Vegetation type," and "Human land-use," according to the habitats studied, namely: habitats with agricultural activities only, habitats with natural vegetation only, and habitats with both agricultural activities and natural habitats. Similarly, "Succession" studies were those investigating natural succession, and "Restoration" studies were those evaluating different rehabilitation techniques, such as succession following managed restoration efforts.

We used McGeoch [7] as a reference to decide if the ants were used as environmental, ecological, or biodiversity indicators in the reviewed papers (see McGeoch's [7] definition in the introduction section). Moreover, we defined ant species as indicators when there was a species list in the paper showing the occurrence of ants in specific sites or when the author considered the ant species to be an indicator elsewhere in the paper. If the ant species occurred in just one habitat, we considered the species to be an indicator of the specific habitat.

We verified the most frequent responses of ants to disturbance, summarizing responses, and relating the most frequent responses to the most frequently used sampling methodologies to determine if there were any trends. To study this relationship, we considered only methodologies that had been used in at least three papers.

3. Results

We analyzed 58 papers, which encompassed a span of almost 25 years (from 1987 to 2010). Among the papers, only one was not classed as an "indication paper" or "Potential indication paper" [83]. The others specifically mentioned the intention to use ants as indicators (either explicitly, using the word "indicator," or implicitly, using ants as a tool or model to indicate the ecological and environmental parameters) (38 papers) or at least have the Potential to do so (17 papers) (Table 1). Two papers [84, 85] were not included in the table because the scope of the papers was not to analyse ants as indicators but to suggest new tools to simplify their use as indicators.

From the 58 papers, exactly half (29) were published in English and the other half in Portuguese. Among the "Potential indication papers," 11 were published in English and six in Portuguese, while among the "indication papers" the number of papers written in Portuguese (22) was higher than the papers written in English (18).

Papers directly concerned with the use of ants as bioindicators began almost 10 years after the development of "Potential indication papers", in which the main focus was the response of ant communities to several disturbances (e.g., logging and land use). Only in the last decade has there been a positive trend of papers using ants as model organisms for bioindication in Brazil (Figure 1).

Regarding ant sampling procedures, 34 studies used only a single sampling method: 14 used two, six used three, and four opted for more than three methods. The methodologies used to capture ants were baits, beating, Berlese extraction,

Table 1: Papers reviewed regarding ants as indicators in Brazil, indicating the main disturbance investigated (Disturbance), the aim of the paper (Aim), if the paper was explicitly, implicitly or has the Potential to be used in indicator studies (Indication), the environmental parameters analyzed/sampled (Environmental parameters), the responses of ant community to disturbance that were found to be significant (Effects on ant community), the indicator type used (Indicator type) and the paper (Reference).

Disturbance	Aim	Indication	Environmental parameters	Effects on ant community	Indicator type	Reference
Agriculture	Evaluate the effect of different soil tillage and crop management systems on soil fauna groups	Yes (implicit)	Soil tillage and crop management systems	Change in species dominance (discriminant and correspondence analysis)	Environmental	Baretta et al. [28]
Agriculture	Evaluate the ant diversity in fig crops under different managements	Yes	Types of soil cover plants	Change in density of species ($P < 0.05$, Tukey test)	Environmental	Merlim et al. [29]
Agriculture (forestry practices in *Eucalyptus*)	Use the ant guild concept to evaluate changes in *Eucalyptus* plantations following control of leaf-cutting ants	Yes	Forestry practices	Change in species composition—observed frequency of species and guilds (non-statistical test)	Environmental	Lacau et al. [30]
Agriculture (preceded by deforestation and fire)	Assess the recolonization by fauna in areas cleared and burned to plant corn and beans	Yes	Human land-use and resting time	Increase in abundance in the less-disturbed areas (non-statistical test)	Environmental	Nunes et al. [31]
Agriculture (formicid granulated baits)	Evaluate the effect of different applications of formicide baits on nontarget ant community	Yes	Forms and timing of application of formicid-granulated baits	No effect of bait type on ant species richness ($P > 0.05$, ANOVA) Reduction in species richness observed only in control method, systematic application being more harmful ($P < 0.05$, ANOVA)	Environmental	Ramos et al. [32]
Anthropogenic activities	Quantify heavy metals in worker ants of *Camponotus rufipes* collected in different environments	Yes	Observed human interference	Three groups of ants with different heavy metal concentrations (PCA analyses)	Environmental	Silva et al. [33]
Conservation status	Create an inventory of epigaeic ant species that occur in vine forest and use them to indicate the level of conservation of this ecosystem	Yes	None	Inventory (nonstatistical test)	—	Carvalho et al. [34]
Conservation status	Verify the impact of human use in mangroves	Potential	Observed levels of human use	Reduction on species richness ($R^2 = 0.53$, $P = 0.007$)	Environmental	Delabie et al. [35]
Conservation status	Inventory the ant community in the Baturité hills	Yes	None	Inventory (nonstatistical test)*	—	Hites et al. [36]
Conservation status	Study the ant communities in preserved and impacted savanna sites	Yes	Observed human interference	Reduction of diversity in impacted sites ($F = 101.62$, $P < 0.0001$)*	Environmental	Ramos et al. [37]
Fire	Test the negative effect of fire in *Restinga* environments on the ant community	Potential	Presence of fire	Increase in species richness with presence of fire (mean and confidence intervals of estimated species richness)	Environmental	Endringer et al. [38]

Table 1: Continued.

Disturbance	Aim	Indication	Environmental parameters	Effects on ant community	Indicator type	Reference
Fire	Test the hypothesis that ant species richness and composition change after burning sand dunes	Yes	History of fire	More ant species and distinct species composition in the unburned area (non-statistical test)	Environmental	Teixeira et al. [39]
Fragmentation	Verify the responses of ants nesting in twigs in the litter layer to habitat changes associated with forest fragmentation	Potential	Distance to forest edge, remnant isolation, leaf-litter depth, density of dead twigs, and vegetation (three parameters measured)	Higher species richness ($F = 8.56,\ P = 0.006$); most ant species had greater nest densities in continuous areas than in remnants, change in species composition ($F = 8.14,\ P = 0.001$) with forest edge	Ecological	Carvalho and Vasconcelos [40]
Fragmentation	Determine the effect of forest fragmentation on ant communities	Yes	Remnant area, distance to forest edge, vegetation cover of matrix, and vegetation (three parameters measured)	No effect of many fragment characteristics on ant species richness: area ($F = 8.22,\ P = 0.77$), distance core-border ($F = 64.86,\ P = 0.42$). Only tree density had an effect ($F = 46.30,\ R^2 = 23.32,\ P = 0.02$)	Ecological	Gomes et al. [41]
Fragmentation	Know the community of ants in forest fragments	Yes	Remnant area	No change in species richness with remnant area ($R^2 = 0.02,\ F = 0.22,\ P = 0.64$)*	Environmental	Santos et al. [42]
Forestry systems	Describe the epigaeic ant communities in *Eucalyptus* plantations	Yes	*Eucalyptus* age	No change in species richness with *Eucalyptus* age ($P = 0.58$)	Environmental	Fonseca and Diehl [43]
Human land-use	Compare the ant community structure between a crop and a secondary forest	Potential	Land use	Reduction of diversity and equitability and change in species composition (non-statistical test)	Environmental	Castro and Queiroz [44]
Human land-use	Compare the impact of different agroecosystems on ant species richness	Yes	Land use	Higher species richness in forest edges and pasture (non-statistical test); coffee crop presented reduced estimated richness ($h = 10.85,\ P > 0.05$)	Environmental	Dias et al. [45]
Human land-use	Survey of ant and termite fauna in four patches with different vegetation structures and in one open field	Potential	Land use	Change in species richness and composition (non-statistical test)	Environmental	Diehl et al. [46]
Human land-use	Test the effects of *Restinga* soil characteristics on ant communities	Yes (implicit)	Land use, physical and chemical soil properties, and microbial activity	Change in species richness (non-statistical test) and composition (canonical correspondence analysis)	Ecological	Gomes et al. [47]
Human land-use	Elucidate ant species richness and community structure associated with the micro basin of Sanga Caramuru-Chapecó	Yes	Habitat type, temperature, and rainfall	Change in species composition (Bray-Curtis Cluster Analysis indicated higher similarity for disturbed areas) and higher richness (observed and estimated) in the native area (sample-based accumulation curves)*	Environmental	Ilha et al. [48]

Table 1: Continued.

Disturbance	Aim	Indication	Environmental parameters	Effects on ant community	Indicator type	Reference
Human land-use	Determine the level of similarity of ant communities in forest areas (three native forest remnants) and an *Eucalyptus* reforestation	Yes	Land use	Change in species composition (Jaccard index—$C_J = 0.29 \pm 0.02$ among *Eucalyptus* crops *versus* forest remnants and $C_J = 0.40 \pm 0.06$ among forest remnants)	Environmental	Lapola and Fowler [49]
Human land-use	To inventory the ant fauna in a Cerrado area and in *Eucalyptus* plantations with five classes of understory ages	Yes	Eucalypt age	Higher density of species in Cerrado areas than in *Eucalyptus* (non-statistical test) and estimated species richness similar between areas ($h = 1.6$, $P > 0.05$)	Environmental	Marinho et al. [50]
Human land-use	Investigate the effect of structural characteristics of the environment on ant communities	Yes	Habitat type	Change in species richness and composition (non-statistical test)	Environmental	Santana-Reis and Santos [51]
Human land-use	Test the hypotheses that there was a decrease in ant species richness and a change in the species composition in habitats with more intense soil use	Yes	Land use	Sites with distinct soil use host a differential ant species composition (cluster analysis-Euclidean distance)	Environmental	Schmidt and Diehl [52]
Human land-use	Evaluate the effect of collection time (day and night) on ant fauna attracted to baits in areas of *Eucalyptus cloeziana* (Myrtaceae) and Cerrado (savanna vegetation)	Potential	Land use	Collection time effect was more important to ant fauna structure than the vegetation effect (ordination analyses)	Environmental	Tavares et al. [53]
Human land-use and succession	Compare ant diversity under different land-use systems	Yes	Land use and age of succession	Change in density of species (non-statistical test), species richness (sample-based accumulation curves and $\chi 2$), and composition (cluster analysis)*	Environmental	Braga et al. [54]
Inundation	Document the ant fauna in three different forest types (one annually inundated and two on terra firme)	Potential	Vegetation (several parameters measured)	Change in diversity, similarity, and proportion of different nesting and feeding habitats (non-statistical test)	Ecological	Majer and Delabie [55]
Logging	Test the hypothesis that logging affects forest ant fauna by reducing the species richness and changing the composition of ground-foraging ant communities	Yes	Canopy openness, abundance of understory vegetation, and leaf-litter depth	Change in species composition proportion of *Pheidole* was reduced from 21.4% and 26% in unlogged forest and low-impact logging, respectively, to 14.8% in high-impact logging ($F = 4.99$, $P < 0.05$)	Ecological	Kalif et al. [56]

Table 1: Continued.

Disturbance	Aim	Indication	Environmental parameters	Effects on ant community	Indicator type	Reference
Mining	Determine the levels of heavy metals in plants and identify soil organisms of the mesofauna that could be biological indicators of soil quality	Yes	Physical and chemical soil properties and heavy metal content	Decrease in abundance and increase in lead (Pb) accumulation (non-statistical test)	Environmental	Barros et al. [57]
Mining	Ant fauna survey and community structure, analyses of the ground-dwelling ants in native vegetation and areas with different inferred copper levels	Yes	Areas with different inferred copper levels	Decrease in species richness with inferred copper levels (non-statistical test)	Environmental	Diehl et al. [58]
Restoration (agriculture)	Investigate the recolonization profile of the restored Atlantic Forest	Yes	Age after planting	Increase in species richness ($P < 0.05$, ANOVA) and change in species composition (ANOSIM, $P < 0.01$)*	Environmental	Pais and Varanda [59]
Restoration (anthropogenic disturbance)	Test the hypothesis that ant fauna is closely related to the structural complexity of habitat	Yes	Age of restoration	Change in species composition (non-statistical test)	Environmental	Coelho et al. [60]
Restoration (dredging disturbance)	Evaluating ant bioindication of impacted habitats	Yes	Time since restoration, distance from the impact, and physical properties of soil	Change in species richness: higher in cerrado than in the restoration habitats, and also higher in the ecotone and intermediate zones than on the beach ($F = 3.95$, $P < 0.05$) and change in abundance ($F = 1.9$, $P < 0.046$) and composition (non-statistical test)	Environmental	Costa et al. [61]
Restoration (mining)	Investigate which ants recolonized reclaimed areas in subtropical regions and evaluate the effect of different rehabilitation techniques, comparing results with Australia	Yes	Age of rehabilitation, soil penetrability, number of logs, litter and vegetation measures (three and five parameter, resp.)	Increase in species richness (non-statistical test) and change in composition (PCoA)	Ecological	Majer [62]
Restoration (mining)	Evaluate the efficacy of rehabilitation procedures in mining sites on facilitating ant recolonization and compare it with other tropical regions and climatic zones	Yes	Age of restoration, soil penetrability, litter depth, percentage of litter, grass, and herb cover, and vegetation (several parameters measured)	Species richness increased in early ages but slowed in late ages and was smaller than control site (non-statistical test). Distinct species composition in sites at early ages, intermediate ages, and control sites (ordination analyses)	Ecological	Majer [63]
Restoration (mining)	Investigate the community structure changes of different rehabilitation techniques	Yes	Rehabilitation technique	Change in species richness (non-statistical test) and composition (cluster analysis)	Ecological	Pereira et al. [64]
Road	Test the hypothesis that dirt roads are favourable landing sites for *Atta laevigata* founding queens. Analyze the importance of litter cover as a proximate cue in nest-site selection	Potential	Presence of dirt roads	The number of colonization attempts in roads was 5 to 10 times greater than that in the adjacent vegetation ($P < 0.001$)	Environmental	Vasconcelos et al. [65]

Table 1: Continued.

Disturbance	Aim	Indication	Environmental parameters	Effects on ant community	Indicator type	Reference
Seasonality	Investigate ant diversity and species composition on an island	Yes	Seasonality	Change in species richness and composition with seasonality (non-statistical test)*	Environmental	Schmidt et al. [66]
Succession	Examine whether secondary forests of the Brazilian Atlantic Forest act as refugia for forest-adapted species	Yes (implicit)	Age of succession and soil type	Richness ($P < 0.001$) and composition ($P < 0.004$) of ant assemblages in secondary forests have recovered slowly and have not approached conditions typical to old-growth forests	Environmental	Bihn et al. [67]
Succession	Examine bait preferences of litter ants along a successional gradient of forest	Potential	Age of succession	Preference of ants for the type of bait changed along the successional gradient ($F = 5.52,\ P = 0.02$). In young successional stages, N baits attracted more ants than CHO baits, whereas in late successional stages, CHO baits attracted more ants	Environmental	Bihn et al. [68]
Succession	Investigate how functional diversity profile changed in a successional gradient	Potential	Age of succession	Increased diversity and change in functional groups (non-statistical test)	Environmental	Bihn et al. [69]
Succession	Verify patterns in the structure of ant communities along a successional gradient	Potential	Age of succession	Increased diversity and equitability (non-statistical test)	Environmental	Castro et al. [70]
Succession	Compare ant diversity among sites in different successional stages	Potential	Age of succession	Higher diversity in intermediary stage and change in composition (non-statistical test)	Environmental	Leal et al. [71]
Succession	Compare the diversity and composition of tree-dwelling ants in different successional stages of a seasonal deciduous forest	Potential	Age of succession	Increase in species abundance ($F = 9.26,\ P = 0.003$) and change in species composition (PCA analysis)	Environmental	Neves et al. [72]
Succession	Compare the ant species diversity related to successional stage and seasonality	Yes	Age of succession, tree richness and density	Change in species composition (DCA deterrent correspondence analysis, $P < 0.001$)	Ecological	Neves et al. [73]
Succession	Evaluate the long-term effect of fire on ant species richness	Potential	Presence of fire 15 years before	Change in species composition (cluster analyses-Euclidean distance)	Environmental	Santos et al. [74]
Succession	Assess the changes in species richness and composition between relatively pristine habitat and along a forest regeneration gradient	Yes	Age of succession	Increase in species richness (sample-based accumulation curves) and distinct species composition between pristine area and areas at regeneration (ANOSIM, $R = 0.79,\ P < 0.001$)*	Environmental	Silva et al. [75]
Succession	Compare the structure of the ground ant communities in areas at different levels of restoration	Yes	Age of succession	Increase in species richness ($F = 5.1,\ P = 0.01$) and decrease in abundance ($F = 8.1,\ P < 0.001$), change in species composition (ordination analysis)	Environmental	Vasconcelos [76]

Table 1: Continued.

Disturbance	Aim	Indication	Environmental parameters	Effects on ant community	Indicator type	Reference
Succession	Determine experimentally the effects of selective logging on ground-living ants	Yes	Logging age, canopy cover, litter depth, and understory density	Species richness, evenness, and abundance per plot did not vary among treatments ($P > 0.05$). Most of the species found in the control plots were also present in the logged plots	Ecological	Vasconcelos et al. [77]
Urbanization	Compare the thermal tolerances of leaf-cutter ants (*Atta sexdens*) from colonies inside and outside an urban area	Potential	Temperature	Urban ants support higher temperatures better than rural ones, which present higher rates of mortality ($\beta = 20.54.237,\ P = 0.02$)	Environmental	Angilletta et al. [78]
Vegetation type	Inventory ants	Yes	Habitat type	Change in species richness (non-statistical test)*	Environmental	Diehl et al. [79]
Vegetation type	Test how the diversity of one taxa can be a good surrogate of all diversity	Yes	Habitat type	Correlation with other taxa (Pearson correlation coefficients)	Biodiversity	Leal et al. [80]
Vegetation type	Compare ant diversity in three different forest stages (primary, reforestation, and secondary)	Potential	Habitat type	Change in diversity and exclusive species (non-statistical test)*	Environmental	Lopes et al. [81]
Vegetation type	Compare the ant fauna from forests and nearby patches of savanna (Cerrado) in the Brazilian Amazon. Assess whether there is a difference in the fauna between the ground and lower vegetation strata in both habitats	Potential	Habitat type	Forests host twice as many species as savanna (sample-based rarefaction curves). In both habitats, the ground hosted more species than vegetation ($P < 0.005$). Distinct species composition between forest and savanna and between ground and vegetation within the same habitat; ant species fidelity and specificity is given by IndVal (see Table 2)	Environmental	Vasconcelos and Vilhena [82]

* papers with sample-based accumulation curves.

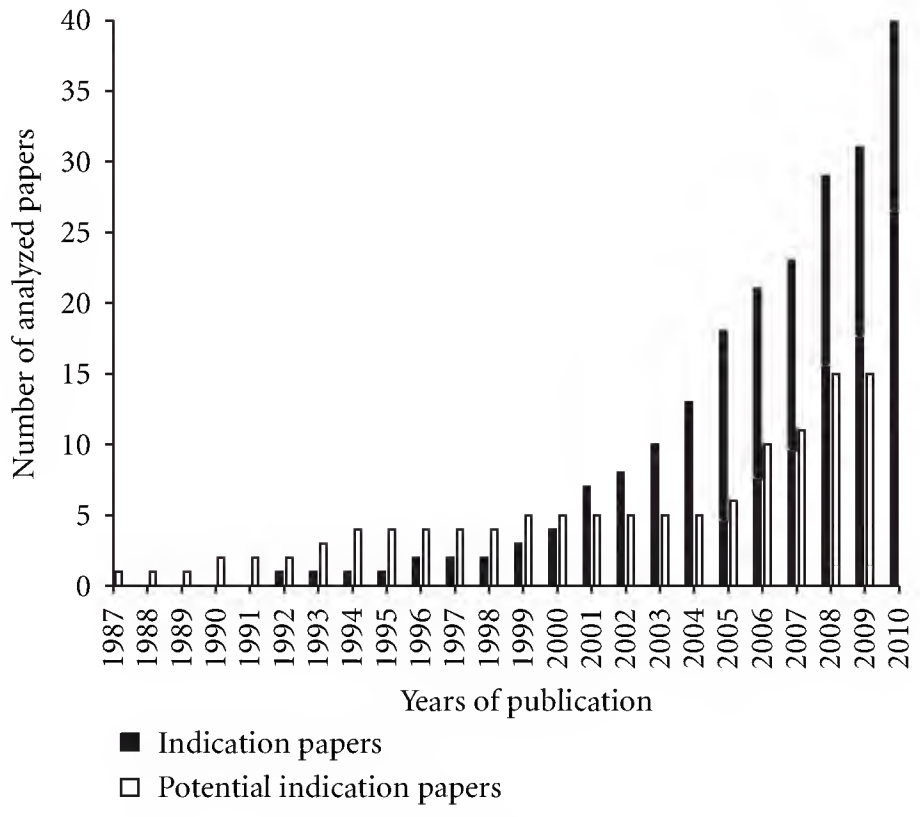

FIGURE 1: Trend of the number of analyzed papers regarding ants as indicators in Brazil. Indication papers—paper specifies (explicitly or implicitly) the intention of analyzing ants as indicators in the aim or introduction. Potential indication paper—the above criteria was not met, but the paper presents results that could potentially enable the use of ants as indicators.

hand collecting, pitfall traps, sweeping, Tretzel traps and Winkler's extractors. Among these methodologies, the most commonly used were baits (used in 26 studies), followed by hand collecting, pitfall traps and Winkler's extractors (used in 20 studies each), and Berlese extraction (used in five studies).

The majority of studies sampled ants at the soil surface (44), but some studies also considered the soil surface together with other habitats, including litter (10), vegetation (7), combination of the above (6). Some other studies did not sample ants at the soil surface, but only in the litter (11), vegetation (two), or in twigs (one), respectively.

The main impacts studied were succession (12), human land-use (11), restoration (6), and agriculture (5). Just a few papers (13) analyzed other environmental parameters besides disturbance (Table 1).

The parameters of the ant faunas that were most commonly related to the disturbance type were ant species richness or diversity indexes (42) and species composition (35) (Table 1). In these papers, if we considered only those that analyzed ant species diversity and composition rigorously (i.e., with statistical tests), the actual number of papers that analyzed ant species diversity decreased to 28, and those that analyzed ant species composition dropped to 22.

Regarding species composition, in 33 papers this parameter was sensitive to disturbance, although if we considered only those papers with statistical analyses, the number decreases to 21. Summarizing the papers that analyze species richness or diversity, the responses found were species richness or diversity increased with disturbance (1), decreased with disturbance (18), changed with disturbance (when there is any clear trend in the response of ants to disturbance) (11), and not affected by disturbance (12). If we considered only papers that tested ant species richness or diversity statistically, the numbers changed to increase with disturbance (1), decrease with disturbance (11), change with disturbance (5), and not affected by disturbance (11).

By connecting the main responses found in the papers (ant species richness, diversity, or ant species composition) to the main methodologies used to sample ants, we can verify some trends (Figure 2). First, species composition was sensitive to disturbance in the majority of papers in which this parameter was tested, irrespective of the sampling methodology, namely, baits plus hand collecting, multiple sampling methods, or pitfall traps. Second, most papers that analyzed species richness or diversity showed that these metrics were also responsive to disturbance, although the sole use of baits or the Winkler did not show any trend, while only using pitfall traps revealed a positive response of ant species richness or diversity to disturbance. Nevertheless, when we considered only those papers with statistical tests (Figure 3) or without statistical tests (Figure 4), the trend for species composition remained the same, but for species richness the use of multiple methods to sample ants showed a higher number of responses to disturbance.

The ants were used as environmental indicators in the majority of studies (42 out of 55) but were also used as ecological indicators (10 papers) and as biodiversity indicators in only one paper. In 20 papers there was a species list, and; therefore, we could determine some of the ant species that served as indicators of certain habitats. The parameters used in the papers to define a species as an indicator were frequency of ant occurrence (11 papers), presence or absence of ant species (8 papers), and the indicator value (IndVal) (1 paper). Irrespective of the parameter used by the authors, 187 ant species were defined as indicators and linked to specific habitats (Table 2). The genera with higher numbers of indicator species were *Camponotus* (18), *Pseudomyrmex* (12), *Pachycondyla* (11), *Ectatomma* (9), *Gnamptogenys* (9), *Acromyrmex* (8), and *Cephalotes* (8). The sites with the most indicator species were forest (39 species), *Eucalyptus* (37), savanna (34), control or undisturbed sites (nonburnt) (29), primary forest (25), early succession sites (19), disturbed sites (15), secondary forest (14), intermediate succession sites (13), burnt sites, low human land-use-impacted sites and pasture (9), late succession (8), and strong human land-use-impacted sites (5).

4. Discussion

It has been possible to determine the history of research carried out in Brazil by searching for the use of ants as indicators over the last 25 years (Figure 1). From 1987 to 1991, there were only "Potential indication papers." In 1992 the first "Indication papers" were published, which increased in the following years and exceeded the "Potential indication papers" in 2001.

Regarding the idiom of the papers, it is interesting to observe that half of the papers are still published in Portuguese. In spite of the growing internationalization of Brazilian

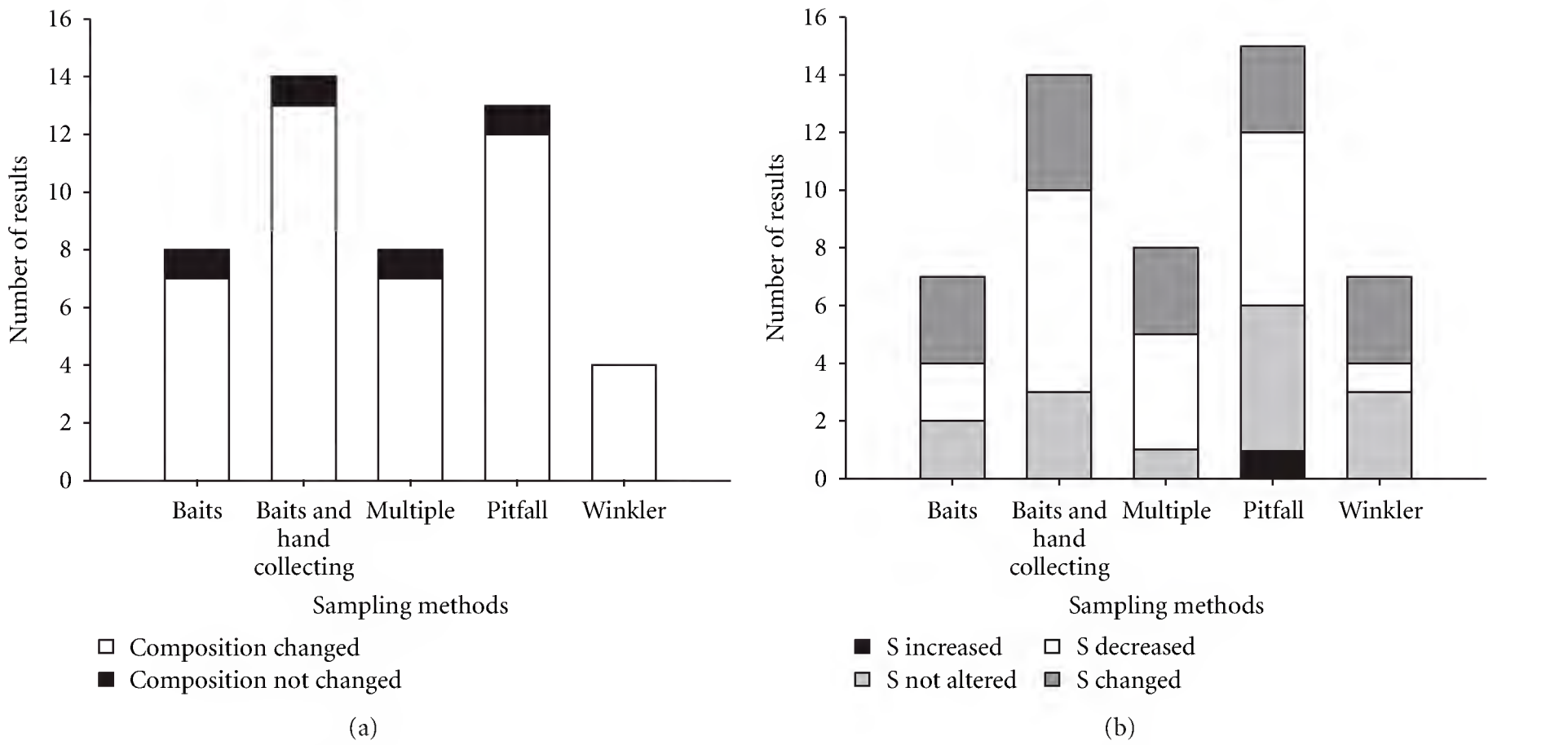

FIGURE 2: Papers that analyzed (a) ant species composition and/or (b) ant species richness/diversity with and without statistical tests and their responses to habitat disturbance through the use of different ant sampling methodologies. Composition changed—species composition altered by disturbance. Composition not changed—species composition not altered by disturbance. S increased—species richness or diversity increased with disturbance. S not altered—species richness or diversity not affected by disturbance. S decreased—species richness or diversity decreased with disturbance. S changed—species richness or diversity changed with disturbance when there is any clear trend in the response of ants to disturbance.

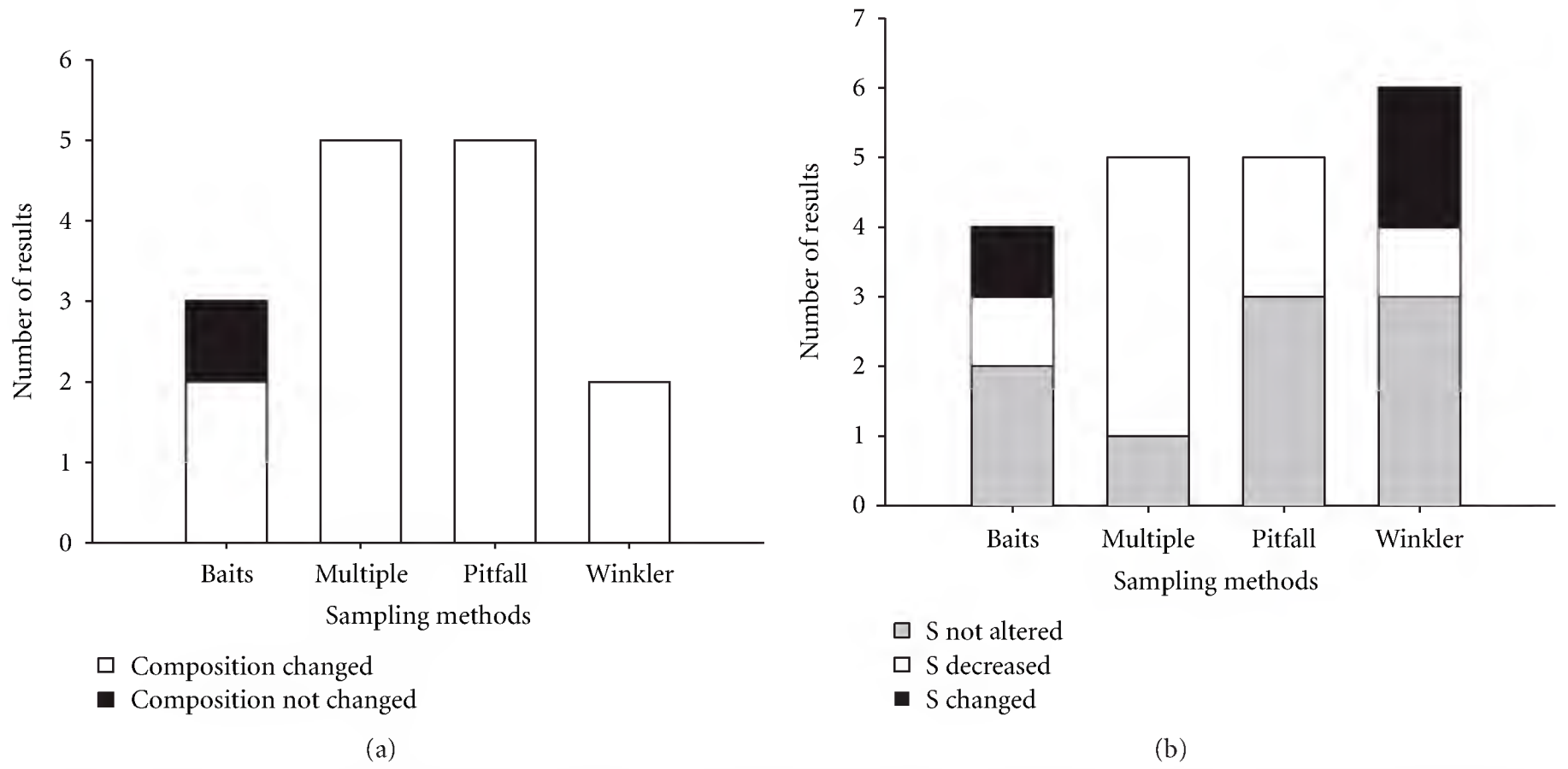

FIGURE 3: Papers that analyzed (a) ant species composition and/or (b) ant species richness/diversity with statistical tests and their responses to habitat disturbance through the use of different ant sampling methodologies. Composition changed—species composition altered by disturbance. Composition not changed—species composition not altered by disturbance. S increased—species richness or diversity increased with disturbance. S not altered—species richness or diversity not affected by disturbance. S decreased—species richness or diversity decreased with disturbance. S changed—species richness or diversity changed with disturbance when there is any clear trend in the response of ants to disturbance.

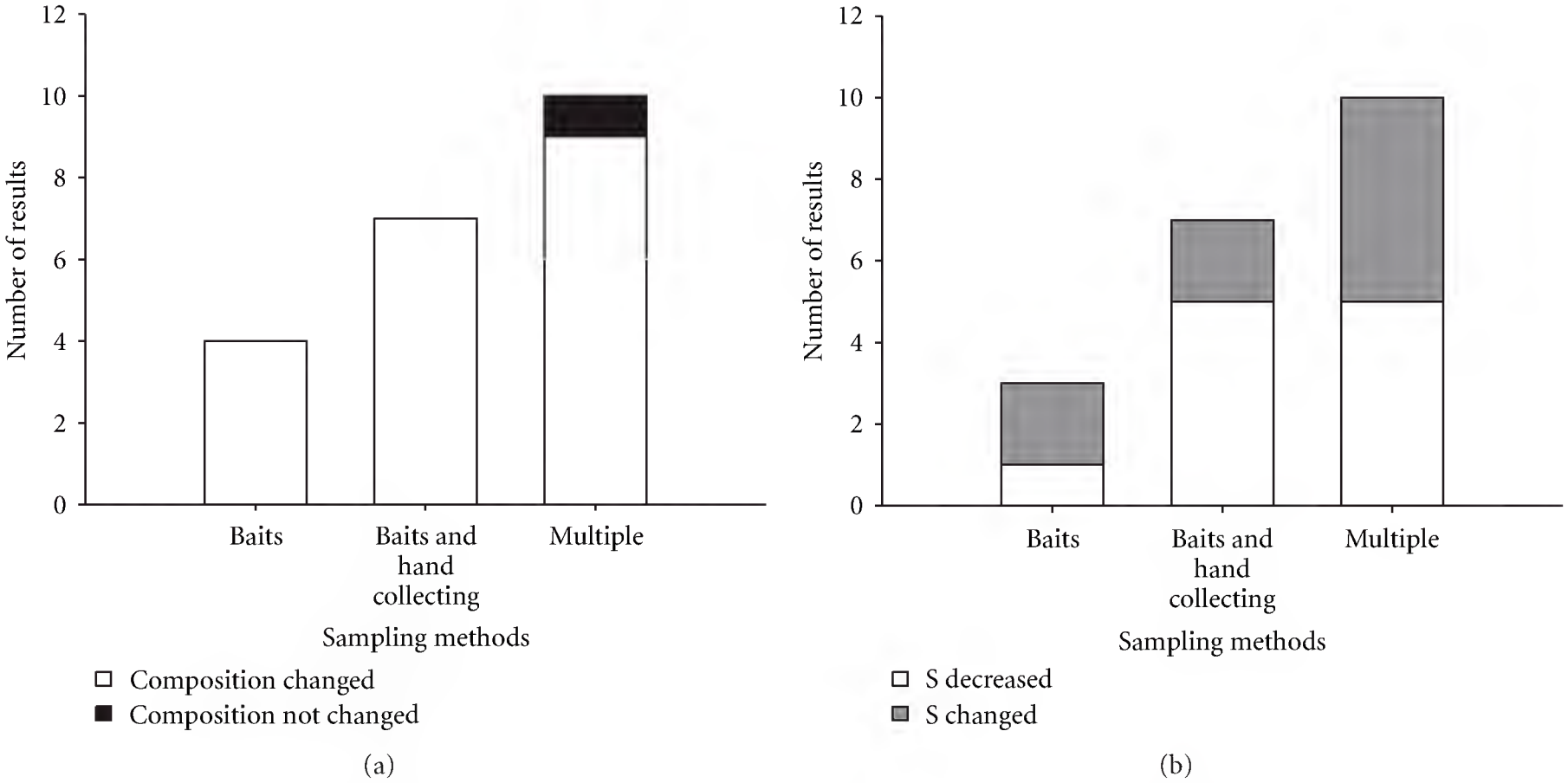

FIGURE 4: Papers that analyzed (a) ant species composition and/or (b) ant species richness/diversity without statistical tests and their responses to habitat disturbance through the use of different ant sampling methodologies. Composition changed—species composition altered by disturbance. Composition not changed—species composition not altered by disturbance. S increased—species richness or diversity increased with disturbance. S not altered—species richness or diversity not affected by disturbance. S decreased—species richness or diversity decreased with disturbance. S changed—species richness or diversity changed with disturbance when there is any clear trend in the response of ants to disturbance.

research [86, 87], many Brazilian studies that use ants as bioindicators cannot have an international impact since they are in Portuguese. We determined at least two main reasons for this. The first is the "*publish or perish*" policy in Brazilian (and worldwide) science, which demands the publication of as many papers as possible in the shortest feasible time span, in which case publishing in Portuguese can be a way to speed up publication time. The second explanation may be that, due to problems with the style of writing of the papers, many international journals reject Brazilian papers. Despite these two issues, in this historical scenario, there is an improving and maturing of bioindication studies using ants, which is shown by the explicit use of the term "indication" in these papers. Furthermore, the increasing knowledge exchange with researchers from other countries reinforces the maturation of this area of research. Examples include Brazilian scientists that complete their Ph.D. studies abroad the possibility for doctorate students to undertake international exchange programs, and the internationalization of the Brazilian Symposium of Myrmecology.

However, it is important to clarify that although some authors explicitly used the term indicator in the introduction or in the aim of their papers (our criteria defined these papers as "Indication papers"), the authors did not always in reality use ants as indicators, either because they did not sample properly (i.e., sampling in just one habitat, without different levels of the disturbance/restoration and control sites) or because they did not analyze their results rigorously (i.e., did not include a satisfactory statistical analysis). Conversely, some authors did not use the term indicator in their papers, but they did test the Potential use of ants as indicators, and were cautious in the above points.

The majority of articles that used ants as environmental indicators (sensu [7]) may be due to the fact that this is the simplest way to detect a change in the environmental state of the habitat but not necessarily the best one. The use of ecological indicators has the advantage of encompassing a broad response as they demonstrate the disturbance effect on the biota, not only for ants [6].

Moreover, the sampling of different environmental parameters and their correlation with the biota is essential, because their inclusion increases the predictive power of the study. If we recognize the environmental parameters that are most sensitive to disturbance and their effect on the biota, we may be able to more accurately monitor the effects of disturbance. Consequently, we may be able to choose the restoration effort according to the most appropriate or effective environmental parameters in order to promote the recovery of the biota [6, 7].

Regarding the number of ant sampling techniques used, although the majority of the papers used only one method, several studies (e.g., [52, 77, 88]) have highlighted the fact that ant communities show a pronounced vertical stratification, and ant faunas specific to each microhabitat may present specific ecological traits and distinct sensitivity to the same environmental impact [67, 89–91]; therefore, more than one sampling method must be considered [13]. On the other hand, the use of several sampling methods increases the financial costs and the time needed to collect, sort, and process the data [13]. Thus, since environmental monitoring

programs usually have short-term goals, it is desirable to balance the benefits and costs of using several types of sampling methods compared to using only one sampling method which could achieve similar reliable results (Figures 2(a) and 2(b)), compare multiple sampling and pitfall outcomes) about the patterns and aims under investigation.

The most used sampling method in the studies was attractive baits, which are more suitable for behavioural questions [92] and are useful for verifying the presence and population trends of invasive and keystone ant species [13]. However, this sampling method results in biased information about ant diversity (e.g., species richness and composition) because many ants have selective diets, and some ants can dominate the baits to the exclusion of a broad range of other ant species [92]. This notion concerning the use of baits in bioindication papers is confirmed in Figures 3(a) and 3(b), which shows that the sole use of baits revealed apparently unchanging species composition and no trend in species richness. Thus, Underwood and Fisher [13] recommend the use of pitfall traps and litter sampling (The Winkler and/or Berlese extractors) as effective ant sampling methods for monitoring goals related to the effect of habitat disturbance and transformation on ant diversity, which is corroborated in Figures 3(a), 3(b), 4(a), and 4(b).

Species richness and diversity and species composition are the parameters of ant communities most commonly analyzed in the papers. However, species richness and diversity should be used as an evaluative method with caution, since several studies have shown that these parameters were not affected by disturbance (Table 1), and only a narrow number of papers showed a trend in the response of ant species richness to disturbance (see Figures 3 and 4). This coarse relationship of species richness to disturbance is probably because ants are generalists, so the loss of some sensitive species to disturbance is compensated by the invasion of other opportunist species or more generalists. Moreover, in dynamic sites under frequent habitat transformation and disturbance, there is no change in species richness among sites at different restoration times, because perturbation events "reset" the ant community to the same stage [93].

In this way, as Hoffmann [94] has highlighted, the disturbance induced changes in species composition, but not necessarily in species richness. Moreover, the recovery of species composition takes longer than species richness [95] and has a strong relation to the vegetation structure [19, 64, 96–99], which changes with disturbance events. Thus, species composition should be a better parameter to evaluate the effect of disturbance on ant communities, even in areas with frequent perturbations, as described by Gollan et al. [93].

Using the same argument, the quantification of the relationship between each ant species and different disturbances (or level of disturbance) or habitats should be very useful, as it is important to decrease the time spent in indication studies. The general public and stakeholders need to know rapidly if the habitat is impacted or recovering, so recognizing which species can be associated positively or negatively with disturbance or restoration is a very desirable tool.

Several of the papers we analyzed described species occurring exclusively or more frequently in specific habitats (Table 2), but we are concerned with the lack of rigour with which this has been carried out in most studies (exception in [82]), as there is no control about the specificity and fidelity of these ant species and few statistical analyses to validate the results. This lack of rigour may explain why there are some ant species with contradictory patterns of occurrence, such as species being present in disturbed *versus* undisturbed sites, such as *Acromyrmex balzani*, *Camponotus trapezoideus*, *Dorymyrmex pyramicus*, *Ectatomma tuberculatum*, *Odontomachus haematodus*, and *Pseudomyrmex tenuis* (see Table 2). Moreover, these ant species might also be generalists, and the choice of better criteria should enable us to distinguish between inappropriate sampling design and truly generalist ant species. The use of the IndVal index [8], mentioned below, is one option to overcome this drawback.

The Indicator Value (IndVal) suggested by Dufrêne and Legendre [8] combines a measure of the habitat specificity of a species to a level of disturbance, or to a disturbance state, with its fidelity within that state. The random reallocation procedure of samples within sample groups can be used to test the significance of the IndVal measure for each species. The use of this method has increased (e.g., [100–105]) and has a number of advantages over other methods [6].

Some species seem to have more consistent responses to disturbance or specificity to some habitats, but this consistency is very difficult to assert due to the lack of rigour with which the ants were related to disturbance or habitats (presence or frequency of occurrence) and the lack of standardization regarding the level of disturbance in the papers. The habitats sampled in one paper may be defined as undisturbed, which may be different from the habitats studied in another paper that are defined as more degraded (or less) and should also be defined as an undisturbed habitat. In our paper (including Table 2), we used the definition of disturbed or undisturbed given by the original authors.

Thus, following the disturbance definition used by the authors, some species are present in disturbed habitats in more than one paper, and, therefore, could be indicators of disturbed habitats, such as *Atta sexdens rubropilosa*, *Camponotus crassus*, *Camponotus melanoticus*, *Camponotus novogranadensis*, *Odontomachus meinerti*, *Pachycondyla villosa*, *Pseudomyrmex termitarius*, and *Solenopsis saevissima*. In the same way, some species could be indicators of undisturbed habitats, such as *Labidus coecus*, *Pachycondyla arhuaca*, *Pachycondyla stigma*, and *Sericomymex bondari*. There are also some species that are indicators of specific habitats, such as indicators of forests (*Discothyrea sexarticulata*, *Ectatomma lugens*, *Labidus coecus*, and *Typhlomyrmex major*) and indicators of savannas (*Camponotus latangulus*, *Pheidole fimbriata*, and *Strumigenys perparva*).

One of the major mistakes related to the use of a taxon as an indicator is the personal motivation of the researchers. There are two ways of avoiding this mistake; several taxa should be rigorously tested *a priori* to select the best one [4] or studied *a posteriori* to validate the response of the indicator [7]. Very few studies have compared how different taxa, including ants, perform under different disturbances (see [89, 101, 106–109]).

[t!]

Table 2: Species of ants defined as indicators, indicating the parameter used by the authors (Parameter) when linking each ant species to each habitat type (Habitat) and the paper (Reference).

Ant species	Parameter	Habitat	Reference
Acanthognathus brevicornis	Frequency of occurrence	Secondary forest and area at early succession	Silva et al. [75]
Acanthognathus ocellatus	Frequency of occurrence	Primary forest	Silva et al. [75]
Acanthognathus rudis	Frequency of occurrence	Primary forest	Silva et al. [75]
Acanthoponera mucronata	Frequency of occurrence	Native forest remnant	Ilha et al. [48]
Acromyrmex balzani	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Presence/absence	Low human land-use-impacted sites	Delabie et al. [35]
	Presence/absence	Undisturbed sites—control site	Diehl et al. [58]
Acromyrmex coronatus	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Acromyrmex lobicornis	Presence/absence	Undisturbed sites—control site	Diehl et al. [58]
Acromyrmex lundi	Frequency of occurrence	Secondary forest	Schmidt and Diehl [52]
Acromyrmex niger	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Acromyrmex rugosus	Frequency of occurrence	*Turnera ulmifolia* field	Santana-Reis and Santos [51]
Acromyrmex striatus	Presence/absence	Undisturbed sites—control site	Diehl et al. [58]
Acromyrmex subterraneus	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Acromyrmex subterraneus brunneus	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Acromyrmex subterraneus subterraneus	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Acropyga decedens	Frequency of occurrence	Pasture	Dias et al. [45]
Amblyopone armigera	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
	Presence/absence	Savanna—cerrado sensu stricto	Marinho et al. [50]
	Frequency of occurrence	Secondary forest and area at early succession	Silva et al. [75]
Amblyopone elongata	Frequency of occurrence	Primary forest	Silva et al. [75]
Anochetus diegensis	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Anochetus mayri	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Anochetus neglectus	Frequency of occurrence	Pasture	Dias et al. [45]
Anochetus targionii	Frequency of occurrence	Pasture	Dias et al. [45]
Apterostigma acre	Frequency of occurrence	Forest fragment	Dias et al. [45]
Apterostigma bolivianum	Frequency of occurrence	Forest fragment	Dias et al. [45]
Atta robusta	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Atta sexdens rubropilosa	Presence/absence	Area at early succession	Coelho et al. [60]
	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Azteca alfari	Presence/absence	Area at late succession stage—dry season	Neves et al. [73]
Azteca muelleri	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Basiceros disciger	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Blepharidatta brasiliensis	Frequency of occurrence	Area at late succession stage	Vasconcelos [76]
Brachymyrmex coactus	Frequency of occurrence	Secondary forest and area at early succession	Silva et al. [75]
Camponotus arboreus	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Camponotus atriceps	Presence/absence	Reforestated area at intermediate succession stage	Coelho et al. [60]
Camponotus bidens	Presence/absence	Low human land-use-impacted sites	Delabie et al. [67
Camponotus burtoni	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Camponotus claviscapus	Presence/absence	Undisturbed sites	Delabie et al. [35]
Camponotus crassus	Presence/absence	Burned restinga	Endringer et al. [38]
	Frequency of occurrence	Burnt site	Teixeira et al. [39]
	Frequency of occurrence	Disturbed sites	Diehl et al. [58]
	Frequency of occurrence	Secondary forest and forest edge	Leal et al. [71]

Table 2: Continued.

Ant species	Parameter	Habitat	Reference
	IndVal	Savanna—vegetation and ground stratum	Vasconcelos and Vilhena [82]
Camponotus fastigatus	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Camponotus latangulus	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
	Presence/absence	Savanna—cerrado sensu stricto	Marinho et al. [50]
Camponotus leydigi	Frequency of occurrence	Area at early succession	Vasconcelos [76]
Camponotus melanoticus	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Frequency of occurrence	Pasture	Dias et al. [45]
	Presence/absence	Area at early succession	Coelho et al. [60]
Camponotus novogranadensis	Frequency of occurrence	Area at early succession	Vasconcelos [76]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	IndVal	Forest—vegetation and ground stratum	Vasconcelos and Vilhena [82]
	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
Camponotus punctatus minutior	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Camponotus renggeri	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Camponotus rufipes	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Camponotus sericeiventris	Frequency of occurrence	Native forest remnant	Ilha et al. [48]
Camponotus trapezoideus	Frequency of occurrence	Burnt site	Teixeira et al. [39]
	Frequency of occurrence	Forest fragment	Dias et al. [45]
	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Camponotus vitatus	Presence/absence	Low human land-use-impacted sites	Delabie et al. [35]
Camponotus westermanni	Presence/absence	Strong human land-used-impacted sites	Delabie et al. [35]
Cardiocondyla obscurior	Presence/absence	Area at intermediate succession	Coelho et al. [60]
Carebara urichi	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Cephalotes atratus	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
	IndVal	Forest—vegetation stratum	Vasconcelos and Vilhena [82]
Cephalotes grandinosus	Presence/absence	Forest	Lopes et al. [81]
Cephalotes minutus	Presence/absence	Area at early succession—dry season	Neves et al. [73]
	Presence/absence	Low human land-use-impacted sites	Delabie et al. [35]
Cephalotes pallidicephalus	Presence/absence	Low human land-use-impacted sites	Delabie et al. [35]
Cephalotes pavonii	Frequency of occurrence	Burnt site	Teixeira et al. [39]
Cephalotes pellans	Presence/absence	Area at intermediate succession—wet season	Neves et al. [73]
Cephalotes pusillus	IndVal	Savanna—vegetation and ground stratum	Vasconcelos and Vilhena [82]
Cephalotes simillimus	IndVal	Savanna—vegetation stratum	Vasconcelos and Vilhena [82]
Crematogaster brasiliensis	IndVal	Forest—vegetation and ground stratum	Vasconcelos and Vilhena [82]
Crematogaster erecta	Presence/absence	Area at intermediate succession	Coelho et al. [60]
	IndVal	Savanna—vegetation and ground stratum	Vasconcelos and Vilhena [82]
Crematogaster limata	IndVal	Forest—vegetation and ground stratum	Vasconcelos and Vilhena [82]
Crematogaster minutissima	IndVal	Forest—ground stratum	Vasconcelos and Vilhena [82]
Crematogaster nigropilosa	Frequency of occurrence	Native forest remnant	Ilha et al. [48]

Table 2: Continued.

Ant species	Parameter	Habitat	Reference
Crematogaster quadriformis	IndVal	Savanna—ground stratum	Vasconcelos and Vilhena [82]
Cyphomyrmex laevigatus	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Cyphomyrmex major	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Cyphomyrmex olitor	Frequency of occurrence	Primary forest	Silva et al. [75]
Cyphomyrmex peltatus	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Frequency of occurrence	Forest fragments	Dias et al. [45]
Cyphomyrmex plaumanni	Frequency of occurrence	Primary forest	Silva et al. [75]
Cyphomyrmex salvini	Frequency of occurrence	Area at early succession	Vasconcelos [76]
	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Cyphomyrmex transversus	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Discothyrea sexarticulata	Frequency of occurrence	Forest fragment	Dias et al. [45]
	Frequency of occurrence	Primary forest	Silva et al. [75]
Dolichoderus attelaboides	IndVal	Forest—vegetation stratum	Vasconcelos and Vilhena [82]
Dolichoderus bispinosus	IndVal	Forest—vegetation stratum	Vasconcelos and Vilhena [82]
Dolichoderus schulzi	Presence/absence	Undisturbed sites	Delabie et al. [35]
Dolichoderus voraginosus	Presence/absence	Area at early succession—dry season	Neves et al. [73]
Dorymyrmex guianensis	IndVal	Savanna—ground stratum	Vasconcelos and Vilhena [82]
Dorymyrmex pyramicus	Frequency of occurrence	Burnt site	Teixeira et al. [39]
	Presence/absence	Undisturbed sites	Delabie et al. [35]
Dorymyrmex thoracicus	IndVal	Savanna—ground stratum	Vasconcelos and Vilhena [82]
Eciton quadriglume	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Ectatomma brunneum	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Frequency of occurrence	Area at early succession	Braga et al. [54]
	Presence/absence	Low human land-use-impacted sites	Delabie et al. [35]
Ectatomma edentatum	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Ectatomma lugens	Frequency of occurrence	Area at late succession stage	Vasconcelos [76]
	IndVal	Forest—ground stratum	Vasconcelos and Vilhena [82]
Ectatomma muticum	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Ectatomma opaciventre	IndVal	Savanna—ground stratum	Vasconcelos and Vilhena [82]
Ectatomma permagnum	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
	Frequency of occurrence	*Eucalyptus* forestry	Braga et al. [54]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Ectatomma planidens	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Ectatomma quadridens	Frequency of occurrence	Area at early succession	Vasconcelos [76]
Ectatomma tuberculatum	Frequency of occurrence	Area at early succession	Silva et al. [75]
	Presence/absence	Burned restinga	Endringer et al. [38]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Frequency of occurrence	Area at late succession stage	Braga et al. [54]
	Frequency of occurrence	Secondary forest and forest edge	Leal et al. [71]
Forelius maranhoensis	IndVal	Savanna—ground stratum	Vasconcelos and Vilhena [82]
Gnamptogenys acuminata	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]

Table 2: Continued.

Ant species	Parameter	Habitat	Reference
Gnamptogenys continua	Frequency of occurrence	Primary forest	Silva et al. [75]
Gnamptogenys horni	Frequency of occurrence	Area at late succession stage	Vasconcelos [76]
Gnamptogenys mediatrix	Frequency of occurrence	Forest fragment	Dias et al. [45]
Gnamptogenys moelleri	Frequency of occurrence	Pasture	Dias et al. [45]
	Frequency of occurrence	Secondary forest and area at early succession	Schmidt and Diehl [52]
Gnamptogenys reichenspergeri	Frequency of occurrence	Primary forest	Silva et al. [75]
Gnamptogenys striatula	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	IndVal	Forest—ground stratum	Vasconcelos and Vilhena [82]
Gnamptogenys sulcata	Presence/absence	Area at early succession—wet season	Neves et al. [73]
Gnamptogenys tortuolosa	Frequency of occurrence	Intermediate disturbed area	Vasconcelos [76]
Heteroponera flava	Frequency of occurrence	Forest fragment	Dias et al. [45]
Heteroponera microps	Frequency of occurrence	Disturbed habitat (*Eucalyptus*)	Ilha et al. [48]
Hylomyrma balzani	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Hylomyrma reitteri	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Hypoponera foeda	Frequency of occurrence	Native forest remnant	Ilha et al. [48]
Hypoponera foreli	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Hypoponera opacior	Frequency of occurrence	Disturbed habitat (*Eucalyptus*)	Ilha et al. [48]
Labidus coecus	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
	Presence/absence	Undisturbed sites	Delabie et al. [35]
Labidus praedator	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Frequency of occurrence	Secondary forest	Schmidt and Diehl [52]
Leptogenys pusilla	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Leptothorax asper	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Leptothorax spininodis	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Linepithema humile	Frequency of occurrence	Burnt site	Teixeira et al. [39]
	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Megalomyrmex goeldii	Frequency of occurrence	Area at early succession	Silva et al. [75]
Mycetagroicus cerradensis	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
Mycetarotes paralelus	Presence/absence	Area revegetated with native species	Pereira et al. [64]
Mycetophylax conformis	Presence/absence	Strong human land-used-impacted sites	Delabie et al. [35]
Myrmicocrypta foreli	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Neivamyrmex orthonotus	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Nesomyrmex spininodis	Presence/absence	Strong human land-used-impacted sites	Delabie et al. [35]
Octostruma balzani	Frequency of occurrence	Area at intermediate succession	Braga et al. [54]
Octostruma jheringhi	Frequency of occurrence	Forest fragment	Dias et al. [45]
	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Odontomachus affinis	Frequency of occurrence	Secondary forest	Silva et al. [75]
Odontomachus bauri	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Odontomachus brunneus	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Odontomachus caelatus	Frequency of occurrence	Area at late succession stage	Vasconcelos [76]
Odontomachus chelifer	Frequency of occurrence	Pasture	Braga et al. [54]
	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
	Frequency of occurrence	Secondary forest and area at early succession	Schmidt and Diehl [52]
	Frequency of occurrence	Secondary forest and area at early succession	Silva et al. [75]

Table 2: Continued.

Ant species	Parameter	Habitat	Reference
Odontomachus haematodus	Frequency of occurrence	Area at intermediate succession	Braga et al. [54]
	Presence/absence	Burned restinga	Endringer et al. [38]
	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
	IndVal	Forest—ground stratum	Vasconcelos and Vilhena [82]
Odontomachus meinerti	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Oxyepoecus plaumanni	Frequency of occurrence	Primary forest	Silva et al. [75]
Oxyepoecus rastratus	Frequency of occurrence	Primary forest	Silva et al. [75]
Pachycondyla apicalis	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Pachycondyla arhuaca	Frequency of occurrence	Area at late succession stage	Braga et al. [54]
	Presence/absence	Primary restinga	Endringer et al. [38]
Pachycondyla bucki	Frequency of occurrence	Primary forest	Silva et al. [75]
Pachycondyla crassinoda	IndVal	Forest—ground stratum	Vasconcelos and Vilhena [82]
Pachycondyla ferruginea	Frequency of occurrence	Secondary forest and area at early succession	Silva et al. [75]
Pachycondyla gilberti	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Pachycondyla harpax	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	IndVal	Forest—ground stratum	Vasconcelos and Vilhena [82]
	Presence/absence	Low human land-use-impacted sites	Delabie et al. [35]
Pachycondyla obscuricornis	Frequency of occurrence	*Eucalyptus* (reforestation)	Lapola and Fowler [49]
Pachycondyla stigma	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
	Frequency of occurrence	Primary forest	Braga et al. [54]
Pachycondyla striata	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
	Presence/absence	Savanna—cerrado sensu stricto	Marinho et al. [50]
	Frequency of occurrence	Secondary forest and area at early succession	Schmidt and Diehl [52]
	Frequency of occurrence	Secondary forest and area at early succession	Silva et al. [75]
Pachycondyla villosa	Presence/absence	Burned restinga	Endringer et al. [38]
	Frequency of occurrence	Disturbed savanna	Ramos et al. [37]
Paratrechina longicornis	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	Presence/absence	Undisturbed sites	Delabie et al. [35]
Pheidole diligens	Presence/absence	Area at intermediate succession	Coelho et al. [60]
Pheidole embolopyx	Frequency of occurrence	Area at late succession stage	Vasconcelos [76]
Pheidole exigua	IndVal	Forest—ground stratum	Vasconcelos and Vilhena [82]
Pheidole fimbriata	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
	Presence/absence	Savanna—cerrado sensu stricto	Marinho et al. [50]
Pheidole fracticeps	IndVal	Forest—ground stratum	Vasconcelos and Vilhena [82]
Pheidole radoszkowskii	Presence/absence	Low human land-use-impacted sites	Delabie et al. [35]
Pheidole scalaris	Presence/absence	Area at early succession—wet season	Neves et al. [73]
Pogonomyrmex abdominalis	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Pogonomyrmex naegelii	Presence/absence	Area at intermediate succession	Coelho et al. [60]
Prionopelta punctulata	Frequency of occurrence	Secondary forest and area at early succession	Silva et al. [75]
Pseudomyrmex elongatus	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Pseudomyrmex filiformis	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Pseudomyrmex flavidulus	IndVal	Savanna—vegetation stratum	Vasconcelos and Vilhena [82]

Table 2: Continued.

Ant species	Parameter	Habitat	Reference
Pseudomyrmex gracilis	Presence/absence	Area at intermediate succession	Coelho et al. [60]
	IndVal	Savanna—vegetation stratum	Vasconcelos and Vilhena [82]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Pseudomyrmex kuenckeli	Presence/absence	Undisturbed sites	Delabie et al. [35]
Pseudomyrmex oculatus	Presence/absence	Area at intermediate succession	Coelho et al. [60]
	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	IndVal	Forest—vegetation stratum	Vasconcelos and Vilhena [82]
Pseudomyrmex schuppi	Presence/absence	Area at intermediate succession	Coelho et al. [60]
Pseudomyrmex sericeus	Presence/absence	Undisturbed sites	Delabie et al. [35]
Pseudomyrmex simplex	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Pseudomyrmex spiculus	Presence/absence	Undisturbed sites	Delabie et al. [35]
Pseudomyrmex tenuis	Frequency of occurrence	Area at early succession	Vasconcelos [76]
	IndVal	Forest—vegetation and ground stratum	Vasconcelos and Vilhena [82]
	Presence/absence	Strong human land-use-impacted sites	Delabie et al. [35]
	Frequency of occurrence	Area at intermediate succession	Braga et al. [54]
	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Pseudomyrmex termitarius	Presence/absence	Area at early succession—dry season	Neves et al. [73]
	Frequency of occurrence	Pasture	Braga et al. [54]
	IndVal	Savanna—vegetation stratum	Vasconcelos and Vilhena [82]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Pyramica appretiata	Frequency of occurrence	Primary forest	Silva et al. [75]
Pyramica denticulata	Frequency of occurrence	Area at early succession	Silva et al. [75]
Pyramica lygatrix	Frequency of occurrence	Primary forest	Silva et al. [75]
Pyramica rugithorax	Frequency of occurrence	Primary forest	Silva et al. [75]
Pyramica schulzi	Presence/absence	Strong human land-use-impacted sites	Delabie et al. [35]
Pyramica subdentata	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
	Frequency of occurrence	Secondary forest and area at early succession	Silva et al. [75]
Pyramica zeteki	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Sericomymex bondari	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
	Presence/absence	Low human land-use-impacted sites	Delabie et al. [35]
Simopelta curvata	Frequency of occurrence	Pasture (edge)	Dias et al. [45]
Solenopsis geminata	Frequency of occurrence	Area at early succession	Vasconcelos [76]
	Frequency of occurrence	*Caesalpinia echinata* forest	Santana-Reis and Santos [51]
Solenopsis saevissima	Frequency of occurrence	Disturbed sites	Ilha et al. [48]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
Solenopsis substituta	Frequency of occurrence	Burnt site	Teixeira et al. [39]
	Presence/absence	*Eucalyptus* forestry	Marinho et al. [50]
	IndVal	Savanna—ground stratum	Vasconcelos and Vilhena [82]
Sphinctomyrmex stali	Frequency of occurrence	Primary forest	Silva et al. [75]
Stegomyrmex vizzotoi	Frequency of occurrence	Primary forest	Silva et al. [75]
Strumigenys denticulata	Frequency of occurrence	Intermediate disturbed area	Vasconcelos [76]
Strumigenys elongata	Frequency of occurrence	Area at early succession	Silva et al. [75]
	Frequency of occurrence	Preserved savanna	Ramos et al. [37]

Table 2: Continued.

Ant species	Parameter	Habitat	Reference
Strumigenys perparva	Presence/absence	Savanna—cerrado sensu stricto	Marinho et al. [50]
	Frequency of occurrence	Preserved savanna	Ramos et al. [37]
Tapinoma melanocephalum	Frequency of occurrence	Disturbed habitat (*Eucalyptus*)	Ilha et al. [48]
	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
Trachymyrmex cornetzi	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Trachymyrmex fuscus	Frequency of occurrence	Pasture (edge)	Dias et al. [45]
Trachymyrmex zeteki	Frequency of occurrence	Primary forest	Silva et al. [75]
Typhlomyrmex major	Frequency of occurrence	Forest fragments	Lapola and Fowler [49]
	Frequency of occurrence	Primary forest	Silva et al. [75]
Typhlomyrmex pusillus	Frequency of occurrence	Primary forest	Silva et al. [75]
Wasmannia auropunctata	Presence/absence	Area at intermediate succession	Coelho et al. [60]
	Frequency of occurrence	Control site (nonburnt)	Teixeira et al. [39]
Wasmannia rochai	Presence/absence	Area at early succession	Coelho et al. [60]

The majority of studies end at the seventh step of the "Procedural steps in bioindicator studies" according to McGeoch [7], which is "Based on the nature of the relationship, either accept or reject the species, higher level taxon or assemblage as an indicator," and just investigate the nature of the relationship between the disturbance and the indicator. To validate the organism as a suitable indicator, we must move to step eight (Establish the robustness of the indicator by developing and testing appropriate hypotheses under different conditions)—establish the robustness of the indicator by testing the same relationship in other areas or at different times (to validate the indicator) [6, 14, 103].

We would like to flag some issues to improve and validate the use of ants as indicators in environmental monitoring programs, including consideration of robust criteria for the validation of ants as indicators, sampling in different seasons and under different disturbances with comparable methodologies, collecting ants with different sampling methodologies in order to recognize that the responses of different ant life styles could be different for the same disturbance, and evaluation of different environmental parameters (biotic and abiotic) to correlate with the ants' response along the disturbance/restoration gradients. The search for indicator ant species should be with analyses that consider their fidelity and specificity to the habitats (e.g., "IndVal" index), in order to more quickly achieve monitoring goals. Finally, evaluating the functional loss of ant species in disturbed habitats will improve predictions about the functional implication of the disturbance.

Moreover, incorporating new approaches that efficiently simplify the study may help to decrease the problems related to time spent identifying ant species, as suggested by Groc et al. [85]. In this study, the authors introduced a new method based on mixed-level taxonomic sufficiency, highly focused on higher-taxon surrogacy. Under this method, only ant species pre established as "indicator taxa" must be taxonomically identified to species level, while other species may be identified to higher (and easier to identify) levels, such as genera. By using this mixed-level approach, the authors argue that a considerable improvement in cost effectiveness can be achieved, mainly by reducing the necessity for well-trained taxonomists to be involved in the study. This is highly desirable in monitoring programs, where time and budget are key limiting factors.

Also, species which have been identified as possible indicators because they occur in specific habitats or conditions still require validation for reliable use as bioindicators, and their presence or frequency in specific conditions can generate testable hypothesis about their relationship with these habitats and conditions, which in turn can validate the use of these species as bioindicators.

In conclusion, we point out that the use of ants as indicators in Brazil has been improving each year. Ants are a useful tool not only because they are sensitive to environmental changes, as related in the papers we reviewed but also because they are keystone species in several ecological processes and, therefore, provide reliable inferences about the ecological and functional implications of disturbances.

We should continue to study ants in Brazil, with proper *a priori* hypothesis tests and sampling designs, statistical analysis and standardized methods, in order to reach the same widespread acceptance of ants as indicators that is common in Australia, as well as to improve our understanding of ant dynamics for predictive frameworks. Moreover, we need to build an effective bridge between our accumulated knowledge (almost 25 years of research) of ants as bioindicators and monitoring programs developed to examine natural resources and areas.

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Review Article

Current Understanding and Future Prospects of Host Selection, Acceptance, Discrimination, and Regulation of Phorid Fly Parasitoids That Attack Ants

Kaitlyn A. Mathis[1] and Stacy M. Philpott[2]

[1] *Department of Environmental Science, Policy and Management, University of California, Berkeley, 137 Mulford Hall, Berkeley, CA 94720, USA*
[2] *Department of Environmental Sciences, The University of Toledo, 2801 W. Bancroft Street, MS 604, Toledo, OH 43606, USA*

Correspondence should be addressed to Kaitlyn A. Mathis, kamathis@berkeley.edu

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Phorid fly parasitoids (Diptera: Phoridae) have evolved a diverse array of cues used to successfully parasitize their ant hosts. Successful parasitism often involves (a) host habitat location, (b) host location, (c) host acceptance, (d) host discrimination, and (e) host regulation. In this paper we discuss our current understanding of how phorid flies use each of these steps to successfully parasitize ant hosts. We examine the wide variety of strategies and cues used by a multiple species of phorid flies within three separate genera that most commonly parasitize ants (*Apocephalus*, *Pseudacteon*, *and Neodohrniphora*) and discuss future directions within this field of study.

1. Introduction

Parasitoids have evolved effective and efficient methods of successful parasitism, many of which involve utilization of multimodal cues [1]. Many dipteran parasitoids in the family Phoridae use social insects as hosts due to the reliability of their intraspecific chemical communication signals that make for effective host selection cues [2–5]. Phorid fly adults parasitize ants by hovering over insect hosts and then diving down to insert an egg beneath the insect's exoskeleton [3, 6–8]. Phorid flies have direct parasitic effects on ants (i.e., cause ant mortality) and also significantly change ant foraging behavior by limiting host resource acquisition behavior, modifying ant competitive hierarchies, and dampening ant effects on herbivores [9–14]. There are phorids that attack ants from at least 22 genera across 5 subfamilies. Likewise, more than 20 genera of phorids attack ant hosts [3]. With such taxonomic diversification in ant-phorid relationships, the types of cues used by phorids to locate, select, and successfully parasitize ant hosts are also quite diverse.

Successful parasitism requires a series of interactions between a parasitoid and its host. The process can be categorized into five general and sometimes overlapping steps: (a) host habitat location, (b) host location, (c) host acceptance, (d) host discrimination, and (e) host regulation [1].

For phorid parasitoids, host location involves the use of both habitat and host cues. Host habitat location is the use of environmental cues by the parasitoid to select areas to search for potential hosts. These cues may be directly related to the preferred environment of the host itself (e.g., volatiles from plants commonly used by hosts) or related to the parasitoid's general habitat preferences (light, temperature, and humidity conditions within a given area) [1]. The host location process also requires that a parasitoid use long-range cues to be directed to its' host. However, unlike host habitat location cues, these cues come directly from the host itself. Ants communicate interspecifically by using complex pheromones. These pheromones often act as host location cues for parasitoids as they can be both reliable (with volatile pheromones highly conserved within a species or

genus) and detectable (ants, being eusocial, live in relatively high densities, and can produce large volumes of volatile pheromones) for the parasitoid [15]. Once a phorid parasitoid has located a potential host through long-range cues, the parasitoid requires host acceptance cues to trigger the parasitoid's oviposition behavior. Short-range cues such as movement, host size, and contact chemical cues have all been implicated in triggering phorid fly oviposition [7, 16–26].

In addition to the cues that are required for overall host selection, host discrimination cues, used by parasitoids to detect and reject potential hosts that have been previously parasitized, can be present. While these cues are not necessary for parasitism, they can increase the likelihood of offspring success [1]. Parasitoids can also increase the success rate of their offspring through host regulation, whereby parasitoids manipulate their hosts to promote the development of the next generation of parasitoids. Host regulation can involve altering the physiology of the host to facilitate growth and development of egg, larvae, or pupae of the parasitoid or altering host behavior to optimize nutrient intake or location within the external environment [27].

This paper focuses on our current understanding of the process by which phorid flies successfully parasitize ants. We examine the wide variety of strategies and cues used by multiple species of phorid flies within three separate genera (*Apocephalus, Pseudacteon, and Neodohrniphora*) to successfully parasitize ant hosts.

2. Host Habitat Location

Parasitoid habitat preference is a major factor that determines where parasitoids will search for hosts and therefore which hosts will be successfully parasitized. Some hosts are selected not because they have a greater degree of inherent suitability but because they happen to be in an environment where parasitoid abundance is greater or where parasitoids are better able to detect cues released by their hosts [1]. Light levels affect attack rates of several species of phorid flies. For example, *Neodohrniphora tonhascai* and *Neodohrniphora elongate* both attack *Atta sexdens* at significantly higher rates when in high-light-level laboratory conditions [28]. Field experiments with *Pseudacteon litoralis* and *Pseudacteon tricuspis* which attack ants in the *Solenopsis saevissima* complex show that these species prefer lower light levels (i.e., just after sunrise and before sunset) and higher light levels (midday sun), respectively [23]. Analogously, lab experiments with *Pseudacteon curvatus* show that the flies attack *Solenopsis* spp. ants on darker backgrounds at greater rates than ants on white or light backgrounds [29]. *Pseudacteon* spp. phorids that attack the *Solenopsis saevissima* also display habitat preferences based on environmental factors such as temperature, rainfall, photoperiod length, sugar availability, wind, humidity, and number of days with frosts [30–32].

Habitat complexity also affects phorid fly attack rates. Two species of phorid flies, *Apocephalus sp. 8* and *Apocephalus sp. 25* attack their host ants (*Pheidole diversiphilosa* and *Pheidole bicarinata,* resp.) at higher rates when leaf litter is less complex, most likely because the ants are able to take better refuge in more complex leaf litter [33]. Further, *Pseudacteon* spp. attack rate on *Azteca instabilis* is higher in coffee plantations with lower shade tree canopy complexity although the exact set of habitat variables that create a preference for lower shade complexity remain unclear [34].

3. Host Location

The long-range cues used by phorid flies to hone in on potential hosts have been examined in several phorid-ant relationships. Some phorids travel at least 10–20 m to reach hosts and possibly up to 50 m, thus host location cues are likely generally volatile compounds, which can be detected by parasitoids well beyond the visual range of their hosts [42]. While sound cues have the potential to be long range and have been documented in some non-phorid parasitoid-insect interactions, to date no phorid flies have been recorded to use sound as a cue in ant host location [3, 43]. Paralleling the rich diversity of volatile ant pheromones, chemical host location cues used by phorid flies can vary widely in structure, glandular origin, and purpose in ant-phorid relationship (Table 1). Long-range cues for phorids derive from several glands (mandibular, pygidial, etc.) and represent a wide array of pheromone types (trail, alarm, etc.). Several specific examples of these cues for different ant-phorid relationships follow.

The first set of host location cues documented for phorids were in the "giant tropical ant" *Paraponera clavata* attacked by the phorid, *Apocephalus paraponerae*. Parasitism of *P. clavata* by *A. paraponerae* was first observed in 1958 by C. W. Rettenmeyer on Barro Colorado Island, Panama. Rettenmeyer originally suspected that the flies were attracted to audible stridulations made by *P. clavata* individuals when alarmed. However, field observations showed that *A. paraponerae* were attracted to mandibular gland extracts of *P. clavata* that contain alarm pheromone [2, 4]. The two major products of the mandibular glands of *P. clavata*, 4-methyl-3-heptanol and 4-methyl-3-heptanone (characterized in [35]), were tested individually and both attract *A. paraponerae* [4].

Another species of phorid fly that utilizes the alarm pheromones of its host is *Pseudacteon brevicauda*. Studies show that these phorid flies are attracted to mandibular gland extracts of their host, *Myrmica rubra* [36]. Within these glands are 3-octanone, 3-nonanone, and 3-octanol [37]. The two ketones were found to attract *P. brevicauda* from a distance [36]. While the alcohol, 3-octanol, did not attract flies from long distances, it was found to increase the "alertness" of the flies at a closer range, possibly indicating its synergistic role in host location or a possible role in host acceptance; however, further observations are needed to confirm the role of this compound [36].

Formic acid, a relatively common alarm and defense compound from the venom glands of ants, is the primary host location cue attracting *Pseudacteon formicarum* to the ants *Lasius niger* and *Lasius emarginatus* [38]. The use of formic acid is relatively common in ants, and previously *P. formicarum* was thought to be one of the only phorid flies with multiple hosts because these flies frequently arrive to areas where a wide variety of ants using formic acid are aggregated. However, it was recently discovered that *P. formicarum* is specific to ants in the genus *Lasius*, rather than all

Table 1: Chemical host location cues used by phorid flies in search of ant hosts.

Phorid species	Ant species	Cue	Source	Ant use	Ref.
Apocephalus paraponerae	*Paraponera clavata*	4-Methyl-3-heptanol and 4-methyl-3-heptanone	Mandibular glands	Alarm pheromone	[2, 4, 35]
Pseudacteon brevicauda	*Myrmica rubra*	3-Octanone and 3-nonanone	Mandibular glands	Alarm pheromone	[36, 37]
Pseudacteon formicarum	*Lasius niger* and *Lasius emarginatus*	Formic acid	Venom glands	Alarm/defense pheromone	[38]
Pseudacteon spp.	*Azteca instabilis*	1-Acetyl-2-methylcyclopentane	Pygidial Gland	Alarm pheromone	[17, 39]
Pseudacteon litoralis	*Solenopsis saevissima* complex	Unknown	Unknown	Unknown use at disturbed mounds	[20]
Pseudacteon wasmanni	*Solenopsis saevissima* complex	Unknown	Unknown	Unknown use at disturbed mounds	[20]
Pseudacteon obtusus	*Solenopsis saevissima* complex	Unknown	Unknown	Unknown use on trails	[20]
Pseudacteon borgmeieri	*Solenopsis saevissima* complex	Unknown	Unknown	Unknown use on trails	[20]
Pseudacteon nuicornis	*Solenopsis saevissima* complex	Unknown	Unknown	Unknown use on trails	[20]
Pseudacteon solenopsidis	*Solenopsis saevissima* complex	Unknown	Unknown	Unknown use on trails	[20]
Pseudacteon tricuspis	*Solenopsis saevissima* complex	2-Ethyl-3, 6-dimethylpyrazine	Mandibular glands	Alarm pheromone	[40, 41]

ants that use formic acid, which indicates that these phorid flies must use other shorter-range cues in addition to formic acid to locate their hosts [44].

Three species of *Pseudacteon* phorid flies [45] use compounds from the pygidial gland of their host *Azteca instabilis* as long-range host location cues. The pygidial gland of *A. instabilis* is the source of the alarm pheromone. At least one compound present within the pygidial gland of *A. instabilis*, 1-acetyl-2-methylcyclopentane, attracts one or more of these phorid fly species to their host [17, 39], but further research is necessary to determine if all three phorid species are attracted to the same compound or suite of compounds.

The *Solenopsis saevissima* complex has one of the largest groups of congeneric parasitoids recorded, with more than 18 *Pseudacteon* spp. known to parasitize this host group. However, despite significant research on these interactions, the details of the host location cues used in these interactions have remained somewhat elusive. In an early study, several of these phorids were categorized based on whether they were more likely to be found near disturbed ant mounds or trails—with the general hypothesis that phorid flies attacking ants near disturbed mounds must use alarm or defense compounds released by the ants as host location cues, and trail pheromone as a cue if they attack near trails. *Pseudacteon litoralis*, *P. tricuspis*, and *P. wasmanni* were all found attacking predominately near disturbed mounds or, in a few circumstances, trails where aggressive interspecies interactions were taking place between the ants. *Pseudacteon obtusus*, *Pseudacteon borgmeieri*, *Pseudacteon nuicornis*, and *Pseudacteon solenopsidis* were more often found attacking ants on trails [20, 46]. In another set of studies, *P. tricuspis* was attracted to the midden (consisting primarily of dead workers) of *Solenopsis invicta*, lending further evidence to the hypothesis that its host location cue is a volatile chemical from the ants themselves [47, 48]. Additionally shaken workers both elicit an alarm response in other workers and attract phorid flies [49]. Electroantennogram (EAG) experiments with *P. tricuspis* show that the flies are attracted to whole body extracts of workers, ant heads (including, to some extent, the mandible alone), and abdomens [49]. The same study confirmed that *P. tricuspis* is not attracted to the trail pheromone of *Solenopsis invicta*, (E,E)-α-farnesene [49]. The mandibular glands located within the head of *Solenopsis* spp. ants are the source of the ant's alarm pheromone, providing evidence that *P. tricuspis* likely uses a set of (rather than an individual) alarm pheromone compounds as a host location cue [50, 51]. Recently, 2-ethyl-3,6-dimethylpyrazine has been confirmed as an active alarm pheromone component from within the

mandibular glands of *S. invicta* and EAG experiments shows that this compound elicits a response in *P. tricuspis*, though the compound has yet to be tested in the field [40, 41].

Yet, not all ant-phorid relationships appear to involve long-range chemical cues. In behavioral observations of *N. elongata* phorid flies and *A. sexdens* ants using a 50 cm^3 observation chamber, Gazal et al. (2009) concluded that these phorids do not have a volatile chemical cue involved in host location [18]. However, it is possible that these cues are essential when phorids are at a greater distance from potential hosts and behavioral observations of ants and phorids in small and contained areas underestimate phorid specificity [52].

4. Host Acceptance

Short-range cues used by phorid flies to inspect potential hosts and determine whether they are suitable for oviposition can be visual or chemical or in some cases both (Table 2). Visual cues are often multifaceted, including several simultaneous or sequential features such as movement, host size, and host shape. The chemical cues used in host acceptance are generally less volatile compounds that can only be detectable at close range.

Movement of target ants is a common visual cue frequently used by the *Pseudacteon* spp. phorid flies that attack both *A. instabilis* and ants in the *Solenopsis saevissima* complex as well as by *N. elongata* phorid flies attacking *A. sexdens* [16–19]. *A. paraponerae* attacking *P. clavata*, however, prefer stationary ant hosts [5].

Size is also an important factor in phorid host acceptance. Variation in size preferences between phorid species attacking the same host is generally seen as an effective method of niche partitioning [16, 20, 22, 25]. Within the guild of phorids that attack the *Solenopsis saevissima* complex, *P. curvatus*, *P. nudicornis,* and *P. obtusus* attack small workers, *P. tricuspis* and *P. wasmanni* prefer medium-sized workers, *P. borgmeieri,* and *P. solenopsidis* tend to attack medium to large workers, and *P. litoralis* attacks large workers [7, 16, 20–24]. Size of the phorid fly is to a great extent a function of host body size [25, 55]. In the case of *P. obtusus*, the small and large biotypes that are otherwise morphologically identical proved to be genetically distinct enough to be different species likely due to a variation in host size preference [56]. Moreover, in *P. litoralis* and *P. tricuspis*, sex ratio is determined by the body size of the host, where larger host ants yield female offspring and smaller host ants yield male offspring [55]. Phorid flies in other genera also use size cues in host acceptance. *N. elongata* only attack *A. sexdens* foragers with a minimum head width of 1.6 mm, and *A. paraponerae* prefer large *P. clavata* workers [5, 53].

The complexity of the visual stimulus related to shape has also been implicated in host acceptance. For example, *N. elongata* will inspect (i.e., hover over) moving visual stimulus of varying degrees of complexity from simple to complex: one model mass sphere, two linked spheres, three linked spheres, a plastic ant model, and the host ant. Yet, the phorids only attack the most complex visual stimulus, which in the experiments was the host ant. Indeed, in this set of experiments, movement was unnecessary to trigger inspection if the visual stimulus was identical to the host, indicating that movement may act as a secondary cue to shape or visual complexity cues in order to enhance the speed and accuracy of attacks in this species [18].

Two classes of short-range chemical cues have been identified in phorid-ant interactions, cuticular hydrocarbons and low volatility venom gland secretions. While *A. paraponerae* flies are equally attracted to untreated ants and ants treated with hexane to remove cuticular hydrocarbons, the flies significantly prefer to lay eggs in ants with cuticular hydrocarbons [5]. Recent work on three *Pseudacteon* spp. phorid flies [45] that attack *A. instabilis* ants also show that these phorid flies may use cuticular hydrocarbons in host acceptance. When cuticular hydrocarbons of other ant species were applied to live *A. instabilis* ants, these *Pseudacteon* spp. phorid flies were much less likely to attack the ants than *A. instabilis* ants that were coated in additional *A. instabilis* cuticular hydrocarbons [54]. In experiments using electroantennograms and y-tube olfactometer bioassays, *P. tricuspis* flies used venom gland secretions of *S. invicta* in host acceptance. These experiments show that several piperidine alkaloids, which are present in the ant's venom glands and used in defense, act as short-range attractants [26].

5. Host Discrimination

The ability for parasitoids to distinguish between unparasitized potential hosts and hosts that have been previously parasitized is evolutionarily favorable as offspring from the same species within a single host are at a competitive disadvantage [1]. In fact, many parasitic hymenoptera can distinguish between parasitized and unparasitized hosts. Hymenopteran parasitoids use a variety of inhibitory cues in host discrimination including internal and external host-marking pheromones, or visual cues such as oviposition wounds [1].

In contrast, dipteran parasitoids, including phorid flies, appear to have high rates of superparasitism within populations [6]. For example, incidences of superparasitism by *Neodohrniphora curvinervis* on *Atta cephalotes* ants are relatively high at 19% in one field study [53]. Superparasitism by *N. elongata* on *A. sexdens* has been reported at 29.4% self-superparasitism and 49.5% conspecific superparasitism in a study conducted under lab conditions [57]. However, behavioral observations also show that once a *A. sexdens* host ant is parasitized, it is significantly less likely to be parasitized again by *N. elongata*, indicating that *N. elongata* are able to discriminate between parasitized and nonparasitized host ants but may in some circumstances (e.g., lab conditions) choose to superparasitize a host. Thus, it appears, however these *N. elongata* do have some, however imperfect, form of host discrimination, that despite the cues [57]. Dipteran parasitoids such as phorid flies do not have the accessory glands commonly used by hymenopteran parasitoids to produce host-marking pheromones [6, 58]. Thus, while more work is needed to determine the mechanism, it seems most likely that at least some phorid flies use visual cues from the ants' oviposition wounds in host discrimination.

Table 2: Host acceptance cues used by phorid flies to choose ant hosts.

Cue modality	Phorid species	Ant species	Cue	Source	Ant use	Ref.
Visual	*Pseudacteon* spp.	*Solenopsis saevissima* complex	Movement	—	—	[16, 19]
	Pseudacteon spp.	*Azteca instabilis*	Movement	—	—	[17]
	Neodohrniphora elongata	*Atta sexdens*	Movement	—	—	[18]
	Apocephalus paraponerae	*Paraponera clavata*	No movement	—	—	[5]
	Pseudacteon nuicornis	*Solenopsis saevissima* complex	Small-sized workers	—	—	[16]
	Pseudacteon obtusus	*Solenopsis saevissima* complex	Small-sized workers	—	—	[16]
	Pseudacteon curvatus	*Solenopsis saevissima* complex	Small-sized workers	—	—	[16]
	Pseudacteon tricuspis	*Solenopsis saevissima* complex	Medium-sized workers	—	—	[16]
	Pseudacteon wasmanni	*Solenopsis saevissima* complex	Medium-sized workers	—	—	[16]
	Pseudacteon borgmeieri	*Solenopsis saevissima* complex	Medium- to Larger-sized workers	—	—	[16]
	Pseudacteon solenopsidis	*Solenopsis saevissima* complex	Medium- to Larger-sized workers	—	—	[16]
	Pseudacteon litoralis	*Solenopsis saevissima* complex	Larger-sized workers	—	—	[16]
	Neodohrniphora elongata	*Atta sexdens*	Minimum head width of 1.6 mm	—	—	[53]
	Apocephalus paraponerae	*Paraponera clavata*	Large workers	—	—	[5]
Chemical	*Apocephalus paraponerae*	*Paraponera clavata*	Cuticular hydrocarbons	—	Nest mate recognition	[5]
	Pseudacteon spp.	*Azteca instabilis*	Cuticular hydrocarbons	—	Nest mate recognition	[54]
	Pseudacteon tricuspis	*Solenopsis invicta*	Piperidine alkaloids	Venom glands	Defense pheromone	[26]

In other ant-phorid fly relationships, superparasitism has only been observed in laboratory experiments where the phorid flies were relatively contained and the phorid fly to individual ant ratio was higher than what would commonly be seen in the field. In a study with *Pseudacteon tricuspis* and *Solenopsis invicta*, laboratory experiments showed the rate of superparasitism to be approximately 15.4%; however, these results do not accurately reflect the rate of superparasitism under natural conditions [8]. Thus, more studies are needed to determine whether superparasitism occurs in the field and whether it is a density-dependent phenomenon potentially affected by colony size or ant behavior.

6. Host Regulation

While relatively little is known about how phorid flies, in general, may manipulate their host's physiology in order to optimize the development of their offspring, strides have been made to understand the role of host regulation of *S. invicta* by *P. tricuspis* phorid flies. Like many other dipteran parasitoids, the developing phorid flies build respiratory structures in order to access fresh air through a hole in the integument of the host ant's head capsule [8]. Additionally, developing *P. tricuspis* is suspected to affect the neurophysiology of its ant hosts, as parasitized ants have

altered behavior whereby they remain safely within the nest until just before the phorid larvae decapitate their hosts. Shortly before decapitation, ants will leave the nest, presumably to find a suitable location for the phorid fly to continue pupation and emerge [59]. However, much remains unknown about the mechanisms by which these behavioral changes manifest in their host. Furthermore, there is nothing known about how any other phorid species are able to affect the behavior or growth of host ant species.

7. Conclusions and Future Prospects

In order to successfully parasitize a host, phorid fly parasitoids must undergo a multistep process to detect and interpret a wide range of cues from their ant hosts. These cocktails of cues, each of which may vary in degrees of host specificity and timing of detection (sequentially or simultaneously), allow the flies to find suitable hosts in a complex environment. Researchers often study the interactions between phorid flies and their ant hosts in order to address the role of phorid flies as potential biological control agents of ants [30, 46, 60–66]. However, understanding these interactions could potentially shed light on evolutionary and ecological processes as well as provide a better understanding of multimodal communication.

Cues used by phorid flies are often traits considered to be highly conserved within the host species. These conserved traits are highly reliable and thus adaptive to phorid flies. Yet, little is known about how phorid use of these cues impacts the adaptive nature of these traits within ants. For example, *P. clavata* was originally thought to have no alarm pheromone responses, as these ants are relatively primitive and therefore independent outside of the nests, not requiring the assistance of their sisters during foraging. However, some studies indicate that *P. clavata* does have fairly developed intraspecific interactions during foraging [67, 68]. As previously mentioned, *A. paraponerae* use the alarm pheromones, 4-methyl-3-heptanol and 4-methyl-3-heptanone, to locate its hosts. Though more intensive investigation is required, it is possible that the use of alarm pheromone by *P. clavata* has been selected against in order to decrease parasitism. On evolutionary timescales, perhaps phorid use of chemical and visual cues has affected ant morphology, behavior, and chemical communication.

While phorid flies are ubiquitous and conspicuous users of ant cues, a wide variety of other organisms are attracted to ants [69, 70]. Considering the context-dependent nature of successful parasitism discussed above, it seems likely that multiple myrmecophiles are utilizing similar cues and may thus affect the parasitism process. Indeed, only considering pair-wise interactions between organisms rather than interactions between a network of multiple parties with distinct cue preferences, perceptions, and responses can be misleading. For example, competitive interactions between male hermit crabs affect mating strategy decisions of how male hermit crabs approach females [71]. Additionally, ant-*Acacia* mutualisms are now better understood based on the overall fitness benefits to the *Acacia* plants via a network of ant species rather than summing the effects of individual ant species separately and, in coffee agroecosystems, the nuances of multiple insect-interactions give insight into the overall effects of coffee pests [72–75]. Thus, a network approach should be taken and future work should be conducted to elucidate how other ant symbionts may affect these phorid-ant interactions. Additionally, as phorid fly behavior is often dependent on a wide array of factors that may be altered in laboratory observations, more studies should be conducted in the field to verify the results of lab experiments.

Finally, phorid flies are often both ecologically relevant species and have remarkably diverse strategies for using diverse arrays of multimodal cues within a complex environment to successfully parasitize host ants [5, 17, 18, 26, 30, 39, 41]. Thus, phorid-ant interactions are ideal systems to bridge the gap between model organisms used in integrated pest management and model organisms used in understanding the behavioral ecology of multimodal cue use.

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Research Article

Competition for Aphid Prey between Different Lady Beetle Species in a Laboratory Arena

Christy Leppanen,[1,2] Andrei Alyokhin,[2] and Serena Gross[2,3]

[1] *U.S. Fish and Wildlife Service, Pacific Islands Fish and Wildlife Office, 300 Ala Moana Boulevard, P. O. Box 50088, Honolulu, HI 96850-5000, USA*
[2] *School of Biology and Ecology, University of Maine, Orono, ME 04469-5722, USA*
[3] *Department of Entomology, Purdue University, 901 West State Street, West Lafayette, IN 47907-2089, USA*

Correspondence should be addressed to Andrei Alyokhin, andrei.alyokhin@umit.maine.edu

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Direct competition for aphid prey (Hemiptera: Aphididae) was evaluated between and among several lady beetle species (Coleoptera: Coccinellidae). The behavior of three native (*Coccinella trifasciata, Coleomegilla maculata,* and *Hippodamia convergens*) and four nonnative (*Coccinella septempunctata*, *Harmonia axyridis*, *Hippodamia variegata,* and *Propylea quatuordecimpunctata*) lady beetles was observed in laboratory arenas. The beetles were kept alone, paired with conspecifics or paired with heterospecifics, and presented with potato aphids (*Macrosiphum euphorbiae*). *Harmonia axyridis* was the most successful aphid predator in our study, being able to find aphids more quickly and consume more of them compared to most other lady beetle species. It was also by far the most aggressive of the tested species. *Coccinella septempunctata, C. trifasciata,* and *C. maculata* generally followed *H. axyridis* in aphid consumption. Prey discovery, consumption, and aggressive behaviors were dependent on which species were present in the arena. Except for the generally superior *H. axyridis*, there was no obvious dominance hierarchy among the other tested species and no dichotomy between the native and non-native species. Asymmetric interactions between lady beetle species may affect their abilities to coexist in the same habitat.

1. Introduction

Lady beetles comprise an ecologically and economically important group of insects that are also charismatic and well known to the general public [1, 2]. Understanding intraguild interactions among lady beetle species is important both for their conservation and for their maximum utilization as biological control agents. For example, the establishment of nonnative lady beetle species often coincides with declines in native lady beetle abundances [3–9] and has been implicated in having profound effects on the populations of pestiferous prey [4, 9, 10].

Competition is often assumed when predatory species consuming the same prey species are found in the same area [11]. Persistent species that share prey and an evolutionary history are often considered to have achieved a compromise over time, allowing them to coexist by differentially exploiting the same prey species [12, 13]; for example, by foraging at different times [14]. When species consuming the same prey are newly brought together, the ability of each to acquire the same necessary resources may allow for their coexistence [15, 16]. Intraguild predation, however, does not mean that a sufficient share goes to each predator [6, 17–19]. Consumption by a more efficient predator may eventually result in the competitive exclusion of the less efficient predator [16, 20].

Most comparative studies of different lady beetle species have either dealt with their relative abundances in the field [3–9, 21] or focused on intraguild predation [3, 6, 7, 17, 22–32]. The recent spread of *Harmonia axyridis* (Pallas) outside of its native range has been the impetus for a number of additional behavioral comparisons [33]. *Harmonia axyridis* has been shown to outcompete other lady beetle species in evaluations of intraguild predation [17, 24, 31], prey utilization [6], pathogen tolerance [34], and in the acquisition of prey tended by aggressive ants [35]. Relatively little research effort has been dedicated to competition for prey items among lady

beetle species. In an extensive field survey, Finlayson et al. [21] documented native and nonnative lady beetle species occurring together in a variety of habitats throughout Maine. A series of experiments [35, 36, and this study] were then conducted to compare behavior between different species. In the present study, we investigated behavior of seven lady beetle species competing for prey in a laboratory arena. We hypothesized that recently introduced species that share habitats with the native species [21], but appear to replace them over time [9], are more aggressive aphid predators.

2. Materials and Methods

2.1. Study Species. Aphidophagous lady beetle species, which were known to be abundant in Maine and were found together in the same habitats [21, 36], were chosen for the present study. Three species are native: the three-banded lady beetle *Coccinella trifasciata perplexa* Mulsant, the twelve-spotted lady beetle *Coleomegilla maculata lengi* Timberlake, and the convergent lady beetle *Hippodamia convergens* Guérin. The native range of *C. trifasciata* is north from New Jersey to Labrador and west to California and Alaska [37]. *Coleomegilla maculata* is native to eastern North America from Georgia to Ontario, and west to Texas and Minnesota [37]. The range of *H. convergens* extends from British Columbia and Ontario to South and Central America and the Antilles [37].

The nonnative lady beetles used in the present study were the seven-spotted lady beetle *Coccinella septempunctata* (L.), the multicolored Asian lady beetle *Harmonia axyridis* (Pallas), the variegated lady beetle *Hippodamia variegata* (Goeze), and the fourteen-spotted lady beetle *Propylea quatuordecimpunctata* (L.). *Harmonia axyridis* is native to Central and Eastern Asia [33, 38]. The other three species are of Palearctic origin [39, 40]. All were inadvertently or intentionally introduced into North America. *Coccinella septempunctata* has been established in the eastern United States since 1979 [41]. *Harmonia axyridis* was first documented as established in North America in 1988 [42, 43] and now occurs throughout much of the continental United States [33]. *Hippodamia variegata* is widespread throughout northeastern North America [44–49]. In Maine, *P. quatuordecimpunctata* was first documented in 1988 in Aroostook, Penobscot, and Kennebec Counties, where it is believed to have expanded its range from populations in Quebec dating to1968 [50].

The potato aphid, *Macrosiphum euphorbiae* (Thomas), served as the prey. *Macrosiphum euphorbiae* is common in Maine and native throughout North America [51]. It is known to feed on over 200 plant species, including potato, apple, aster, and rose [51] and is a common prey item for many lady beetle species [2, 37, 52].

2.2. Insect Origins and Maintenance. Lady beetles were collected 48–72 hours before the initiation of each trial and were provided with water, but no food, for 48 hours before trials began. Beetles were collected in Orono, Maine (44.8835°N, 68.6721°W) from a variety of habitats: mixed shrub (*Solidago* sp., *Rubus* sp., *Prunus* sp., *Rosa* sp., *Cornus sericea*, and *Alnus* sp.), apple (*Malus* sp.), grain (*Hordeum* sp. and *Avena* sp.), mixed organic crops (*Solanum lycopersicon, Allium* sp., *Brassica* sp., *Pisum* sp., and *Phaseolus* sp.), and field (*Phleum pratense, Trifolium* sp., *Cirsium* sp., *Vicia* sp., and *Fragaria* sp.). Potato aphids were obtained from a colony maintained in our laboratory. The colony was originally founded from aphids collected in Presque Isle, Maine (46.6528°N, 68.0109°W) from potato (*Solanum tuberosum*, Family: Solanaceae) fields and then maintained on excised potato foliage in the laboratory. Until they were used in trials, lady beetles and aphid colonies were housed separately in ventilated, 0.95 L ball glass jars (Jarden Home Brands, Inc., Daleville, IN, USA) held within Percival I-33VL Intellus environmental chambers (Percival Scientific, Inc., Perry, IA, USA) at 16 (light) : 8 (dark) hour photoperiod. The temperature was maintained at 20 ± 1°C during both the photophase and scotophase. Trials were conducted from May 16 to September 8, 2006.

2.3. Competition Trials with Paired Lady Beetles. Each trial took place in an observation arena under a clear, ventilated plastic container (8.9-cm diameter and 9.5-cm height), which was turned upside down and placed inside the bottom of a Petri dish. For each container, a cut potato leaf was placed in a small plastic vial with water. Using a paintbrush, 4 adult wingless aphids were placed on the upper surface of the leaf. Aphid number was chosen based on a previous study [36] in which lady beetles consumed between 5.33 ± 0.4271 (*P. quatuordecimpunctata*) and 9.17 ± 0.2039 (*H. axyridis*) adult potato aphids in a 24-hour period. Therefore, we believe that four aphids provided an adequate, but not overabundant, food supply. The vial containing the vegetation and aphids was then placed in an upright position inside the observation arena. Adult lady beetles were transferred to a different observation arena by allowing each lady beetle to crawl on to the tip of a paintbrush and then onto the interior of the arena. After a 10-minute period of adjustment, the cover holding the lady beetle(s) was switched with the cover under which the vial holding the leaf and aphids was housed, simultaneously exposing the lady beetle(s) to the aphids. Trials were conducted for 45 minutes. Time to prey discovery (of the first aphid), number of prey consumed by each beetle (documented to 0.25 aphid when the entire aphid was not consumed), and behavior (as a count of aggression delivered and received by each beetle in each trial) were recorded. The following behaviors were considered aggressive: chasing, grasping, biting, climbing upon, and attempting to or successfully stealing prey. Ten trials were conducted in random order, with individuals of each species and with pairs of all combinations of each species, including conspecific pairings.

2.4. Prey Consumption and Discovery Time by Single Lady Beetles. To serve as a comparison with the paired trials described above, aphid consumption and time to prey discovery was also documented in trials with single lady beetles. These trials were conducted following the same protocol as described above, but with one individual introduced in each arena. Ten trials were conducted with each of the seven lady beetle species.

Table 1: Mean (± SE) aphid consumption (number of aphids), prey discovery time (minutes), and aggression delivered (number of occurrences) by seven lady beetle species during laboratory trials. The data were pooled for all trials conducted with a given species (see text for details). Means in each column followed by the same letter are not significantly different from each other (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

	Aphid consumption			Aggression delivered	Prey discovery time	
	Alone	Same species	Other species	Other species	Same species	Other species
C. trifasciata	1.30 ± 0.34b	1.55 ± 0.21ab	1.78 ± 0.17ab	0.22 ± 0.05b	15.95 ± 3.11ab	16.47 ± 2.02b
C. maculata	1.60 ± 0.37ab	1.55 ± 0.20ab	1.42 ± 0.16bcd	0.23 ± 0.06b	20.30 ± 2.75a	17.80 ± 2.01b
H. convergens	1.20 ± 0.29b	1.35 ± 0.20ab	1.30 ± 0.14bcd	0.20 ± 0.05b	18.40 ± 3.07a	19.18 ± 2.18b
C. septempunctata	1.70 ± 0.42ab	1.50 ± 0.28ab	1.48 ± 0.17abc	0.13 ± 0.04b	18.70 ± 3.75a	20.80 ± 2.33ab
H. axyridis	2.70 ± 0.30a	1.95 ± 0.23a	2.10 ± 0.17a	0.57 ± 0.06a	6.35 ± 1.47b	13.23 ± 1.99b
H. variegata	0.70 ± 0.26b	0.75 ± 0.12ab	0.84 ± 0.12d	0.13 ± 0.04b	24.90 ± 3.33a	28.13 ± 2.13a
P. quatuordecimpunctata	1.10 ± 0.23b	1.03 ± 0.13b	0.94 ± 0.11cd	0.33 ± 0.06b	17.85 ± 3.39a	20 ± 2.18 ab
N	10	20	60	60	20	60
P	0.0146	0.0122	<0.0001	<0.0001	0.0002	<0.0001
F	2.90	2.85	5.99	6.27	4.76	5.56
DF	6, 63	6, 133	6, 413	6, 413	6, 133	4, 413

2.5. Measurements of Lady Beetle Weight and Size. Because differences in predator size have been used in some studies to explain differences in competition [6, 17, 53, 54], the weight and volume of 20 lady beetles of each species were documented. The weight of each beetle was determined to the 0.0001 gram using an electronic Ohaus Adventurer Balance AR2140 (Ohaus Corp., Pine Brook, NJ, USA). Width, length, and height were measured using a ruler mounted in the eyepiece of a Stereoscopic Zoom Microscope SMZ800 (Nikon Instruments Inc., Melville, NY, USA) at 10x magnification. Volume was estimated by multiplying width (across the pronotum, dorsal side), length (from the frons of the head to the end of the elytra, dorsal side), and height (the greatest height below the elytra, laterally).

2.6. Statistical Analyses. The Wilk-Shapiro test (PROC UNIVARIATE; SAS Institute, Inc. 2002) was used to test data normality. Data were transformed using rank transformations [55]. Untransformed data were used to calculate the means and standard errors reported in this paper.

Behavioral data were analyzed using one-way ANOVAs followed by Tukey's HSD tests (PROC GLM, SAS Institute, Inc. 2002). First, we compared the overall differences among the species for beetles that were held alone, paired with conspecifics, and paired with heterospecifics (all species other than the species of interest pooled together). Lady beetle species were used as the main effect (Table 1). Secondly, we tested the effects of the competition context (beetle held alone, paired with conspecifics, or paired individually with each of the heterospecific species) separately for each lady beetle species. Competition contexts were used as the main effect (Tables 2–4). Aphid consumption, prey discovery time, aggression received, and aggression delivered were used as dependent variables in both analyses.

Table 2: Number of aphids (mean ± SE) consumed by *C. trifasciata* and *C. maculata* in different competition contexts (see text for details). Means in each column followed by the same letter are not significantly different from each other (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

Competition context	*C. trifasciata*	*C. maculata*
Alone	1.30 ± 0.34ab	1.60 ± 0.37ab
C. trifasciata	1.70 ± 0.34ab	0.40 ± 0.22b
*C. trifasciata**	1.40 ± 0.27ab	N/A
C. maculata	2.60 ± 0.37ab	1.60 ± 0.31ab
*C. maculata**	N/A	1.50 ± 0.27ab
C. septempunctata	1.00 ± 0.42b	1.80 ± 0.36a
H. axyridis	1.30 ± 0.37ab	1.00 ± 0.27ab
H. convergens	2.60 ± 0.31a	1.55 ± 0.26ab
H. variegata	1.20 ± 0.36ab	1.95 ± 0.51a
P. quatuordecimpunctata	2.00 ± 0.39ab	1.80 ± 0.42ab
P	0.0073	0.0262
F	2.87	2.33
DF	8, 81	8, 81

* When beetles were paired with conspecifics, the data are listed separately for each beetle in the pair.

Correlation analysis (PROC CORR; SAS Institute Inc. 2002) was used to test associations between aphid consumption, prey discovery time, aggression delivered, and aggression received. The analyses were conducted both within each species (e.g., correlation between aphid consumption and prey discovery time for *H. axyridis*), as well as between the two paired species (e.g., correlation between aphid consumption by *H. axyridis* and *C. septempunctata*) or the two

Table 3: Number of aggression events (mean ± SE) delivered by *H. axyridis* and *H. variegata* in different competition contexts (see text for details). Means in each column followed by the same letter are not significantly different from each other (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

Competition context	***H. axyridis***	***H. variegata***
H. axyridis	0.10 ± 0.10b	0.50± 0.17a
H. axyridis*	0.10 ± 0.10b	N/A
C. maculata	0.60 ± 0.16ab	0.10 ± 0.10b
C. septempunctata	0.10 ± 0.10b	0.00 ± 0.00b
C. trifasciata	0.80 ± 0.13a	0.00 ± 0.00b
H. convergens	0.70 ± 0.15ab	0.00 ± 0.00b
H. variegata	0.50 ± 0.17ab	0.00 ± 0.00b
H. variegata*	N/A	0.00 ± 0.00b
P. quatuordecim-punctata	0.70 ± 0.16ab	0.20 ± 0.13ab
P	0.0003	<0.0001
F	4.70	4.72
DF	7, 72	7, 72

*When beetles were paired with conspecifics, the data are listed separately for each beetle in the pair.

individuals of the same species in case of conspecific trials. Most of the correlations between aphid consumption and prey discovery time were statistically significant. Therefore, for the ease of interpretation, their results are reported separately (Table 5) from statistically significant comparisons between all other combinations of variables (Table 6).

Weights and volumes of different lady beetle species were compared using one-way ANOVA (PROC GLM, SAS Institute, Inc. 2002). Means were separated by Tukey's HSD tests.

3. Results

Aphid consumption was significantly different among the species whether the beetles were held alone, paired with conspecifics, or paired with heterospecifics (Table 1). *Harmonia axyridis* generally consumed the most aphids, while *P. quatuordecimpunctata* and *H. variegata* consumed the least. Also, *H. axyridis* was the most aggressive species towards other lady beetles when held with heterospecifics (Table 1). No difference in delivered aggression was detected among the species paired with conspecifics (d.f. = 6, 133, $F = 2.07$, $P = 0.1544$). The overall amount of received aggression was similar among the tested species ($P > 0.15$).

Prey discovery time did not differ among species when the beetles were held alone (d.f. = 6, 63, $F = 1.01$, $P = 0.4273$). However, in the presence of conspecifics, *H. axyridis* found aphids quicker compared to the other species (Table 1). In the trials with heterospecifics, *H. variegata* discovered prey slower than all other species except *C. septempunctata* and *P. quatuordecimpunctata* (Table 1).

Competition context affected aphid consumption for two of the tested lady beetle species (Table 2). *Coccinella trifasciata* consumed fewer aphids when paired with *C. septempunctata* than when paired with *H. convergens*, while *C. maculata* consumed fewer aphids when paired with *C. trifasciata* than when paired with *C. septempunctata* or *H. variegata*. Prey discovery time did not vary within any of the tested species regardless of the competition context ($P > 0.2$).

Harmonia axyridis exhibited significantly more aggression towards *C. trifasciata* than towards the other lady beetle species (Table 3). Interestingly, *H. variegata*, which was a rather peaceful species in our trials, significantly increased its level of aggression when paired with *H. axyridis* (Table 3). *Coccinella trifasciata*, *H. convergens*, *H. variegata*, and *P. quatuordecimpunctata* received different amounts of aggression from different lady beetle species (Table 4). A statistically significant difference was also detected for *C. maculata*, but the effect was relatively weak, inconsistent, and its biological significance is uncertain (Table 4). Beetles from all five aforementioned species received more aggression from *H. axyridis* compared to at least one other species with which they were paired. *Hippodamia variegata* also received as much aggression from *P. quatuordecimpunctata* as from *H. axyridis* (Table 4).

Not surprisingly, aphid consumption was negatively correlated with prey discovery time (Table 5). In other words, the beetles that found their prey the most quickly consumed the most. The only exceptions were *C. trifasciata* paired with *C. maculata*, *H. convergens* paired with *H. axyridis*, and *H. axyridis* paired with *P. quatuordecimpunctata*. Correlation coefficients were marginally significant for *C. maculata* paired with *H. axyridis*, *H. axyridis* paired with *C. trifasciata*, and *P. quatuordecimpunctata* paired with *C. maculata* (Table 5).

Correlation analyses also revealed a number of strong relationships between other measured parameters (Table 6). In six trials, aphid consumption by one species was negatively correlated with aphid consumption by the other species confined in the same arena. Similarly, there were three cases of negative correlations between prey discovery times by two beetles in a pair. In five comparisons, aphid consumption by one species was positively correlated with prey discovery time by the other species. Aggressive behavior increased aphid consumption for *C. maculata* when paired with *C. trifasciata*, and for *H. convergens* when paired with *H. axyridis*. However, prey discovery time for *C. maculata* increased with increased aggression against *C. septempunctata*. Receiving aggression from *P. quatuordecimpunctata* significantly decreased aphid consumption by *C. septempunctata*. Similarly, prey discovery time for three aphid species increased as they received more aggression from another beetle in the pair (Table 6).

Coccinella septempunctata was the largest of the species tested, closely followed by *H. axyridis* (Table 7). *Hippodamia variegata* was the smallest.

4. Discussion

Results of the present study suggest the existence of asymmetric competitive interactions among the tested lady beetle species. There were significant differences in aphid consumption and prey discovery times among the species, and numerous occasions of aggressive encounters among the beetles confined in the observation arenas. The nature and strength

Table 4: Number of aggression events (mean ± SE) received by *C. trifasciata*, *C. maculata*, *H. convergens*, *H. variegata*, and *P. quatuordecimpunctata* in different competition contexts (see text for details). Means in each column followed by the same letter are not significantly different from each other (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

	C. trifasciata	*C. maculata*	*H. convergens*	***H. variegata***	***P.* *quatordecimpunctata***
C. trifasciata	0.20 ± 0.13b	0.30 ± 0.15ab	0.20 ± 0.13ab	0.10 ± 0.10b	0.30 ± 0.15ab
*C. trifasciata**	0.30 ± 0.15ab	N/A	N/A	N/A	N/A
C. maculata	0.20 ± 0.13b	0.00 ± 0.00b	0.20 ± 0.13ab	0.30 ± 0.15ab	0.10 ± 0.10b
*C. maculata**	N/A	0.10 ± 0.10ab	N/A	N/A	N/A
C. septempunctata	0.20 ± 0.13b	0.10 ± 0.10ab	0.30 ± 0.15ab	0.00 ± 0.00b	0.10 ± 0.10b
H. axyridis	0.80 ± 0.13a	0.60 ± 0.16a	0.70 ± 0.15a	0.50 ± 0.17a	0.70 ± 0.15a
H. convergens	0.00 ± 0.00b	0.30 ± 0.15ab	0.10 ± 0.10b	0.20 ± 0.13ab	0.20 ± 0.13ab
*H. convergens**	N/A	N/A	0.10 ± 0.10b	N/A	N/A
H. variegata	0.00 ± 0.00b	0.10 ± 0.10ab	0.00 ± 0.00b	0.00 ± 0.00b	0.20 ± 0.13ab
H. variegata*	N/A	N/A	N/A	0.00 ± 0.00b	N/A
P. quatuordecimpunctata	0.40 ± 0.16ab	0.20 ± 0.13ab	0.30 ± 0.15ab	0.50 ± 0.17a	0.10 ± 0.10b
P. quatuordecimpunctata*	N/A	N/A	N/A	N/A	0.10 ± 0.10b
P	0.0012	0.0298	0.0132	0.0028	0.0170
F	3.89	1.79	2.39	3.22	2.88
DF	7, 72	7, 72	7, 72	7, 72	7, 72

* When beetles were paired with conspecifics, the data are listed separately for each beetle in the pair.

Table 5: Correlations between aphid consumption and prey discovery time for single and paired lady beetles in trials ($N = 10$). Each row represents the relationship between aphid consumption and prey discovery time for the species in the left column when it was alone or paired with the species in the first row of the table. Ct: *Coccinella trifasciata*, Cm: *Coleomegilla maculata*, Hc: *Hippodamia convergens*, Cs: *Coccinella septempunctata*, Ha: *Harmonia axyridis*, Hv: *Hippodamia variegata*, Pq: *Propylea quatuordecimpunctata*. Nonnative species are printed in bold font.

		Alone	Ct	Cm	Hc	**Cs**	**Ha**	**Hv**	**Pq**
Ct	*r*	−0.8698	−0.7745	−0.3644	−0.8675	−0.8541	−0.7642	−0.9107	−0.7571
	P	0.0011	<0.0001	0.3005	0.0011	0.0017	0.0101	0.0002	0.0112
Cm	*r*	−0.9524	−0.7942	−0.8559	−0.9011	−0.6469	−0.6235	−0.8016	−0.7745
	P	<0.0001	0.0061	<0.0001	0.0004	0.0432	0.0541	0.0053	0.0085
Hc	*r*	−0.7994	−0.8708	−0.8199	−0.9091	−0.9039	−0.5518	−0.9431	−0.9184
	P	0.0055	0.0010	0.0037	<0.0001	0.0003	0.0982	<0.0001	0.0002
Cs	*r*	−0.8420	−0.8009	−0.8193	−0.8701	−0.8735	−0.9240	−0.9066	−0.8609
	P	0.0022	0.0054	0.0037	0.0011	<0.0001	0.0001	0.0003	0.0014
Ha	*r*	−0.9389	−0.6010	−0.7980	−0.8140	−0.6836	−0.7743	−0.9708	−0.2439
	P	<0.0001	0.0661	0.0057	0.0042	0.0293	<0.0001	<0.0001	0.4970
Hv	*r*	−0.9447	−0.7891	−0.8894	−0.9322	−0.7487	−0.8316	−0.8647	−0.8033
	P	<0.0001	0.0067	0.0006	<0.0001	0.0127	0.0029	<0.0001	0.0051
Pq	*r*	−0.8818	−0.8734	−0.6182	−0.7900	−0.8852	−0.6361	−0.8284	−0.7502
	P	0.0011	0.0010	0.0568	0.0065	0.0007	0.0480	0.0031	0.0001

of the observed interactions varied depending on the species involved.

Harmonia axyridis was the most successful aphid predator in our study, being able to find aphids quicker and consume more of them compared to most other lady beetle species. Furthermore, *H. axyridis* was by far the most aggressive of the tested species. These observations are consistent with a number of studies that have documented the superior competitive abilities of *H. axyridis* among lady beetle species [6, 17, 24, 26, 28, 31, 56, 57]. A superior competitive ability of invasive species to utilize resources over native species has been also documented in numerous other systems [58–61].

Interestingly, it took about twice as long for *H. axyridis* to find aphids when paired with heterospecifics than when paired with conspecifics (Table 1). It is possible that attacking heterospecifics distracted them from searching for aphids. Indeed, *H. axyridis* attacked heterospecifics 5–8 times more often than conspecifics (Table 3) although the differences were not always statistically significant. The aphid consumption data suggest that such a strategy paid off. Similarly,

Table 6: Additional significant correlations between aphid consumption, prey discovery time, aggression delivered, and aggression received by lady beetles in trials ($N = 10$). Nonnative species are printed in bold font.

Correlation between:	And:		
Aphid consumption	Aphid consumption	r	P
C. septempunctata	*C. trifasciata*	−0.9049	0.0002
C. trifasciata	*H. convergens*	−0.7356	0.0127
C. maculata	***H. axyridis***	−0.7098	0.0112
C. septempunctata	*H. convergens*	−0.8195	0.0053
H. axyridis	*H. convergens*	−0.9133	0.0003
H. axyridis	***P. quatuordecimpunctata***	−0.8497	0.0020
Prey discovery time	Prey discovery time	r	P
C. septempunctata	*C. trifasciata*	−0.7653	0.0085
C. septempunctata	*H. convergens*	−0.8138	0.0030
H. convergens	***P. quatuordecimpunctata***	−0.7001	0.0143
Aphid consumption	Prey discovery time	r	P
C. trifasciata	***C. septempunctata***	0.8350	0.0017
C. septempunctata	*H. convergens*	0.7069	0.0002
C. septempunctata	*C. trifasciata*	0.7665	0.0112
H. convergens	***C. septempunctata***	0.8344	0.0022
P. quatuordecimpunctata	***H. variegata***	0.7107	0.0088
Aphid consumption	Aggression delivered towards	r	P
C. maculata	*C. trifasciata*	0.7994	0.0063
H. convergens	***H. axyridis***	0.7327	0.0029
Aphid consumption	Aggression received from	r	P
C. septempunctata	***P. quatuordecimpunctata***	−0.7812	0.0080
Prey discovery time	Aggression delivered towards	r	P
C. maculata	***C. septempunctata***	0.9225	<0.0001
Prey discovery time	Aggression received from	r	P
C. maculata	***C. septempunctata***	0.8511	0.0017
H. convergens	*C. maculata*	0.8370	0.0002
C. septempunctata	***P. quatuordecimpunctata***	0.8392	0.0028
Aggression delivered by	or Aggression received by	r	P
C. trifasciata	*C. trifasciata*	0.7003	0.0004

Michaud [6] found *H. axyridis* to be a highly evolved interspecific competitor in the Florida citrus ecosystem.

Harmonia axyridis data generally agree with our hypothesis that recently introduced lady beetle species that replace native species over time are more aggressive aphid predators. However, we did not observe the same situation for the other three nonnative species. There was no distinct dichotomy between supposedly more aggressive nonnative species and supposedly more docile native species. Also, except for the generally superior *H. axyridis*, there was no obvious dominance hierarchy among the other tested species.

The native lady beetle species used in the current study, *C. maculata*, *C. trifasciata*, and *H. convergens* are currently numerous in Maine [21]. Native species, *Coccinella transversoguttata* (Brown) and *Hippodamia tredecimpunctata tibialis* (Say), that have experienced declines in abundance since nonnative lady beetle introductions [9] were excluded because they were not easily found in numbers sufficient for testing [21]. It would be interesting and valuable to pair native species once numerous in Maine with both the now common nonnative species and the native species that still persist.

Among the species tested, *C. septempunctata*, *C. trifasciata* and *C. maculata* generally followed *H. axyridis* in aphid consumption. *Coccinella septempunctata* and *H. axyridis* were also the heaviest and largest species among the seven species tested (Table 7). Despite *C. septempunctata*'s large size and being among the species consuming the most aphids, *C. septempunctata* generally did not deliver or receive more aggression than other species. Larger lady beetle species have been shown to be competitively favored over smaller ones [6, 17, 53, 54], possibly because they are able to consume more due to their larger size, or perhaps because their size is advantageous in direct fighting. *Coccinella septempunctata* has also been documented to deter aggression by ants

Table 7: Mean (± SE) weight (mg) and volume (mm^3) of lady beetle species ($N = 20$) used in laboratory trials. Means in each column with the same letter are not significantly different (Tukey's HSD tests, $P < 0.05$). Nonnative species are printed in bold font.

	Weight	Volume
C. trifasciata	1.04 ± 0.0007c	20.41 ± 1.2005d
C. maculata	0.91 ± 0.0008c	15.10 ± 0.8356de
H. convergens	0.87 ± 0.0009c	32.43 ± 1.8409c
C. septempunctata	2.25 ± 0.0017a	78.87 ± 2.6835a
H. axyridis	1.68 ± 0.0015b	66.30 ± 2.4081b
H. variegata	0.40 ± 0.0004d	8.64 ± 0.5435e
P. quatuordecim-punctata	0.63 ± 0.0005dc	12.87 ± 0.8090e
P	<0.0001	<0.0001
F	38.63	280.85
DF	6, 133	6, 133

chemically by producing a defensive alkaloid and bleeding reflectively [62, 63]. It is possible that chemical defense is also used by *C. septempunctata* to prevent aggression from other coccinellids.

It is worth noting that *H. axyridis*, *C. septempunctata*, *H. convergens*, *H. variegata*, and *P. quatuordecimpunctata* showed no difference in aphid consumption and prey discovery time whether they were kept alone or paired with any other species tested in the study, including conspecifics (data not shown). Perhaps if a given species is an efficient predator that can find and consume aphids quickly, its ability to acquire prey may not be significantly hindered by the presence of other lady beetles. Prey consumption by *C. trifasciata* and *C. maculata*, on the other hand, differed depending on which species they were paired with.

Significant negative correlations between the numbers of aphids consumed and prey discovery times in paired trials (Table 6) confirm the existence of competitive interactions. Furthermore, we detected a number of significant positive correlations between the number of aphids consumed by one beetle in a pair and prey discovery time by the other beetle in the pair. In other words, the longer it took a beetle to discover the prey, the more aphids its competitor could consume.

Increased aggression delivered by *C. maculata* and *H. convergens* (Table 6) was correlated with increased aphid consumption by those species in trials with *C. trifasciata* and *H. axyridis*, respectively. In those cases, aggression may have helped deter other species from consuming prey. On the contrary, increased aggression by *C. maculata* was correlated with its own increased prey discovery time, suggesting that it was distracted from foraging.

Receiving aggression from *P. quatuordecimpunctata* increased prey discovery time and decreased aphid consumption for *C. septempunctata* (Table 6). Similarly, prey discovery time increased for *H. convergens* with the increase in aggression it received from *C. maculata*, and for *C. septempunctata* with the increased aggression it received from *P. quatuordecimpunctata*. In a conspecific pairing of *C. trifasciata*, aggression received by one conspecific was correlated with the aggression it delivered, meaning that aggressive interactions were not one sided, but equally met by the other conspecific.

Overall, our results confirm that behavioral interactions between different lady beetle species affect their ability to secure prey items, with *H. axyridis* generally having a competitive advantage over the other species. Our study was conducted in a relatively simple setting of a laboratory arena with a limited number of aphids. Furthermore, prey choice was limited to a single aphid species. Increased environmental complexity, including variations in prey species and their abundances (including relative abundances of winged and wingless morphs), may modify competitive abilities of and interactions between certain species. Nevertheless, our findings support the idea that behavioral differences in prey discovery, consumption, and intraguild aggressiveness may, in part, lead to reductions in native lady beetle species following the establishment of *H. axyridis*.

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Research Article

Effect of Air Humidity on Sex Ratio and Development of Ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae)

Oldřich Nedvěd[1,2] and Plamen Kalushkov[3]

[1] *Faculty of Biological Sciences, University of South Bohemia, 370 05 České Budějovice, Czech Republic*
[2] *Institute of Entomology, Biology Center, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic*
[3] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Boulevard Tzar Osvoboditel, 1000 Sofia, Bulgaria*

Correspondence should be addressed to Oldřich Nedvěd, nedved@prf.jcu.cz

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Length of development of larvae and pupae of the invasive alien ladybird beetle *Harmonia axyridis*, their survival rates, sex ratio, and fresh mass of the emerged adults were measured at three contrasting levels of relative air humidity: 30, 60, and 90%, 25°C and photoperiod 16L : 8D. Overall sex ratio was 51%, but there was a strong trend for higher proportion of males at low humidity and higher proportion of females at high humidity. Survival rate, larval developmental time, and adult mass were all differently influenced by air humidity depending on the food type. In individuals fed with aphid *Acyrthosiphon pisum* there was a trend for better survival, shorter development, and higher mass gained at higher humidity. These trends were opposite or nonsignificant in individuals fed with frozen eggs of moth *Ephestia kuehniella*.

1. Introduction

The multicoloured Asian lady beetle or harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is native to eastern temperate Asia. It has a long history of use as a classical biological control agent of aphids and coccids in North America and has been deliberately introduced and has subsequently spread in four continents at a very fast rate during the last 23 years [1]. A CLIMEX model has been set up [2] to show the potential geographic distribution of *H. axyridis* that has been subsequently largely proven by new records. Besides several environmental temperature parameters and conditions for diapause, the model takes into account moisture parameters for the prediction of successful establishment in new areas. However, there are some regions where the prediction was not accurate. In Greece, *H. axyridis* was temporally established on orange trees heavily infested with aphids in 1993-1994 [3], but failed to survive in following years despite continued releases [4]. Another case of a failure in establishment of *H. axyridis* was observed [5] on the Azores islands. According to these studies, the released populations failed to establish due to ecological factors such as the maladaptation to the local conditions, mainly high temperature during diapause, and functional diversity saturation. Anyway, a conspicuous environmental trait that differs in these two regions from those where *H. axyridis* was successful is air humidity: very low in Greece and very high in Azores.

Among ambient factors controlled in laboratory experiments measuring development time and other life history traits of ectotherms, temperature and photoperiod are the most commonly and best studied, while moisture, for example, as relative air humidity, is often neglected: either not properly controlled or set to one value (e.g., [6]). More attention attracted moisture of substrate in soil arthropods [7] and of food or controlled atmosphere in stored product insects [8].

Developmental time of *Ophraella communa* (Coleoptera: Chrysomelidae) at different stages shortened along with the increasing relative air humidity (60%, 75%, and 90% RHs).

Table 1: Survival of developmental stages of *Harmonia axyridis* reared on two diets and in three levels of relative air humidity at 25°C and 16L : 8D photoperiod. *N*: initial number of second instar larvae, S3, S4, P and A: percentage survival to particular larval instar, pupal, or adult stage relative to the initial number of individuals, M: sex ratio (percentage of males). Chi-square tests with their probabilities were calculated for three-dimensional contingency table (food × humidity × survival/sex).

Food	RH %	*N*	S3 %	S4 %	P %	A %	M %
Acyrthosiphon	30	100	74	55	26	24	71
Acyrthosiphon	60	80	84	70	36	36	48
Acyrthosiphon	90	90	83	67	36	34	39
Ephestia	30	50	84	72	60	58	62
Ephestia	60	50	82	70	50	50	52
Ephestia	90	40	75	60	28	28	18
χ^2 ($d = 2$)			6.59	9.22	23.58	24.25	18.21
P			0.037	0.010	$<10^{-4}$	$<10^{-4}$	10^{-4}

The survival rates during the egg, larva, and entire immature stage were significantly higher at 75% RH and 90% RH than at 60% RH [9]. Low RH (20%) prolonged developmental duration of eggs and larvae reduced egg hatching and larval survival and reduced the body mass and body length of pine caterpillar, *Dendrolimus tabulaeformis* (Lepidoptera: Lasiocampidae), compared to 40%, 60%, and 80% RH [10]. As vapour pressure deficit decreased from 2.8 to 0.009 kPa, median life expectancy increased from 1.1 to 9.0 days for plum curculio *Conotrachelus nenuphar* (Coleoptera: Curculionidae) without food supply [11]. Complete development of *Ahasverus advena* (Coleoptera: Silvanidae) at 70% RH took 67, 58, and 48 days at 20, 22.5, and 25°C, respectively, while it was shorter (46, 31, and 26 days) at 90% RH [12].

The present study shows the effect of ambient humidity on some life history parameters of the ladybird *H. axyridis* that might play role in different establishment ability during its invasion to new areas.

2. Material and Methods

Adult beetles of the colour form *succinea* were collected outdoors and placed in laboratory, temperature 20°C, photoperiod 16L : 8D, and RH 60%, and fed with aphids *Acyrthosiphon pisum*. Eggs were removed from parents and placed to the experimental incubators at 25°C, 16L : 8D, and three contrasting levels of relative humidity: 30, 60, and 90%. They were checked daily for hatching. Neonate larvae were fed either with aphids *Acyrthosiphon pisum* or frozen eggs of *Ephestia kuehniella*. After moulting to the second instar, larvae were placed in groups of ten into 0.5 L glass jars covered with nylon mesh to provide air flow. They were continuously fed with either diet and provided with water in a vial that enabled limited evaporation. The food of larvae was offered in abundance to minimize their cannibalism. Survival and time of moulting to subsequent larval instars, pupa, and adult were recorded daily to calculate development time. Developmental time of individual instars was based on all individuals that survived to that particular instar even if they subsequently died. Adults were sexed one day after emergence, and fresh mass was recorded on electronic balances with precision 0.1 mg.

Low humidity (30%) was achieved by placing dishes with sodium hydroxide to the incubator; moderate and high humidity was made thanks to small or large evaporation surface of tissue dipped to dish with tap water. Temperature and humidity were measured with electronic equipment placed in the incubator among the jars with beetles and recorded daily. Precize environmental conditions measured twice daily in the three experimental incubators were (mean ± SD) 24.9 ± 0.1°C and 29 ± 6% RH, 24.9 ± 0.2°C and 59 ± 5% RH, and 25.0 ± 0.2°C and 87 ± 1% RH with no difference between morning and evening.

3. Results

3.1. Survival. Overall, survival of larvae from the second to the third instar (S3) was 80%, to the fourth instar, it was 65%, to pupa 37%, and to adult 36%. Individual treatments (food and humidity) slightly differed from each other (Table 1) in survival to the third and fourth larval instars, but there was much better survival to pupa and adult in individuals fed with *Ephestia* eggs than those fed *A. pisum* (χ^2 ($d = 1$) = 9.35, $P = 0.0022$). Survival to pupa and adult on *Ephestia* eggs was lower at 90% RH than in the two lower humidities (χ^2 ($d = 2$) = 8.71, $P = 0.013$). Survival to pupa and adult on *A. pisum* was lower at 30% RH than in the two higher humidities but this difference was not significant (χ^2 ($d = 2$) = 3.81, $P = 0.15$).

3.2. Sex Ratio. Overall, sex ratio in newly emerged adults was 51%, but there was a significant (χ^2 ($d = 2$) = 10.06, $P = 0.0065$) trend for higher proportion of males at low humidity and higher proportion of females at high humidity in both food treatments (Table 1).

3.3. Developmental Time. Development time of the first instar was two days except in larvae fed with *Acyrthosiphon* at 30% RH, where it was slightly but significantly prolonged (two-way ANOVA, $F_{2,404} = 4.76$, $P = 0.009$) (Table 2). There were opposite trends in the development time of the second instar, shortening with increasing humidity in larvae fed with

Table 2: Developmental time (days) and adult fresh mass (mg) of *Harmonia axyridis* reared on two diets and in three levels of relative air humidities at 25°C and 16L : 8D photoperiod. D1 to D4, DL and DP: development time of particular larval instar, entire larval stage, and pupa, FMM: fresh mass of males, FMF: fresh mass of females. Means that do not differ significantly are marked with the same letter in each column.

Food	RH %	D1 days	D2 days	D3 days	D4 days	DL days	DP days	FMM mg	FMF mg
Acyrthosiphon	30	2.1 a	3.6 a	3.4 bc	7.5 a	15.1	5.0	18.2	20.7
Acyrthosiphon	60	2.0 b	3.3 ab	3.5 bc	6.2 b	14.1	5.0	18.3	25.1
Acyrthosiphon	90	2.0 b	3.1 b	3.1 c	6.4 ab	13.5	5.0	19.2	25.4
Ephestia	30	2.0 b	3.1 ab	3.8 ab	6.3 b	14.6	5.0	19.4	23.0
Ephestia	60	2.0 b	3.3 ab	4.0 a	6.9 ab	15.4	5.0	19.4	23.5
Ephestia	90	2.0 b	3.5 ab	3.1 c	7.3 a	14.3	5.0	14.5	21.8

Acyrthosiphon and prolonging with increasing humidity in larvae fed with *Ephestia* (two-way ANOVA, $F_{2,323} = 4.01$, $P = 0.019$). In the third instar, there was high effect of humidity (one-way ANOVA, $F_2 = 13.4$, $P = 3.10^{-6}$) with shorter development at high humidity and a separate effect of food (one-way ANOVA, $F_1 = 10.4$, $P = 0.001$) with longer development time in larvae fed with *Ephestia* eggs than with *A. pisum*. In the fourth instar, there were again opposite trends in the development time ($F_{2,147} = 5.4$, $P = 0.005$) with shortening of time with increasing humidity in larvae fed with *Acyrthosiphon* and vice versa. When analyzing the entire larval development, the opposite trends were still present but not significant (two-way ANOVA, $F_{2,147} = 2.56$, $P = 0.08$); no difference accountable to either food, humidity, or their interaction was found. Pupal development time was identical in all treatments (two-way ANOVA, $F_{2,143} = 0.08$, $P = 0.92$).

3.4. Body Size. There was a trend for increasing adult body mass with increasing humidity in larvae fed with *A. pisum* but not in those fed with *Ephestia* eggs but generally, there was overall no significant difference (two-way ANOVA, $F_{2,143} = 2.37$, $P = 0.097$). When males and females were included in the analysis of variance, the interaction (opposite trends) of food and humidity became significant (three-way ANOVA, $F_{2,143} = 4.06$, $P = 0.02$). Females were much heavier (23.8 mg) than males (18.8 mg) in all treatments (three-way ANOVA, $F_1 = 40.6$, $P < 10^{-6}$) but the differences were greater at 90%. The ratio of body mass of females over males was 1.26.

4. Discussion

4.1. Survival. Since we started the experiment with second instar larvae, and there is often high mortality in the first instar, our data are not fully comparable to others. Anyway, our survival rates are much lower than those of Berkvens et al. [13]. They reared each larva individually, while we started with groups of ten. Cannibalism rate is high in this ladybird species and accounted for a part of the mortality, although we have not precise data. At least, the cannibalism recorded in this study did not occur due to the lack of food. Earlier, we observed increased larval cannibalism when larvae did not have an access to liquid water for drinking, so we expected higher mortality at low humidity which was not confirmed.

4.2. Developmental Time. In a previous study [14], at 26°C on a diet of *Acyrthosiphon pisum*, the mean duration of each stage was as follows: egg 2.8 days, first instar 2.5 days, second instar 1.5 days, third instar 1.8 days, fourth instar 4.4 days, and pupa 4.5 days. In our experiments, the development was longer for the second through the fourth larval instars (3.3, 3.5, and 6.8 days). Similarly, we measured complete larval development time (12.7 days) on *A. pisum* diet in a previous study [15] which was shorter than the time measured here (14.5 days). The total developmental time was 20.5 days in *succinea* morph whose parents were wild-caught in Belgium and larvae fed with *A. pisum* at 23°C [16], while in our study, the total development time was estimated as 22.5 days (providing egg stage was 3 days). Part of the difference might be explained by molesting of the satiated larvae by aphids crawling over the container. In some of the previous studies, aphids were provided with their host plants and thus did not disturb resting larvae. In the present study, plants were not included because they would increase the humidity in containers, and aphids were walking all the time over the containers, disturbed resting satiated larvae which on turn also walked around the container resulting in energy expenditure and subsequently longer development.

There was generally no significant difference in the development time between the "natural" food, aphid *A. pisum* and supplement food, and frozen eggs of the flour moth *E. kuehniella*. Similarly, Berkvens et al. [16] found similar developmental times when feeding several morphs or populations by these two foods.

4.3. Body Size. While higher temperature shortens the development, it often decreases adult weight. Ladybird larvae reared at higher temperatures produced smaller adults than larvae reared at lower temperatures [17]. Among our treatments, there were cases when shorter developmental time resulted in larger adults but also vice versa. Fresh body mass was smaller in our experiments than in those of Berkvens et al. [16] (about 35 mg) for both foods.

4.4. Interaction between Food Type and Humidity. Three important life history parameters, that is, survival rate, larval developmental time, and adult mass were all differently influenced by diverse ambient humidity depending on the food type. In individuals fed with aphid *A. pisum*, there

was a trend for better survival, shorter development, and higher mass gained at higher humidity. These trends were opposite or nonsignificant in individuals fed with eggs of *E. kuehniella*. Such a difference might be explained by the food quality. While aphids survived longer and in a better condition at high air humidity when they were without their food plant, moth eggs became soon mouldy and rejected at the highest humidity. It seems that the effect of different humidities was mainly mediated through the food and did not strongly influence the life parameters directly. Since *H. axyridis* performed comparatively well in all levels of humidity, it seems that it is not a strong single limiting factor for the further spread of this alien ladybird species to new areas.

4.5. Sex Ratio. The only measured parameter that showed a consistent trend regardless of food treatment was the sex ratio. It was close to 1 : 1 (0.5) at the medium humidity, while there were more males at low humidity and more females at high humidity. It emerged as differential larval mortality, maybe different rates of larval cannibalism. The only similar case found was the sex ratio of *Attagenus fasciatus* (Coleoptera: Dermestidae) which was male biased at 30°C at 40 and 60% RH, but not at 80% RH [18].

From the evolutionary perspective, sex ratio biased towards females is better, resulting in higher population growth, so that areas with high humidity might be colonized more quickly than those with low air humidity.

5. Conclusion

The effect of air humidity is context dependent. In our study, we used only one constant experimental temperature and constant humidity, while in the field, these factors fluctuate on a daily basis. The effect might be different in the field where plant material or other resources provide water for drinking or higher-humidity shelter in generally dry conditions or exposure to sun may generally reduce high humidity. The only report from the field conditions was a negative correlation between *H. axyridis* abundance and air relative humidity (70–90%) in noncitric plants in Brazil [19]. We recommend to monitor the sex ratio in field populations in regions with contrasting humidity although they must be checked for the occurrence of male killing bacteria [20].

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Research Article

The Dark Side of the Light Show: Predators of Fireflies in the Great Smoky Mountains

Sara M. Lewis,[1] Lynn Faust,[2] and Raphaël De Cock[3]

[1] *Department of Biology, Tufts University, Medford, MA 02155, USA*
[2] *Emory River Land Company, 11828 Couch Mill Road, Knoxville, TN 37932, USA*
[3] *Evolutionary Ecology Group, University of Antwerp, 2610 Antwerp, Belgium*

Correspondence should be addressed to Sara M. Lewis, sara.lewis@tufts.edu

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In the Great Smoky Mountains of East Tennessee, the Light Show is a popular seasonal attraction created by thousands of courting male *Photinus carolinus* fireflies (Coleoptera: Lampyridae) that flash in synchrony to locate females. This study was undertaken to provide a temporal snapshot of whether invertebrate predators are active within these dense and conspicuous firefly breeding aggregations. In addition, we examined whether female *Photuris* fireflies, which are specialist predators on other fireflies, show any feeding preferences within the diverse local firefly fauna. A field survey revealed a surprisingly diverse suite of generalist insectivores feeding on fireflies within *P. carolinus* breeding aggregations. In addition, laboratory studies revealed major differences in prey consumption rates when *Photuris* predators were given access to several lampyrid taxa. This suite of generalist and specialist predators appears to create a complex selective landscape that is predicted to be a powerful force shaping the evolution of firefly defenses.

1. Introduction

Animals with conspicuous courtship displays that breed in dense aggregations are expected to be targeted by many predators [1, 2]. Fireflies (Coleoptera: Lampyridae), however, have a reputation for being distasteful to many potential predators [3, 4]. Several lampyrid taxa have been shown to contain chemicals that confer protection against generalist insectivores such as birds, spiders, and ants [5–7]. While lists have published tallying instances of observed predation on various fireflies [3, 8], no study has described the predator guild active within a firefly breeding aggregation at a single location and season.

In North America, *Photuris* fireflies are specialist predators that eavesdrop on the courtship signals of other fireflies [9–12]. *Photuris* females have been shown to be voracious predators of certain *Photinus* fireflies [3, 13, 14], from which they sequester defensive compounds known as lucibufagins [6]. Lloyd [3, 10] reviewed numerous field observations of *Photuris* females preying upon several firefly species. Eisner et al. [6] reported lab studies in which 6 *Photuris* females each ate 2 *Photinus ignitus* males, and a study by Gronquist et al. [15] found that 5 *Photuris* females each ate 3 *Lucidota atra* fireflies, a diurnally active species that also contains lucibufagins. To date, however, no systematic study has been made of the feeding proclivities of these predatory *Photuris* fireflies.

The Great Smoky Mountains in East Tennessee host a diverse and abundant lampyrid fauna, including both diurnal and nocturnal species [16, 17]. Among these are *Photinus carolinus*, a species in which thousands of males gather in dense aggregations and flash synchronously to locate females [18, 19]. In the Great Smoky Mountains National Park (GSMNP), this phenomenon is popularly known as the Light Show. During their 2-week mating season in June, these fireflies attract close to 30,000 park visitors. Such aggregations might be expected to attract many predators as well. Faust [19] reported that *P. carolinus* males were often caught in webs of Araneidae spiders, and harvestmen (Opiliones: Phalangiidae) was found carrying dead *P. carolinus*.

Another abundant nocturnal firefly, *Phausis reticulata*, is also active in this rich alluvial montane habitat at the same time of night. Commonly known as blue ghost fireflies, these males fly slowly over the forest floor emitting a blue-green flickering glow. However, to date there has been no systematic survey describing the common predators of these two firefly species which are so popular with park visitors.

This study was conducted during the *P. carolinus* mating season with the goal to survey invertebrate predators of *P. carolinus* and *Phausis reticulata* adults and also to determine whether specialist *Photuris* predators differentially prey on various firefly taxa.

2. Methods

2.1. Field Surveys of Firefly Predators. Field observations were conducted at GSMNP by walking along a ~4 km path through *P. carolinus* breeding aggregations from 2000 to 2400 h during the peak display season (4–19 June 2011). Our surveys were conducted in Sevier Co. at Elkmont, Tennessee (35°39′13″N, 83°34′50″W), although this species is found throughout the park in second growth hardwood forests at about 750 m elevation [19]. Male courtship signals in *P. carolinus* consist of flash trains containing 4–8 pulses given at 0.5 sec intervals, followed by 6–9 sec of darkness; females respond to male advertisements by emitting a doublet flash approximately 3 sec following final pulse in a male's flash train [19]. We detected predation by looking along the ground and on vegetation for the distress flashes given by *P. carolinus*; these distress flashes consist of consistent, rhythmic single flashes repeated every 1.5–3 sec [19] and are easily distinguished from firefly courtship flashes. We also looked for continuous stationary glows emitted from the light organ of injured fireflies. Whenever predator-prey interactions were observed, they were recorded and photographed (Sony Cybershot DSC-T20). Prey captured by orb-weaving spiders was monitored by counting firefly and other captured prey nightly in webs at ~2400 h, toward the end of the *P. carolinus* flight period. Since webs were less likely to contain glowing prey towards the end of the firefly season, web surveys were made with spotlights.

Similar observations focusing on invertebrate predators of nocturnal fireflies were also made in other areas of GSMNP. Birds and other potential diurnal predators were not covered by our surveys, as nocturnal fireflies such as *P. carolinus* disperse during the day to rest on or under vegetation, and thus their interactions with diurnal predators are quite difficult to observe. Similarly, it was logistically impossible to include bat predators in our field survey.

2.2. Laboratory Tests of Photuris Feeding Preferences. While most adult fireflies do not feed, some *Photuris* females are specialist nocturnal predators that hunt *Photinus* males using a combination of stalking, aerial hawking, and aggressive mimicry of prey females [9, 10, 13, 20]. To determine whether predaceous *Photuris* females show preferences among males of different lampyrid taxa, we conducted laboratory trials using as prey several different firefly species that overlapped spatially and/or temporally with *Photuris* spp. Because *Photuris* is a taxonomically problematic group currently in need of revision, it is not possible to provide definitive species identifications for these predators. These females included *Photuris hebes* and *P. lucicrescens*, while others were in the *Photuris versicolor* complex (J. E. Lloyd, *personal communication):* here we refer to them collectively as *Photuris*.

All fireflies were kept on a 14 : 10 light cycle (this was shifted from natural by 9 h). Predatory *Photuris* females were housed individually in 1-quart (14 cm height × 10 cm diameter) plastic containers with damp paper towel and a silk plant, and prey was added at dusk. Because prey could move about and avoid attacks, this experimental setup provided considerably more natural conditions than the 9 cm petri dishes assays that have previously been used in lab studies of *Photuris* predation [6, 15]. *Photuris* behavior was observed for the first hour under blue light (many lampyrids show reduced retinal sensitivity for these wavelengths [21]), and trials were checked periodically for 24 h. These laboratory trials were conducted between 6 and 21 June 2011. Most prey was offered in pair-choice trials, which allowed us to test several species during their short breeding seasons. Some prey was offered in single-choice trials: 4 (of 8) *Phausis reticulata*, and 8 (of 40) *Photinus pyralis*.

Because they are lampyrid specialists, none of the 11 *Photuris* females we tested consumed any of the "palatable" prey we offered them in these experiments (these included *Tribolium* beetle larvae, as well as various flies, click beetles, grasshoppers, and bugs that were collected from the field). We therefore confirmed that *Photuris* predators were hungry, following trials in which no prey was eaten, by offering them prey shown to be highly desirable (*P. carolinus* or *L. atra*) in our preliminary experiments.

3. Results

3.1. Field Surveys of Nocturnal Firefly Predators. Several predators were found actively hunting in the midst of *P. carolinus* mating aggregations (Figure 1). Orb-weaving spiders including *Cyclosa conica* (Figure 1(a)) and *Neoscona arabesca* constructed webs at dusk that captured mainly *P. carolinus* males, which constituted 72% of prey items; on a single night one web contained 7 *P. carolinus* males. In addition, 2 *Phausis reticulata* males (blue ghost fireflies) along with 1 *P. carolinus* female were found trapped in webs. Thus, of the 25 total prey items found in these webs, the vast majority were male fireflies. Many of the males continued to flash rhythmically after they were wrapped in silk. We also noticed that fireflies were often positioned at the center of spider webs, although we did not quantify how often this occurred.

Remains were collected the next morning below marked web locations, and these silk-wrapped fragments suggested active predation on *P. carolinus* by orb-weavers. Some *P. carolinus* males had been partially consumed, while others were largely intact but had a large puncture wound at the anterior corner of their wing cover.

Additional predators included several *Leiobunum* spp. harvestmen (Opiliones: Phalangiidae) that we observed feeding on *P. carolinus* males. One had captured a newly eclosed *Photuris* which struggled unsuccessfully to escape (Figure 1(b)). In addition to preying on live fireflies, harvestmen were also observed feeding on silk-wrapped fireflies that they apparently scavenged from beneath the webs of orb-weaving spiders. An assassin bug, *Zelus luridus* (Hemiptera: Reduvidae), was found perched on a hickory tree leaf ~10 ft off the ground, feeding on a *P. carolinus* male that

(a)

(b)

(c)

(d)

FIGURE 1: Some invertebrate predators of fireflies in the Great Smoky Mountains (photos by R. De Cock). (a) Orb-weaving spider (Araneidae) *Cyclosa conica* attacking a *Photinus* male that has been caught and wrapped. (b) Harvestman *Leiobunum* spp. (Opiliones: Phalangiidae) attacking a newly eclosed *Photuris* firefly. (c) *Zelus luridus* assassin bug (Hemiptera: Reduviidae) feeding on a male *Photinus carolinus*. (d) *Bittacus* spp. hangingfly (Mecoptera: Bittacidae) consuming a male *Phausis reticulata*.

was flashing periodically. When brought into the lab, this bug recommenced feeding on the same *P. carolinus* male by piercing the intersegmental membrane between the fireflies' thorax and abdomen with its proboscis (Figure 1(d)). Two small *Theridion* spp. cobweb spiders were each found eating a *Phausis* male wrapped in silk. In addition, two *Bittacus* spp. hangingflies (Mecoptera: Bittacidae) were each found consuming still-glowing *Phausis* males (Figure 1(d)).

When we surveyed four webs at the end of the *P. carolinus* flight season, we found only a single nonfirefly prey item at the study site. However, two webs were found nearby that each contained a single firefly (one web captured a *P. carolinus* male and the other a *Phausis reticulata* male). Thus, web capture efficiency appeared to be dependent on firefly population density, with fewer captures as firefly abundance declined.

3.2. Laboratory Tests of Photuris Feeding Preferences. All *Photuris* females fed readily under these experimental conditions (Figures 2(a) and 2(b)); one predator consumed 8 out the 11

(a) (b) (c) (d)

Figure 2: Laboratory tests of *Photuris* feeding preferences choice. (a) Trial setup with single *Photuris* spp. female in 1-quart container with artificial plant. (b) Female *Photuris* attacking an *L. atra* firefly. (c) Remnants of a male *Photinus carolinus* (arrow indicates dried hemolymph on the elytra). (d) A surviving male *Photinus pyralis* (arrow indicates puncture wound on left elytra).

prey she was offered over 7 days of testing. During the light photoperiod both predators and prey generally rested on the upper or lower surface of artificial leaves or sides of the container. Within 1 hr of dark phase, fireflies started walking, flashing, and occasionally flying. When prey fireflies were contacted by the *Photuris* female, they rapidly withdrew or dropped to the bottom of the container. During a predator attack, the *Photuris* female grasped the firefly with her front legs and then bit into the prey, often between the elytral shoulder and the pronotum, with her mandibles. Prey reflex bleeding (originally described by Blum and Sannasi [22] for *Photinus pyralis*) was often observed. When they were bitten, both *P. pyralis* and *P. carolinus* males released copious amounts of thick white fluid; we often observed that this fluid rapidly coagulated into a sticky mass that coated the predator's mouthparts. Although this appeared to temporarily prevent the *Photuris* female from continuing her attack, under laboratory conditions most predators eventually returned to continue feeding on the wounded prey. Notably, *Phausis reticulata* males did not exhibit reflex bleeding although they typically showed prolonged thanatosis. After 24 hours, prey that had been successfully attacked had been reduced to scattered bits of exoskeleton, including pronotum, eyes, elytrae, and wings (Figure 2(c)).

We found marked differences in consumption rates when various firefly species were offered to captive *Photuris* females (Figure 3). In three *Photinus* species, *P. carolinus*, *P. macdermotti*, *and P. marginellus*, 60–76% of males were eaten within 24 h; in contrast, only 12.5% of *Photinus pyralis* males were eaten. Microscopic examination of the surviving *P. pyralis* males revealed that many had been attacked, as bite marks and dried blood were seen on their pronotum or elytra (Figure 2(d)). Most *Phausis reticulata* also remained uneaten over 24 h, although again close examination of the surviving males revealed bite marks on their elytra or abdomen. We also tested two diurnally active species of *Lucidota* fireflies, *L. atra* and *L. punctata*, both of which were readily consumed by *Photuris* females (Figure 3).

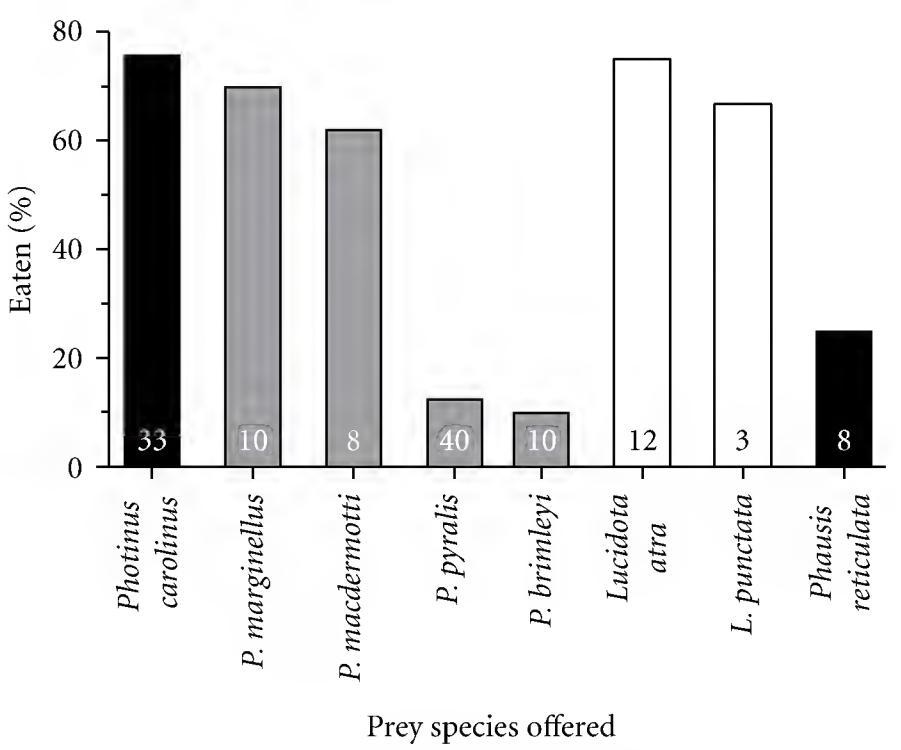

Figure 3: Differences among firefly taxa in the percentage of individuals consumed by predatory *Photuris* femmes fatales during 24 h laboratory trials. Sample sizes (number of trials) are shown within bars, and shading indicates the activity period of each species (black: fully nocturnal; grey: dusk-active; white: diurnal).

We incidentally noted very few parasitoids among the fireflies collected for this study: about 5% of *Photuris* females appeared to die from tachinid parasitism (*Strongygaster triangulifera* Loew), and less than 5% of prey (all species combined) died due to phorids (*Apocephalus antennatus* Malloch).

4. Discussion

Previous reviews have described the general diversity of firefly predators [3, 4]. Our study focused on providing a temporal snapshot of predation upon Smokies fireflies during the brief but explosive breeding season of the synchronous firefly, *Photinus carolinus*. Our field survey revealed a surprisingly diverse suite of predators feeding on fireflies

within these aggregations, including the first record of hangingflies (Mecoptera) eating fireflies, here *Phausis reticulata*. Our results confirm that harvestmen are firefly predators as well as scavengers. This supports previous observations by Lloyd [3], who also noted that harvestmen (including *Leiobunum*) ate live fireflies (*Photuris* spp. and *Photinus scintillans*) on three occasions. It is not known whether these opilionid arachnids are able to visually detect light-emitting prey, but the capacity to orient toward light has been demonstrated for two cave-dwelling harvestmen that feed exclusively on the bioluminescent dipteran, *Arachnocampa luminosa* [23]. Our observation of predation on *Photinus carolinus* fireflies by the reduviid bug *Zelus luridus* confirms Lloyd's [3] reports of *Zelus spp.* attacking a female *Photuris congener*, and an unsuccessful attack by another reduviid on a male *Pteroptyx* firefly. Spiders and other invertebrates have also been reported to consume various Japanese fireflies [8]. It seems likely that such predators use a combination of substrate vibrations and visual cues to detect fireflies walking on leaf litter or vegetation, as shown for lycosid spiders [24].

Orb-weaving spiders are a notable source of male-biased mortality for fireflies, as they mainly capture flying males that are searching for females [3]. We found this to be especially true for the males of two fully nocturnal fireflies, *P. carolinus* and *Phausis reticulata*. While diurnal (*Lucidota atra*, *L. punctata*) and dusk-flying fireflies (*Photinus macdermotti* and *P. marginellus*) were also active at this site, these species were less susceptible to predation by nocturnal orb-weavers because their webs were constructed after dark and then dismantled before the next morning. Our field survey also supports previous observations that once fireflies are captured in a web, many continue to glow or flash rhythmically [3, 8, 25]. Previous authors have suggested that this behavior acts as a bioluminescent lure to attract additional prey to the web; so it would repay further investigation to determine whether and how spiders are able to induce their prey's bioluminescence. We also noticed that captured fireflies were quite often positioned at the center of webs, which might also serve to maximize a spider's chance of capturing additional prey that had been attracted.

Consumption of *Photinus carolinus* and *Phausis reticulata* by these diverse generalist predators remains somewhat surprising because firefly taxa have been shown to contain a variety of defensive steroidal pyrones collectively known as lucibufagins (LBGs). First isolated from *Photinus ignitus* and *P. marginellus* [5], LBGs have also been found in *P. pyralis* [26] and *Lucidota atra* [15], as well as in larvae of the European glow-worm, *Lampyris noctiluca* [27]. LBG deters predation by at least two generalist predators: *Hylocichla* thrushes [5] and *Phidippus* jumping spiders [6]. As *Photinus carolinus* and *Phausis reticulata* have not yet been examined, it is possible that these species lack chemical defenses. Alternatively, it may be that the suite of generalist predators active within these breeding aggregations is able to circumvent firefly chemical defenses.

Our lab results confirm previous field observations indicating that nocturnally active *Photuris* females are specialist predators upon other fireflies[9]. Eisner et al. [6] demonstrated that *Photuris* fireflies are incapable of producing LBG on their own but rather must rely on acquiring these compounds from their prey to gain protection against their own predators. Thus, we expect *Photuris* predation to select for very different defensive strategies than those that might be effective against generalist insectivores.

Although our results indicate that *Photuris* females readily consume a broad range of lampyrid prey, including males of the synchronous species *Photinus carolinus*, firefly taxa differed markedly in their susceptibility to predation by *Photuris* fireflies. What factors might account for such differences? It might be predicted that those prey species whose activity period overlaps with the fully nocturnal *Photuris* would show reduced susceptibility. However, observed *Photuris* predation rates did not follow this prediction: low consumption rates were seen for the fully nocturnal blue ghost firefly, *Phausis reticulata*, but also for two dusk-active fireflies, *Photinus pyralis* and *P. brimleyi*. In addition, *Photuris* females readily consumed some dusk-active, some fully nocturnal, and two diurnal species.

Another reasonable prediction is that *Photuris* consumption rates might be positively correlated with LBG content across lampyrid taxa. Unfortunately, this cannot currently be tested because the defensive chemistry of most firefly taxa remains unexamined. However, two firefly species shown in our study to be highly palatable are known to contain LBG: *Photinus marginellus* [5] and *Lucidota atra,* [15]. Thus, these species and others such as the synchronous firefly *Photinus carolinus* could be especially targeted by *Photuris* predators that are seeking to obtain LBG.

Several explanations may be considered for the very low *Photuris* predation rates we observed on three firefly species. Many firefly taxa exhibit reflex bleeding when disturbed, emitting droplets of hemolymph from their elytra and pronotum [22]. The released hemolymph rapidly coagulates, and this lampyrid bloodbath has previously been shown to deter predation by ants [7, 8, 22]. Our observations indicate that reflex bleeding may also help some fireflies escape predation by *Photuris* females, as we observed predators that were incapitated when their mouthparts became coated by sticky, coagulated blood. *Photinus pyralis* males are presumably desirable prey as they contain LBG [26], and they might use such copious reflex bleeding to gain mechanical protection against *Photuris* predators. The same may be true for *Photinus brimleyi*, although its defensive chemistry is unknown. Strong selection is expected for a prey's ability to glue shut a predator's mouthparts, as under natural conditions this would almost certainly allow the prey to escape. An alternate explanation is that additional chemical deterrents, or different and perhaps less desirable forms of LBG [26], make these particular *Photinus* species less attractive as prey for *Photuris*. Finally, in spite of their lack of reflex bleeding, *Phausis reticulata* males were often attacked yet not eaten. This suggests that these blue ghost fireflies also may have additional chemical deterrents and/or may lack the particular LBG required by *Photuris* females.

In summary, this temporal snapshot of predators active within Smokies firefly aggregations has revealed a surprisingly diverse suite of generalist insectivores. In addition,

laboratory studies in which specialist *Photuris* predators were given access to several lampyrid taxa revealed major differences in prey consumption rates. The predator-prey interactions described here suggest that the evolution of firefly defenses occurs within a complex selective landscape involving both generalist and specialist predators. Testing evolutionary hypotheses concerning firefly chemical defenses and their effectiveness against both types of predators should prove a powerful approach for future investigations.

Additional Information

See Supplementary Material available online at doi: 10.1155/2012/634027. It is a short video that illustrates common predators on fireflies and shows attacks by predatory *Photuris* fireflies.

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Research Article

The Influence of Abiotic Factors on an Invasive Pest of Pulse Crops, *Sitona lineatus* (L.) (Coleoptera: Curculionidae), in North America

O. Olfert,[1] R. M. Weiss,[1] H. A. Cárcamo,[2] and S. Meers[3]

[1] *Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK, Canada S7N 0X2*
[2] *Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403 1st Avenue South, Lethbridge, AB, Canada T1J 4B1*
[3] *Alberta Agriculture and Rural Development, Brooks Research Centre, 131 S.S. No.4 Brooks, AB, Canada T1R 1E6*

Correspondence should be addressed to O. Olfert, owen.olfert@agr.gc.ca

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Pea leaf weevil, *Sitona lineatus* (L.), native to Europe and North Africa, has been introduced into many other countries around the world, including the USA and Canada. Adults are oligophagous pests on leguminaceous plants. *Sitona lineatus* was first recorded in Canada in 1997, near Lethbridge, Alberta. Since then, it has spread north in Alberta and west into Saskatchewan in 2007. Bioclimatic simulation models were used to predict the distribution and extent of establishment of *S. lineatus* in Canada based on its current geographic range, phenology, relative abundance, and empirical data. The study identified areas in Canada that are at risk for future establishment of *S. lineatus* and developed a better understanding of climate effects. Climate change projections (General Circulation Models) were then imposed on the bioclimatic model of *S. lineatus*. Bioclimatic model output varied for each of the three General Circulation Models. In terms of suitability for pest establishment (Ecoclimatic Index), the NCAR273 CCSM climate data resulted in the most significant shift northward.

1. Introduction

Pea leaf weevil, *Sitona lineatus* (L.), native to Europe and North Africa, has been introduced into many other countries around the world, including North America. Adults are oligophagous pests on leguminaceous plants. *Sitona lineatus* was first recorded in Canada in 1997, near Lethbridge, Alberta. [1–3]. The adults are oligophagous pests on leguminaceous plants but prefer and maximize their reproductive potential on peas and faba beans [4]. The species has one generation per year [5]. Adults overwinter in a variety of locations, particularly sites containing perennial legumes and weeds. In spring, adults leave overwintering sites in search of pea fields. Eggs are laid in the soil, near developing pea plants. Larvae feed on root nodules and develop through five instars. Pupation occurs in the soil. In late summer adults leave pea fields in search of late season pulse crops before overwintering [6]. Adults feed on leaf margins of legume seedlings. Larval feeding on nodules can result in partial or complete inhibition of nitrogen fixation [7].

Sitona lineatus was first collected in Canada in 1997, near Lethbridge, Alberta [4]. Since then, it has spread northward in Alberta and east into Saskatchewan in 2007 [8, 9]. The introduction of *S. lineatus* into this region presents a risk to pea production in Northern Great Plains of Canada and USA [10, 11].

Abiotic factors, primarily climate, constrain population growth and survival that ultimately affect species distribution and abundance [12]. Bioclimatic simulation models have been used successfully to predict the distribution and extent of insect establishment in new environments [13–17]. Bioclimatic modelling software, such as CLIMEX [14], enables the development of models that describe the potential distribution and seasonal abundance of a species based on climate. Inferential models infer a species response to climate, based on its geographic range, phenology, seasonal abundance, and empirical data. CLIMEX models allow researchers to develop an overview of climatic factors that affect species distribution and abundance and permit identification of nonclimatic factors that limit species distribution [14]. Sensitivity

analysis can be used to test hypotheses related to the effect of varying climate variables (i.e., warmer/cooler or wetter/dryer than normal conditions) on the species distribution and abundance [14].

The objectives of the study were to develop a bioclimatic model to predict potential range and relative abundance of *S. lineatus*, to identify areas in Canada that are at risk for future establishment of the pea leaf weevil, and to use the model to develop a better understanding of how a changing climate might potentially influence *S. lineatus* populations across North America.

2. Methods

The bioclimatic modeling process has been previously described [15, 18, 19]. CLIMEX models derive Ecoclimatic Index (EI) values that describe the climatic suitability, in terms of insect survival and reproduction, of specific locations. The respective Growth Indices and Stress Indices (with related parameters) are illustrated in Table 1. The EI value integrates annual growth (GI) with annual stress (heat, cold, dry, wet) to produce a single value (between 1 and 100) for each location. Ecoclimatic Index values near zero indicate that the location's climate is not suitable for long-term establishment of the species. An EI value greater than 20 indicates a "Very Favourable" climate.

Initial model parameter values were based on published data that resulted from laboratory and field studies [3, 6, 20–24] and are defined in Table 1. Climatic requirements were inferred from known distributions of pea leaf weevil in Europe. The model for *S. lineatus*, using CLIMEX 3.0 [14], was developed by iteratively adjusting parameter values to produce mapped results that closely approximated observed distribution for *S. lineatus* in Europe [1–3]. Model parameterization was conducted for Britain, Denmark, France, Germany, Switzerland, Norway, and The Netherlands. The remaining European countries were treated as an independent dataset and used for model validation. Once the European distribution was defined, based on a visual comparison of model output with observed distribution, EI values were compared to reported data on relative abundance. Published results related to abundance were used to refine parameter values so that highest EI values occurred where *S. lineatus* was known to cause damage and lower values occurred when the species was less prevalent.

The model was validated by comparing output to reported distributions and seasonal phenology and tested for consistency with empirical data. Three methods were used to validate the model. The model was then applied to predict the population distribution of *S. lineatus* in eastern Europe (Bulgaria, Czech Republic, Hungary, Poland, Romania, Slovakia, Ukraine, and Yugoslavia), Asia, Washington, Oregon, and Idaho. Model output for these regions was compared to known distributions as reported by Schotzko and Quisenberry [25], Fauna Europaea Web Service [1], and Hoebeke and Wheeler Jr. [3]. Second, model output for phenology and life history was compared to published reports for Europe [22, 26]. Third, model results which related to insect phenology were based on weather data and insect population data collected from southern Alberta [27].

The CLIMEX model required five meteorological inputs: temperature (maximum and minimum), precipitation, and relative humidity (09 : 00 and 15 : 00 hours). The *Compare Locations* function required monthly long-term average climatic variables. Climate data was used as an input for the Compare Locations function. The dataset represents a splined 0.5° world grid dataset [28]. Models were run for Europe ($n = 6416$ grids) and Canada (south of 65°N latitude, $n = 4472$ grids). The moisture index (MI) is based on a calculated soil moisture value. CLIMEX used a hydrological submodel to compute a weekly soil moisture balance. Soil moisture balance was based on soil moisture from the previous week, current week values for precipitation and evapotranspiration. CLIMEX used a degree-day model, based on the algorithm published by Baskerville and Emin [29], to compute the temperature index (TI) and the potential number of generations per year.

Climate change projections were obtained from the Intergovernmental Panel on Climate Change [30] as monthly means for three General Circulation Models (GCMs), based on current climate, 30-year average (1961–1990) dataset (A1B emission scenario) (CRU: Climate Research Unit, East Anglia, UK). The GCMs used were CSIRO Mark 3.0 (CSIRO, Australia), NCAR273 CCSM (National Centre for Atmospheric Research, USA), and MIROC-H (Centre for Climate Research, Japan). All three had the requisite climatic variables at a temporal resolution appropriate for CLIMEX and were pattern-scaled to develop individual change scenarios relative to the base climatology [31]. The GCMs cover a range of climate sensitivity, defined as the amount of global warming for a doubling of the atmospheric CO_2 concentration compared with 1990 levels [32]. The respective sensitivities are CSIRO Mark 3.0 (2.11°C), NCAR-CCSM (2.47°C), and MIROC-H (4.13°C).

In order to query the resulting database at a regional scale, a geographic rectangle, 4° latitude by 7° longitude, was used to delineate a regional template consisting of 112 grid cells. Specific regions, based on latitude and longitude coordinates, were defined and output (averaged across the region) was generated for detailed analysis. The datasets permitted comparison of variables, both spatially and temporally (weekly intervals). Analyses were based on values centered on six locations including Peace River, Alberta (56.25°N; 117.25°W), Lethbridge, Alberta (49.75°N; 112.75°W), Red Deer, Alberta (52.25°N; 113.75°W), Saskatoon, Saskatchewan (52.25°N; 106.75°W), Regina, Saskatchewan (50.25°N; 104.75°W), and Winnipeg, Manitoba (49.75°N; 97.25°W).

Sensitivity analysis was conducted to quantify the response of *S. lineatus* to changes in precipitation and temperature. Incremental scenarios were developed to reflect the possible range of temperature and precipitation values that could be expected to occur in Europe and Canada, based on current climate. Scenarios were selected, based on potential variation of present climate. EI values, based on current climate, were compared to scenarios that differed by −2, −1, +1, and +2°C from current temperatures (maximum

Table 1: Descriptions of CLIMEX parameters and parameter values used to predict the potential distribution and relative abundance of *Sitona lineatus* in North America.

Index	Parameter	Description	Value
Temperature	DV0	Limiting low temperature	7.0°C
	DV1	Lower optimal temperature	16.0°C
	DV2	Upper optimal temperature	25.0°C
	DV3	Limiting high temperature	32.0°C
Moisture	SM0	Limiting low soil moisture	0.10
	SM1	Lower optimal soil moisture	0.40
	SM2	Upper optimal soil moisture	1.00
	SM3	Limiting high moisture	1.50
Diapause	DPD0	Diapause induction day length	14 h
	DPT0	Diapause induction temperature	11.0°C
	DPT1	Diapause termination temperature	3.0°C
	DPD	Diapause development days	120
	DPSW	Diapause indicator for winter diapause	0
Cold Stress	TTCS	Cold stress threshold	−14.0°C
	THCS	Cold stress temperature rate	−0.00025
Heat Stress	TTHS	Heat stress temperature threshold	34.0°C
	THHS	Heat stress temperature rate	0.002
Dry Stress	SMDS	Dry stress threshold	0.02
	HDS	Dry stress rate	−0.003
Wet Stress	SMWS	Wet stress threshold	2.0
	HWS	Wet stress rate	0.01
Day-degree accumulation above DV0			
	DV0		7.0
	DV3		32.0
Day-degree accumulation above DV3			
	DV3		32.0
	DV4		100
Day-degree accumulation above DVCS			
	DVCS		8.0
	DV4		100
Degree-days per generation			
	PDD	Minimum degree days above DV0 to complete generation	450

and minimum monthly values) and precipitation values (monthly total) that were −40, −20, +20, and +40% of current values. The comparison was conducted for five locations within the major pulse crop production region of western Canada (Table 2). The locations were selected to provide a range of EI values (EI = 22–36).

Contour maps were generated by importing CLIMEX output into ArcView 8.1 [33]. Ecoclimatic Index values were displayed in five categories: "Unfavourable" (0–5), "Suitable" (5–10), "Marginal" (5–10)"Favourable" (15–20), and "Very Favourable" (>20). The "Suitable" and higher categories represent areas that may experience pest outbreaks of *S. lineatus*. Actual densities will be dependent on meteorological conditions that differ from long-term climate normals. The "Favourable" and "Very Favourable" categories describe meteorological conditions, similar to long-term climate normals, in which outbreaks resulting in crop damage may occur.

3. Results and Discussion

3.1. Model Development. In Europe, Hans [23] reported that overwintered *S. lineatus* adults become active when temperatures exceed 4.5°C. Flight was found to occur when temperatures were greater than 12.5°C [6]. In North America, Prescott and Reeher [22] observed that overwintered adults began spring flight in March, when maximum temperatures were 57°F (13.9°C) or greater. In Idaho, USA, Fisher [21] reported that adult flight occurred between April 25 and May 19 and adult flights, out of the host crop, occurred in late July and August.

Table 2: Effect of changes in mean annual precipitation (−40% to +40%) and temperature (−2 to +2) from current values on Ecoclimatic Indices for *Sitona lineatus* at five locations.

			Current	Change in mean precipitation (%)				Change in mean temperature (C)			
Location	Latitude	Longitude	Climate	−40	−20	+20	+40	−2	−1	+1	+2
Lethbridge, Alberta	49.69°	−112.83°	11	3	7	16	19	10	11	11	10
Red Deer, Alberta	52.27°	−113.80°	21	10	17	22	22	14	18	24	26
Regina, Saskatchewan	50.44°	−104.61°	11	3	7	15	19	10	11	10	9
Saskatoon, Saskatchewan	52.15°	−106.65°	10	2	6	14	19	10	9	9	8
Winnipeg, Manitoba	49.89°	−97.15°	20	8	14	22	22	18	20	20	18

In Europe, oviposition was found to occur when the daily mean temperature is 12°C and the daily temperature must rise above 13°C for some hours [34]. In Idaho, USA, oviposition occurred during May [21]. Prescott and Reeher [22] reported that in the Pacific Coastal region of North America oviposition can occur between February and May. Lerin [26] reported that it took 70 days for eggs to hatch at 8°C and 6.2 days at 29°C. Development was linear up to 25°C and only one day difference between 25 and 30.5°C. Egg mortality was negligible at 30°C, 26% at 32°C, and 100% at 33°C. In England, larvae were collected on May 21 and in Scotland larvae were collected as late as July 24 [24]. In Idaho, USA, pupae required 14–18 days to complete development and pupae occurred from early July to early August [21]. In England, late summer flights began soon after adults emerged from pupal cells during late July, with flights continuing until mid-October [6].

The model was developed to produce output that fitted with reported results, based on phenology and distribution for Britain, Denmark, France, Germany, Switzerland, Norway, and The Netherlands. Limiting lower temperature (DV0) values between 4 and 12°C were iteratively tested and a value of 7°C provided the best fit with distributions and phenologies in Europe. Similarly, values for optimal and limiting high temperatures (DV1, DV2, DV3) were incrementally adjusted in order to develop a model that matched reported distributions and phenologies for Europe (Table 1).

Soil moisture indices (SM0, SM1, SM2, SM3) reflected the assumption that soil moisture is a significant factor that is related to plant moisture content and microclimatic conditions [14]. The moisture index was based on computed weekly soil moisture levels. This species, particularly larval stages, appears to prefer moist conditions. High temperatures and dry soil resulted in mortality rates of eggs and larvae reaching 85% [21]. Andersen [34] reported that survival of first instar larvae was 5.5 days at 100% RH and 9°C but dropped to 1.5 days at 100% RH and 26°C. When relative humidity dropped below 90% (15°C), all larvae died within 5 hours. Limiting low soil moisture (SM0) was set at 0.1. The lower optimal moisture was increased to 0.4 from 0.3 and the upper optimal moisture level (SM2) was set at 1.0 and SM3 was set at 1.5 to permit saturation that may occur in irrigated fields (Table 1).

CLIMEX uses both photoperiod and temperature as inputs for determination of induction and temperature for termination of diapause. Simulations indicated that an induction day length of 14 hours provided the best fit to results reported for Britain, Denmark, Germany, and Idaho (USA) [21, 23, 24]. Final diapause values for induction day length (DPDO), diapause induction temperature (DPTO), diapause termination temperature (DPT1), and required days for diapause development (DPD) were set at 14, 11, 3, and 120, respectively. The known distribution of *S. lineatus* seemed to indicate a greater diversity of diapause behaviour than could be accounted for with the model parameters. As a result, the parameters were adjusted to reflect the weevil's patterns in its core distribution (i.e., north of 45° latitude in Europe) (Table 1).

Stress values, related to the ability of the species to survive adverse conditions, were set to limit geographical distributions. Cold stress limits were assigned at a level to reflect the occurrence of *S. lineatus* in northern countries such as Denmark, Finland, and Sweden. Selected values were similar to values for the pollen beetle, *Meligethes viridescens* (Fabricius) [19]. *Sitona lineatus* occurs across southern Europe, central Asia, and Africa. The rate of heat stress accumulation (THHS) was set in order to permit distribution across these regions.

3.2. Model Validation. Lower limiting temperature (DV0) values between 4 and 12°C were iteratively tested and a value of 7°C provided the best fit with distribution and growing season phenology in Europe. Similarly values for DV1, DV2, and DV3 were incrementally adjusted in order to develop a model that matched reported distributions and phenologies for Europe.

Predicted distribution of *S. lineatus* in Europe (Figure 1) agreed with the extensive distribution data reported from Fauna Europaea Web Service [1], Botha et al. [2], and Hoebeke and Wheeler Jr. [3]. The model did not predict that *S. lineatus* would occur in Egypt or Saudi Arabia. Output suggested that soil moistures were too dry and that diapause would not occur, resulting in EI = 0. Application of irrigation scenarios indicated that soil moisture values could be raised to suitable values. Diapause (based on day length) still proved to be limiting. The model predicted that some locations with climates where *S. lineatus* does not currently occur are suitable for establishment of this species. For example, the model predicted that climates in Australia, New Zealand, China, Ethiopia, Kenya, and Tanzania could support *S. lineatus* populations.

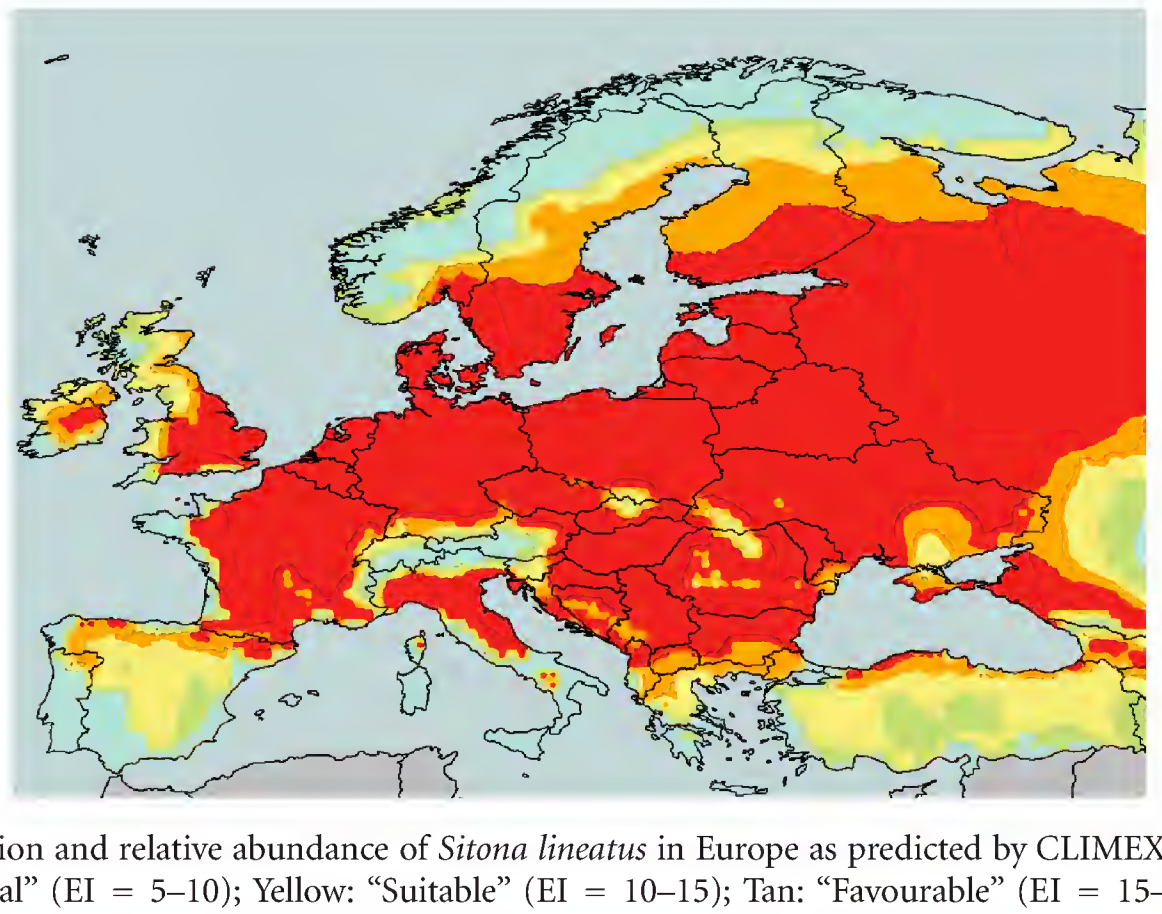


Figure 1: Potential distribution and relative abundance of *Sitona lineatus* in Europe as predicted by CLIMEX. Light blue: "Unfavourable" (EI = 0–5); Green: "Marginal" (EI = 5–10); Yellow: "Suitable" (EI = 10–15); Tan: "Favourable" (EI = 15–20); Red: "Very Favourable" (EI > 20).

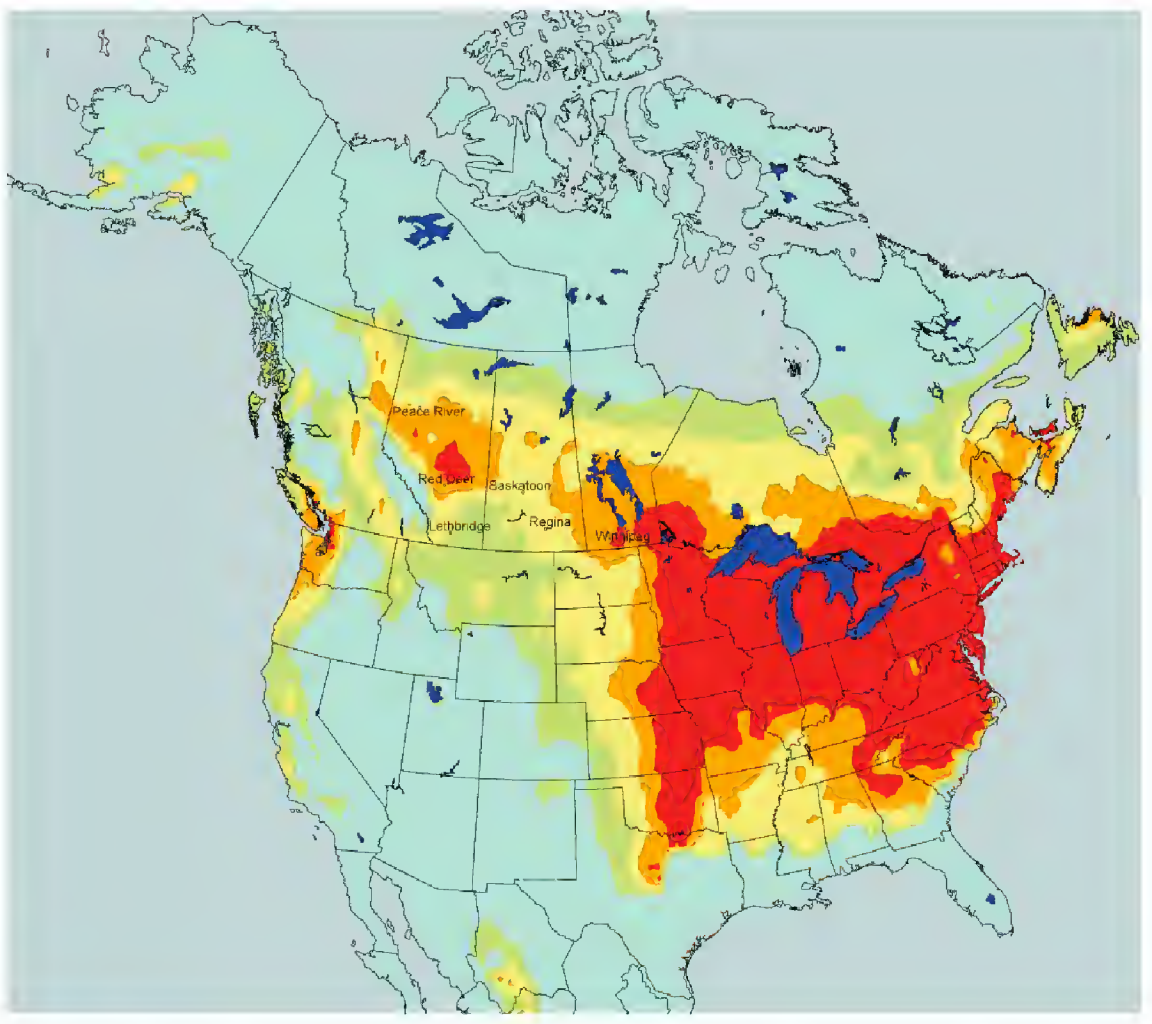


Figure 2: Potential distribution and relative abundance of *Sitona lineatus* in North America as predicted by CLIMEX under current climate conditions. Dark blue: lakes; Light blue: "Unfavourable" (EI = 0–5); Green: "Marginal" (EI = 5–10); Yellow: "Suitable" (EI = 10–15); Tan: "Favourable" (EI = 15–20); Red: "Very Favourable" (EI > 20).

In North America, predicted distributions (Figure 2) in the province of British Columbia, Canada, and the States of Washington, Oregon, Idaho, California, and Virginia in the USA agree with reported distributions [3]. Model output also predicted that *S. lineatus* could become established in the Prairie Ecozone of western Canada and agreed with population surveys in Alberta and Saskatchewan that have been conducted since 2001 [27]. Further, the model indicated that EI values would be greater in regions north of the current geographic range of *S. lineatus*. Moisture Index (MI) values were shown to be less than optimal, indicating that precipitation in southern Alberta was less than optimal. Rainfall, between late June and August, was minimal, relative to the species requirements. Ecoclimatic Index values near Red Deer were higher. The increased EI values were associated with higher MI values. Rainfall

amounts for the period of June to August were greater than those that were reported for Lethbridge. Dry moisture conditions could have a negative impact on both larval and pupal survival [4].

Model predictions for phenology agreed with published reports. Early season activity of *S. lineatus* is of particular interest. At Rothamsted, England, adults were first collected from late March until mid-April [6]. Our model predicted that first flights would occur in early April. In Kent, England adults were observed in peas in late March [24] and these dates were similar to the model prediction of March 22. Jackson [24] also stated that adult appearance was delayed by two weeks (April 8) during a cool spring. The model was run with a scenario in which the temperature was reduced by 1°C and adults were predicted to first become active on April 12. In Denmark, the model predicted that adults would become active in mid-April. This result was in agreement with data presented by Nielson and Jensen [20]. In Canada, adults first appeared in pea fields in June of 2007, and peak larval counts occurred on June 8 at Lethbridge, Alberta. [8]. It is likely that adults were active before this time. The weekly growth index suggested that *S. lineatus* would be highest in early to mid-June.

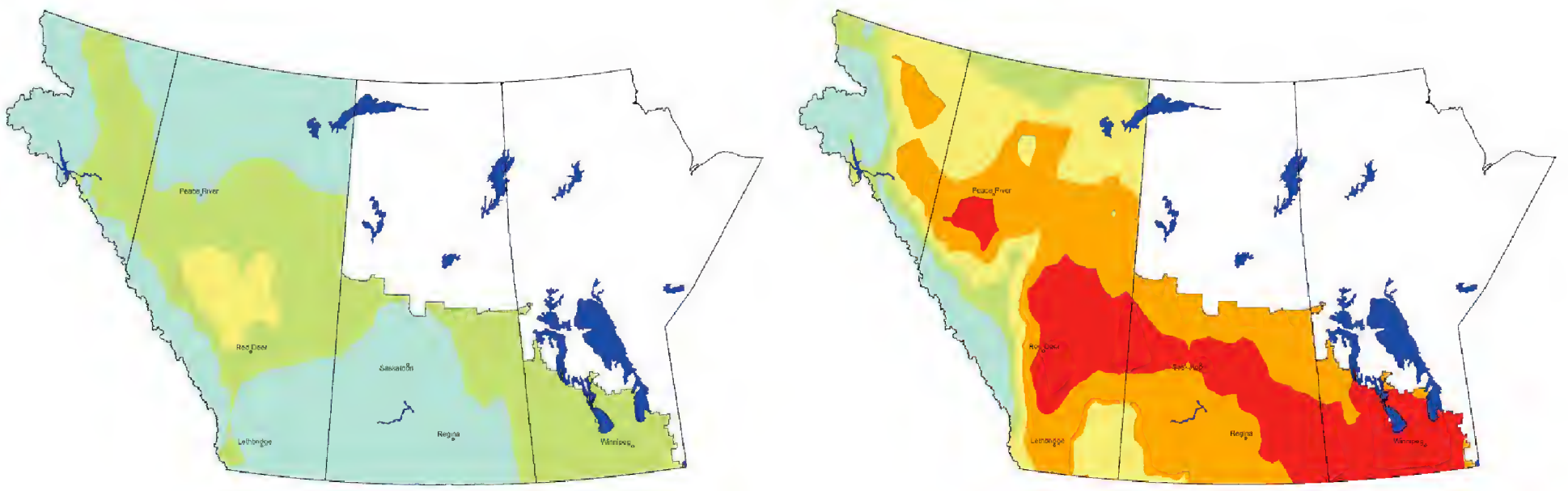
The model predicted that the potential range of *S. lineatus* could extend well beyond current distributions along western and eastern seaboards in North America. Areas in southern Ontario, Quebec, and eastern USA were also predicted to be at risk. Current areas of Canadian pulse production include Quebec and Ontario (a wide selection of coloured beans and the white navy bean), Manitoba (white and coloured beans, pea, and lentil), Saskatchewan (pea, lentil, and chickpea and some bean), and Alberta (beans, pea, lentil, and chickpea) [35].

3.3. Sensitivity Analysis. Sensitivity analyses were conducted to measure EI response to changes in temperature and precipitation. Model output indicated that *S. lineatus* was more sensitive to changes in precipitation (Figure 3) than temperature, indicating that the five locations (climate) were dryer than optimal moistures and temperatures within the Prairie Ecosystem were generally suitable for this species. The model also indicated that sensitivity was location specific. Varying temperatures from −2 to +2°C from current long-term normals revealed that the Lethbridge, Regina, Saskatoon, and Winnipeg locations were not sensitive to temperature changes (Table 2). That is, EI values showed marginal changes. Temperatures at these locations are between lower and upper optimal temperature parameters (DV1 and DV2). Ecoclimatic Index values at Red Deer did show a linear effect with increasing temperatures, increasing from EI = 14 to EI = 26 with incremental temperature increases. The results suggest that *S. lineatus* populations may increase, in areas north of Calgary, in warmer growing seasons. The *S. lineatus* model also demonstrated a linear response in EI values to increased precipitation amounts (Table 3). The model predicted that EI values near Saskatoon would increase from EI = 2 (40% less than long-term normal climate data) to EI = 19 with wetter than average weather (+ 40%).

Sensitivity analysis was also conducted to compare spatial response of EI values to variations in temperature and precipitation. For current climate, EI values were relatively low. Categories were set for EI = 10 (low), 15, and 20. This analysis was conducted for all five locations within the Prairie Ecosystem (n = 3420 grid cells) and also specifically for areas in central and southern Alberta (n = 456 grid cells). Responses were similar across both scales and agreed with location specific trends (Tables 3 and 4). Ecoclimatic Index values increased from temperatures that were 2°C below long-term normals back up to long-term normal levels. Temperatures warmer than long-term normals appeared to have little effect on EI values. Results suggest that moist conditions would be conducive to large populations (i.e., compared to cool or dry conditions). Under climate conditions that were 40% wetter than long-term normals, the model predicted that 47% (Prairie Ecosystem) and 39% (Alberta) of the spatial area could expect to have EI = 20 or greater (Tables 3 and 4; Figure 3). Across southern Saskatchewan and Alberta, the model predicted that EI values would be reduced for dryer than normal conditions (Figure 3(a)).

Compared to climate data (long-term normals), sensitivity analysis results suggest that *S. lineatus* should respond less favourably in dry seasons and more favourably in wetter seasons. This conclusion may be based on conditions that occurred in the previous summer. That is, late May to July rainfall may be an important factor that determines mid-summer survival and potential number of adults available for the following season. For example, the number of notches per plant (>27) from locations near Lethbridge was greatest in 2006 [8]. Though the 2006 growing season (April–August) was dry, April and May were wetter than normal [36]. Also, the 2005 growing season was much wetter than normal. The model would predict that these conditions would be conducive for population increase. Across most of Alberta, numbers declined dramatically between 2007 and 2008 [37, 38]. This may have been due to exceptionally dry periods during June, July, and August in 2007. In 2008, April and early May were exceptionally dry near Lethbridge [36]. Dry conditions may have reduced larval and pupal survival. Though *S. lineatus* was first collected in fields around Lethbridge in 1997, only sporadic damage was reported in the early 2000s. The region experienced a severe drought between 2001 and 2003 [36]. Increasing outbreak levels and geographic expansion throughout southern Alberta were experienced in 2006 and 2007 (reported in Saskatchewan as well) when several thousands of hectares of field peas were sprayed [8]. Low densities and associated sporadic damage may have been associated with the hot dry conditions and increased damage/range expansion may be explained by increased moisture levels experienced between 2004 and 2008.

3.4. General Circulation Model Analyses. Bioclimatic model output varied for each of the three GCMs (Figures 4, 5 and 6). In terms of EI values, the NCAR273 CCSM climate data resulted in the most significant increase in northern regions. Application of this GCM predicted that *S. lineatus* would



(a) (b)

Figure 3: Predicted Ecoclimatic Index (EI) values with precipitation 40% less (a) and 40% greater (b) than current climate. Dark blue: lakes; Light blue: "Unfavourable" (EI = 0–5); Green: "Marginal" (EI = 5–10); Yellow: "Suitable" (EI = 10–15); Tan: "Favourable" (EI = 15–20); Red: "Very Favourable" (EI > 20).

Table 3: Effect of changes in temperature and precipitation from current climate on Ecoclimatic Index (EI) values for *Sitona lineatus* across all locations within the Prairie Ecosystem (n = 3420 grid cells). The values are expressed as a percentage of total geographic area.

Variable	Scenario	EI ≥ 10	EI ≥ 15	EI ≥ 20
	Current climate	**85.7%**	**51.1%**	**13.5%**
Temperature	−2°C	79.9%	20.5%	0.1%
Temperature	−1°C	86.3%	37.9%	3.9%
Temperature	+1°C	82.3%	54.7%	17.0%
Temperature	+2°C	78.8%	51.2%	15.8%
Precipitation	−40%	2.8%	0.0%	0.0%
Precipitation	−20%	52.3%	10.4%	0.2%
Precipitation	+20%	98.4%	81.5%	32.4%
Precipitation	+40%	99.8%	93.7%	47.4%

Table 4: Effect of changes in temperature and precipitation from current climate on Ecoclimatic Indices (EIs) for *Sitona lineatus* in southern and central Alberta (n = 456 grid cells). Values are % of total area. The values are expressed as a percentage of total geographic area in this region.

Variable	Scenario	EI ≥ 10	EI ≥ 15	EI ≥ 20
	Current climate	**56.6%**	**15.1%**	**2.2%**
Temperature	−2°C	49.1%	2.6%	0.0%
Temperature	−1°C	57.0%	9.0%	0.0%
Temperature	+1°C	53.1%	17.5%	2.6%
Temperature	+2°C	48.2%	15.1%	3.3%
Precipitation	−40%	0.9%	0.0%	0.0%
Precipitation	−20%	15.6%	1.8%	0.0%
Precipitation	+20%	92.8%	51.3%	11.4%
Precipitation	+40%	99.6%	82.0%	39.0%

be very abundant north of 53°N. Similar, though slightly lower, EI values were predicted for the CSIRO MARK 3.0 and MIROC-H GCM climates. The three GCMs also resulted in varying output across the Prairie Ecosystem. NCAR273 climate data resulted in suitable to very favourable EI values while the CSIRO MARK 3.0 data resulted in some areas being categorized as marginal to suitable. The model predicted that south eastern Alberta and a large area of southern Saskatchewan would be marginal when the MIROC-H GCM was applied. Results of this study suggest that species responses are specific not only to GCM but also to specific regions across North America. Olfert et al. [39] assessed the impact of GCMs on *Melanoplus sanguinipes* (Fabricius) distribution and abundance. Their study was based on

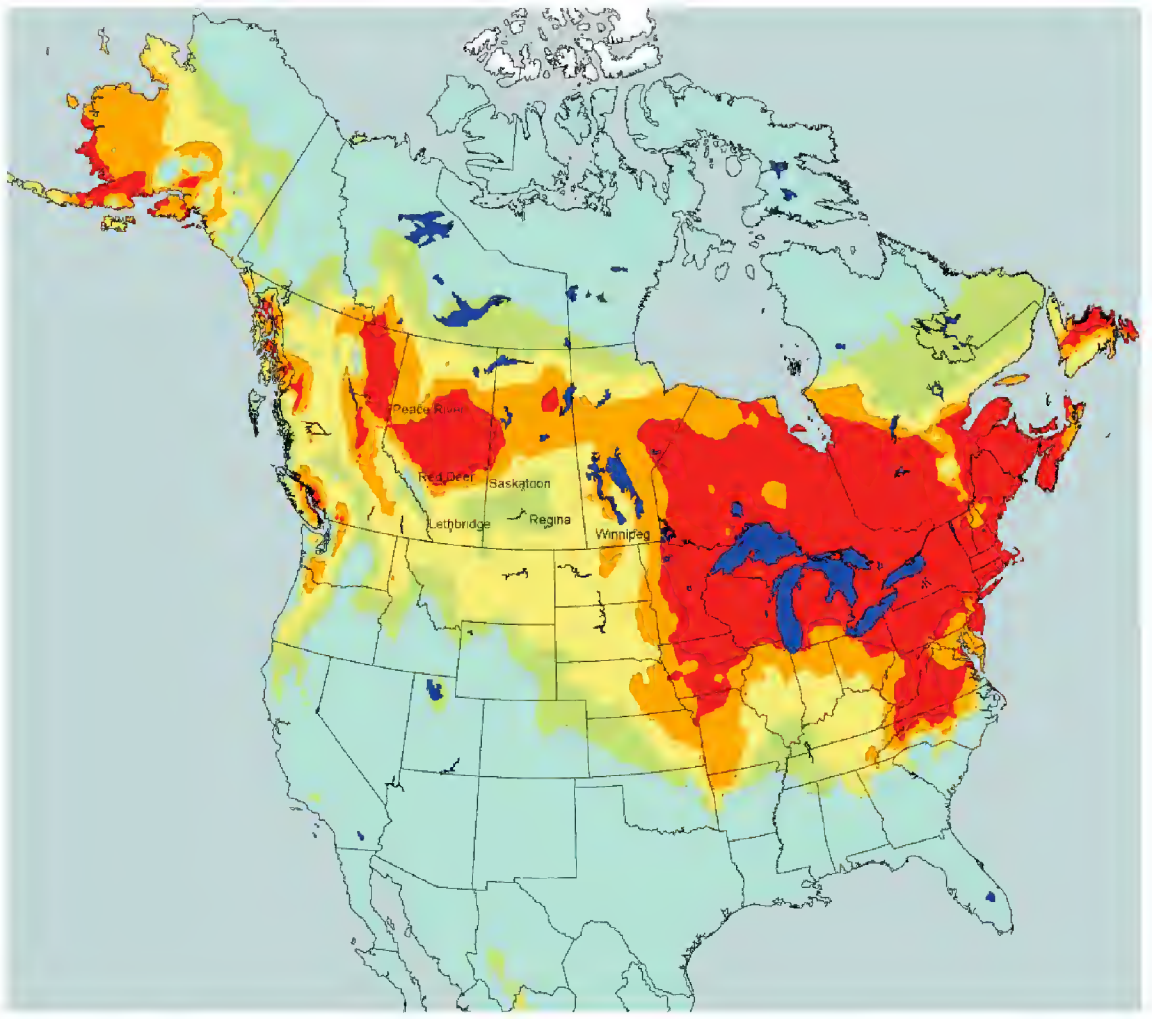


FIGURE 4: Potential distribution and relative abundance of *Sitona lineatus* in North America for 2080 as predicted by CLIMEX and the CSIRO Mark 3.0 climate change projection. Dark blue: lakes; Light blue: "Unfavourable" (EI = 0–5); Green: "Marginal" (EI = 5–10); Yellow: "Suitable" (EI = 10–15); Tan: "Favourable" (EI = 15–20); Red: "Very Favourable" (EI > 20).

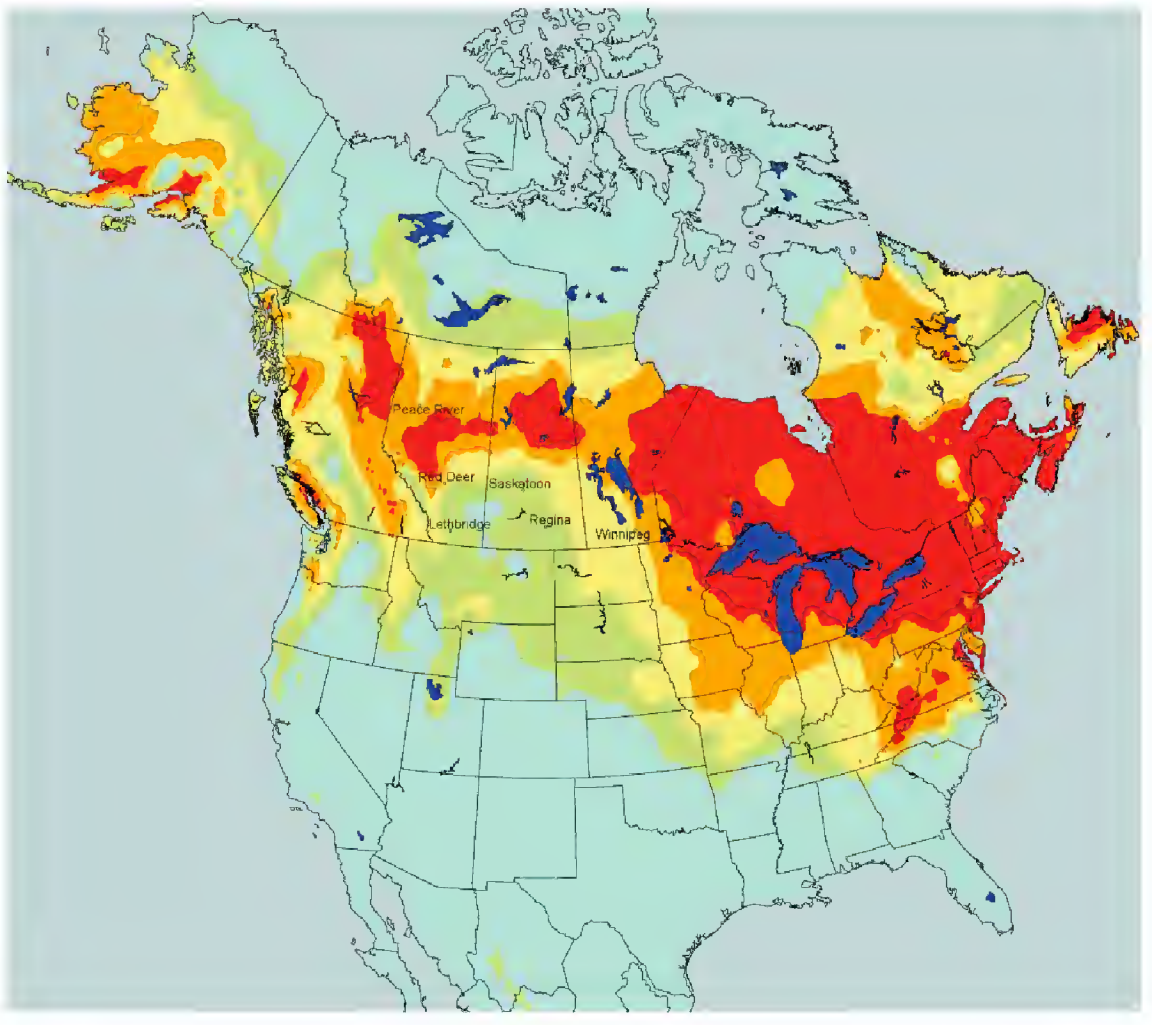


FIGURE 5: Potential distribution and relative abundance of *Sitona lineatus* in North America for 2080 as predicted by CLIMEX and the MIROC-H climate change projection. Dark blue: lakes; Light blue = "Unfavourable" (EI = 0–5); Green: "Marginal" (EI = 5–10); Yellow: "Suitable" (EI = 10–15); Tan: "Favourable" (EI = 15–20); Red: "Very Favourable" (EI > 20).

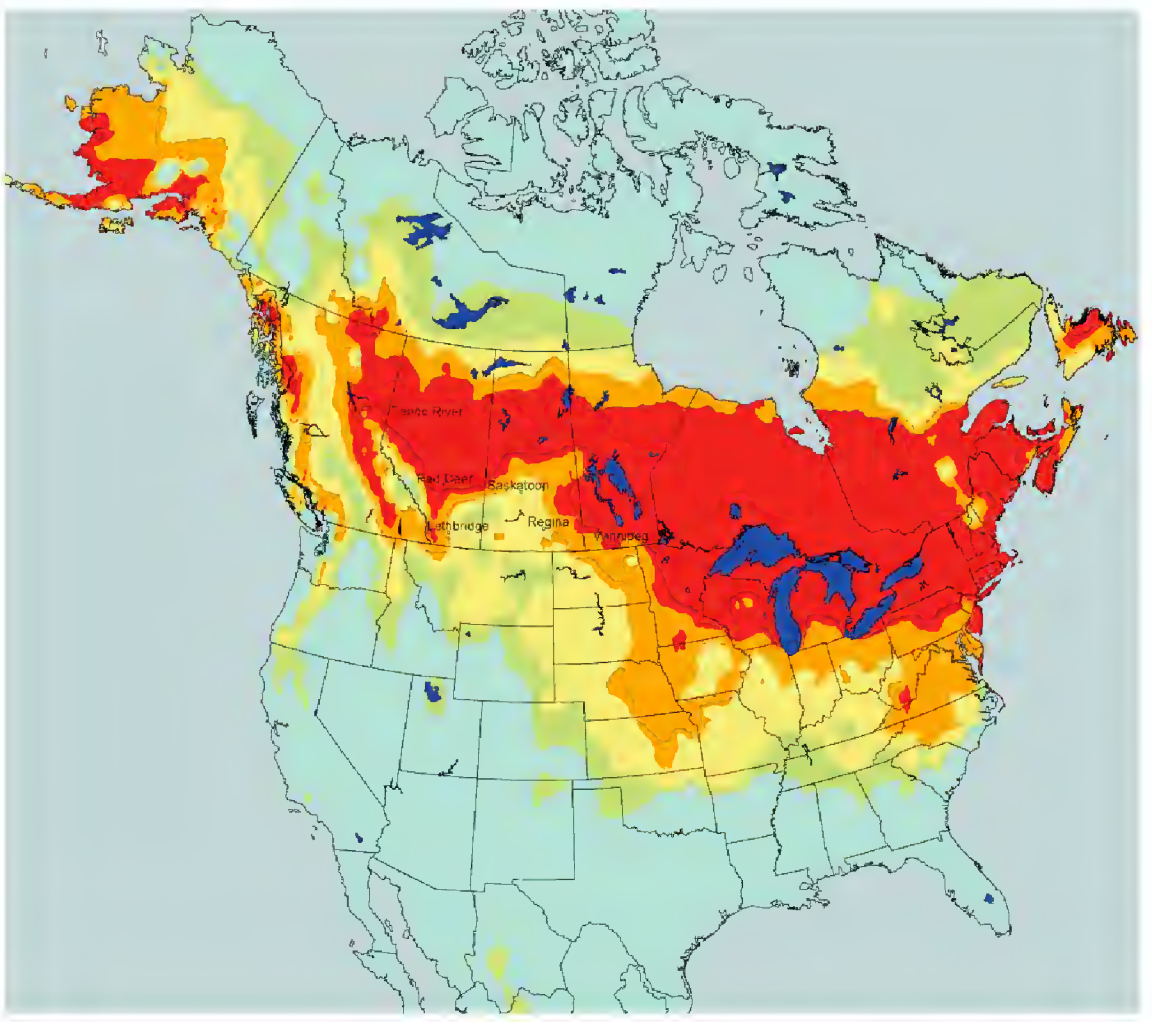


Figure 6: Potential distribution and relative abundance of *Sitona lineatus* in North America for 2080 as predicted by CLIMEX and the NCAR273 CCSM climate change projection. Dark blue: lakes; Light blue: "Unfavourable" (EI = 0–5); Green: "Marginal" (EI = 5–10); Yellow: "Suitable" (EI = 10–15); Tan: "Favourable" (EI = 15–20); Red: "Very Favourable" (EI > 20).

Table 5: Baseline (Current climate: CRU) and General Circulation Model scenarios (CSIRO MARK 3.0, MIROC-H, NCAR273 CCSM) and resulting changes to mean Ecoclimatic Index (EI) values for *Sitona lineatus* at six defined regions (geographic rectangle, 4° latitude by 7° longitude) in western Canada.

Location	Latitude	Longitude	Current climate (CRU)	CSIRO Mark 3.0	MIROC-H	NCAR273 CCSM
Lethbridge Alberta	49.75°	−112.75°	9	13	6	13
Peace River Alberta	56.25°	−117.25°	13	14	13	21
Red Deer Alberta	52.25°	−113.75°	20	21	13	28
Regina Saskatchewan	50.25°	−104.75°	10	8	6	12
Saskatoon Saskatchewan	52.25°	−106.75°	11	8	6	11
Winnipeg Manitoba	49.75°	−97.25°	20	14	14	22

the three GCMs that were used in the current study and they found that response of *M. sanguinipes* not only varied by GCM but also was region specific. Mika et al. [13] reported that effect of climate change differed strongly between GCMs and that EI differences for *Contarinia nasturtii* were greatest for regions that were categorized as "very favourable" (EI = 30).

The relational database was queried to analyze the impact of climate change for six locations in western Canada (Table 5). Model output based on NCAR273 CCSM resulted in EI increases at each of the locations with the greatest increases at Red Deer and Peace River. The CSIRO MARK 3.0 resulted in EI increases for Lethbridge, Red Deer, and Peace River and decreased EI values for the remaining three locations. Model output based on MIROC-H climate data resulted in reduced EI values for five of the six locations. Peace River was predicted to have an EI value that was the same as for current climate.

Table 6: Effect of changes in precipitation (expressed as a percentage of total geographic area) from current values on Ecoclimatic Indices for *Sitona lineatus* across North America for CRU (current climate) and general circulation model (CSIRO MARK 3.0, MIROC-H, NCAR273 CCSM) scenarios.

Scenario	EI ≥ 10	EI ≥ 15	EI ≥ 20
Current climate (CRU)	25.2%	17.1%	9.8%
CSIRO Mark 3.0	37.5%	25.5%	15.2%
MIROC-H	34.6%	22.9%	13.1%
NCAR273 CCSM	37.4%	26.2%	17.3%

Analysis was conducted to compare changes in EI values, as a result of climate change, across North America (Table 6). Compared to current climate, model output indicates that the area of the continent that will have EI values greater than 10 will increase by 37–48%. Model runs showed that areas

with EI >20 could increase by 33% (MIROC-H), 54% (CSIRO MARK 3.0), and 76% (NCAR273 CCSM). These results were similar to values reported by Olfert and Weiss [40] who indicated that a + 3°C increase in temperature would result in 19.7% to 47.1% increase in areas with EI >20 for *Ceutorhynchus obstrictus* (Marsham), *Oulema melanopus* L., and *Meligethes viridescens* (Fabricius).

4. Conclusions

Some cautions have been expressed regarding the utilization of bioclimatic models for investigating the potential impact of climate on insect populations. For example, adaptation is likely to occur with the result that biotic interactions may not remain the same over time, and genetic and phenotypic composition of populations may change [41]. In addition, most insect species have some limitation to dispersal [42]. In the instance of *S. lineatus*, the impact of biotic factors such as natural enemies (e.g., diseases, parasites, predators) and host plant resistance and other abiotic factors, such as intercropping and chemical insecticides, must also be considered [4]. So even though model results suggest conditions in some regions to be conducive to *S. lineatus* populations under climate change, these additional biotic and abiotic factors could result in population decline. In these instances, bioclimate and GCMs may not account for changes in population and may overestimate populations.

To address these naturally occurring phenomena, bioclimatic modeling of *S. lineatus* populations would benefit from multitrophic studies (host plants—*S. lineatus*—natural enemies). For example, Cárcamo et al. [9] suggested that when adults lay eggs on plants past the 5th node stage, larval recruitment was lower compared to oviposition at the 2nd node stage. As a result, cooler growing conditions in the spring may delay *S. lineatus* invasions into fields until the crops are more advanced. Warmer temperatures may improve the synchrony between insect and plant.

Sitona lineatus is expected to continue to expand its range; as a result Vankosky et al. [4] suggested that an integrated approach of intercropping, host plant resistance, predators, parasitoids, pathogens, and chemical insecticides would be required to successfully manage this recently introduced pest species. In an effort to provide advance warnings of continued range expansion a region wide monitoring program has been initiated across western Canada [43].

Acknowledgments

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Research Article

Life History of *Aricoris propitia* (Lepidoptera: Riodinidae)—A Myrmecophilous Butterfly Obligately Associated with Fire Ants

Lucas A. Kaminski[1] and Fernando S. Carvalho-Filho[2]

[1] *Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, CP 6109, Campinas 13083-970, SP, Brazil*

[2] *Laboratório de Ecologia de Invertebrados, Instituto de Biologia, Universidade Federal do Pará, Rua Augusto Corrêa 01, Guamá, Belém 66075-110, PA, Brazil*

Correspondence should be addressed to Lucas A. Kaminski, lucaskaminski@yahoo.com.br

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The immature stages of *Aricoris propitia* (Stichel) are described and illustrated for the first time, using both light and scanning electron microscopy. Females oviposit in at least seven host-plant families, always in the presence of fire ants (*Solenopsis saevissima* (Smith) complex), without being attacked by them. Larvae are tended by ants during all larval and pupal stages. From the fourth instar on, larvae feed at night and rest during the day inside underground shelters constructed by ants on the host plant roots, and where pupation occurs. Several observed features, including ant-mediated oviposition, persistent ant attendance throughout all instars, and high spatiotemporal fidelity indicate that *A. propitia* is a myrmecophile obligately associated with fire ants. We propose *A. propitia* as an extraordinary model for studies on ant-butterfly evolutionary history in the Neotropics.

1. Introduction

Symbiotic associations between butterfly larvae and ants have attracted the attention of early naturalists, both in Europe and North America, since the second half of the 18th century (see references in [1]). Nonetheless, these interactions are historically poorly studied in the Neotropical region despite their richness and abundance [2, 3]. An exception in this scenario is the classic paper by Bruch [4], which describes some aspects of the life history of an Argentinean species of *Aricoris* Westwood. In addition to being the first detailed description of a myrmecophilous larva from the Riodinidae family, the aforementioned study presents the first evidence of a butterfly larva living inside ant nests in the Neotropics. This behavior has been reported for a small number of Lycaenidae clades, such as the charismatic large blue *Maculinea* Van Eecke (*Phengaris* Doherty spp.), which parasitizes ant societies in Eurasia (see [5–7]). But unlike large blue butterflies, which today are model organisms in mutualism and parasitism studies, little progress has been achieved on the biology of *Aricoris* since the initial work by Bruch [4] (but see [8–12]).

The riodinid genus *Aricoris* contains 24 described species [13, 14] typically found in open dry areas of South America [3]. *Aricoris propitia* (Stichel) is widespread in Central and Northern Brazil ([15], C. Callaghan, pers. comm.). Since its original description in 1910, no additional information was published for this species. The purpose of this paper is to fill that gap by presenting the natural history and morphological description of immature stages of *A. propitia*, with emphasis on their obligatory association with fire ants of the *Solenopsis saevissima* (Smith) complex (Formicidae: Myrmicinae).

2. Material and Methods

2.1. Study Sites. Four sites were sampled in central and northern Brazil (Figure 1): (1) cerrado *sensu stricto* and gallery forest areas in Alto Paraíso, Goiás (13°48′S, 47°54′W) (July 2009); (2) suburban areas of the city of Assis Brasil, Acre

(a) (b) (c) (d)

FIGURE 1: Overview of *Aricoris propitia* study sites in Central (a) and Northern ((b)–(d)) Brazil. (a) Cerrado *sensu stricto* area in Alto Paraíso, Goiás; (b) surroundings of Assis Brasil, Acre—note the bordering suburbs, pastures and forest; (c) sandy beach areas along the Xingu river, Porto de Moz, Pará; (d) small home garden in a neighborhood of Belém, Pará—note that within this small space the larvae were able to use three ornamental plant species (see Table 1).

(10°56′S, 69°33′W) (August-September 2006); (3) sandy beach and small-farm cultivation areas along the Xingu river, Porto de Moz, Pará (02°07′S, 52°15′W) (July 2010); (4) house garden in a neighborhood of Belém, Pará (01°25′S, 48°27′W) (several occasions between 2006 and 2009).

2.2. Sampling, Rearing, and Behavioral Observations. Available host-plants in the study sites were visually scanned for the presence of larvae and tending ants (as in [16]). Additionally, some potential host-plants with distinct signs of herbivory and visited by *S. saevissima* ants were excavated in search of larvae and pupae. Plants with immatures (eggs and larvae) were collected for identification, as well as the tending ants. We also recorded the presence of food sources that may promote ant visitation on the plants, such as extrafloral nectaries (EFNs) and/or honeydew-producing hemipterans (HPHs). The immatures of *A. propitia* used for morphological description were collected in the field and reared as follows: eggs were placed in Petri dishes and observed daily until eclosion; newly hatched larvae were reared individually in transparent 250 mL plastic pots under controlled conditions (25 ± 2°C; 12 h L: 12 h D). Branches of the same host-plant on which each larva was found were offered *ad libitum*, and larvae were checked daily for food replacement and cleaning when necessary. Immatures for morphological analysis were separated, fixed in Dietrich's solution, and then preserved in 70% ethanol. Shed head capsules were collected and preserved for measuring. Voucher specimens of the immature stages were deposited at the Museu de Zoologia "Adão José Cardoso" (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Behavioral interactions between *A. propitia* larvae and tending ants were observed *ad libitum* [17] in the field during the day (ca 10:00–16:00 h), and sometimes at night (ca 18:00–06:00 h), for the population of Porto de Moz. Additional observations on larval ant-organs and their role in the interaction with ants were obtained from larvae reared in plastic pots with their host ants or from larvae maintained in a terrarium together with a captive colony of tending ants (from a population of Belém).

2.3. Morphology. Measurements were taken and general aspects of morphology were observed using a Leica MZ7.5 stereomicroscope equipped with a micrometric scale. Egg

Table 1: Summary of recorded host-plants of *Aricoris propitia*, including liquid food source types available for ants (EFNs, extrafloral nectaries; HPHs, honeydew-producing hemipterans) and localities.

Host plants	Sources of liquid food	Localities
Chrysobalanaceae		
Hirtella glandulosa	EFNs, HPHs	Alto Paraíso (Goiás)
Fabaceae		
Senna obtusifolia	EFNs	Belém (Pará)
Malpighiaceae		
Byrsonima sp.	HPHs	Porto de Moz (Pará)
Malvaceae		
Hibiscus rosa-sinensis	HPHs	Belém (Pará)
Verbenaceae		
Aegiphila sp.	EFNs	Assis Brasil (Acre)
Rubiaceae		
Ixora coccinea	HPHs	Belém (Pará)
Simaroubaceae		
Simarouba sp.	EFNs	Alto Paraíso (Goiás)
Turneraceae		
*Turnera ulmifolia**	EFNs	Campinas (São Paulo)

*Lab-accepted host-plant.

size is given as height and diameter. Head capsule width of larvae was considered to be the distance between the most external stemmata; maximum total length for both larvae and pupae corresponded to the distance from head to posterior margin of the tenth abdominal segment in dorsal view (as in [18]). Measurements are given as minimum-maximum values. Scanning electron microscopy (SEM) was conducted using both JEOL JSM-5800 and Carl Zeiss LEO-1430VP microscopes, with samples prepared according to standard techniques (for details, see [19]). Terminology for early stage descriptions follows Downey and Allyn [20] for eggs, Stehr [21] for general morphology of larvae, Mosher [22] for pupae, and DeVries [23] for ant-organs.

3. Results

3.1. Natural History of Aricoris propitia. This butterfly is locally abundant in open areas, where it occurs close to its ant colonies. Adults can be observed flying fast near the ground, perching on the undergrowth where they become almost invisible. Males were observed defending small territories and visiting many wild flowers. Females were seen flying near host-plants infested by host ants (Figure 2(a)), which for all studied populations were ants of the *Solenopsis saevissima* complex. Oviposition occurred in the warmest period of the day, from 11 AM to 2 PM ($n = 15$ oviposition events), a period when ants are more active. Females flew in circles around a host-plant occupied by ants before starting to oviposit (prealighting phase). After landing (postalighting phase), females frequently touched the plant surface with the tip of their abdomen, particularly on ant trails, but were never attacked by the ants. Eggs were laid singly or in small clusters of two to five eggs (Figure 2(b)). Our host-plant records indicate that the larvae of *A. propitia* are polyphagous using at least seven families of plants, including ornamental (nonnative) species cultivated in urban gardens (see Table 1 and Figure 1(d)). Also, in the laboratory, larvae accepted and developed well on leaves of *Turnera ulmifolia* L. (Turneraceae). All observed host-plants of *A. propitia* provided some source of liquid food that could be potentially used by ants, such as honeydew-producing hemipterans and/or extrafloral nectaries (see Table 1). Other potential host-plants without fire ants or visited by other ant species were also examined at some of the study sites ($n = 51$ at Assis Brasil, $n = 15$ at Alto Paraíso), but no larvae of *A. propitia* were found.

All instars are ant-tended, and even the small first instar is equipped with functional tentacular nectary organs (TNOs). From the second instar on, other ant-organs appear or become functional (Figure 3). Ants antennate the larval body intensely, but especially the anterior region where a row of papilliform setae and the openings of the anterior tentacle organs (ATOs) are located (Figure 3(a)). When everted, these organs provoke clear alterations in ant behavior, such as opening of the jaws and a marked increase in activity and aggressiveness. In the early instars (first to third) the larvae can be found during the day feeding on the host-plant leaves (Figures 2(b)–2(d)). From the fourth instar on, they rest during the day inside underground shelters constructed by ants within the host-plant roots, and that is where pupation occurs. When night falls, the larvae leave the underground shelters and climb up to feed on the host leaves (Figure 2(e)), returning to the shelters by dawn. Large quantities of mature larvae and pupae can be found inside the underground shelters, which are permanently patrolled by tending ants (Figure 2(f)).

3.2. Description of the Immature Stages. The reared immatures from the four sites were very similar and went through five instars. Developmental time is based on material from Alto Paraíso, Goiás, reared on *Turnera ulmifolia* leaves. The egg description and measurements are based on material from Assis Brasil, Acre; the larval and pupal description and measurements are based on material from Porto de Moz and Belém, Pará.

3.2.1. Egg (Figures 2(b) and 4). Duration 6-7 d ($n = 5$). Height 0.30–0.32 mm; diameter 0.54–0.58 mm ($n = 3$). Color whitish-cream when laid, changing to beige before hatching. General spherical shape, with convex upper surface and flattened bottom surface; exochorion with smooth surface and hexagonal cells in lateral view (Figure 4(a)). Slightly depressed micropylar area; annulus present, and rosette surrounded by petal-shaped cells; micropyles at center of the micropylar area (Figure 4(b)). Aeropyles in tiny protuberances in the rib intersections (Figure 4(c)).

3.2.2. First Instar (Figures 2(c) and 5(a)–5(c)). Duration 4-5 d ($n = 2$). Head capsule width 0.24–0.26 mm ($n = 3$), total length 2.2 mm. Dark brown head, prothoracic and anal

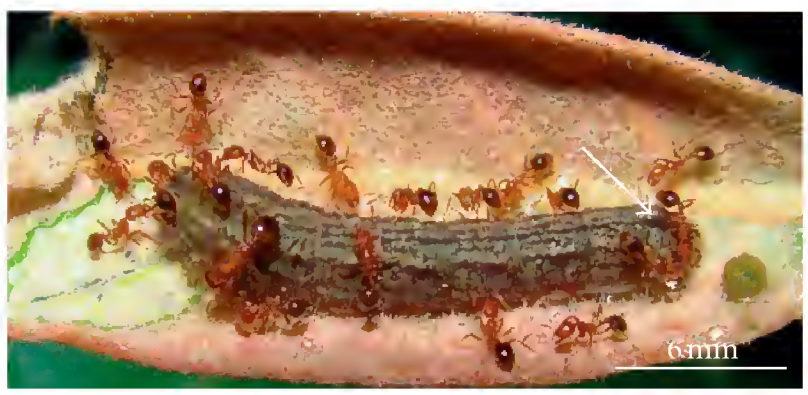
(a)

(b)

(c)

(d)

(e)



(f)

(g)

(h)

FIGURE 2: Life stages of *Aricoris propitia* tended by *Solenopsis saevissima* on *Byrsonima* sp. ((a), (d)–(f)), *Simarouba* sp. ((b), (d) and (e)), and *Hirtella glandulosa* (c). (a) Female at postalighting phase near an ant-tended treehopper aggregation (arrow); (b) eggs (white arrows) and a third instar larva (black arrow); (c) first instar tended by one worker; (d) second instar tended by ants; (e) third instar tended by ants, note that both anterior (black arrow) and tentacle nectary organs (white arrow) are everted; (f) nocturnal fifth (last) instar tended by several ants; (g) nocturnal group of larvae and treehoppers (arrow) tended by ants; (h) diurnal larval group inside a shelter in the host-plant roots.

(a)

(b)

FIGURE 3: Sequence of interactions between *Aricoris propitia* third instar larva and *Solenopsis saevissima* ants. (a) Worker antennating the row of setae on the prothoracic shield (white arrow), note the everted anterior tentacle organ (black arrow); (b) everted nectary tentacle organ (white arrow) after repeated antennation by ant on the A8 segment.

shields; yellowish orange body with beige or translucent setae (Figure 2(c)). Epicranium and frontoclypeus with several setae, pores, and two pairs of perforated cupola organs (PCOs) in the adfrontal areas (Figure 5(a)). Body with long plumose setae in the lateral areas and in the prothoracic and anal shields; the remaining dorsal and subdorsal setae are short and dendritic, and PCOs are associated with these groups of setae. The openings of the anterior tentacle organs (ATOs) are present in the metathoracic segment, but these organs are apparently not functional (Figure 5(b)). Functional tentacle nectary organs (TNOs) are present in the A8 segment (Figure 5(c)).

3.2.3. Second Instar (Figure 2(d)). Duration 5-6 d ($n = 2$). Head capsule width 0.44 mm ($n = 2$), total length 3.1 mm. Dark brown head, prothoracic and anal shields; yellowish green body with two longitudinal light brown bands (Figure 2(d)). All ant-organs present, including ATOs, TNOs, PCOs, dendritic setae, and one pair of vibratory papilla on the anterior border of the prothoracic shield. A dorsal row of papilliform setae is also present on the posterior margin of the prothoracic shield and is maintained in the subsequent instars (Figures 3(a) and 5(d)).

3.2.4. Third Instar (Figures 2(e) and 3). Duration 6 d ($n = 2$). Head capsule width 0.72–0.84 mm ($n = 2$), total length 6.2 mm. Brown head; black prothoracic and anal shields with beige spots; green body with two longitudinal brown bands (Figure 2(e)). General morphology is similar to the second instar's, but with more numerous and enlarged setae.

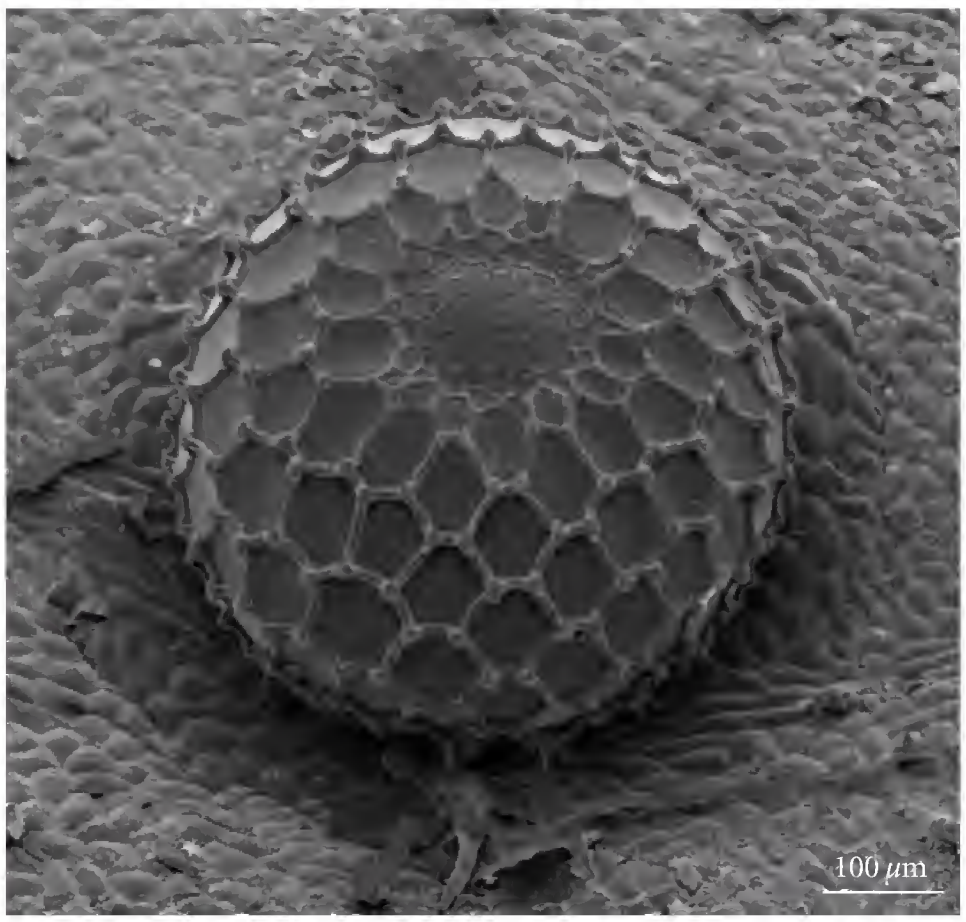
3.2.5. Fourth Instar (Figures 2(g)-2(h) and 5(d)-5(e)). Duration 6 d ($n = 2$). Head capsule width 1.28–1.30 mm ($n = 4$), total length 15.2 mm. Brown head; black prothoracic and anal shields with beige and grey spots; variegated body coloring with frosted brown and beige spots (Figures 2(g) and 2(h)). General morphology is similar to preceding instar's, but with more numerous and enlarged setae (Figures 5(d) and 5(e)).

3.2.6. Fifth (Last) Instar (Figures 2(f)–2(h) and 5(f)–5(h)). Duration 6-7 d ($n = 2$). Head capsule width 1.76–1.87 mm ($n = 5$), total length 2.1 cm. Coloring is similar to fourth instar (Figures 2(f)–2(h)). Mandibles with eight teeth and six setae (Figure 5(f)). Body covered with several types of setae, including prominent setae on the lateral areas, prothoracic and anal shields; two pairs of prominent dorsal setae in the same position as primary setae on the mesothorax to A8 segments; two types of dendritic setae and several perforated cupola organs (Figures 5(g) and 5(h)). The spiracle on the A1 segment is lateroventral, whereas those on segments A2 to A8 are in a dorsal position.

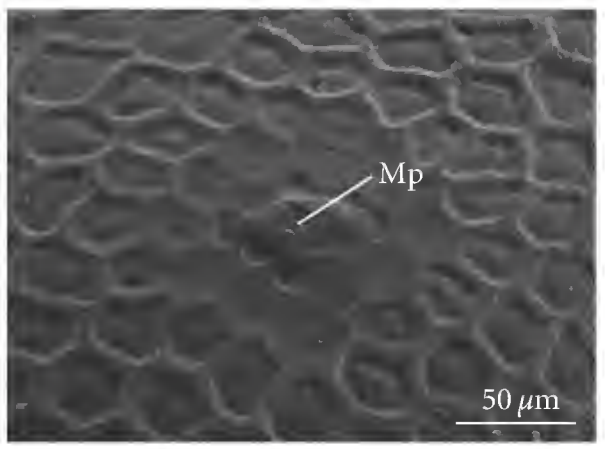
3.2.7. Pupa (Figure 6). Duration 10–12 d ($n = 2$). Total length 1.29 cm, width at A1 0.33 cm. Variegated coloring with brown, beige, and dark spots (Figure 6(a)). Tegument is entirely sculptured, with irregular striations and lacking prominent tubercles (Figures 6(b)–6(e)). Prothorax bears dorsal clusters of papilliform setae (Figure 6(a)). Silk girdle crossing the A1 segment near one pair of small tubercles with several associated dendritic setae and PCOs (Figure 6(b)). Body with some small dendritic setae, and PCOs located in clusters on lateral areas close to spiracles (Figures 6(b)–6(e)); these clusters are absent on the A2 and A7 segments. The intersegmental area between the A4-A5 and A5-A6 abdominal segments features plates and files (Figure 6(f)) that may act as a stridulatory mechanism. The consolidated A9 and A10 segments constitute the ventrally flattened cremaster; with long crochets in a ventral position (Figure 6(g)).

4. Discussion

In general terms, the egg of *Aricoris propitia* resembles those described for other Nymphidiini genera in the Lemoniadina group (such as *Juditha* Hemming, *Lemonias* Hübner, *Synargis* Hübner, and *Thisbe* Hübner), with a semispherical shape, exochorion with hexagonal cells in lateral view, aeropyles in the rib intersections, and micropylar area centered on



(a)



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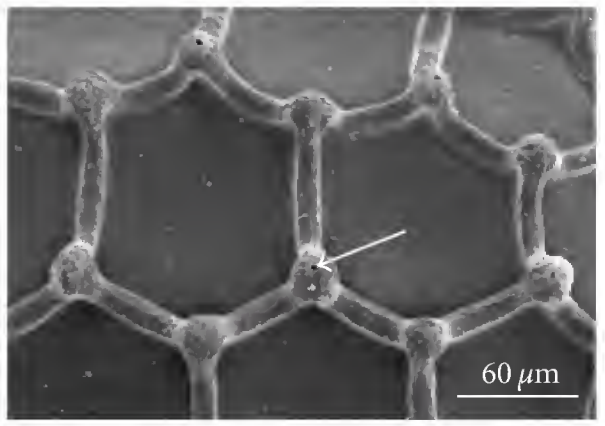

(c)

FIGURE 4: Scanning electron microscopy of *Aricoris propitia* egg (material from Assis Brasil, Acre). (a) Lateral view; (b) micropylar area (Mp); (c) hexagonal cells of the exochorion with aeropyles in the rib intersections (arrow).

the top surface (see [3, 24, 25]). However, it differs in that the limits of the micropylar area are slightly bounded; this pattern is shared with other *Aricoris* and *Ariconias* Hall and Harvey (L.A. Kaminski, unpublished). The first instar presents some characteristics of myrmecophilous larvae, namely, conspicuous perforated cupola organs, functional tentacle nectary organs, and short, dorsally located dendritic setae (see examples of riodinid first instar larvae in [3, 18, 26]).

Larvae of *A. propitia* present the typical pattern of Nymphidiini, with the first abdominal spiracle in a ventral position and vibratory papillae (VPs) on the prothoracic shield [27]. In addition to the sound producing organs (VPs), the mature larvae of *A. propitia* feature two other important types of riodinid ant-organs (see [3, 23, 24]): the anterior tentacle organs (ATOs) and the tentacle nectary organs (TNOs). The larvae also present another putative ant-organ: the row of papilliform setae on the prothorax, which had already been described for other *Aricoris* species [4, 8]. Tending ants frequently antennate these papilliform setae, and usually this palpation is accompanied by eversion of the ATOs. The way the ants react after ATO eversion suggests that the ATOs emit a volatile chemical similar to the ant alarm pheromone, as has been suggested for other Riodinidae [3, 23, 28]. The chemical compositions of ATO emissions by myrmecophilous butterflies are still unknown. In contrast, the chemical ecology of fire ants, including alarm pheromones and their role in interactions with other organisms, is relatively well known (e.g., [29]). Thus, the *A. propitia*/fire ants system may be helpful in answering some outstanding questions about the functioning of ant-organs in myrmecophilous butterflies.

The larvae of *A. propitia* can be considered polyphagous since they feed on at least seven families of host-plants. Polyphagy in obligate myrmecophilous butterflies, including Riodinidae, has been regarded as a consequence of ant-dependent oviposition [3, 12, 25, 30–32], and this seems to be the case for *A. propitia*. Aphytophagy, on the other hand, is quite rare in butterfly larvae [33], but it has been suggested for some species of *Aricoris* [3, 12]. It is believed that the larvae of these species are able to get food directly from ants, through regurgitations (trophallaxis) from ant workers or by preying directly on ant brood. Although *A. propitia* rest during the day inside underground shelters together with their tending ants, we do not have evidence that the larvae get some kind of food from the ants.

All known species of *Aricoris* seem to be engaged in obligatory associations with their tending ants. To date, *Aricoris domina* (Bates) has been associated with *Ectatomma* Smith [11], and seven *Aricoris* species have been associated with *Camponotus* Mayr [3, 8–12, 34]. So far, only *Aricoris hubrichi* (Stichel) and *Aricoris campestris* (Bates) have been reported to be associated with *Solenopsis* Westwood ants ([4], A.V.L. Freitas pers. comm.). Both *Aricoris* species are inserted within the derived "*epulus*-group" sensu Hall and Harvey [35]. Despite the high species richness and ecological prevalence, symbiotic interactions between butterfly larvae and *Solenopsis* are very rare ([36], L.A. Kaminski, unpublished). Apart from the association with fire ants, several natural history and morphological features of *A. propitia* are very similar to those observed for *A. hubrichi* and *A. campestris*, suggesting an evolutionary relationship among these species. As the life history of most species within the "*epulus*-group" is still unknown (see [35]), it is not possible to tell whether

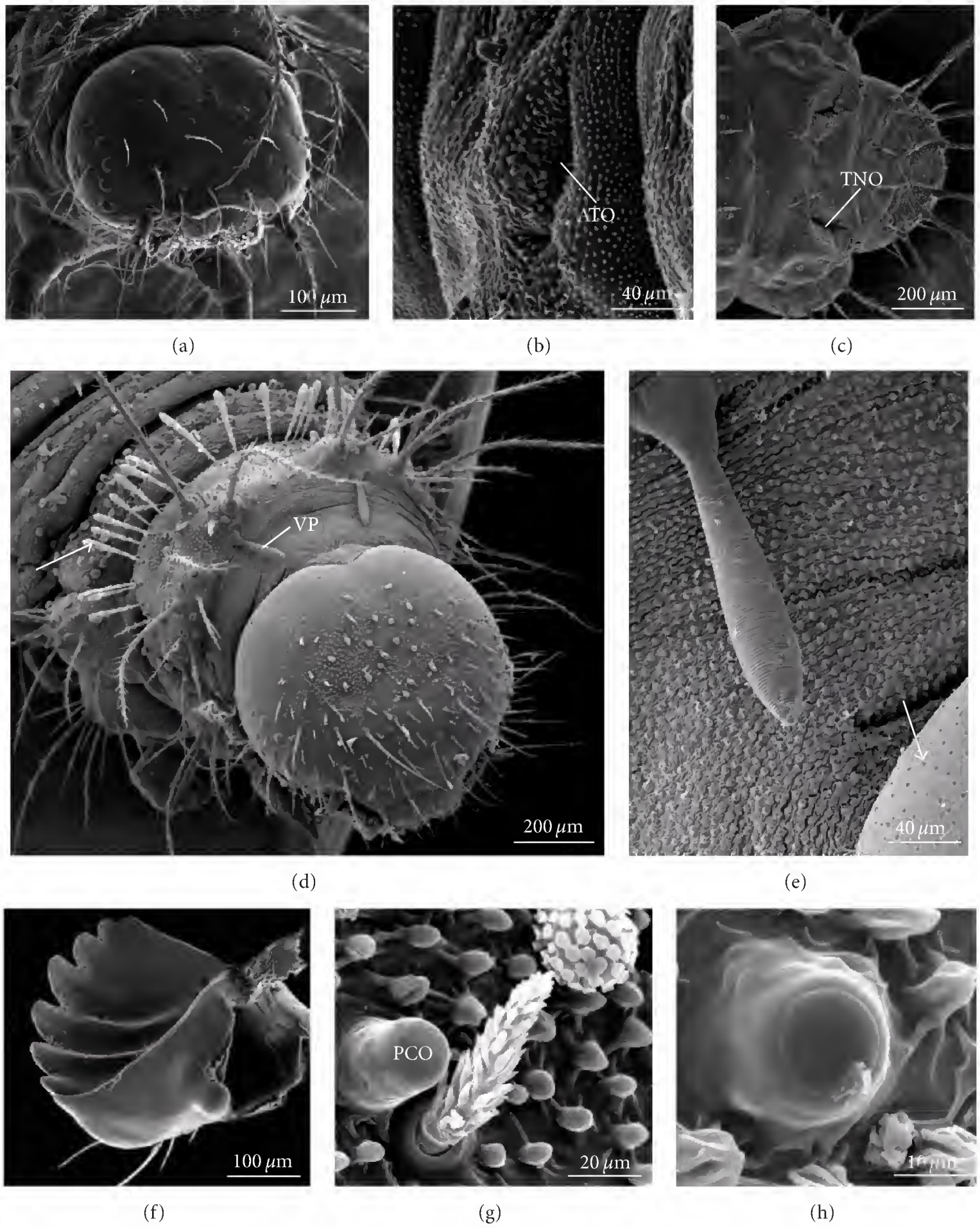

FIGURE 5: Scanning electron microscopy of first ((a)–(c)), fourth ((d) and (e)), and fifth ((g) and (h)) instar of *Aricoris propitia* ((a)–(e) from Assis Brasil, Acre, and ((f)–(h)) from Belém, Pará). (a) Head in frontal view; (b) opening of the anterior tentacle organ (ATO); (c) posterior abdominal segments showing the openings of tentacle nectary organs (TNOs); (d) head and prothorax in dorsofrontal view, note the dorsal row of setae (arrow) and the vibratory papillae (VP); (e) detail of vibratory papilla, note the epicranial granulations (arrow); (f) mandible; (g) two types of dendritic setae and perforated cupola organ (PCO); (h) perforated cupola organ.

interaction with fire ants has a single origin or has arisen more than once in these lineages.

The fire ants are highly dominant organisms and considered one of the most harmful bioinvaders ever known [37]. In their native range, from southern Brazil to Suriname, they are also considered pests in disturbed areas, especially in the Amazon (e.g., [38–40]). Although *A. propitia* occurs naturally in the Amazon (the holotype is from "Amazonas"), continual deforestation over the recent decades—especially in the "arc of deforestation" (see [41])—could be providing a recent range expansion for this butterfly. Recent studies involving several molecular markers and morphological variation have revealed that *Solenopsis saevissima* belongs to a geographically structured complex of cryptic species [40]. How populations of *A. propitia* respond to ant host structure is an interesting and yet unanswered question. A recent study [42], for example, did not find a direct influence of host ants on the population structure of the obligate myrmecophilous

Figure 6: Pupa of *Aricoris propitia* in lateral view (a) and details ((b)–(g)) in scanning electron microscopy ((a), from Alto Paraíso and ((b)–(g)), from Belém, Pará). (b) Laterodorsal tubercle on A1 segment with dendritic setae and perforated cupola organs; (c) dendritic setae on A1 segment; (d) spiracle on A5 segment; (e) dendritic setae; (f) detail of putative stridulatory area between A4-A5 segments; (g) detail of cremaster crochet.

butterfly *Jalmenus evagoras* (Donovan) (Lycaenidae), but showed that biogeographical and host-plant aspects have an effect on that structure. *Aricoris propitia* may be a candidate system to elucidate the effects of ant attendance on the diversification of myrmecophilous butterflies.

The system involving *Aricoris propitia* and their tending fire ants presents several features of a model system, including: (1) it is common and widely distributed; (2) it is found in easily accessible environments (open and/or altered areas); (3) it adjusts well to laboratory conditions; (4) it has a short generation time; (5) the larvae accept many host-plant species; (6) the host fire ants have economic importance and various aspects of their biology are well known. Accordingly, we expect that the basic information provided in this work will encourage further studies on this interesting butterfly-ant system.

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Review Article

Ecto- and Endoparasitic Fungi on Ants from the Holarctic Region

Xavier Espadaler[1] and Sergi Santamaria[2]

[1] *Ecology Unit and CREAF, Autonomous University of Barcelona, 08193 Bellaterra, Spain*
[2] *Botany Unit, Autonomous University of Barcelona, 08193 Bellaterra, Spain*

Correspondence should be addressed to Xavier Espadaler, xavier.espadaler@uab.es

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The ant-specific fungi *Aegeritella, Laboulbenia, Rickia, Hormiscium,* and *Myrmicinosporidium* in the Holarctic region—nine species—are reviewed. Present knowledge is highly biased geographically, as shows the single record for Holarctic Asia, and this is to solve. The phylogenetic position of *Aegeritella, Hormiscium*, and *Myrmicinosporidium* is unknown. Hosts seem to be also skewed phylogenetically although this may be a true pattern.

1. Introduction

Extensive, massive mycoses are an extremely rare instance in ants [1] and involve individuals, rather than whole colonies. A fortiori, documented population level attacks are practically nonexistent. A case concerning *Tetramorium caespitum* [2, 3] seems to be an isolate within ant literature. Here we deal with ecto- and endoparasitic fungi, and we limit our survey to those that are ant specific. We differentiate parasitic fungi, that are not deadly to ants, and pathogenic fungi, which kill the host. Thus, generalist entomopathogenic fungi like *Beauveria* and *Metarhizium* or ant specifics like *Pandora myrmecophaga* (Figure 1) or *Telohannia solenopsae* are not included. Recent revisions of entomopathogens are those from Roy et al. [4], Kleespies et al. [5], Oi and Pereira [6] and, centred in social insects, in the seminal book by Schmid-Hempel [7]. We aim to review the knowledge of taxonomic and geographic distribution and, whenever possible, natural history and/or ecology of selected groups of fungi. The Holarctic is understood as comprising the nontropical parts of Europe and Asia, Africa north of the Sahara, and North America south to the Mexican desert region.

The fungi considered in this paper show a gradient of negative effects on the host. From a seemingly near absolute absence of any measurable—or measured—effect in some cases (*Aegeritella*, *Hormiscium*, and *Laboulbenia camponoti*), to a mild effect in other Laboulbeniales (reduced immunological response in *L. formicarum*; S. Cremer pers. comm.), or a possible strong negative effect in *Myrmicinosporidium*). This effect may concern exclusively infested individual ants (*Myrmicinosporidium*) although in some cases, because of the fungus life cycle and the social nature of ants, with many physical contacts between colony members outside of the nest and in the nest galleries, this may be multiplied and traduced directly to the colony level (Laboulbeniales, or *Aegeritella*). This general absence of strong negative effects indicates probably a very old interaction with ants.

An unfortunate circumstance is the completely unknown phylogenetic position of some of those specific ant fungi, and this is calling for a dedicated, focused study, using molecular techniques. We stress the necessity of enhanced attention from the part of myrmecologists and mycologists towards this interesting group of ectoparasitic fungi. Just remembering their existence, and with a little care and open mind, many more instances of Laboulbeniales, *Aegeritella*, *Myrmicinosporidium*, and pathogenic fungi on ants should surface in ample areas within the Holarctic region.

2. Material and Methods

Apart from our current files, we did a search in the ant data base FORMIS (version 2011) [9]. Search terms are as follow: ectoparasitic, endoparasitic, fungus, fungi, Laboulbeniales, *Laboulbenia, Rickia, Aegeritella, Myrmicinosporidium*, and filtered out a posteriori by geographical region

FIGURE 1: *Pandora myrmecophaga* having killed a worker *Formica rufa*, from The Netherlands, showing the characteristic attachment to the distal part of a grass leave caused by the summit disease [after [8]; Photo by H. Niesen; with permission).

(Holarctic). Within each fungus species, we give the country, ant species attacked, and reference. Taxonomical scheme and terminology follow Index Fungorum [10] (http://www.indexfungorum.org/).

3. Results

3.1. Ectoparasitic Fungi on Ants

3.1.1. Aegeritella Bałazy & J. Wiśn. Anamorphic Pezizomycotina. Those fungi were first noted by Wiśniewski in 1967 [11] although its fungal nature was not proven then. The fungi grow over the cuticle like dark protuberances (= bulbils). On a first sight, they look like dirt, and its form is usually a dome, rounded in perimeter, and up to 400 μm diameter (Figure 2). The number of bulbils may be from a single one to several hundreds. The distribution of bulbils on the body of ants is heterogeneous, being more abundant at the rear part [12–14]. The total number of bulbils is inversely related to ant size, with bigger ants having less bulbils than smaller ants [14]. Bulbils have been detected in workers and queens.

The ant-fungus relationship has not been properly ascertained although a reduced life duration or activity level has been suggested [15, 16]. In a similar vein, Bałazy et al. [17] note some workers with hundreds of bulbils, having immobilized bucal palps, all covered by hyphae. Nothing is known of the dynamics of infestation or transmission mechanisms of those enigmatic fungi, not even its phylogenetic position within the realm of Fungi.

(1) *Aegeritella superficialis* Bałazy & J. Wiś. 1974.

Europe

Czech Republic: *Formica sanguinea* Latreille, *Formica rufa* L., *Formica polyctena* Förster, *Formica pratensis* Retzius, *Formica truncorum* Fabricius, *Formica lugubris* Zetterstedt, *Formica exsecta* Nylander [18, 19].

Germany: *Formica polyctena* Förster [16].

Italy: *Formica lugubris* Zetterstedt [20].

Poland: *Formica polyctena* Förster, *Formica rufa* L., *Formica pratensis* Retzius; *Formica truncorum* Fabricius, *Formica fusca* L. [21–24]; *Formica sanguinea* Latreille [25].

Rumania: *Formica rufa* group [26].

Spain: *Formica decipiens* Bondroit [12].

Switzerland: *Formica rufa* L., *Formica polyctena* Förster, *Formica lugubris* Zetterstedt, *Formica sanguinea* Latreille [15].

(2) *Aegeritella tuberculata* Bałazy & J. Wiś. 1983.

Europe

Czech Republic: *Lasius distinguendus* Emery, *Lasius nitidigaster* Seifert (as *Lasius rabaudi*), *Lasius umbratus* (Nylander) [19].

Poland: *Lasius flavus* (Fabricius), *Formica fusca* L. [27].

Spain: *Lasius umbratus* (Nylander), *Lasius distinguendus* (Emery) [28], *Lasius umbratus* ([29], as *L. distinguendus*); *Formica pressilabris* Nylander [12]; *Formica rufa* L., *Formica rufibarbis* Fabr. [14]. Canary islands: Tenerife, *Lasius grandis* Forel [13].

North America

USA, Alaska: *Lasius pallitarsis* (Provancher) ([30], as *Lasius sitkaensis*).

(3) *Aegeritella roussillonensis* Bałazy, Lenoir & J. Wiś. 1986.

France. On *Cataglyphis cursor* (Fonscolombe) [17].

(4) *Aegeritella maroccana* Bałazy, Espad. & J. Wiś. 1990.

Morocco. On *Aphaenogaster baronii* Cagniant [31].

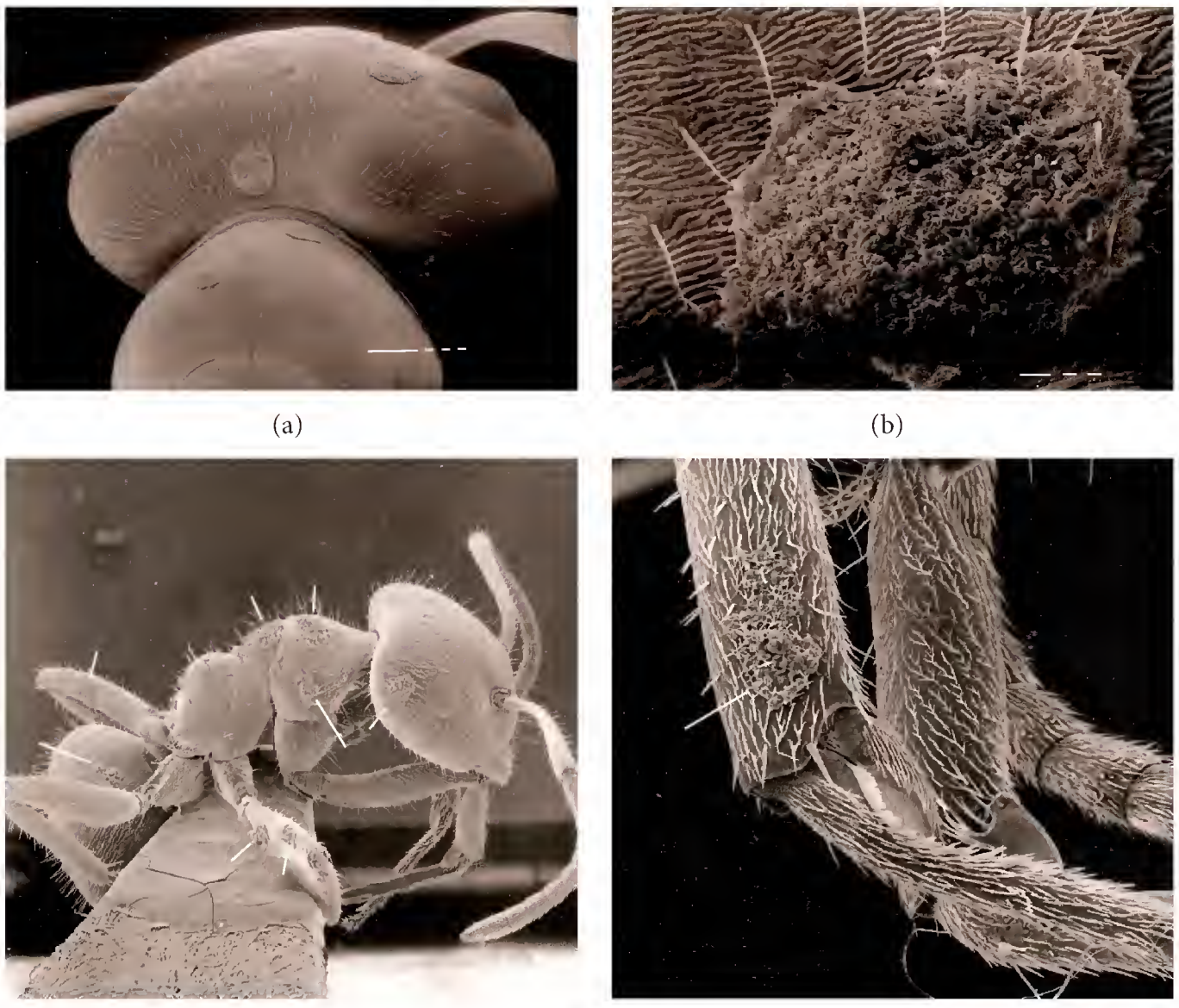
(5) An unidentified *Aegeritella* was noted on two workers

Polyergus breviceps Emery from Arizona [30].

3.1.2. Hormiscium Kunze, Incertae Sedis Pezizomycotina

(1) *Hormiscium myrmecophilum* Thaxter, 1914.

The species was described from an Amazonian *Pseudomyrmex* and remained elusive since its original description until it was found in Europe eighty years later. The filamentous, somewhat dichotomic thallus is undifferentiated and grows directly out of different parts of the ant body, without any apparent attaching structure. Mycelia have a maximum length of 163 μm and constant width of 10 μm. (Figure 3). Spores are unknown.



(a) (b) (c) (d)

FIGURE 2: (a) *Aegeritella tuberculata* on *Formica pressilabris* (Spain). Two bulbils are in the pronotum, one at the back of head, (b) closeup of a bulbil; (c) *A. tuberculata* on *Lasius grandis* from Tenerife, Canary Islands; white arrows indicate bulbils; (d) closeup of bulbils in the first leg.

(a) (b)

FIGURE 3: *Hormiscium myrmecophilum*. (a) hyphae on *Myrmica* sp.; (b) worker *Myrmica sabuleti* with hyphae on the head and lateral pronotum.

Europe

Portugal. On *Myrmica* sp. [32].

Spain. On *Myrmica sabuleti* Meinert (present paper).

3.1.3. Laboulbeniales (Ascomycota). Laboulbeniales are unusual among fungi because of their limited thallus with determinate growth. They are obligate external parasites of arthropods, especially insects. One key peculiarity is the ability to grow on their hosts without inflicting any noticeable injury. Ten orders of insects, in addition with millipedes and acari, may be affected although 80% of some 2000 species are recorded from beetles [33]. Only six are known to date infesting ants from the Holarctic region, and all castes are known to be susceptible to infestation.

(1) *Rickia wasmannii* Cavara, 1899.

The species is extremely characteristic in its microscopic morphological aspect (Figure 4) and is limited to several

(a) (b)

FIGURE 4: (a) *Rickia wasmannii* on *Myrmica scabrinodis* from Slovakia. Each "spatulate hair" is a thallus of *Rickia*. Photo by P. Bezděčka; with permission; (b) two mature thalli. Spores are oozing out of the perithecium on the specimen from the right.

species of *Myrmica*. Infested ants may harbour from a few thalli to several hundred thalli all over the body. Heavy infestations are visible to the naked eye and give a greyish shade, a pulverulent image to living individuals. Worker and queens may be infested.

Europe

Austria: *Myrmica rubra* (L.) [34].

Bulgaria: *Myrmica scabrinodis* Nylander [35].

Czech Republic: *Myrmica slovaca* Sadil, *Myrmica scabrinodis* Nylander [36].

France: *Myrmica scabrinodis* (Nylander) [37].

Germany: *Myrmica rubra* (L.) [38].

Hungary: *Myrmica slovaca* Sadil (as *M. salina*), *M. scabrinodis* Nylander, *M. specioides* Nylander, *M. vandeli* Bondroit [39].

Italy: *Myrmica scabrinodis* Nylander [40].

Luxembourg: *Myrmica rubra* L. [41].

Rumania: *Myrmica scabrinodis* Nylander [39].

Slovakia: *Myrmica scabrinodis* Nylander [42].

Slovenia: *Myrmica sabuleti* [41].

Spain: *Myrmica specioides* Bondroit [28, 43]; *Myrmica spinosior* Bondroit ([43], as *M. sabuleti*).

Switzerland: *Myrmica rubra* (L.) ([44], as *M. laevinodis*).

United Kingdom: *Myrmica sabuleti* Meinert [45, 46].

(2) *Rickia* sp.1.

Greece: On *Messor* (unpublished observation: description is pending).

(3) *Laboulbenia camponoti* S. W. T. Batra 1963.

Under the binocular, the thallus looks like a distorted ant hair (Figure 5) and is found all over the body, albeit more abundant in dorsal surfaces and external surface of legs. Density is much lower than in other ant-specific Laboulbeniales. In the Holarctic, it has been detected exclusively in *Camponotus* species, all six from the subgenus *Tanaemyrmex*.

Asia

Turkey: *Camponotus baldaccii* Emery [47].

Europe

Bulgaria: *Camponotus aethiops* (Latreille), *Camponotus universitatis* Forel, *Camponotus* sp. (as *C. pilicornis*) [35].

Spain: *Camponotus pilicornis* (Roger) [48]; *Camponotus sylvaticus* (Olivier) [49].

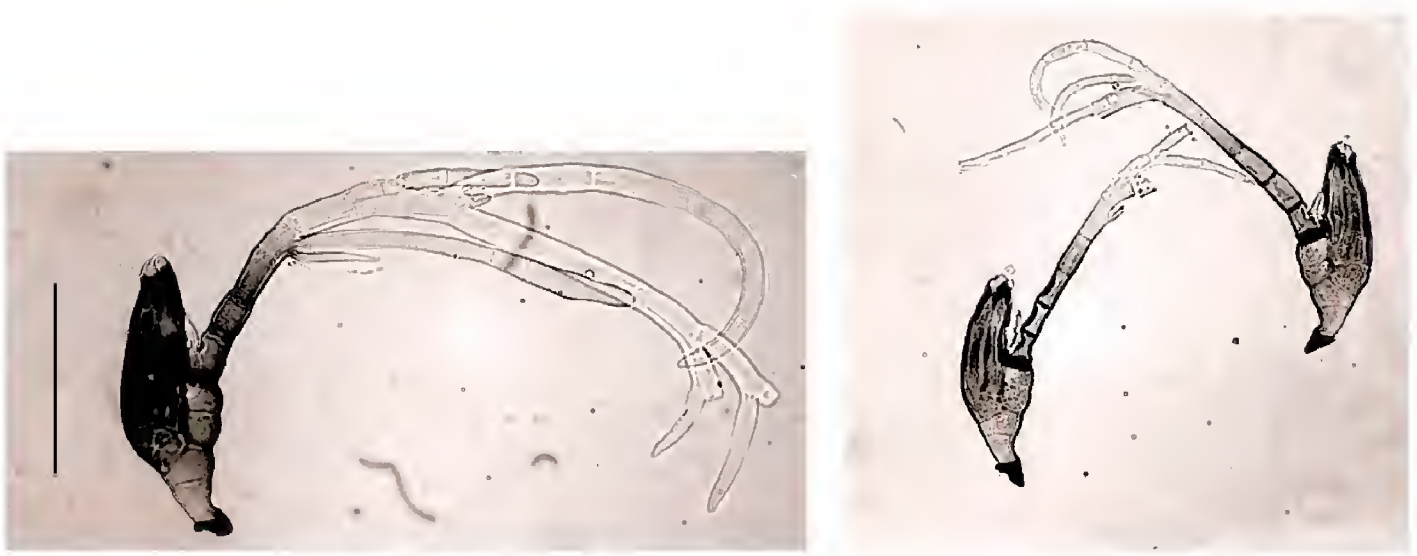
(4) *Laboulbenia formicarum* Thaxt, 1902.

This is one of the smallest Laboulbeniales (up to 0.3 mm total length). Thalli can be extremely abundant on infested workers (Figure 6), which go foraging seemingly unaffected amid noninfested workers.

North America

Canada: *Lasius alienus* (Förster) [50].

USA: *Formica argentea* Wheeler [51]; *Formica aserva* Forel ([52], as *F. subnuda*); *Formica curiosa* Creighton ([53], as *F. parcipappa*); *Formica incerta* Buren [51]; *Formica lasioides* Emery [54]; *Formica montana* Wheeler ([54], as *F. neocinerea*); *Formica neogagates* Viereck [51, 55]; *Formica pallidefulva* Latreille ([54], as *F. nitidiventris*; [56], as *F. schaufussi*); *Formica puberula* Emery [52]; *Formica subintegra* Wheler [54]; *Formica subpolita* Mayr ([52], as *F. camponoticeps*); *Formica subsericea* Say [54]; *Formica vinculans* Wheeler [54]; *Lasius alienus* (Förster) ([55, 57], as *L. americanus*); *Lasius murphyi* Forel [58]; *Lasius neoniger* Emery [51, 59]; *Lasius pallitarsis* (Provancher) ([30], as *L. sitkaensis*); *Myrmecocystus mimicus* Wheeler [60]; *Polyergus breviceps* Emery [54]; *Polyergus lucidus* Mayr [54]; *Prenolepis impairs* (Say) [54].



(a) (b)

FIGURE 5: *Laboulbenia camponoti* from *Camponotus sylvaticus* (Spain); line: 1 mm. (a) A mature specimen; (b) two immature specimens.

FIGURE 6: *Laboulbenia formicarum* on *Lasius grandis*. Worker tibia, showing full-grown thalli and dark spots which indicate attachment point of spores (more than 50 in the viewed side). Inset: one mature (right) and immature (left) specimens of *Laboulbenia formicarum*.

Europe

France: *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy [61].

Portugal (Madeira): *Lasius grandis* Forel [62].

Spain: *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy [63].

3.2. Endoparasitic Fungi on Ants

3.2.1. Incertae Sedis

Myrmicinosporidium durum Hölldobler 1933. Those fungi were first noted by Hölldobler [64, 65] although they were formally described later, in 1933 [66]. Its phylogenetic position is still unknown, and their true fungal nature has been only proved recently [67]. Infested ants are usually well detected because the darker spores are visible through the integument (Figure 7); spores number may be very low, but usually they reach more than one hundred in a single ant. The caveat here is that the fungus may be much difficult to detect in ants having fuscous or black colouration. As a consequence, host range is probably biased. The usual aspect of concave spores, with a bow-like depression, is an artefact of fixation in alcohol [68].

Although the infested workers are almost certainly killed by the fungus when spores begin producing hyphae, life span seems not to be curtailed [67]. Infested workers seem scarcely affected in its normal behaviour [67, 69], and infested queens may participate in swarming flights [69] and show normal fertility [68]. Males have been found infected too [70]. Life cycle and mode on infestation are unknown although reports of *Myrmicinosporidium* from callow workers in *Pogonomyrmex badius* indicate that the infection is carried over from immature stages [71]. It is perhaps significant that the majority of diseased ants were collected in late summer and fall. After hibernation, those infected workers die [69]. Its geographical distribution is ample as is also the range of hosts.

Europe

Austria: *Plagiolepis vindobonensis* Lomnicki [67].

Croatia: *Temnothorax recedens* (Nylander), *Temnothorax affinis* (Mayr), *Temnothorax unifasciatus* (Latreille), *Plagiolepis pygmaea* (Latreille) [67].

France: *Solenopsis fugax* (Latreille), *Pheidole pallidula* (Nylander) [72]; *Temnothorax unifasciatus* (Latreille), *Temnothorax recedens* (Nylander) [68].

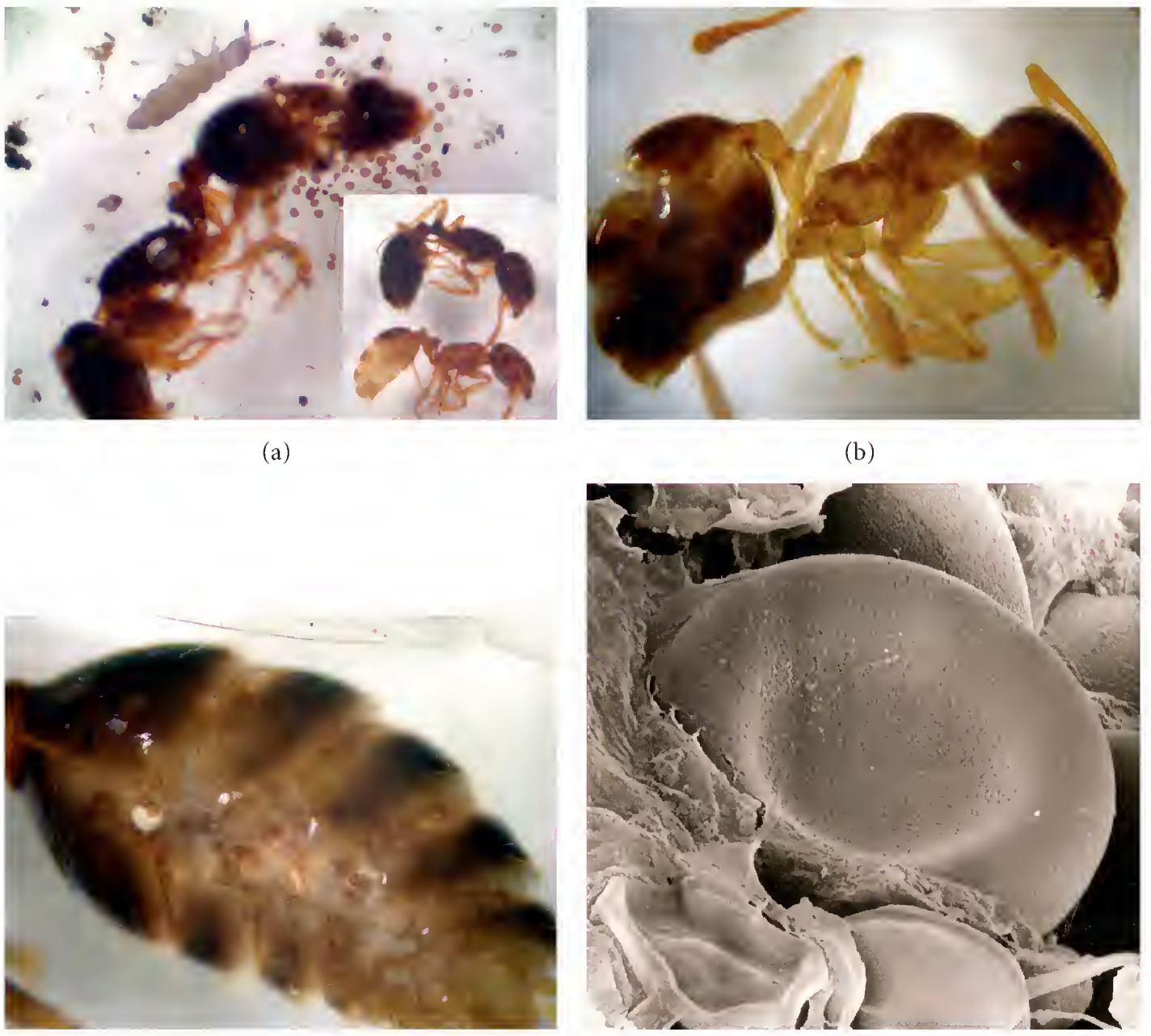
Germany: *Solenopsis fugax* (Latreille) [64, 65]), *Temnothorax tuberum* (Fabricius) [66].

Hungary: *Solenopsis fugax* (Latreille), *Tetramorium caespitum* (L.), *Plagiolepis taurica* Santschi [73].

Italy: *Temnothorax unifasciatus* (Latreille) [67, 69], *Temnothorax albipennis* (Curtis) [67], *Temnothorax angustulus* (Nylander) [67], *Temnothorax exilis* (Emery) [67], *Temnothorax nylanderi* (Forster) [67], *Chalepoxenus muellerianus* (Finzi) [67].

Spain: *Pheidole pallidula* (Nylander), *Solenopsis* sp., *Strongylognathus caeciliae* Forel, *Tetramorium semilaeve* (André), *Plagiolepis pygmaea* (Latreille) [70], *Temnothorax lichtensteini* (Bondroit), *Temnothorax racovitzai* (Bondroit) [72].

Switzerland: *Solenopsis fugax* (Latreille) [68].



(a) (b) (c) (d)

FIGURE 7: (a) *Myrmicinosporidium* mature spores inside workers *Tetramorium semilaeve* (inset: darker, infested worker, and normally coloured worker); (b) *Pheidole pallidula* with many spores on thorax, coxae, and gaster; (c) gaster of a male *Pheidole pallidula* with spores; (d) SEM image of a spore, showing the artifactual characteristic doughnout shaped form resulting from the alcohol fixation.

North America

USA: *Pogonomyrmex barbatus* (F. Smith) [67]; *Solenopsis carolinensis* Forel, *Solenopsis invicta* Buren, *Pheidole tysoni* Forel, *Pheidole bicarinata* Mayr, *Pyramica membranifera* (Emery), *Pogonomyrmex badius* (Latreille) [67]; *Nylanderia vividula* (Nylander) ([67], as *Paratrechina vividula*).

3.2.2. Dubious Cases. Across literature, two cases have been described but not identified. Although unproven, those are highly likely to belong in *Aegeritella* because of the macroscopic description given.

Bequaert ([56], page 74) wrote "*A number of so-called "imperfect fungi"—incompletely developed, conidia-bearing or sterile stages of various Ascomycetes—have been recorded from ants. A nest of* Formica rufa *Linné, at Potsdam, Germany, was heavily infested with fungous growths, about the size of a pinhead and attached mainly to the thorax, more rarely to other parts of the body. The ants were apparently but little hampered by their parasites. From cultures obtained with these fungi, Bischoff concluded that thy belonged to several species, among them a* Mucor, *a* Penicillium *and a yeast. Thaxter also found in the vicinity of Cambridge, Mass., a fungus forming blackish incrustations on various parts of ants and giving rise to a few short, colorless, erect branches; the exact nature of this plant has not been determined, nor is the name of its host mentioned.*"

Donisthorpe ([74], page 235 and Figure 86) commenting on *Lasius umbratus* var. *mixto-umbratus* Forel, [now *Lasius (Chthonolasius)* unrecognisable species] noted "*On August 11th, 1912, when at Weybridge in company with Professor Wheeler, we found two colonies of this variety, very many of the ants of both being infested with a curious dark brown warty growth in patches on parts of the body and legs—this Wheeler thought might be a fungus which was unknown to him. I kept a number of these ants in captivity, and added uninfected workers of* umbrata *from other localities; the growth however did not increase nor spread to the new ants, but rather seemed to decrease. I sent some of the infested ants alive and others in spirit, to Dr. Baylis Elliot, and she considered the patches were colonies of unicellular organisms growing on the outside of the ants; eventually she came to the conclusion that they were not fungoid growths, but probably colonies of an alga.*" Thus, albeit without a named host, *Aegeritella* is probably present too in the United Kingdom. A search with Donisthorpe's collection and/or in the vicinities of Weybridge could confirm this.

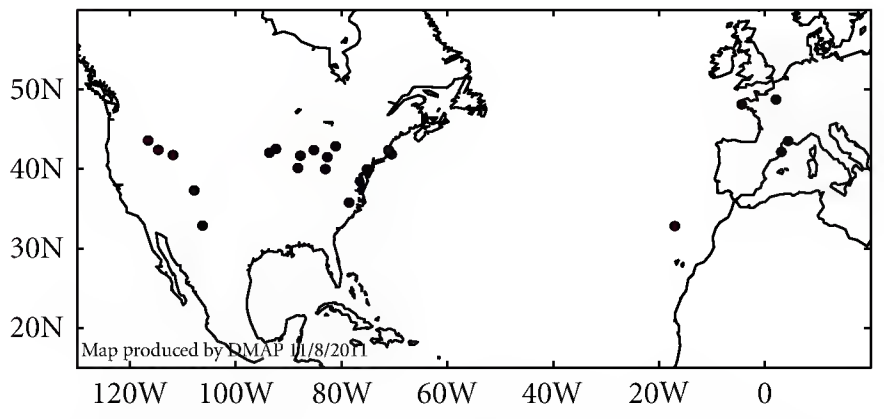


Figure 8: Distribution of *Laboulbenia formicarum*. North American records date from 1902 to 1979 and belong in 24 ant host species of five genera. European records date from 2003 to 2011 and imply two host species of *Lasius*.

4. Discussion

4.1. On Fungus Taxonomy. Laboulbeniales are taxonomically and nomenclaturally stable. There seems to be no major problem in morphological identification of the species involved. Perhaps, only, it would be worth examining the possibility of several species within *Laboulbenia formicarum* since its hosts belong in five genera, from three tribes—Formicini, Lasiini, and Plagiolepidini—in Formicinae.

Aegeritella is an especially difficult situation. Apart from its doubtful position within Fungi, bulbils are usually not in a perfect fruiting condition, and microscopic preparations are not easy to do since the bulbils are tightly attached to the ant's surface, anchored by the pubescence and hairs of the ant. The two most abundant species (*A. superficialis, A. tuberculata*) are well differentiated by the presence of hyphal elements in *A. superficialis* and by its absence in *A. tuberculata* [17].

Myrmicinosporidium is also an unsolved problem. All records but one are based simply on the presence of spores, which have a strikingly similar appearance across the two continents. Although they seem to be close to Chytridiomycetes [67], it remains to be studied where do those fungi belong within the phylogeny, and also the conspecificity of all so-called *M. durum* records. A similar situation is that of *Hormiscium*, from which only hyphae are known.

4.2. Host Phylogeny. A minimum of 13 subfamilies of ants are found in the Holarctic region. Only two (Myrmicinae and Formicinae) are noted with ecto- or endoparasitic fungi. Why should the distribution be so biased? If this is not a sampling artefact, it is noteworthy that the two subfamilies appear close together in the last comprehensive ant phylogenies [75, 76], thus indicating perhaps an ancestral susceptibility for both subfamilies.

Aegeritella is found on *Formica* and *Lasius*. *Laboulbenia* species infest exclusively ants from the subfamily Formicinae and *Rickia* infests Myrmicinae. This host specificity is not rare with Laboulbeniales [33]. Inasmuch *L. formicarum* is hosted by 24 ant species that belong in three tribes (Formicini, Lasiini, and Plagiolepidini), this calls for a dedicated evaluation (molecular and morphological) of the cospecificity of all populations of *L. formicarum*.

Myrmicinosporidium may be found in both ant subfamilies although the majority of cases belong in the Myrmicinae. We may speculate if the generic name is entirely appropriate or there is a detection bias of unknown origin towards Myrmicinae. Infested species belong in six tribes in Myrmicinae (Dacetini, Formicoxenini, Myrmicini, Pheidolini, Solenopsidini, and Tetramoriini), and one tribe in Formicinae (Plagiolepidini), widely scattered within ant phylogeny ([75], Figure 1; [76], Figure 1). Specificity is evidently not to uncritically assume in this fungus.

4.3. Geographical Distribution and Host Number. Knowledge is absolutely fragmentary and skewed. Asia in special, with a single record of ecto- and endoparasitic fungi, is a promising region to explore. The genus *Myrmica* with its many species should be searched for *Rickia*, and the genera *Formica* and *Lasius* for *Aegeritella*. Within Europe, countries such as Ireland, Belgium, The Netherlands, Denmark, Poland, or Portugal are obvious candidates for *Rickia*. The northernmost locale for *Rickia* seems to be Denbies Hillside, at 51°14′N [45]. Some cases, such as *Laboulbenia formicarum* (Figure 8) or *Myrmicinosporidium durum* (Figure 9) agree with the usual worldwide or wide-ranging specific distribution of fungi although others are only known from its original description, from a single locality (*Aegeritella maroccana, Aegeritella roussillonensis*).

With host number, the situation seems to be dichotomous. Some fungi are known from a range of hosts: *A. superficialis* 9 hosts, *A. tuberculata* 10, *L. formicarum* 24, *L. camponoti* 7, *R. wasmannii* 8, and *Myrmicinosporidium* 27, while other fungi are known from single hosts, in parallel with geographical range, likely reflecting a sampling artefact. Horizontal transmission to slave-making ants is possible, as attested by *Aegeritella* [30] and *Laboulbenia formicarum* [54] on *Polyergus*, and by *Myrmicinosporidium* in *Chalepoxenus* [67] and *Strongylognathus* [70].

In the USA, three species (*Pheidole,* and 2 *Solenopsis*) from a single farm in Houston Co., Alabama [71] were noted as infested with *Myrmicinoporidium*. In southern Hungary, three genera (*Plagiolepis, Solenopsis,* and *Tetramorium*) [73] were noted as hosts in a single locality. A similar situation is that of an organic citrus field in Spain [70], in which up to four different genera (*Pheidole, Plagiolepis, Tetramorium,* and *Solenopsis*) have been detected as hosts during several years, their nests being at distances of 5–20 m. The disease may qualify as chronic in the three localities. In this last locality, *Aegeritella* on *Formica rufibarbis* and *Laboulbenia camponoti* on *Camponotus aethiops, C. pilicornis,* and *C. sylvaticus* exist too. The single circumstance we can suggest for this "abnormal" abundance of parasitic fungi in this last site is the intensity—monthly samples—and duration—since 2002 and ongoing—of ecological studies with abundant insect collection. This is suggestive of a general low-prevalence but ample geographic distribution. Thus, we cannot but expect a growth of information if proper attention is directed to those ecto- and endoparasitic fungi of ants. Myrmecologists, please, be aware!

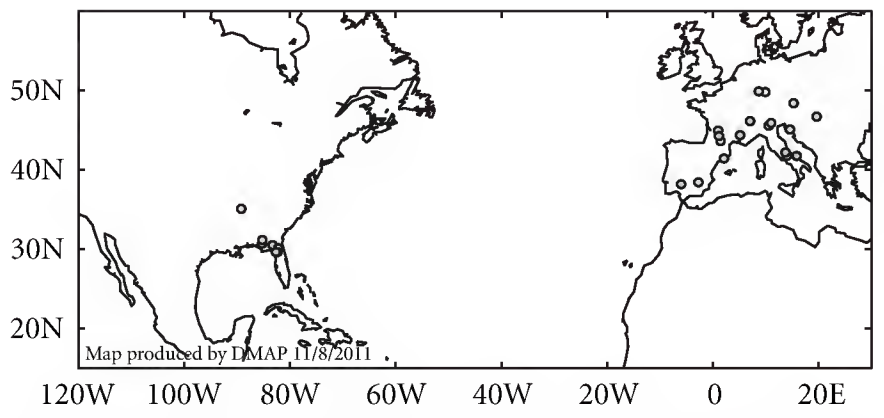


FIGURE 9: Distribution of *Myrmicinosporidium* sp. Eight ant host species are known from USA, and 19 from Europe.

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Research Article

Effects of Environmental Temperature on *Capnodis tenebrionis* Adult Phenology

Carmelo Peter Bonsignore

Dipartimento Patrimonio Architettonico ed Urbanistico (PAU) e Laboratorio di Entomologia ed Ecologia Applicata, Università Mediterranea di Reggio Calabria, Salita Melissari, Feo de Vito, 89100 Reggio Calabria, Italy

Correspondence should be addressed to Carmelo Peter Bonsignore, cbonsignore@unirc.it

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The phenology of *Capnodis tenebrionis* adults was presented with reference to two different climate conditions. In a temperate moderate-warm climate, adult density showed two separate peaks during the year: one in early summer of the overwintering generation and one with beetles emerging in the late summer. In a warmer semiarid climate, the overwintering adults and the new generation overlapped during summer with a continuous increase of adult density. The difference in the average annual temperature between areas during the study period was almost 3°C, and, in the warmer area, the new generation of *C. tenebrionis* emerged at least one month earlier. To make a prediction of adult presence, a model utilizing degree-days was developed from data collected over a five-year period. Models obtained from equations (Logistic 4-parameter, $y(x) = yo + a/(1 + (x/xo)^b)$) of each year were developed to describe the relationship between degree-day accumulation (with a minimal threshold activity temperature of 14.21°C calculated in the laboratory) and the cumulative percentage of adult presence. According to the overall model, the 50% of overwintering beetles occurred at 726 degree-days (Biofix: 1st March) and the emerging beetles occurred at 801 degree-days (Biofix: 1st July). The results show that a change in temperature is an important aspect that highlights the adaptability of this species.

1. Introduction

Abiotic factors including climate may limit the abundance of poikilothermic species and affect their distribution. In particular, variations in the ambient temperature have a dramatic impact on a range of fundamental biological processes including reproduction [1, 2]. Similarly, the relationship between biological events and temperature may provide useful information for predicting the same events, to define the most appropriate time for pest control using presence simulation and seasonal dynamics in regressive models [3].

The buprestid beetle *Capnodis tenebrionis* L. seriously damages *Prunus* spp. L., especially apricot, cherry, and plum [4–6], and it is capable of constraining the organic cultivation of these tree species [7]. Presence of the beetle has serious effects in orchards, and trees can be rapidly killed by the destructive action of its endophytic larvae. The species is widespread in the Mediterranean region, even in areas where their presence has been considered sporadic [8]. Outbreaks in areas previously not affected by the insect have allowed for the presence of the host, possibly as a consequence of global warming [9]. The adults of this species can live longer than one year and some hibernate twice (C. P. Bonsignore, unpubl. data). The females of *C. tenebrionis* are larger and heavier than males, with a sex ratio of 1 : 1 in the population [10]. During the reproductive phase of their lifecycle, the adults of beetle make male-biased aggregations and mated females lay their eggs at the base of host plants, after which, the larvae penetrate the roots of the tree. When the summer months are cold and wet, the number of eggs laid by one female drastically decreases [11]. The species overwinters in the adult stage or with different larval instars that can be simultaneously present on trees (range: 1–7 cm). The adult is active during day and flies during warm days [12], seriously damaging the plant by feeding on the young bark of shoots, buds, and at the base of the petiole of leaves, which drop off.

The presence of overwintering adults in fields starts in spring and is characterized by a gradual emergence when the temperature rises [7]. The presence of the new generation of adults of the beetle takes place gradually over the summer, with variation in different areas relating to climatic conditions. The timing of adult occurrence is not always predictable, which can make pest control difficult, as measures are generally targeted at the adult stage, eggs, and emerging larvae [13, 14].

Developing models of population phenology can enhance decision-making processes around pest control and provide greater opportunity to control the pest within integrated pest management programs [3, 15]. There are not many references concerning the phenology of *C. tenebrionis*, perhaps due to the cryptic habits of the juveniles and the long life cycle that characterizes the species.

The relationship between temperature and adult beetle activity has been partially explored [12]. In the present study, the movement of the insect has been considered a fundamental feature of the initial activity and presence of the adults in orchards. The objective of this study was to verify the influence of temperature on adult phenology and to describe a development and phenology model able to predict the presence of *C. tenebrionis* adults.

2. Materials and Methods

2.1. Study Site and Experimental Design. Studies on *C. tenebrionis* were conducted on apricot plantations in two different regions of southern Italy. One was in Sicily, in the hilly area of Serradifalco (CL) (37°25′52″N, 13°52′37″E 500 m above sea level), and the second was in the coastal area of Gioiosa Ionica (RC) in Calabria (38°11′16″N, 13°11′56″E 50 m above sea level).

The climate of the Sicilian orchard, according to the climatic index of De Martonne [16], is defined as "moderate warm," described here as temperate, with an annual mean temperature of 15-16°C [17]. The second site, in Gioiosa Ionica (RC), according to the climatic index of De Martonne, falls into the category of "subhumid" with a tendency towards semiarid, with an annual mean temperature of 17-18°C [18]. Moreover, this is one of Calabria's driest areas, owing to its orographic characteristics. The minimum precipitation is near the coastline as a consequence of the shielding effect of the mountains, because Mediterranean storms usually impact on Calabria from west to east [19, 20].

In the study areas, tree crops are predominantly grown, such as peach, grape, and apricot in Sicily and citrus and olive groves in Calabria. Over the past few decades, *C. tenebrionis* has been abundant in these areas. Observations were conducted in 2005, 2006, and 2007 in Sicily and in 2008 and 2009 in Calabria. The apricot orchards grafted onto Mirabolano (*Prunus cerasifera* Ehrh.) were, respectively, 11 and 9 years old, and the former had been organically managed since planting. No phytoiatric interventions against insects and mites were made in the orchards during the study years. The trees were arranged in a 4 × 4 m layout and grown in the form of a vase 2.5–3.0 m high.

2.2. Sampling of C. tenebrionis. Each year, observations were made weekly, or at least every 10 days. Observations of overwintering adults started in early spring and continued until their disappearance. The new adult generation of *C. tenebrionis* was assumed to start when the adult beetles began to emerge during summer. All insects detected on the sampled trees were manually collected, counted and identified to sex and generation then released back into the tree.

To identify which generation adults belonged to, the mandibles were examined. They were sharp and pointed in the specimens of the newly emerged generation and more blunt in the overwintered generation [6]. To evaluate possible difference between the populations of the two areas, body size measurements were taken for ~60 male and 60 female specimens. At least 24 plants were sampled in the orchard in Sicily and 48 plants in the orchard in Calabria. The observations concerned the number, sex, and generation of adults on each plant.

2.3. Laboratory Experiment. Laboratory trials were carried out in a climatic chamber to investigate the relationship between adult beetle movement and temperature to find the threshold temperature (t0) at which the movement rate was zero. At this thermal threshold, the insect would not be expected to carry out activities and therefore would not be detected in the field. Experimental adults were sourced from the field population by collecting overwintering adults a week before the start of trials. Captured individuals were separated by sex and then provided with *ad libitum* apricot tree shoots. Twelve hours before the observations began, each adult was placed in a cage measuring 25 × 25 × 35 cm. An apricot shoot with at least 12 leaves was placed in the middle of the cage. The base of each shoot was placed in a plastic tube containing water. The cages were kept at 15, 20, 25, 30, 35, 40, or 45 ± 1°C, relative humidity of 50 ± 5% and a photoperiod of 13L : 11D. Seven observations of 60 min each, with an interval of one hour, were carried out at each temperature. Each temperature was replicated eight times. During each observation period, the number of 5-min sequences in which the insect was stationary was recorded.

The rate of movement activity of the insect was calculated using the formula $1/(n+1)$, with n = the number of sequences with no movement for five minutes. This transformation made it possible to use periods of inactivity of any duration with a maximum value of 1, which corresponds with continual movement and also stabilized the variance of the data [21]. The regression method was used to find the threshold temperature (t0) at which the movement rate was zero, estimated by the x-intercept based on linear regression models [22]. The threshold temperature (t0) at which the movement rate was zero was used to calculate degree-day accumulation.

2.4. Data Analysis. The average number of adults per plant was calculated for each monitoring date, and the date of new generation adults emerging was noted for each experimental area and for each year of observation.

Paired t-tests were used to compare the body size of each sex between the two locations. The mean daily temperature

was obtained from data loggers (Hobo, Onset Computer Corporation) and was calculated from bihourly data. The daily degree-day was calculated with summation of the difference in mean daily temperature and the minimum temperature threshold of beetle movement. In each year, the biofix starts on the 1st of March for overwintering beetles and 1st of July for new emerging adults. To compare the difference between areas, the annual mean temperatures were calculated.

A Logistic equation (4-parameter) commonly used for phenology modeling [23–25] was applied to the cumulative percentage of overwintering and emerging adult beetles and was fitted for each year of the data:

$$y(x) = yo + \frac{a}{1 + (x/xo)^b}, \quad (1)$$

in which y is the cumulative percentage of adults, x is the accumulated degree-days, and a, b, xo, and yo are the constants. From these, the parameter xo has biological meaning and represents the degree-days of 50% of beetle presence. Moreover, two Logistic overall equations were constructed to find 50% of the cumulative percentage of overwintering and emerging adults considering all years and the two areas. The predicted values of each year and area obtained from overall equations were linearly regressed against the observed cumulative percentages of the adult to verify the fit between observed and predicted data [26].

The values of degree-days at 50% of cumulative percentage obtained with the nonlinear regression were also compared with values obtained from the ordinary least square (OLS) method, where the cumulative percentage of the observed beetle was used as independent variable versus degree-days.

For each year, the time between the appearing and subsequent increase of adults in each generation was calculated using degree-days. This was defined as time between the onset of generations (TBG). For each area, this was calculated by the average of TBG. All analyses were performed with SigmaPlot and SPSS software.

3. Results

3.1. Laboratory Results. Using data from the movement activity of the beetle, a minimum threshold temperature for movement was calculated. The increase in temperature in relation to the rate of insect movement followed a sigmoidal function (Figure 1). Given the average daily temperature trend in the experimental areas is long periods under 25°C, we considered the first three temperatures used (up to 25°C). The movement rate was almost linear, and the linear regression method ($y = a + bx$) calculated the temperature threshold to be 14.21°C. From this temperature, the value of the accumulated degree-days was calculated for overwintering and emerging adults.

The different thermal conditions of the two areas are shown in Figure 2. In the warmer area (Calabria), the temperature is higher in the first and last months of the year. The average annual temperature estimates were 15.81 ± 0.34°C for the temperate area and 18.79 ± 0.33°C for the semiarid area. These temperatures are consistent with the reported data available for the two areas [17, 18]. Usually, females were larger than males, but paired t-test found no difference in the size of each sex between the two experimental areas: male $t = 1.221$, $n = 61$, $P = 0.227$ or female $t = 1.111$, $n = 64$, $P = 0.271$.

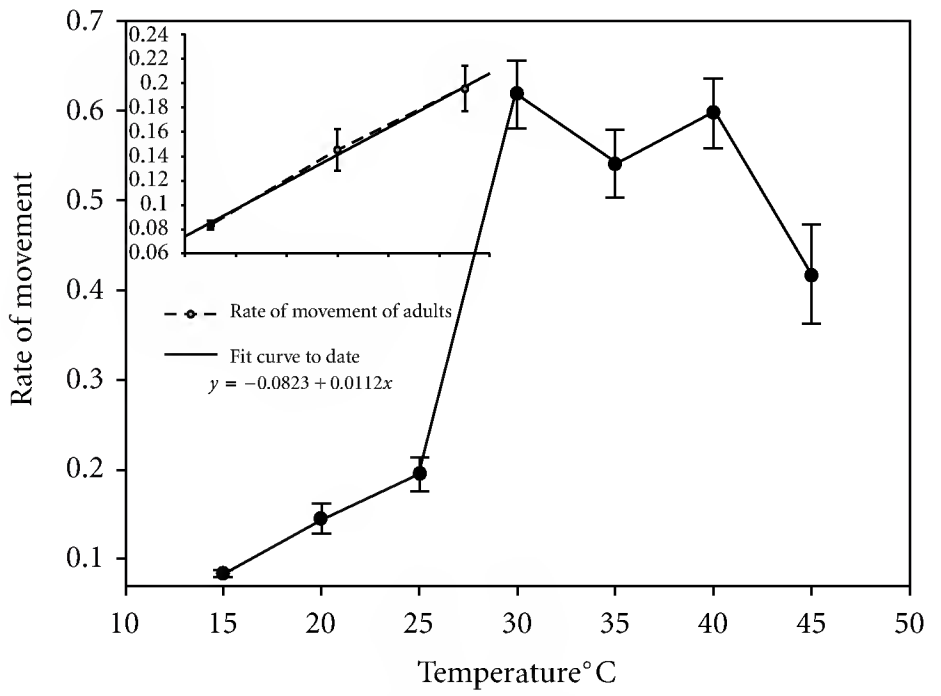

Figure 1: Rate of movement of *Capnodis tenebrionis* adults at different temperatures. Linear rate of movement at the first three temperatures can be observed ($R^2 = 0.996$; $F = 302.37$; df = 1, 2; $P = 0.037$).

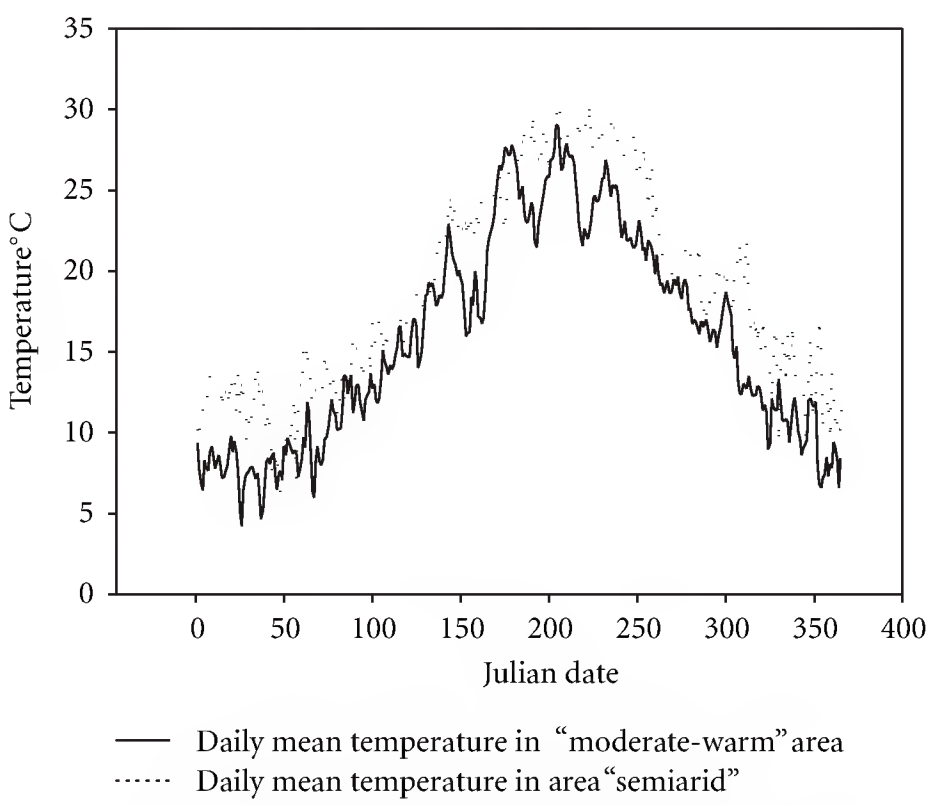

Figure 2: Daily mean temperature of temperate climate (Sicily), years 2005, 2006, and 2007; daily mean temperature of semiarid climate (Calabria), years 2008 and 2009.

3.2. Beetle Phenology. The presence of *C. tenebrionis* adults, although it has shown some variation in density over the years, has been widely documented in the two areas of investigation.

In the temperate area, the maximum density of adults in the field is reached with the overwintering generation,

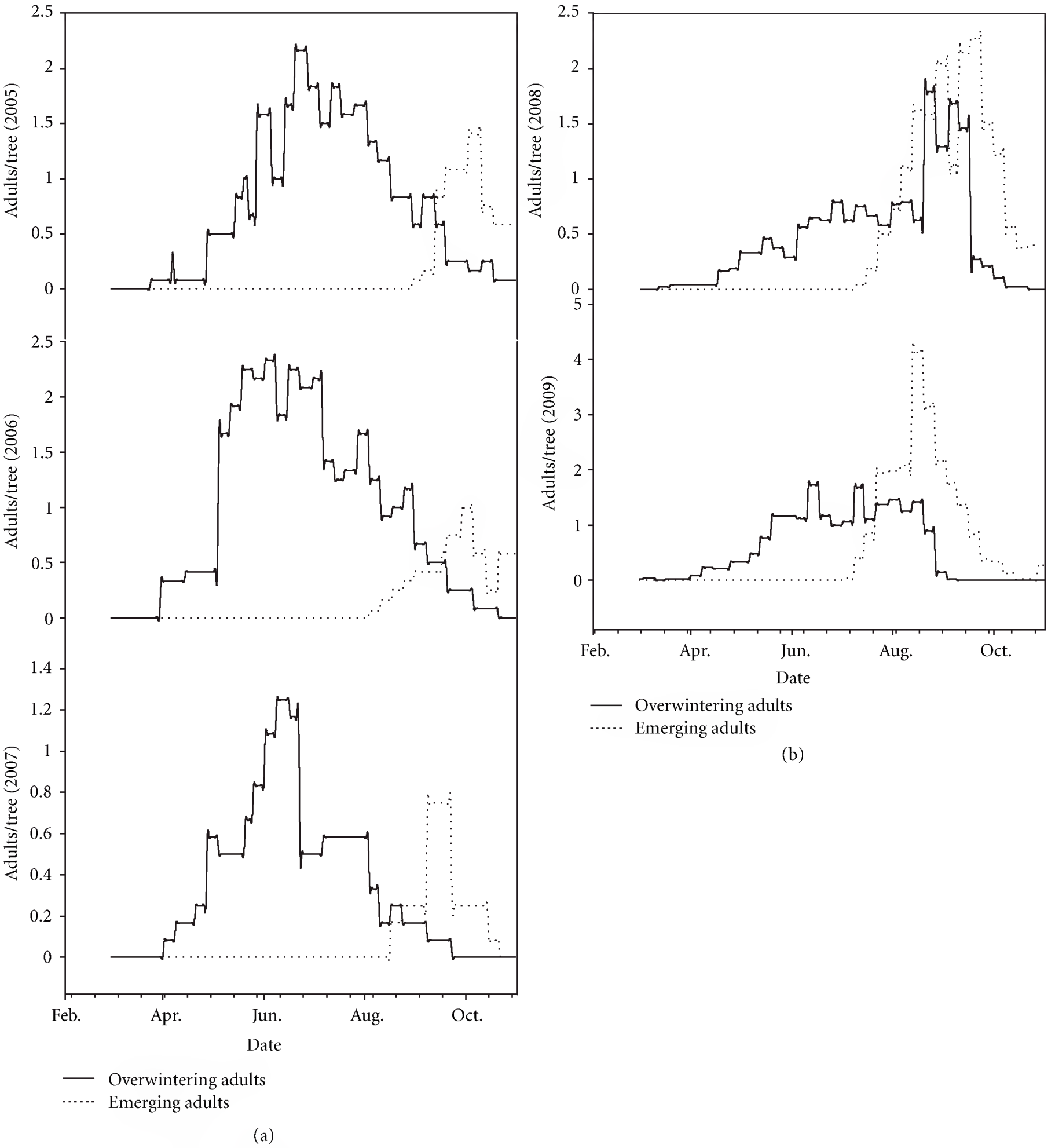

FIGURE 3: Adult presence of *Capnodis tenebrionis* in a temperate area (*a*) and in a semiarid area (*b*). The early onset and the overlapping of generations of adults are evident in the warmer area.

and the lowest density of adults was found in 2007 (1.25 adults per tree), which was connected with the disappearance of plant resources due to *C. tenebrionis* attacks. The first adult maximum density in the temperate climate was reached in the middle of June in 2005 and in the first 10 days in June in 2006 and 2007. In the semiarid area of investigation in 2008 and 2009, the adult maximum density was reached in the middle of August (Figure 3), at which time the overwintering generation overlapped with the newly emerged adults. Research in the Calabrian orchard was suspended in 2010 due to the disappearance of the host resource and, subsequently, the disappearance of beetles. The overwintering generation appeared in open fields from late March and gradually spread as temperatures rose.

The appearance of adult beetles in 2005, 2006, and 2007 varied slightly in the temperate area, with the new adults emerging in August. In 2008 and 2009, the new generation emerged in the first 20 days of July, in accordance with the more elevated temperatures of this area. The emergence of adults in the two areas differed by around one month (Figure 4).

The analysis of the parameters obtained with logistic equations for overwintering gave the value of the parameter *xo* (time of 50% adult emergence), which ranged from 320

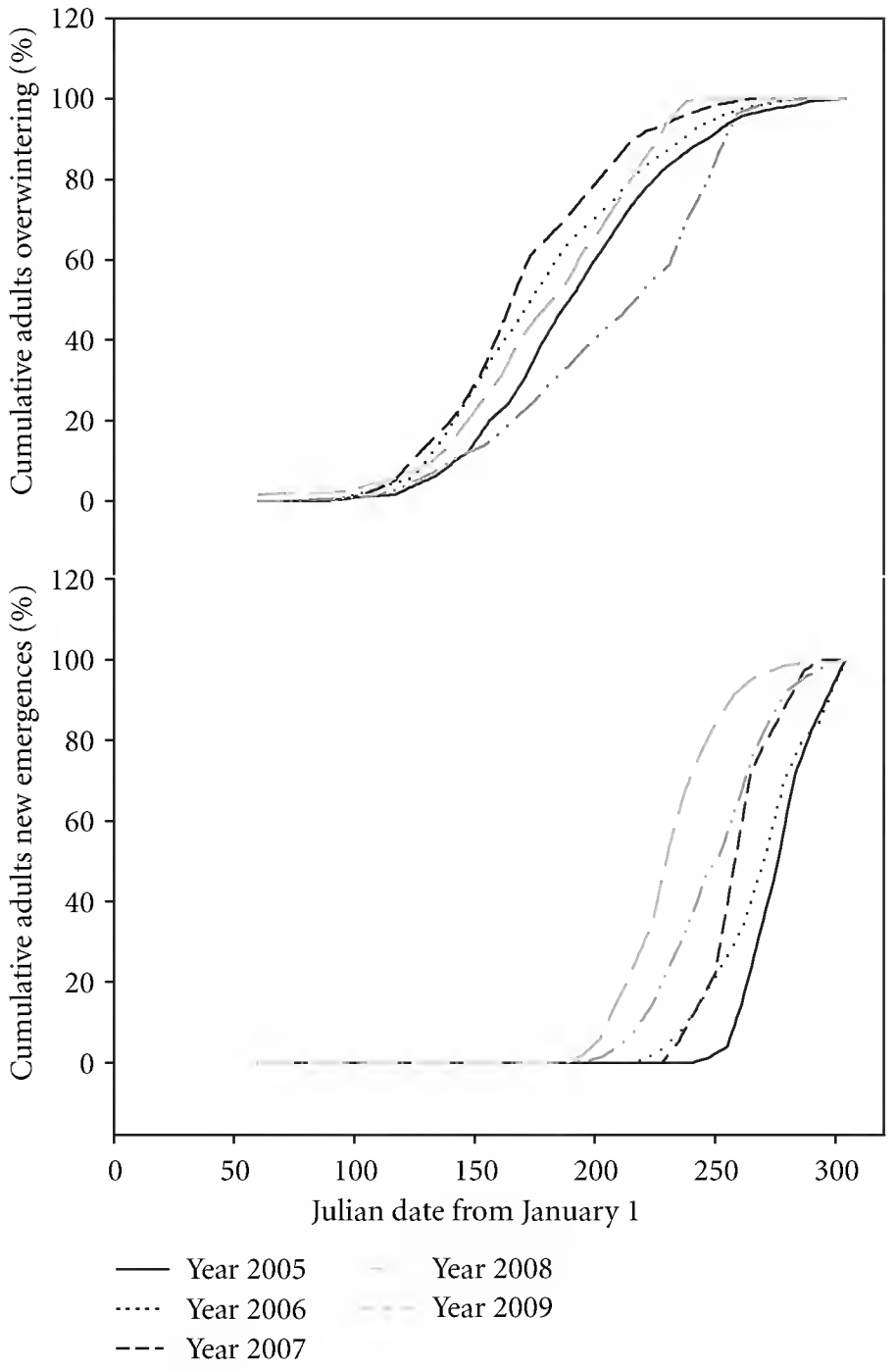

FIGURE 4: Cumulative percentage of *Capnodis tenebrionis* (overwintering and emerging) adults. The grey line refers to semiarid climate, and the black line refers to temperate area.

degree-days (DD) (2007) to 930 DD (2005) for the temperate area. In the semiarid climate, *xo* ranged from 1163 DD (2008) to 768 DD (2009) (Table 1). The overall equation for the analysis of data allowed us to obtain a value of 726 DD for 50% of cumulative adults. It should be noted that, for 2008, the logistic equation of the data was not adjusted to the programmed iterations of the software, so the Boltzmann equation was utilized.

For the emerging generation of *C. tenebrionis* whose onset is in midsummer, the values obtained were a minimum of 812 DD (2005) and a maximum of 1199 DD (2006). In the semiarid climate, the values were 1385 (2008) and 722 DD (2009). The overall analysis of data allowed us to obtain the figure of 801 DD for 50% accumulation of adults emerging (Table 1).

The comparison with the linear regression for each year of the observed cumulative percentage (overwintering and emerging adults) versus the predicted values with the overall logistic equation showed a close fit between the model and observed data (Table 1). The comparison of the DDs at 50% of cumulative adults obtained with the nonlinear regression with values from the OLS method has been highlighted in Table 1. In this case, only the overall values of DDs are similar, and, in general, the value for this linear method is lower, with differences reduced between years in the OLS method.

The average time between the appearance of each generation (TBG) and calculated degree-days showed in the temperate area that for the three years (2005–2007), the TBG value was greater (DD = 858 ± 51 SE, $n = 3$), while in the semiarid area, the value remained the lowest (DD = 631 ± 46 SE, $n = 2$).

4. Discussion

This study of *C. tenebrionis* showed the crucial role of temperature in the emergence of adult beetles. The thermal differences between the two different locations affected the development of species and caused the early onset of the new generation in the summer in the semiarid area. In contrast, overwintering adults emerged in spring in both areas, though the timing appeared to be more uniform in the temperate area. Interestingly, initial emergence of *C. tenebrionis* at both sites coincided with the opening of the earliest flowers at each site; however, the increase of adults at each location was slow, and the maximum density occurred when plants were fully vegetative. Thus, the emergence of beetles in spring appears not to be bound by the phenology of the plant, perhaps because its feeding is independent of the flowering plant.

The temperature seems to be responsible for spring adult appearance, and this strong dependence was expected, because the average daily temperatures for the first four months often remain below the threshold calculated (see Figure 2). It is necessary to obtain more years of monitoring dates of appearance to verify whether climatic fluctuations are primarily responsible for the interannual variability in spring appearance phenology. An early onset brought about by increasing temperature is seen in other species of insects, such as *Apis mellifera* (L.) and *Pieris rapae* (L.) [27]. However, other factors may influence the phenology of *C. tenebrionis* and their subsequent appearance in orchards. For these, we can refer to the microhabitats of orchards, which can influence the activity of adults. For example, in some orchards, tillage of the soil is not carried out, leaving a ground layer of grassy vegetation. In this case, the heating process of the basal part of the plant is reduced, which may result in retardation of the emergence of overwintering adults and formation of new adults. The effects of the grass, as discussed by Snyder et al. [28], could explain the delayed appearance of the overwintering adults in the semiarid orchard compared with the temperate orchard. Although during winter in the semiarid area specimens were rarely present at the base of the plants, they would be unable to feed due to the absence of plant resources.

The difference of recorded temperature in the two study areas was nearly 3°C, and this made it possible to identify the effects of temperature on the phenology of the species in the field, but a manipulation of temperature in the laboratory is desirable for further investigation. *Capnodis tenebrionis* can be reared in the laboratory, although certain technical issues make it a difficult species to culture [29].

Table 1: The parameter estimate of a nonlinear model describing *Capnodis tenebrionis* adult phenology. The comparison with the linear regression for each year of the observed cumulative percentage versus the predicted values with the global logistic equation. Degree-day values at 50% of cumulative adults calculated with ordinary least squares regression for *Capnodis tenebrionis* adults (overwintering and emerging adults of the year) for two different Mediterranean areas (S: Sicily; C: Calabria).

		Parameter estimates (SE) with logistic equations and regression statistics									
		Overwintering adult					Emerging adult				
		Log. equat. parameter	Log. regression values versus observed cumulative		OLS regression		Log. equat. parameter	Log. regression values versus observed cumulative		OLS regression	
2005 (S)	*a*	167.99 (1.7)	$y = 0.91x + 2.22$	$R^2 = 0.997$		**2005 (S)**	*a*	109.70 (1.21)	$y = 0.831x + 6.33$	$R^2 = 0.922$	
	b	−1.22 (0.01)		$(N = 245)$			*b*	−24.75 (0.48)		$(N = 123)$	
	x0	**930.95** (15.22)			**560.73**		*x0*	**832.96** (0.98)			**727.41**
	y0	0.49 (0.07)			$(r^2 = 0.9829)$		*y0*	0.06 (0.1)			$(r^2 = 0.5147)$
	R^2	0.9998					R^2	0.9984			
2006 (S)	*a*	161.56 (4.04)	$y = 0.93x - 2.49$	$R^2 = 0.991$		**2006 (S)**	*a*	571.93 (180.13)	$y = 0.942x + 0.31$	$R^2 = 0.978$	
	b	−0.92 (0.02)		$(N = 245)$			*b*	−6.21 (0.23)		$(N = 123)$	
	x0	**753.58** (41.16)			**517.23**		*x0*	**1198.71** (83.18)			**725.70**
	y0	−0.83 (0.27)			$(r^2 = 0.9360)$		*y0*	0.11 (0.14)			$(r^2 = 0.7166)$
	R^2	0.9982					R^2	0.9970			
2007 (S)	*a*	117.52 (1.35)	$y = 1.01x - 4.00$	$R^2 = 0.973$		**2007 (S)**	*a*	108.88 (1.13)	$y = 0.876x + 1.52$	$R^2 = 0.997$	
	b	−1.22 (0.02)		$(N = 245)$			*b*	−13.22 (0.31)		$(N = 123)$	
	x0	**319.92** (7.12)			**488.05**		*x0*	**812.03** (1.93)			**690.76**
	y0	0.511 (0.29)			$(r^2 = 0.8845)$		*y0*	0.13 (0.14)			$(r^2 = 0.7416)$
	R^2	0.9974					R^2	0.9979			
2008 (C)	*a*	120.79 (2.67)	$y = 1.007x$+13.90	$R^2 = 0.904$		**2008 (C)**	*a*	228.14 (23.74)	$y = 1.074x + 2.60$	$R^2 = 0.896$	
	b	402.77 (11.41)		$(N = 245)$			*b*	−3.28 (0.14)		$(N = 123)$	
	x0	**1163.03**			**923.60**		*x0*	**1385.63** (79.62)			**842.25**
	y0	*			$(r^2 = 0.9908)$		*y0*	0.044 (0.24)			$(r^2 = 0.9213)$
	R^2	0.9847					R^2	0.9945			
2009 (C)	*a*	123.01 (2.05)	$y = 0.991x + 3.89$	$R^2 = 0.985$		**2009 (C)**	*a*	114.20 (1.00)	$y = 1.066x - 13.13$	$R^2 = 0.926$	
	b	−1.67 (0.04)		$(N = 245)$			*b*	−3.53 (0.06)		$(N = 123)$	
	x0	**768.28** (17.47)			**736.34**		*x0*	**721.70** (4.91)			**682.0**
	y0	3.33 (0.30)			$(r^2 = 0.9582)$		*y0*	0.22 (0.13)			$(r^2 = 0.9731)$
	R^2	0.9965					R^2	0.9984			
2005–2009	*a*	137.42 (6.72)				**2005–2009**	*a*	93.65 (1.27)			
	b	−1.18 (0.06)					*b*	−12.94 (0.66)			
	x0	**726.74** (63.03)			**636.55**		*x0*	**801.08** (3.49)			**754.64**
	y0	0.8499 (0.71)			$(r^2 = 0.8608)$		*y0*	1.06 (0.38)			$(r^2 = 0.7807)$
	R^2	0.9231					R^2	0.9176			

* Boltzmann equation $Y(x) = a/1 + e^{((-x-xo)/b)}$.

As in other agroecosystems, in the cultivation of stone fruits, the joint action of different factors (variety, agricultural choices, fertilizer, etc.) can influence the development rates and population dynamics of pests. Among other climatic factors that may affect the adaptability of pests, it is possible to consider photoperiod, but in this case, the difference of this factor between the areas was very low. Moreover, factors such as moisture availability, competition, and extreme weather events could affect phenology, potentially modulating the effects of cumulative heat units. Even the effect of heat on development rate might be nonlinear [30, 31] or can vary according to the life stage of the insect [23].

In early July, the new generation of adults causes more adverse effects on vegetation (decrease of photosynthetic activity and disorders) in accordance with the negative action of the beetle on leaves and buds. As these effects are added, the opportunity for adults to reach sexual maturity before winter is reduced (C. P. Bonsignore, unpubl. data). This advantage of early onset, however, may be accompanied by the inability to survive a second winter, as for some individuals in a temperate climate. In fact, emergence of the new generation in late summer could lead to a second overwintering (C. P. Bonsignore, pers. observation). Also, the average time between the appearances of each generation (TBG) showed in the temperate area for the three years yielded a greater value (DD = 858), while the semiarid area yielded the lowest value (DD = 631). This difference showed a shorter interval between the two generations and a greater overlap of generations in the semiarid area. In confirmation of this species' thermophilic preferences, other activities (e.g., egg laying, egg hatching, and feeding activity) require high thermal optimal condition [12, 32], and some of these events also need to be associated with drought conditions, such as low soil humidity being preferable for newly hatched larvae to reach plant roots [14].

The information collected here suggests new possibilities for the pest to expand its range of distribution under drought and warmer conditions. The various reports of the presence of the species, in areas such as the south of France [8], are generally not considered preferential for the presence of *C. tenebrionis*, which suggests that its expansion may be linked to global warming. In these new areas, the simple, single-parameter degree-day models of phenology may not fit well and could have little power to predict phenology under new conditions. Greater understanding of these possible complexities should permit better forecasting of the phenology of species.

The possibility to recognize, in the orchard, the susceptible stages of the pest and their seasonal predictability is crucial for the application of control measures. This may be even more important if the natural antagonist is unable to exert, either individually or together, a satisfactory control of root-borer populations [33–35].

The values in degree-days calculated for each generation in the five years of study show a close correspondence with the values observed annually for the adoption of a defense strategy that takes into account the emergence of adults. Given the length of adult life of *C. tenebrionis*, it is necessary to take the first action against adults of the overwintering generation when the DD reaches 50% of the value calculated with the equation. Generally, half of this value coincides with the first peaks of the presence of adults, and the first treatment would reduce the number of eggs laid in the field. Understanding the life history of *C. tenebrionis* and its population will be necessary for improving its management and further understanding the spread of this beetle, which is heavily influenced by abiotic climatic factors.

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Review Article

Parasitoid Guilds of *Agrilus* Woodborers (Coleoptera: Buprestidae): Their Diversity and Potential for Use in Biological Control

Philip B. Taylor,[1] Jian J. Duan,[1] Roger W. Fuester,[1] Mark Hoddle,[2] and Roy Van Driesche[3]

[1] *Beneficial Insects Introduction Research Unit, USDA-ARS, Newark, DE 19713, USA*
[2] *Department of Entomology, University of California Riverside, Riverside, CA 92521, USA*
[3] *Department of Plant, Soil and Insect Sciences, University of Massachusetts, Amherst, MA 01003, USA*

Correspondence should be addressed to Jian J. Duan, jian.duan@ars.usda.gov

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Literature studies in North America (US and Canada), Europe, and Asia (particularly Russia, China, Japan, and the Korean peninsula) were reviewed to identify parasitoid guilds associated with *Agrilus* woodborers. There are at least 12 species of hymenopteran parasitoids attacking eggs of *Agrilus* beetles and 56 species (36 genera), attacking *Agrilus* larvae infesting various host plants in North America, Asia, and Europe. While most of the egg parasitoids (9 species) belong to the family Encyrtidae, a majority of the larval parasitoids are members of five families: Braconidae (24 species/11 genera), Eulophidae (8 species/4 genera), Ichneumonidae (10 species/9 genera), and Eupelmidae (6 species/5 genera). The highest rate of *Agrilus* egg parasitism (>50%) was exerted by encyrtid wasps (4 species) in North America, Asia, and Europe. In contrast, the highest rate of *Agrilus* larval parasitism (>50%) was caused by species in two genera of braconids: *Atanycolus* (North America) and *Spathius* (Asia), and one eulophid genus, *Tetrastichus* (Asia and Europe). Reported rate of *Agrilus* larval parasitism ichneumonids was frequent in North America, but generally low (<1%). Potential for success in biological control of emerald ash borer (*Agrilus planipennis* Fairmaire) in the USA with North American native parasitoids and old-association Asian parasitoids is discussed.

1. Introduction

Agrilus is the largest genus within the family Buprestidae (Coleoptera), with nearly 3,000 described species worldwide [1]. Generally, *Agrilus* spp. only attack angiosperms and do not develop in conifers [2]. Moreover, they tend to be specialists, most species being confined to a single genus or species of host plant. While most *Agrilus* species are not considered to be serious pests of agriculture or forests, at least two species have recently become seriously damaging in forests in their newly invaded areas in North America: the emerald ash borer (EAB), *A. planipennis* Fairmaire, and the gold spotted oak borer (GSOB), *A. auroguttatus* Shaefer. EAB was accidentally introduced to Michigan in late 1990s from its native range (northeast Asia, in parts of China, Russia, and Korea) possibly via wooden crates or pallets for cargo shipment [3]; it has since spread to 14 additional US states and two Canadian provinces and killed millions of North American ash (*Fraxinus* spp.) since its detection in 2002 [4, 5]. By contrast, GSOB is native to the oak forests of southwestern Arizona, and while its damage to oak trees in its invaded range has been on a smaller scale, it has killed more than 25,000 oaks in the oak savannahs of California since first discovered there in 2002 [6–8]. A few other exotic *Agrilus* species have also been recently detected in the United States (e.g., soapberry borer—*A. prionurus* Chevrolat in Texas [9]) and Canada (e.g., European oak borer—*A. sulcicollis* Lacordaire in Ontario [10]). Although some of the recently detected, exotic *Agrilus* species have not become as widespread or damaging as EAB and GSOB, the pest status of *Agrilus* borers as a whole along with other woodborers appears to have increased in recent years [11].

Table 1: Summary of online database search for *Agrilus* and associated parasitoids.

Search database	Years searched	*Agrilus* hits	Search hits combined with parasitoid (parasite, natural enemy, or biological control)
Agricola	1970-date	302	38
BioAbstracts (BIOS)	1926-date	507	43
Biological and Agricultural Index Plus	1983-date	30	2
Biological Sciences Set	1982-date	273	23
CSA Illumina		265	22
CAB Abstracts	1985-date	354	94
ISI Web of Science	1900-date	211	25
	No duplicates[a]		−105
	Total publications	1,942	142

[a] We did not review all 1,942 to exclude duplicates.

Management for the invasive (exotic) *Agrilus* woodborers (EAB and GSOB) in the United States initially focused on attempted eradication but changed to integration of several approaches when eradication failed to reduce the pests' populations in infested areas and slow spread of the pests to the noninfested areas [12, 13]. In some cases, control methods being used include delimitation of infested areas, regulatory restriction of movement of pest-infested wood or plant materials, insecticide treatment or physical destruction of infested trees [12–14], and biological control via introduction and release of natural enemies collected from pests' native ranges [7, 15–18]. Although none of these approaches individually is adequate, biological control, which relies on self-propagating and dispersing natural enemies, has potential to reduce invasive pest populations, particularly in forests [19–21].

Agrilus adults normally lay their eggs under loose bark or in crevices of host plant tissues and rarely cause significant damage; in contrast, *Agrilus* larvae typically bore into the living tissue (stems, trunks, branches, or roots) of their host plants, interrupt the translocation of water and nutrients as they feed, and can kill plants within one or a few years of infestation (e.g., EAB [22]; GSOB [6]; *A. prionurus* [9]). In their native habitats, *Agrilus* populations are generally suppressed by a diverse group of natural enemies and/or host tree resistance and only occasionally become serious pests. However, when introduced into ecosystems where host plants lack coevolutionary resistance, or where appropriate specialized natural enemies are absent, they can become severe pests. The recent invasions of North America by EAB from northeast Asia and GSOB from southwestern Arizona are excellent examples of this. For example, EAB is considered a sporadic pest of ash stands in its native range in Asia [23–26] but has become a serious pest threatening the existence of North American ash trees since it was accidentally introduced there [22]. Similar observations have been made for GSOB in its home range. Field studies in Asia found that a complex of natural enemies (primarily parasitoids) and host plant resistance by Asian ash trees appear to be the factors responsible for suppressing EAB populations and preventing them from frequently causing ash mortalities [15, 19].

Deliberate efforts have been recently undertaken in the United States to achieve biological control of EAB and GSOB through introduction of natural enemies (parasitoids) from the native ranges of these pests [7, 17]. These classical biological control efforts for EAB have led to the discovery and introduction of several egg and larval parasitoids that have the potential to establish and suppress the pests' populations in the newly introduced regions [19–21, 27]. Similar programs for GSOB commenced in 2010 and are too immature to reach tentative conclusions about natural enemy diversity and impacts. In reviewing the literature, we found that many groups of parasitoids and other natural enemies have reported attacking *Agrilus* beetles. An overview of the composition of the parasitoid guild attacking this group of woodborers will contribute to the current and future development of biological control programs to manage these pests, particularly those *Agrilus* that have invaded new regions or environments. In the present study, we first review the diversity of natural enemy complexes in particular, hymenopteran parasitoid guilds associated with egg and larval stages of *Agrilus* species, and then discuss the potential of those parasitoids for use as agents of classical biological control against this group of pests.

2. Literature Reviewed

We searched seven major online data bases using the key word "*Agrilus*" either alone or in combination with any of the key words "parasitoid," "parasite", "natural enemy", or "biological control" to locate relevant literature. Databases examined were (1) Agricola, (2) BioAbstracts (BIOS), (3) Biological Sciences Set, (4) Biological and Agricultural Index Plus, (5) CSA Illumina, (6) CAB Abstract, and (7) ISI Web of Sciences set. The key word "*Agrilus*" alone resulted in 1942 articles (Table 1), of which 142 articles remained when combined with "parasitoid or parasite, natural enemies, or biological control." It must be noted that database searches concluded in March 2011. For this paper, we included only

Table 2: Parasitoid guilds associated with *Agrilus* woodborers in North America and Asia.

Parasitoid guilds	Order: Family	Species	Recorded *Agrilus* host	Habitat	Native range in distribution	Level of parasitism	Reference sources
Egg parasitoids	Hym: Aphelinidae	*Ablerus sp.*	*A. anxius*	Birch trees	Northeastern USA/Canada	<0.2%	[28]
		Avetianella sp.	*A. anxius*; *A. subcinctus*	Birch trees; ash trees	Northeastern USA/Canada	<3.5%	[28, 34]
	Hym: Encyrtidae	*Coccidencyrtus sp.*	*A. liragus*	Poplar trees	Northeastern USA/Canada	~55%	[35]
		Ooencyrtus erionotae	*A. sexsignatus*	Eucalyptus trees	Southeast Asia (Philippines)	32–57%	[31, 32]
		Ooencyrtus sp.	*A. anxius*	Birch trees	Northeastern USA/Canada	<2.4%	[28]
		Oobius agrili	*A. planipennis*	Ash trees	China/northeast China-Jilin province	>50%	[15, 36]
		Oobius agrili	*A. planipennis*	Ash trees	United States/Michigan	Not reported	[19]
		Oobius zahaikevitshi	*A. viridis and A. planipennis*	Hazelnut and ash trees, resp.	Northern Italy/Russian	8 –58%	[37, 38]
		Orianos brazai	*A. sexsignatus*	Eucalyptus trees	Southeast Asia (Philippines)	0–47%	[39]
		Signichorini tribe	*A. anxius*	Birch trees	Northeastern USA/Canada	<1%	[28]
		Ptinobius magniflcus	*A. ruficollis*	Raspberry, Blackberry, Dewberry	North America	Not reported	[40, 41]
	Hym: Signiphoridae	*Thysanus* sp.	*A. liragus*	Poplar trees	Northeastern USA/Canada	~12%	[35]
	Hym: Eulophidae	*Pediobius* sp.	*A. planipennis*	Ash trees	United States/Michigan	Not reported	[42]
Larval Parasitoids	Hym: Braconidae	*Atanycolus charus*	*A. anxius* and *A. liragus*	Birch and poplar trees	Northeastern USA/Canada	0.3–52%	[29, 35]
		Atanycolus cappaerti	*A. planipennis; A. liragus and A. bilineatus*	Ash trees; poplar and chestnut trees	Northeastern USA/Canada	9–71%	[33]
		Atanycolus disputabilis	*A. planipennis* and other North American native woodborers	Oak trees	Northeastern USA/Canada	<1%	[43]
		Atanycolus simplex	*A. planipennis; A. liragus and A. bilineatus*	Ash trees; poplar and chestnut trees	Northeastern USA/Canada	<1%	[35, 44]
		Atanycolus hicorie	*A. planipennis and* other native *Agrilus* woodborers	Ash trees	Northeastern USA/Canada	<2%	[45, 46]
		Atanycolus nigropopyga	*A. planipennis* and other North American native woodborers	Ash trees	Northeastern US/Canada	<3%	JJD (unpublished)

Table 2: Continued.

Parasitoid guilds	Order: Family	Species	Recorded *Agrilus* host	Habitat	Native range in distribution	Level of parasitism	Reference sources
		Atanycolus picipes	*A. planipennis*	Ash trees	Vladivostok, Russia	<5%	JJD (unpublished), [25]
		Doryctes farthus	*A. anxius* and *A. liragus*	Birch and poplar trees	Northeastern US/Canada	<0.1%	[44]
		Doryctes rufipes	*A. anxius* and *A. liragus*	Birch and poplar trees	Northeastern USA/Canada	<0.1%	[44]
		Doryctes atripes	*A. anxius*	Birch tree	Northeastern USA/Canada	<0.1%	[44]
		Iphiaulaz impostor	*A. biguttatus*	Poplar trees	Europe	~13%	[47]
		Leluthia astigma	*A. planipennis; A. difficilis* and other *Agrilus* spp.	Ash trees; honey locust trees	USA	~2.1%	[48, 49]
		Spathius agrili	*A. planipennis*	Ash trees	China	60–90%	[50–56]
		Spathius agrili	*A. planipennis*	Ash trees	USA/Michigan	Not reported	[21, 57]
		Spathius agrilivorus	*A. planipennis*	Ash trees	Vladivostok, Russia	~64%	JJD (unpublished), [25]
		Spathius curvicaudis	*A. biguttatus*	Oak trees	Europe	~25%	[47, 58]
		Spathius floridanus	*A. planipennis* and other North American native woodborers	Ash trees	USA	<0.5%	JJD (unpublished)
		Spathius laflammei	*A. planipennis* and other North American native woodborers	Ash trees	USA	<1%	JJD (unpublished)
		Spathius simillimus	*A. anxius and A. liragus/; A. planipennis*	Birch and poplar tree; ash trees	USA/Canada	<0.5%	[18]
		Wroughtonia (Helconidea) ligator	*A. anxius, A. liragus and A. bilineatus*	Birch, poplar and chestnut trees	northeastern USA/Canada	<1%	[29, 44]
		Ecphylus sp.	*A. subcinctus*	Ash trees	USA	Not reported	[34]
		Heterospilus sp.	*A. subcinctus*	Ash trees	USA	Not reported	[34]
		Pareucorystes varinervis	*A. viridis*	Hazelnut	Europe/Russia	Not reported	[59]
		Monogonogastra agrili	*A. arcuatus*	Hickory, pecan	North America	Not reported	[60]
		Microbracon xanthostigmus	*A. ruficollis*	Raspberry, blackberry, dewberry	North America	Not reported	[40, 41]
	Hym: Chalcididae	*Phasgonophora sulcata*	*A. anxius, A. bilineatus and A. liragus; A. planipennis*	Birch, chestnut and poplar tree; ash trees	USA/Canada	2–20%	[28–30, 35]

Table 2: Continued.

Parasitoid guilds	Order: Family	Species	Recorded *Agrilus* host	Habitat	Native range in distribution	Level of parasitism	Reference sources
	Hym: Eulophidae	*Tetrastichus nr.rugglesi*	*A. anxius and A. liragus; A. planipennis*	Birch and poplar trees; ash trees	USA/Canada	<0.1%	[29, 35]
		Tetrastichus heeringi	*A. sinuatus and A.aurichalceus*	Pear trees and raspberries, resp.	Europe	55–75%	[61–63]
		Tetrastichus heeringi	*A. ribesi*	Black current	Europe	Not reported	[64]
		Tetrastichus sp.	*A. sexignatus*	Eucalyptus trees	Southeast Asia (Philippines)	2–50%	[31, 32]
		Tetrastichus planipennisi	*A. planipennis*	Ash trees	Northeastern China/Russian Far East	22–40%	[15, 65, 66]
		Tetrastichus planipennisi	*A. planipennis*	Ash trees	USA Michigan	0.80%	[27, 57, 67]
		Baryscapus agrilorum	*A. aurichalceus*	Raspberry	Europe/Hungary	Not reported	[62, 68]
		near Hadrotrichodes	*A. subcinctus*	Ash trees	USA	Not reported	[34]
		Entodon epicharis	*A. surorovi/*	Poplar	China	Not reported	[69]
		Entodon zanara	*A. surorovi*	Poplar	China	Not reported	[69]
	Hym: Eupelmidae	*Balcha indica*	*A. planipennis* and other Asia and North American woodborers	Ash trees	Southeast Asia/North America	<4%	[70–72]
		Calosota elongata	*A. auroguttatus*	Oak trees	USA/Mexico	15%	[6]
		Eupelmus pini	*A. planipennis*	Ash trees and weevils	North America	<0.2%	[70]
		Metapelma sp.	*A. subcinctus*	Ash trees	USA	Not reported	[34]
		Calosota agrili	*A. salicis*	Willow	Europe/Poland and Russia	Not reported	[73, 74]
		Pentacladia hatayensis	*Agrilus* sp.	Fig	Turkey	Not reported	[75]
	Hym: Eurytomidae	*Bephratoides agrili*	*A. anxius*	Birch trees	North America	<1%	[29]
		Eurytoma rosae	*A. rubicola* and *A. bilineatus*	Rose and chestnut trees		Not reported	
		Eurytoma sp.	*A. anxius*	Birch trees	North America	<1%	[29]
		Eurytoma sp.	*A. subcinctus*	Ash trees	North America	Not reported	[34]
	Hym: Ichneumonidae	*Cunocephalus sp.*	*A. planipennis*	Ash trees—to be confirmed	North America	<0.2%	[70]
		Dolichomitus messorperlongus	*A. anxius* and *A. liragus*	Birch trees	North America	<0.4%	[29]
		Dolichomitus vitticrus	*A. planipennis* and other native woodborers	Ash trees	North America	<0.2%	[70]
		Ephialtes sp.	*A. anxius* and *A. liragus*	Birch trees	North America	<0.4%	[35]

Table 2: Continued.

Parasitoid guilds	Order: Family	Species	Recorded *Agrilus* host	Habitat	Native range in distribution	Level of parasitism	Reference sources
		Glypta sp.	*A. anxius*	Birch trees	North America	<1%	[44]
		Ichneumon sp.	*A. anxius*	Birch trees	North America	<1%	[44]
		Olesicampe sp.	*A. anxius*	Birch trees	North America	<1%	[44]
		Unknown spp.	*A. suvorovi-populneus*	Poplar trees	Europe/Hungary	4-5%	[76]
		Orthizema sp.	*A. anxius; A. planipennis*	Birch trees; ash trees	North America	<1%	[44, 70]
		Pimploterus sp.	*A. anxius*	Birch trees	North America	<1%	[44]
		Labena apicaulis	*A. arcuatus*	Hickory, pecan	North America	Not reported	[60]
	Hym: Stephanidae	*Foenatopus sp.*	*A. sexsignatus*	Eucalyptus trees	Southeast Asia (Philippines)	2–50%	[31, 32]
	Hym: Pteromalidae	*Zatropus* sp.	*A. arcuatus*	Hickory, pecan	North America	Not reported	[60]
		Oodera sp.	*A. subcinctus*	Ash trees	USA	Not reported	[34]
	Hym: Bethylidae	*Sclerodermus pupariae*	*A. planipennis*	Ash trees	China	Not reported	[77]

those original research articles that provide information on parasitoid identity at the family, genus, or species levels (Table 2).

In addition to searching databases, we contacted colleagues who work on invasive EAB and GSOB beetles and their biological control in the United States and Canada for information on parasitoid guilds of these species. All relevant studies were read and analyzed for mention of *Agrilus* species, associated parasitoids, known host associations, host plants, and geographic distributions. If available, parasitism rates by each group, guild, or species of parasitoids were noted.

3. Results and Discussion

At the genus level, the guilds of egg and larval parasitoids of *Agrilus* species were similar in North America, Europe, and Asia. While several families of North American parasitoids (including Braconidae, Chalcididae, Ichneumonidae, and Eupelmidae) are capable of utilizing larvae of the newly introduced emerald ash borer (*A. planipennis*) as a novel host, some Asiatic species of parasitoids appear to be more specific and only utilize Asian *Agrilus* species as hosts. From the geographic distribution point of view, it appears that there is more diversity in the parasitoid complex associated with *Agrilus* beetles in North American than in Asia and Europe. However, this geographic difference in parasitoid diversity may actually reflect different levels of research activities on the subject. For example, the invasion of North America by EAB has certainly resulted in much more research activities on the parasitoid complex of this group of woodborers in North America.

There are at least 12 species of hymenopteran parasitoids that attack eggs of *Agrilus* beetles and 56 parasitoid species that attack *Agrilus* larvae in various plants in North America, Asia, or Europe (Table 2). While most of these egg parasitoids (9 species) belong to the family Encyrtidae, a majority of the larval parasitoids are members of five families: Braconidae (24 species/11 genera), Eulophidae (8 species/4 genera), Ichneumonidae (10 species/9 genera), and Eupelmidae (6 species/5 genera). One species of larval parasitoid (*Phasgnophora sulcata* Westwood) (Chalcididae) is frequently associated with native *Agrilus* woodborers in North America [28–30]. In addition, there is one larval parasitoid (*Foenatopus* sp.) in the family Stephanidae that was reported attacking *A. sexsignatus* (Fisher) infesting Eucalyptus trees in southeast Asia [31, 32].

The highest rates of *Agrilus* egg parasitism (>50%) occurred with four species of encyrtid wasps reported in North America, Asia, and Europe (Table 2). In contrast, the highest rates of *Agrilus* larval parasitism (>50%) were caused by two groups of braconid wasps: *Atanycolus* spp. (in North America) and *Spathius* spp. (in Asia), and three species of eulophid wasps (in Asia and Europe). Although ichneumonid wasps were frequently reported attacking *Agrilus* woodborers in North America, the reported rate of parasitism was very low (<1%) for all the ichneumonid species.

It is interesting to note that several species of North American native parasitoids, *Atanycolus* spp., *Spathius floridanus* Ashmead, *S. laflammei* Provancher, *S. simillimus* Ashmead, *Phasgonophora sulcata* Westwood, and one accidentally introduced Asiatic wasp *Balcha indica* (Mani and Kaul), have been recently reported attacking the invasive emerald ash borer. One group of native parasitoids, *Atanycolus* spp., has recently become the dominant mortality factor associated with emerald ash borer, attacking >50% of *A. planipennis* larvae at some forest sites in Michigan (USA) [19, 33]. The potential of both the native (new-association) parasitoids and the introduced (old-association) parasitoids

(e.g., *Oobius agrili* Zhang and Huang, *Tetrastichus planipennisi* Yang, and *Spathius agrili* Yang) for biological control of EAB, in the USA, needs further investigation.

A diverse group of hymenopteran parasitoids attacks eggs and larvae of *Agrilus* woodborers in North America, Asia, and Europe. Literature review of this genus, in regards to its parasitoid guild, has interest due to the introduction of two species in North America (GSOB and EAB). In biological control, parasitoid species of invasive pests are often introduced from the land of origin, if proved to be safe (not become a pest themselves). In addition, new-association parasitoids that inhabit the region prior to the pest introduction sometimes exert pressure on this newly arrived pest and offer opportunity for research and augmentation of indigenous parasitoid populations. Our literature review has provided documentation of research activities for 12 egg parasitoid species and 56 larval parasitoid species. These parasitoids are identified from 19 species of *Agrilus*, a small representation of almost 3000 described, that attack 18 recorded plant types (13 hardwoods, 5 shrubs). Being a diverse genus, these results show a wealth of research opportunities for further work on *Agrilus* parasitoids worldwide. Nearly two thirds (64.3%) of the literature found was published after year 2000. Twenty-seven of 83 entries (32.5%), in Table 2, reference *A. planipennis*. These findings are results of EAB postdetection in 2002.

Although *Agrilus* species are relatively host specific, because of larvae's concealed nature, early stages and damage are difficult to assess and take much effort to obtain. This has implications on finding and identifying parasitoid complexes for biocontrol and may be a reason for so little literature. Of those parasitoid species found in association with EAB, some are ectoparasitoids and known to attack woodborers in different families (e.g., Cerambycidae). While data from the current literature do not show any particular relationship between host specificity and mode of parasitization (endo- versus ectoparasitoids), further research is needed to investigate such relationship.

Some species occur on multiple *Agrilus* spp., such as egg parasitoid *Oobius zahaikevitshi*. *Atanycolus cappaerti* is known to attack *A. planipennis*, *A. liragus*, and *A. bilineatus*, while *Leluthia astigma* attacks *A. planipennis*, *A. difficilis*, and other *Agrilus* spp. These may provide better access of parasitoids where poplar, chestnut, honey locust, and ash occur together.

The parasitoid guild of *Agrilus* in China, Russia, and North America and EAB distribution may provide species for introduction or augmentation. Though most of the parasitism rates are low (<10%), a few worthy candidates not yet used for introduction or augmentation include egg parasitoid, *O. zahaikevitshi* from Russia, and larval parasitoids *Atanycolus cappaerti*, *Spathius agrilovorus*, and *Spathius floridanus*. These species in the USA have not yet been reared in large numbers, and further studies on rearing methods need pursuing. It appears also that braconids and eulophids have provided the best potential for biological control, and the number of studies the last five years bear this out. It also indicates that species size and morphology (ovipositor length) for accessing the host from outside the host plant are important for success.

Finally, parasitoid work in biological control efforts often lack taxonomic expertise to provide accurate identifications. Some of these newly known parasitoid species are not well understood. Egg parasitoids are often disregarded due to size and inaccessibility of host eggs. These hamper ongoing biological control of invasive or cyclic native pest populations. A concluding question is should work be done now on conspecifics that have the potential to be invasive (e.g., *A. coxalis* attacks oaks in Mexico—California has a history of acquiring pests from MX, could *A. coxalis* be another threat to CA's besieged oaks forests?).

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Research Article

Genetic Diversity of *Melipona mandacaia* SMITH 1863 (Hymenoptera, Apidae), an Endemic Bee Species from Brazilian Caatinga, Using ISSR

Elder Assis Miranda,[1] Henrique Batalha-Filho,[2] Priscila Santos Oliveira,[1] Rogério Marcos Oliveira Alves,[3] Lucio Antonio Oliveira Campos,[4] and Ana Maria Waldschmidt[1]

[1] *Departamento de Ciências Biológicas, Universidade Estadual do Sudoeste da Bahia, Avenida José Moreira Sobrinho s/n, 45206-190 Jequié, BA, Brazil*
[2] *Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, USP, Rua do Matão 277, Cidade Universitária, 05508-090 São Paulo, SP, Brazil*
[3] *Escola Agrotécnica Federal de Catu, Rua Barão de Camacari 118, 48110-000 Catu, BA, Brazil*
[4] *Departamento de Biologia Geral, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs s/n Campus Universitário, 36571-000 Viçosa, MG, Brazil*

Correspondence should be addressed to Elder Assis Miranda, elderuesb@gmail.com

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In order to evaluate the genetic diversity and structure of *Melipona mandacaia,* we analyzed 104 colonies collected in 12 localities in Bahia state, northeastern Brazil, using ISSR-PCR. A total of 109 bands were obtained with a significant polymorphism of 72.47%. Estimates of genetic diversity indicated low values of heterozygosity (H_e and H_B values were 0.2616 and 0.2573, resp.). These reduced values have been reported in other studies in stingless bees and maybe justified by dispersion process in the origin of new nests. AMOVA revealed that the higher percentage of variation is within localities (70.39%). The Φ_{ST} and θ_B values were, respectively, 0.2961 and 0.3289, thereby indicating a moderate population structuring. The correlation between genetic and geographic distances ($r = 0.4542$; $P < 0.01$) suggests isolation by distance. Our study contributes to describing the genetic diversity of endemic organisms from Caatinga and may help future efforts to preserve this threatened biome.

1. Introduction

The bee species *Melipona mandacaia* SMITH 1863 belongs to the subtribe Meliponina, which comprises the stingless bees. This species is endemic to Caatinga biome, being widespread in the Brazilian states of Piauí, Ceará, Bahia, Paraíba, and Pernambuco, usually close to São Francisco River [1, 2]. It plays an important role in Caatinga, acting as specific pollinators of this biome, besides presenting a great potential in meliponiculture [3].

The Caatinga is an exclusively Brazilian biome, comprising a wide drought belt in South America. This biome encompasses about 800,000 km^2 (8.6% of Brazilian territory) being surrounded by Atlantic Forest to the east, Amazon Forest to the west, and Brazilian savannah (Cerrado) to the south [4].

The genetic resources from Caatinga suffer accentuated pressure by continuous deforestation and expansion of agricultural frontiers. Consequently, the number of trees used for nesting of stingless bees is becoming more and more scarce [5], leading to population decreases or even local extinction of some bee species [6]. Studies in Caatinga bees have shown several peculiarities, such as endemism and specific interactions between bees and local flora [7]. Therefore, knowledge about this ecosystem and its potentialities, as well as the conservation of original covers, is essential.

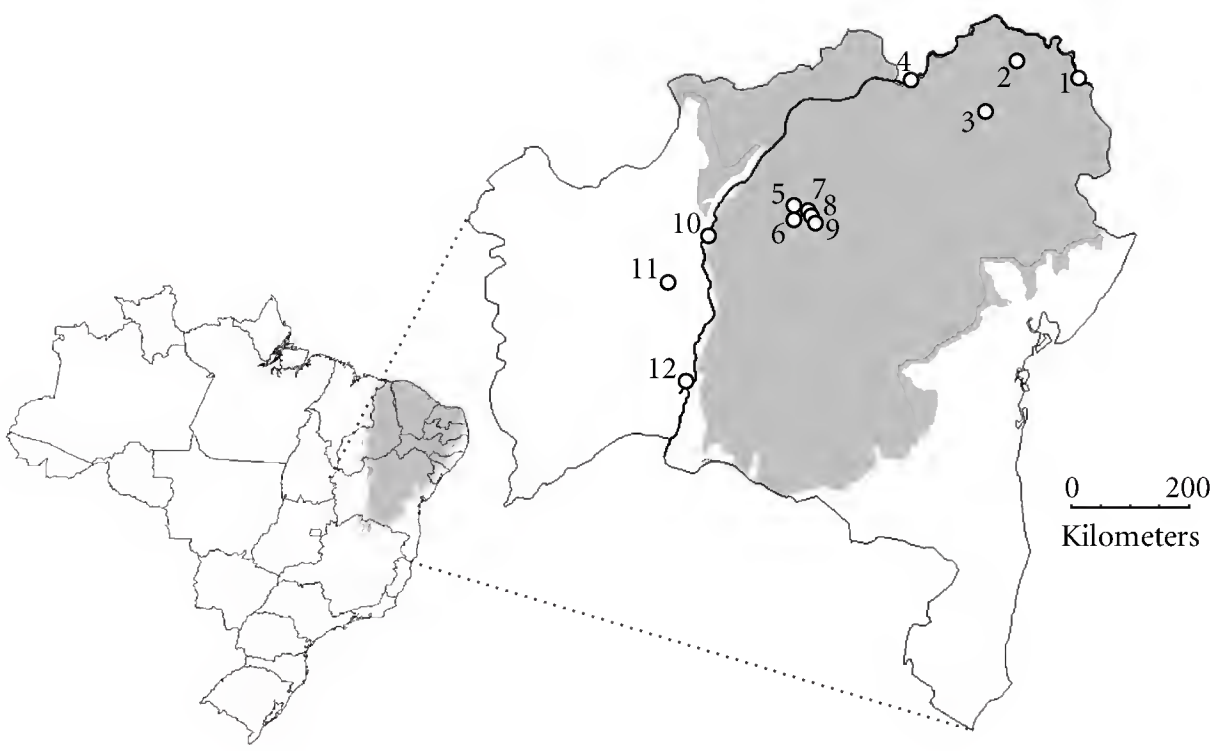

Figure 1: Map of collection sites of *M. mandacaia*. The grey area represents the Caatinga biome and the black thick line the São Francisco River. The circles indicate the sampled localities: 1 (Paulo Afonso), 2 (Macururé), 3 (Uauá), 4 (Juazeiro), 5 (Lapão), 6 (Irecê), 7 (São Gabriel), 8 (Central), 9 (Uibaí), 10 (Morpará), 11 (Muquém do S. Francisco), and 12 (Serra do Ramalho).

However, little is known about the genetic population structure of endemic species from Caatinga, and the conservation and the importance of its biota to local biodiversity remain overlooked [8]. Studies of genetic diversity in natural populations usually refer to quantification of levels of variation within and among populations [9]. When combined to other biological aspects such as reproductive behavior and dispersion process, these studies are able to provide valuable insights for defining conservation programs of a given species [10].

Amongst the several molecular markers available for genetic studies, the Inter Single Sequence Repeats (ISSRs) stands out as an efficient technique in the genome characterization of plants, fungi, vertebrates, and insects [11]. This methodology allows detecting polymorphism in DNA regions flanked by microsatellites without requiring previous isolation and sequencing of specific DNA fragments.

Moreover, the estimates of genetic diversity of *M. mandacaia* might contribute for defining further management and conservation strategies. Therefore, the goal of the present study was to estimate the genetic diversity and structure of *M. mandacaia* throughout Bahia state, focusing on three main questions. (i) How much genetic diversity is there in *M. mandacaia*? (ii) How this diversity is structured? (iii) Is the genetic variation correlated to geographic distribution?

2. Material and Methods

2.1. Sampling and DNA Extraction. Samples of 104 colonies of *M. mandacaia* were collected in 12 localities in Caatinga along Bahia state between 2003 and 2010 (Figure 1 and Table 1). The collected specimens were stored in absolute ethanol and kept at −20°C prior analyses. The total DNA was extracted from one worker bee per colony, based on the methodology proposed by Waldschmidt et al. [12]. The DNA samples were quantified in 0.8% agarose gel to verify their concentration and integrity.

Table 1: Sampled localities of *Melipona mandacaia*, number of colonies per locality (*N*), altitude, and geographic coordinates.

Code/Locality	*N*	Altitude (m)	Longitude (W)	Latitude (S)
PA—Paulo Afonso	10	243	38° 12′ 52″	9° 24′ 22″
MC—Macururé	12	357	39° 03′ 26″	9° 10′ 03″
UA—Uauá	14	439	39° 28′ 53″	9° 50′ 29″
JU—Juazeiro	14	368	40° 29′ 55″	9° 24′ 43″
LA—Lapão	3	775	41° 49′ 54″	11° 22′ 60″
IR—Irecê	6	721	41° 51′ 20″	11° 18′ 16″
SG—São Gabriel	8	692	41° 54′ 43″	11° 13′ 46″
CE—Central	3	698	42° 06′ 45″	11° 08′ 09″
UI—Uibaí	15	582	42° 07′ 56″	11° 20′ 13″
MO—Morpará	6	405	43° 16′ 51″	11° 33′ 31″
MU—Muquém do S. Francisco	6	560	39° 31′ 59″	12° 18′ 60″
SR—Serra do Ramalho	15	497	43° 35′ 48″	13° 33′ 45″

2.2. ISSR-PCR. Firstly, 70 ISSR primers (manufactured by UBC) were tested and 15 were selected based on their reproducibility, definition, and number of bands. Afterwards, the effects of concentration of primer (0.05; 0.10, and 0.15 μM), DNA (10 and 50 ng), and annealing temperature (48 to 60°C) were analyzed. After optimizing the PCRs, 10 primers were selected for population analyses (Table 2). The amplification reactions were adjusted based on the methodology proposed by Eiadthong et al. [13].

The PCRs comprised a final volume of 25 μL including 10 ng of template DNA, 2.5 μL of 10x buffer (Biotools), 2.0 μL of dNTPs at 200 μM each, 0.5 U of Taq polymerase (Biotools) and 0.4 pmoles of primer. The PCR conditions involved an

Table 2: Selected ISSR primers with their respective sequences, number of bands, and percentage of polymorphism.

Primers	Sequence (5′ → 3′)	Total number of bands	Number of polymorphic bands	%
UBC 807	AGAGAGAGAGAGAGAGT	10	10	100
UBC 808	AGAGAGAGAGAGAGAGC	10	5	50.0
UBC 811	GAGAGAGAGAGAGAGAC	10	6	60.0
UBC 813	CTCTCTCTCTCTCTCTT	9	6	66.6
UBC 815	CTCTCTCTCTCTCTCTG	13	10	77.0
UBC 835	AGAGAGAGAGAGAGAGYC	14	11	78.6
UBC 841	GAGAGAGAGAGAGAGACTC	8	6	75.0
UBC 855	ACACACACACACACACCTT	10	9	90.0
UBC 857	ACACACACACACACACYG	11	7	63.6
UBC 889	AGTCGTAGTACACACACACACAC	14	8	57.1

initial denaturation step at 94°C for 3 minutes, followed by 40 cycles at 92°C for 1 minute, 53°C for 2 minutes, and 72°C for 2 minutes; plus a final extension step at 72°C for 7 minutes. All reactions included a negative control with all PCR components but without DNA. The PCR products were separated by electrophoresis in 1.2% agarose gel with 0.2 μg/ml of ethidium bromide, immersed in 1X TBE buffer (Tris-Borate 90 mM, EDTA 1 mM, and pH 8.0). The DNA fragments (bands) were visualized under ultraviolet light and photodocumented.

2.3. Data Analysis. The amplification products were transformed into binary data according to the presence (1) or absence (0) of bands. The genetic diversity (H_e) [14] and the percentage of polymorphic loci were estimated using the software TFPGA v1.3 [15].

The analysis of molecular variance (AMOVA) [16] and the estimate of structuring index among localities (Φ_{ST}) were performed using the software Arlequin v3.5.1.2 [17]. The test significance was based on 1,000 permutations.

Genetic diversity and population structure were also estimated using the Bayesian approach [18] available in the software HICKORY 1.1 [19]. The H_B and θ_B indexes, analog with H_e and Φ_{ST}, respectively, were estimated as well. The analyses were carried out using four *a priori* models: full model, $f = 0$ model, $\theta = 0$ model, and f free model. The best model was determined based on the deviance information criterion (DIC) according to software's authors, in which the lowest DIC values indicate the best adjusted model. A total of 100,000 MCMC (Markov Chain Monte Carlo) generations and a burn-in of 20% were implemented. The analyses were run twice in order to check the convergence of parameters.

Mantel's test was also performed to check the correlation between geographic distance and Φ_{ST} values amongst localities. This test was applied in order to verify whether distance isolation between localities was present or not. The analyses were carried out using the software Arlequin v3.5.1.2 [16] and the statistical significance was based on 1,000 permutations.

3. Results

The 10 selected ISSR primers yielded 109 bands with a mean polymorphism of 72.47% (Table 2). The primers UBC-889 and UBC-835 presented the highest number of bands (14) while UBC-841 had the lowest number (8). The primer UBC 807 presented the highest polymorphism (100%). The mean number of bands per primer was 10.9. The direct estimate of gene diversity (H_e) was equal to 0.2616.

The genetic structure based on AMOVA partitioned in two hierarchical levels showed that 70.39% of genetic variation was within localities whereas 29.61% of variation was related to variation among localities. The Φ_{ST} value was 0.2961 ($P < 0.000001$; Table 3). When three hierarchical levels were considered (assuming localities between left and right bank of the São Francisco River as groups in the third hierarchical level) in AMOVA, no genetic variation among groups was revealed, resulting in 70.43% of variation observed among localities within groups and 29.67% within localities (Table 3). The Φ_{ST} obtained using three hierarchical levels was equal to 0.29568. The pairwise Φ_{ST} estimate among localities showed sites with moderate genetic structure and others with extremely low Φ_{ST} values (Table 4).

When genetic diversity was estimated using Bayesian analysis, the full model presented the best adjustment based on DIC (Table 5). The estimates of genetic diversity (H_B) and genetic structure (θ_B) in this analysis were 0.2573 (highest posterior density (HPD) 97.5%: lower 0.2397, upper 0.2759) and 0.3289 (HPD 97.5%: lower 0.2746, upper 0.3809), respectively.

Mantel's test showed a positive correlation between genetic and geographic distances ($r = 0.4542$; $P < 0.01$), indicating isolation by distance among localities.

4. Discussion

The significant percentage of polymorphism and the mean number of bands obtained were 72.47% and 10.9, respectively. These values differ from previous reports in other species of Hymenoptera. Nascimento et al. [20] using 11 ISSR primers observed 13.36 bands per primer, on average,

Table 3: Analysis of molecular variance (AMOVA) with two hierarchical levels and with three hierarchical levels, testing the São Francisco River as a geographic barrier in *M. mandacaia*.

Source of variation	d.f	Sum of squares	Variance components	Variation (%)	*P* value
Two hierarchical levels					
among populations	11	340.738	2.85713 Va	29.61	<0.001
within populations	92	624.724	6.79048 Vb	70.39	
Total	103	965.462	9.6476		
Fixation index		Φ_{ST} = 0.2961			
Three hierarchical levels					
Among groups	1	39.166	−0.00989 Va	0	<0.001
Among populations within groups	10	301.571	2.86056 Va	29.67	
within populations	92	624.724	6.79048 Vb	70.43	
Total	103	965.462	9.64115		
Fixation index		Φ_{ST} = 0.29640			

Populations refer to sampled localities.

Table 4: Matrix of Φ_{ST} values for each pairwise combination among specimens from 12 localities based on 109 ISSR loci.

	PA	MC	UA	JU	LA	IR	SG	CE	UI	MO	MU	SR
PA	0											
MC	**0.55677**	0										
UA	0.32338	0.34428	0									
JU	**0.46979**	0.15931	0.24881	0								
LA	**0.49314**	**0.4858**	0.28199	0.29895	0							
IR	0.38122	0.32182	0.23765	0.19623	0.09091	0						
SG	0.33165	0.32086	0.16698	0.15168	0.09663	0.10783	0					
CE	**0.55384**	**0.46495**	0.29448	0.29988	0.18367	0.16749	0.2235	0				
UI	0.36049	0.29423	0.20965	0.2022	0.20736	0.18062	−0.0254	0.28913	0			
MO	**0.54598**	**0.45746**	0.35337	0.35786	**0.39932**	0.28588	0.30723	0.35402	0.3406	0		
MU	0.35722	**0.49369**	0.32324	**0.41837**	**0.41837**	0.33353	0.28858	**0.4856**	0.32608	**0.40166**	0	
SR	0.36774	0.32721	0.21991	0.2491	0.24141	0.22776	0.20532	0.33568	0.22515	0.34242	0.33757	0

The pairs with highest structuring levels are shown in bold.

with 59.18% of polymorphism in *M. quadrifasciata* from Minas Gerais state. Paplauskiené et al. [21] observed a mean number of six bands per primer using 11 ISSR primers for a subspecies of *Apis mellifera*. Borba et al. [22], studying lineages of *Trichogramma*, a group of small parasitic wasps, using 11 ISSR primers reported a mean number of 16 bands per primer and a high polymorphism (96%).

The low value of genetic diversity (H_e = 0.2616 and H_B = 0.2573) herein observed has been reported in other studies based on molecular markers in stingless bees. Tavares et al. [23], using RAPD markers, observed an H_e = 0.21 for *M. mondury* and H_e = 0.23 for *M. rufiventris*. Borges et al. [24], based on studies of microsatellite markers in *Partamona helleri* that reported a genetic diversity (H_e) of 0.18. Nascimento et al. [20], analyzing the genetic variability of *M. quadrifasciata* with ISSR markers, reported a genetic diversity of H_e = 0.20. Such reduced values of genetic diversity might be related to the swarming behavior for formation of new nests. In this process, the new nest can only be funded within short distances since it will depend on both food and workers from the mother colony up to its full establishment, which favors endogamy [25].

The two-hierarchical level AMOVA revealed a higher percentage of variation within localities (70.39%). The high concentration of genetic variability within localities is usually associated with lack or restrictions to gene flow among individuals from different localities, which might potentially lead to increased inbreeding within localities. When we partitioned the AMOVA into three hierarchical levels, placing São Francisco River as a geographic barrier among groups, no variation was observed, while the variation within localities remained high (70.43%).

The variation percentage detected by AMOVA and both Φ_{ST} (0.2961) and θ_B (0.3289) values showed that *M. mandacaia* is moderately structured through sampled localities when compared to other studies in stingless bees, where higher Φ_{ST} indexes suggest high genetic structuring ([26], Φ_{ST} = 0.90; [27], Φ_{ST} = 0.76 and 0.77; [20], Φ_{ST} = 0.59), even though these studies were based on distinct molecular markers.

Table 5: Model comparison of posterior means for Bayesian H_B and θ_B by Hickory. Comma values for H_B and θ_B represent lower and upper limits for 97.5% of high posterior density. Best fit model chosen based on DIC is indicated in bold.

Model	H_B	θ_B	DIC
Full	**0.2573 (0.2397–0.2759)**	**0.3289 (0.2746–0.3809)**	**2460.29**
$f = 0$	0.2730 (0.2634–0.2826)	0.2859 (0.2546–0.3183)	2512.8
theta = 0	0.3585 (0.3506–0.3664)	0.2837 (0.2277–0.3486)	4573.75
f free	0.2519 (0.2417–0.2623)	0.3490 (0.3138–0.3842)	2567.54

Moreover, the pairwise Φ_{ST} matrix among localities also confirms the genetic differentiation of *M. mandacaia* in Bahia state, revealing localities with high values of genetic structure, close to those reported by Nascimento et al. [20], while others presented low levels of structure (Table 5).

Mantel's test ($r = 0.4542$; $P < 0.01$) indicated a positive correlation among geographic and genetic distances, revealing an isolation by distance among sampled localities. Nascimento et al. [20] reported $r = 0.3998$ ($P < 0.05$) and also inferred that isolation by distance was present in *M. quadrifasciata*. This evidence corroborates the results of genetic structure obtained by AMOVA, Φ_{ST}, and Bayesian analysis.

Mantel's test values and the pairwise Φ_{ST} matrix among localities showed that the longer the geographic distance between them, the higher the genetic differentiation is. According to the Φ_{ST} matrix (Table 4), three groups can be distinguished: group 1 (1 (Paulo Afonso), 2 (Macururé), 3 (Uauá) and 4 (Juazeiro)); group 2 (5 (Lapão), 6 (Irecê), 7 (São Gabriel), 8 (Central) and 9 (Uibaí)); group 3 (10 (Morpará), 11 (Muquém do S. Francisco) and 12 (Serra do Ramalho)). The second group presented the lowest genetic differentiation among localities due to the increased geographical distance, when compared to groups 1 and 3, it presents intermediary Φ_{ST} values, as expected according to geographical distances. The groups 1 and 3 presented high levels of genetic differentiation when compared to group 2. However, the differentiation within groups 1 and 3 is as high as the differentiation between groups. This result might be related either to a preexisting differentiation among analyzed samples or to the geographic distance among the localities in this group.

The moderate genetic structure observed in this work could be explained due to the short period of separation among analyzed localities or else to a restricted gene flow. The restriction or lack of gene flow might be a consequence historical habitat fragmentation of biome Caatinga. Phylogeographic analyses using DNA sequences can help elucidate this scenario.

The environmental degradation has threatened bee populations worldwide [28], once woody and large trees, one of the main resources for nesting of bees, have decreased [29]. In spite of its continuous deforestation, there are only 11 protection areas (including national parks, ecological stations, and biological reserves) in Caatinga that, together, represent less than 1% of the original biome [30]. Taking into account the relevance of *M. mandacaia* as pollinators to the maintenance of Caatinga and the increased environmental impacts on this biome, our data suggest that further genetic studies, such as phylogeographic ones, are necessary to depict how the shape of genetic variation in this species across its geographic distribution is organized.

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Research Article

Classification, Natural History, and Evolution of Tarsosteninae (Coleoptera: Cleridae)—Part I: Generic Composition of the Subfamily and Key and Phylogeny of Genera

Weston Opitz

Department of Biology, Kansas Wesleyan University, 100 East Claflin Avenue, Salina, KS 67401-6196, USA

Correspondence should be addressed to Weston Opitz, opitz@kwu.edu

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Four new genera and one new species of the subfamily Tarsosteninae (Coleoptera: Cleridae) are described. The new genera are: *Agapetilus* Opitz, gen. nov., *Fallopylus* Opitz, gen., nov, *Globoclava* Opitz, gen. nov., and *Pseudopylus* Opitz, gen. nov. The new species involves *Agapetilus vietus* Opitz. sp. nov. *Liostylus* Fairmaire is synonymized with *Rhophaloclerus* Fairmaire. New combinations, *Fallopylus pallipes* (MacLeay, 1872), comb. nov., *Globoclava quadrimaculata* (Chevrolat, 1876), comb. nov., *Parapylus sedlaceki* (Kolibáč, 2003), comb. nov., *Pseudopylus okei* (Elston, 1929), comb. nov., and *Rhophaloclerus pictus* (Fairmaire, 1902), comb. nov., are established. A key and phylogeny of the genera of Tarsosteninae is provided.

1. Introduction

According to Opitz [1] there are six subfamilies in the Cleridae whose specimens have the fourth tarsomere reduced. The elucidation of the generic composition of these subfamilies, and their intrasubfamilial relationships, is the focus of the research program of the author. This contribution involves Tarsosteninae. It is the fourth of a series of works that makes known the generic composition of the six subfamilies referenced above. The first three contributions involve Epiphloeinae Kuwert [2], Neorthopleurinae Barr [3], and Korynetinae Laporte [4]. The revisions of the remaining two subfamilies, the Peloniinae Opitz and Enopliinae Gistel, are in various stages of preparation.

2. Taxonomic History

The majority of the generic taxa herein classified in Tarsosteninae were originally grouped under other subfamilies: *Rhophaloclerus* Fairmaire in Tillinae [5]; *Abeliella* Peracchi, *Curacavi* Solervicens, *Apteropilo* Lea, and *Neopylus* Solervicens in Enopliinae [5]; *Apopylus* Kolibáč, *Blackburniella* Chapin, *Parapylus* Blackburn, *Pylus* Newman, *Tarsostenodes* Blackburn, *Thriocera* Gorham; *Riotenerus* Pic in Peloniinae [1]; *Tarsostenosis* Heller and *Thriocerodes* Wolcott & Dybas in Korynetinae [6]. It is likely that the monotypic *Pallenothriocera* Pic, presently classified in Korynetinae, also belongs in Tarsosteninae. However, the specimen representing this nominal genus has not been found.

3. Material and Methods

For the most part the entire inventory of species of each genus was examined and several nonconspecific specimens of genera were disarticulated to examine the more cryptic structures of the integument. Methods and concepts involving dissection, measurements, terminology, specific and generic

delimitations, and preparation of illustrations were similar to those implemented in [1].

4. Systematics

4.1. Phylogenetics of Genera. The concepts of Hennig's phylogenetics were implemented in this treatise [7]. This involved the preparation of a suite of character states and a character matrix (Table 1), which was analyzed via NONA [8] in combination with Winclada version 1.00.08 [9]. The analysis generated 58 trees, with 46 steps, index of consistency of 58, and an index of retention of 74. The 58 trees were examined and the one selected (Figure 24) most closely approximates a tree prepared manually. Heuristic analysis (maximum trees (hold) = 100, number of replications 9 (mult) = 100, and multiple TBR (mult max)) was used.

4.2. Character States. Twenty-seven character states were used to analyze the phylogenetic relationships among the genera of Tarsosteninae. Outgroups included taxa of Korynetinae [4]. Character states valued "0" are considered plesiotypic, whereas those assigned a value of "1" are interpreted as apotypic (Table 1). The methods by which the phylogenetic state of a characteristic is determined are well documented [10, 11].

Character 0

Unguis denticle: (0) absent; (1) present

Character 1

Pronotal tubercle: (0) absent; (1) present

Character 2

Pronotal tubercle: (0) slightly developed; (1) highly developed

Character 3

Asetiferous punctations: (0) present; (0) absent

Character 4

Ninth row of elytral asetiferous punctations: (0) not reduced; (1) reduced

Character 5

Elytral 2°: (0) present; (1) absent

Character 6

Terminal maxillary palpomere: (0) digitiform; (1) somewhat securiform

Character 7

Terminal maxillary palpomere: (0) subsecuriform; (1) securiform

Character 8

Tibial spur formula: (0) 2-2-2; (1) 0-1-1

Character 9

Tibial spur formula: (0) 2-2-2; (1) 1-2-1

Character 10

Tibial spur formula: (0) 2-2-2; (1) 0-0-0

Character 11

Tarsal pulvillar formula: (0) 3-3-3; (1) third pulvillus reduced

Character 12

Ommatidia: (0) large; (1) small

Character 13

Capitulum: (0) compact; (1) lax

Character 14

Pronotal sides: (0) smooth; (1) crenulated

Character 15

Eye: (0) large; (1) small

Character 16

Ocular plate: (0) small; (1) large

Character 17

Elytral asetiferous punctations nodes: (0) absent; (1) present

Character 18

Pronotal indentations: (0) absent; (1) present

Character 19

Pronotal collar: (0) not extended; (1) extended

Character 20

Elytral asetiferous punctations: (0) to elytral apex; (1) to elytral half

Character 21

Pronotal disc: (0) without glabrous elevations; (1) with glabrous elevations

Character 22

Pronotal disc: (0) without glabrous spots; (1) with glabrous spots

Character 23

Pronotal disc: (0) without narrow glabrous streaks; (1) with narrow glabrous streaks

Character 24

Gular process: (0) not confluent; (1) confluent

Character 25

Pronotal commissure: (0) absent; (1) present

Character 26

Capitulum: (0) not much shorter than rest of antennal length; (1) much shorter than rest of antenna.

4.3. Key to Genera of Tarsosteninae.

1 Unguis with denticle (Figure 15(j))	2
1′. Unguis without denticle (Figure 15(i))	4
2(1). Last antennomere globose, about three times larger than penultimate antennomere (South Africa)	***Globoclava* gen. nov**
2′. Last antennomere only slightly larger than penultimate antennomere	3
3(2′). Body form oblong narrow (Figure 22(b)), pronotum oblong (Tanzania)	***Agapetilus* gen.nov**
3′. Body form oblong broad (Figure 23(h)), pronotum transverse (Democratic Republic of the Congo, Kenya, Mozambique, South Africa, Tanzania)	***Thriocera* Gorham**
4(1′). Pronotal sides without vestige of tubercle (Figure 14(b))	5
4′. Pronotal sides with shallow (Figure 1(d)) or with well defined projecting tubercle (Figure 4(c))	7
5(4). Pronotum subquadrate (Madagascar)	***Rhophaloclerus* Fairmaire**
5′. Pronotum distinctly oblong	6
6(5′). Elytral disc with 8 rows of punctations; pronotal disc uniformly scabrous (Bolivia, Brazil, Uruguay)	***Abiliella* Peracchi**
6. Elytral disc with 10 rows of punctations; pronotal disc with glabrous streaks (Cosmopolitan)	***Tarsostenus* Spinola**
7(4′). Pronotal sides with shallow tubercle (Figure 1(d))	8
7′. Pronotal sides with well defined projecting tubercle (Figure 4(c))	14
8(7). Last maxillary palpomere distinctly securiform (Figure 12(a))	9
8′. Last maxillary palpomere subsecuriform (Figure 6(c))	13
9(8). Pronotal side margins serrulated (Chile)	***Curacavi* Solervicens**
9′. Pronotal side margins not serrulated	10
10(9′). Metabasitarsal pulvillus well developed	11
10′. Metabasitarsal pulvillus not well developed or absent	12
11(10). Pronotum distinctly oblong (Australia)	***Tarsostenodes* Blackburn**
11′. Pronotum quadrate (Australia)	***Blackburniella* Chapin**
12(10′). Elytral asetiferous punctations nodulated (Australia)	***Apopylus* Kolibáč**
12′. Elytral asetiferous punctations not nodulated (New Caledonia).	***Tarsostenosis* Heller**
13(8′). Pronotum distinctly oblong (Argentina)	***Riotenerus* Pic**
13′. Pronotum subquadrate (Australia)	***Thriocerodes* Wolcott & Dybas**
14(7′). Apical maxillary palpomere distinctly securiform	15
14′. Apical maxillary palpomere subsecuriform	17
15(14). Rows of elytral asetiferous punctations not defined (Chile)	***Neopylus* Solervicens**
15. Rows of elytral asetiferous punctations clearly defined	16
16(15). Pronotum with glabrous tumescences (Australia)	***Apteropilo* Lea**
16. Pronotum without glabrous tumescences (Australia)	***Pseudopylus* gen.nov.**
17(14′). Elytral base with tumescence (Australia)	***Parapylus* Blackburn**
17′. Elytral base without tumescence	18
18(17′). Tibial spur formula 2-2-1 (Australia)	***Pylus* Newman**
18′. Tibial spur formula 1-2-2 (Australia)	***Fallopylus* gen. nov.**

4.4. Description of Tarsosteninae

Type Genus. Tarsostenus Spinola [12].

Diagnosis. These beetles have a reduced 4th tarsomere, do not have a pair of pronotal trichobothria, and have long capitate antennae in which the funicular antennomeres are filiform and the length of the capitulum is not as long as the combined length of the remainder of the antennomeres. The incomplete dorsolateral pronotal carina is confluent with the pronotal hem at the posterior angles of the pronotum.

Description. Shape: ranges from narrow rectangulate to short rectangulate. *Size*: length 2.2–14.0 mm; width 0.6–6.0 mm. *Integumental color*: Varies from uniformly reddish-brown to multicolored conditions where the integument is mostly dark brown and the elytral disc shows a paler fascia, in very few cases the integument may be shiny blue or shiny multicolored with red, yellow and brown. *Head*: transverse, strongly deflexed, usually narrower than pronotum, surface usually finely punctated; epistomal suture faintly indicated; internal epistomal ridge poorly developed; clypeus bipartite, comprised of pigmented upper region and nonpigmented

Table 1: Character matrix for 27 morphological characters of Tarsosteninae genera.

Taxa	Characters																										
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Abeliella	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1
Agapetilus	1	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Apopylus	0	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1
Apteropilo	*0*	1	1	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	0	0	0	1
Blackburniella	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Curacavy	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1
Fallopylus	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Globoclava	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Neopylus	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Parapylus	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Pseudopylus	0	1	1	0	1	1	1	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Pylus	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1
Rhophaloclerus	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Riotenerus	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Tarsostenodes	0	1	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
Tarsostenosis	0	1	0	0	0	0	1	1	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1
Tarsostenus	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1
Thriocera	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Thriocerodes	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1

lower region; antenna comprised of 11 antennomeres, capitate, capitulum shorter than length of combined other antennomeres, noncapitular antennomeres filiform; frontal preantennal angle not acute; eyes coarsely to finely faceted, slightly notched anteriorly; labrum shallowly incised, transverse tormal processes fused contiguous; epipharynx not complex; last palpomere of maxillary and labial palpus boldly or slightly securiform; mandible with well-developed dens, basal notch not large; gula large, gular processes widely separated, gular sutures strongly converging. *Thorax*: pronotum usually transverse-quadrate, or elongate, lateral tubercle absent or strongly developed, anterior transverse depression present or not, dorsolateral carina incomplete or complete, always posteriorly confluent with pronotal hem, pronotal commissure absent; pronotal projections vary in lengths, prointercoxal process linear or expanded distally; pronototergosternal suture complete; procoxal cavity open, procryptosternum incomplete; metendosternite with furcal lamina; elytral form usually elongate rectangulate or short rectangulate, anterior margin with carina, disc with asetiferous punctations, 1° and 2° setae usually present, epipleural fold laterally positioned, gradually narrowing to elytral apical four-fifths, elytral punctations, plain, or bimodal or tetranodal; metathoracic wings present or not; legs, tarsal formula 5-5-5, cursorial, tibial spur formula 2-2-2, 2-2-1, 1-2-2, 1-2-1, 0-2-2, or 0-0-0, tarsal pulvillar formula 3-3-3 or 3-3-2; unguis with (Figure 15(j)) or without denticle (Figures 2(f) and 15(i)); wedge cell of metathoracic wing present or not, when present closed or open. *Abdomen*: comprised of 6 visible sternites, 6th visible sternite usually beneath 5th, robust and compact; pygidium quadrate or scutiform; aedeagus sometimes inverted, well sclerotized, tegmen tubular very sclerotized or lightly sclerotized, bilobed distally, tegminal lobes usually fimbriate, phallobasic rod variously developed, phallobasic apodeme well developed, phallic plates variously developed; spicular fork well developed, intraspicular plate linear, spicular apodeme variously fused; ovipositor not longer than abdomen, with multilobed dorsal and ventral lamina; oblique and ventral bacculi well developed. *Alimentary canal* (Figure 18(m)): stomodaeum short, proventricular valve comprised of 4 primary lobes (Figure 18(j)); ventriculus well developed, ventricular crypts poorly developed; 4 cryptonephridial Malpighian tubules; proctodaeum short in males and long in females. *Mesodermal male reproductive organs*: typically with two pairs of accessory glands, rarely with one pair of glands; testes comprised of multiple follicles. *Mesodermal female reproductive organs*: spermathecal capsule from faintly to highly sclerotized, spermathecal gland attached to apex or subapex of spermathecal capsule; saccular bursal copulatrix well developed bursal sclerite present or not; ovaries comprised of multiple follicles.

4.5. Descriptions of Genera of Tarsosteninae

4.5.1. Abiliella Peracchi (Figures 1, 2, and 22(a)). *Abiliella* Peracchi [13]. *Type species: Abiliella fasciata* Peracchi [13]. By monotypy.

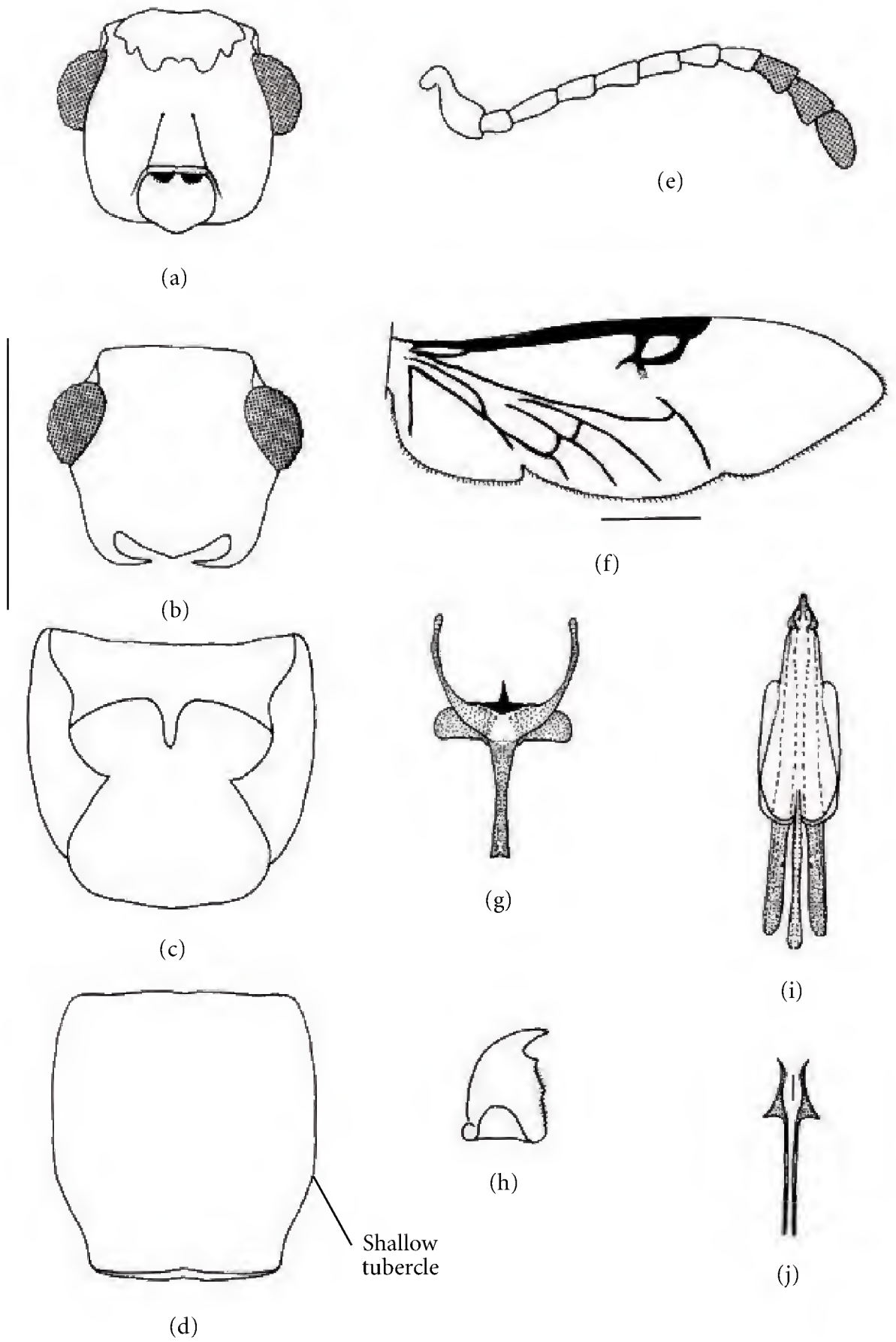


FIGURE 1: Various organs of *Abiliella fasciata*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metathoracic wing. (g) Metendosternite. (h) Mandible. (i) Aedeagus. (j) Spiculum.

Synapotypic Characteristics. Pronotum elongate, elytral punctations binodal, epipleural margin serrulated, unguis without denticle, phallic plates very broad, and phallobasic lobes not fimbriate.

Diagnosis. Specimens of *Abiliella* are distinguishable from the superficially similar specimens of *Tarsostenus* by having only eight rows of elytral punctations; the elytral disc of *Tarsostenus* specimens have 10.

Description. Size: length 4.0–9.0 mm; width 1.2–2.8 mm. *Form* (Figure 22(a)): oblong rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with white setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 1(a), 1(b), and 2(a)): cranium quadrate, frons wider than or narrower than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 1(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, medial incision shallow, transverse tormal processes curvate, not confluent, epipharyngeal plate very small; mandible (Figure 1(h)), body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 2(b)), laterolacinia present, terminal palpomere securiform; labium (Figure 2(b)), ligula not deeply incised, terminal palpomere

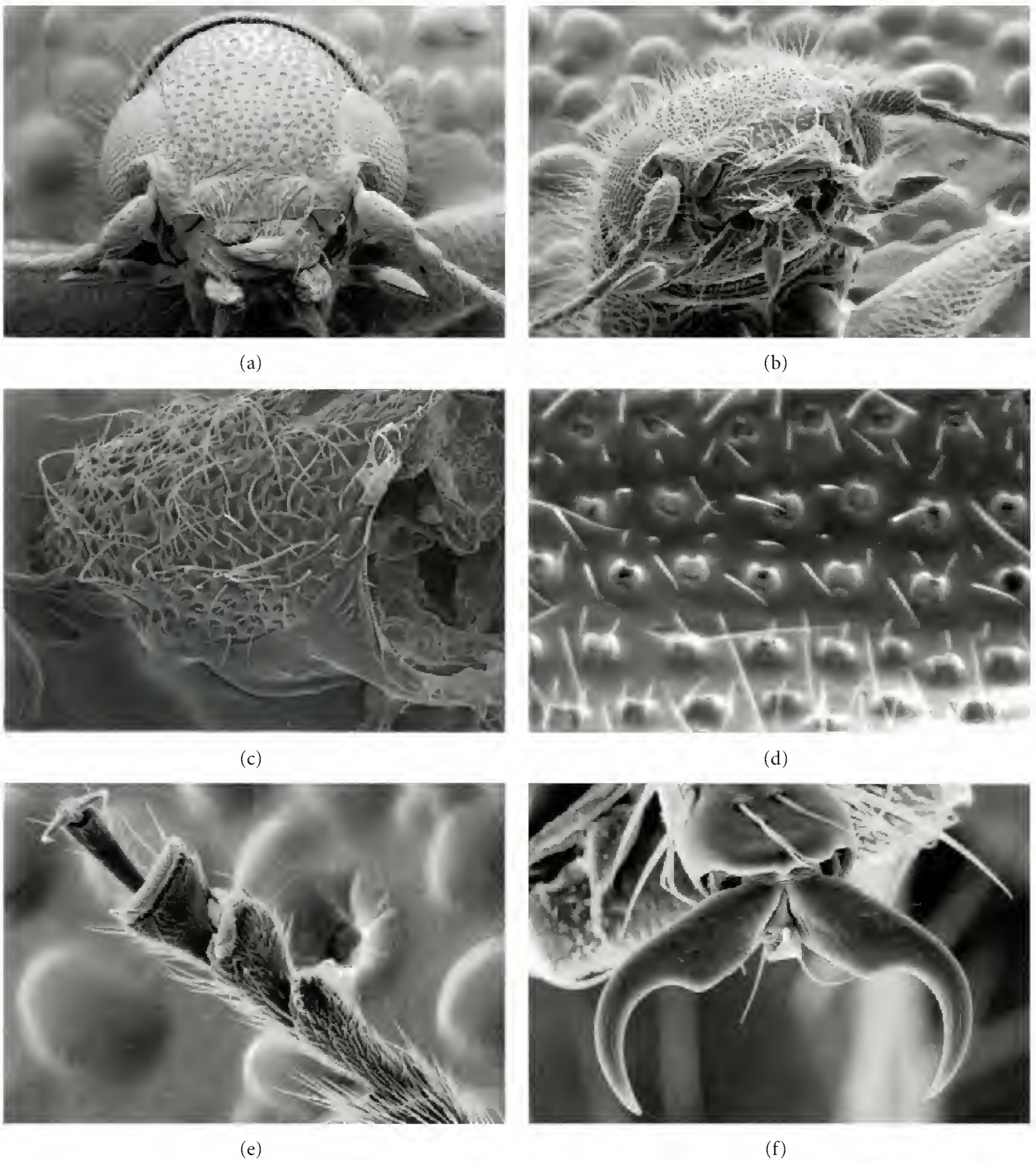


FIGURE 2: Various organs of *Abiliella fasciata*. (a) Head. (b) Mouthparts. (c) Pronotum (posterolateral angle). (d) Elytral surface (shows binodal asetiferous punctations). (e) Metatarsus. (f) Metatarsal unguis (shows absence of denticle).

securiform; eyes small or large, coarsely faceted, ocular notch large; antenna (Figure 1(e)), capitate, capitulum lax and narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres narrow, antennomeres 9 and 10 subrectangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 1(c), 1(d), and 2(c)), elongate, convex, side margins slightly sinuous, sculptured with large round setiferous punctations, dorsolateral ridge extends from posterior angle to anterior angle (Figure 2(b)), surface smooth and not fractured by coarse punctations, prebasal fissure well developed, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations, latter seriate and binodal (Figure 2(d)), 1° setae always adjacent to asetiferous punctations, 2° setae present, arranged serially, epipleural fold laterally positioned, extended to elytral apex, margin minutely serrulated, anterior margin carinate; metathoracic wing (Figure 1(f)), wedge cell open; metendosternite (Figure 1(g)), with furcal lamina, furcal anterior plate diminutive, acuminate; legs, tibial spur formula 0-1-1, tarsal pulvillar formula 3-3-3 (Figure 2(e)), unguis without denticle. *Abdomen*: aedeagus (Figure 1(i)), shorter than length of abdomen, phallobase lobate distally, lobes not fimbriate; phallic lateral plates very broad, spicular plates triangular, acuminate, rarely, spicular apodemes not fused (Figure 1(j)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised,

distal margin of male 6th sternite slightly incised. *Alimentary canal*: no information available. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. The members of this genus have been found only in Brazil and Bolivia.

Species Examined. Abiliella fasciata Peracchi and one new undescribed species.

4.5.2. Agapetilus gen. nov. (Figure 22(b)). Type species: Agapetilus vietus Opitz, sp. nov. Herein designated.

Synapotypic Characteristics. Pronotal wrinkles.

Diagnosis. The pronotal disc is profusely sculptured with wrinkles.

Description. Size: length 5.2 mm; width 1.2 mm. *Form* (Figure 22(b)): oblong, narrow rectangulate, about 5 times longer than broad. *Vestiture*: disc of cranium and pronotum densely vested with pale setae, elytral disc vested with 1° setae, 2° setae absent. *Head*: cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, tormal processes not examined, epipharyngeal plate not examined; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus not verified; maxilla well developed, laterolacinia not verified, terminal palpomere subsecuriform; labium, ligula deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch large; antenna, capitate, capitulum compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres slightly expanded, antennomeres 9 and 10 subtriangular, antennomere 11 ovoid. *Thorax*: pronotum oblong, disc sculptured with many wrinkles, prebasal fissure shallow, prointercoxal process not expanded distally, pronotal projections long; elytron sculptured with small setiferous punctations, basal tumescences present, asetiferous punctations absent, 1° setae present, 2° setae concentrated into medial fascia, interstitial spaces smooth, epipleural fold laterally positioned, very narrow to elytral apex, anterior margin not carinate; metathoracic wing not examined; metendosternite not examined; legs, tibial spur formula 0-2-2, tarsal pulvillar formula 3-3-3, unguis with denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, not lobate nor fimbriate; phallic lateral plates broad, phallobasic rod absent, phallic apex robust, spiculum not examined; ovipositor not examined. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. This monotypic genus is known only from Tanzania.

Species Examined. Agapetilus vietus Opitz, sp. nov.

Etymology. The generic name Agapetilus stems from the Latin petilus (=slender) and the intensive prefix *aga-* (=very). I refer to the slender body form of the type species.

4.5.3. Agapetilus vietus Opitz, sp. nov.

Type Material [Holotype ♂]. Tanzania, Tanga, Lushoto Dist., Mazumbai For. Res. 4°49′S 38°29′E, 1650–1730 m, 27. XI.1995, Fog 29 II, Zmuc Denmark (Institute Royal des Sciences Naturelles de Belgique).

Description. Form: oblong slender. *Size*: length 5.2 mm; width 1.2 mm. *Integumental color*: Antenna, legs, and posterior half of elytral disc, and abdomen yellow-brown, forebody, pterothorax, and anterior half of elytral disc brown, with white fascia across middle of elytral disc. *Male genitalia*: Aedeagus very short, tegmen without lobes and posterior limit not fimbriate; phallic plates broad and apex pronounced.

Distribution. Known only from Tanzania.

Etymology. The specific epithet *vietus* (=wrinkled) is a Latin adjective. I refer to the extensive wrinkling on the pronotal disc.

4.5.4. Apopylus Kolibáč (Figures 4(h) and 22(c)). Apopylus Kolibáč [6]. *Type species: Apopylus unumgarensis* Kolibáč [6]. By monotypy.

Synapotypic Characteristics. The restriction of the tetranodal punctations to the posterior four-fifths of the elytral disc is a uniquely derived characteristic of this genus.

Diagnosis. The restriction of the tetranodal punctations to the posterior four-fifths of the elytral disc will conveniently distinguish the members of this genus within Tarsosteninae.

Description. Apopylus Kolibáč and its type species were adequately described by Kolibáč [6]. The metendosternite of *Apopylus unumgarensis* specimens have well-developed laminae.

Species Examined. Apopylus unumgarensis Kolibáč and one undescribed species.

4.5.5. Apteropilo Lea (Figures 5(a)–5(h), 6(e), 6(f), and 22(d)). Apteropilo Lea [14]. Type species*: Apteropilo pictipes* Lea [14]. By monotypy. Corporaal [5]. Kolibáč [6] (*Pylusopsis* Elston), Bartlett [15].

Synapotypic Characteristics. Pronotal disc with two glabrous tumescences, pronotal sides with two extraordinarily large setiferous punctations, metendosternite without furcal anterior plate.

Diagnosis. The combination of glabrous tumescences on the pronotal disc present, elytral punctations binodal, and each side of the pronotum with two large setiferous punctations will conveniently distinguish the members of this genus within Tarsosteninae.

Description. Size: length 3.5–6.0 mm; width 1.2–2.0 mm. *Form*: Figure 22(d) oblong short rectangulate, rarely hind body suboval, about 2.5 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 5(a) and 5(b)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance, or cranial indentations small and widely separated; gula (Figure 5(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes not confluent, epipharyngeal plate very small; mandible (Figure 5(d)), body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere securiform; labium, ligula not deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch small; antenna (Figure 5(h)), capitate, capitulum lax or not, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, antennomeres 9 and 10 triangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 5(c) and 5(g)), transverse, disc with two glabrous tumescences that sometimes are confluent with other elevations, side margins with distinct tubercles, usually two round punctations particularly large near sides, rest of disc with large round setiferous punctations, dorsolateral ridge extends from posterior angle to anterior angle, surface coarse and fractured by coarse punctations, sclerotized region above pronotal projection glabrous, prebasal fissure well developed, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations, latter seriate and binodal, 1° setae always adjacent to asetiferous punctations, 2° setae densely distributed, not arranged serially, epipleural fold laterally positioned, extended to elytral apex, anterior margin carinate; metathoracic wing (Figure 5(f)), rarely absent, wedge cell open; metendosternite (Figure 5(e)), with furcal lamina, furcal anterior plate absent; legs, tibial spur formula 0-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle (Figure 6(f)). *Abdomen*: Aedeagus shorter than length of abdomen, phallobase reduced, lobate distally, lobes fimbriate; phallic lateral plates very broad, spicular plates narrowly triangular, acuminate, spicular apodemes not fused, intraspicular plate rod-shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary Canal*: Not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: Spermathecal capsule well sclerotized, tubular, elongate, spermathecal gland attached to apex of spermathecal capsule.

Distribution. The members of this genus have been found in southeastern and Western Australia and on Kings Island, off the Bass Strait.

Species Examined. Apteropilo chrysocome (Elston), *A. pictipes* Lea, *A. raldae* Bartlett, and *A. volans* Bartlett.

Notes. Bartlett [15] provided habitus illustrations of each species and drawings of male genitalia.

4.5.6. Blackburniella Chapin (Figures 7(a)–7(h), 12(a), 12(b), and 22(e)). Blackburniella Chapin [16]. *Type species: Thanasimomorpha intricta* Blackburn [17]. By original designation. Corporaal [5]. Matthews [18]. Kolibáč [6].

Erolestus Wolcott [19]. Synonymized by Wolcott, 1947.

Synapotypic Characteristics. Spermathecal duct very long, tuft of setae on elytral umbo, phallic apex extended.

Diagnosis. The tuft of black setae on the elytral umbo will distinguish the members of this genus from others in the subfamily.

Description. Size: length 4.2–8.0 mm; width 1.2–2.0 mm. *Form*: Figure 22(e), oblong long rectangulate, about 4 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae, elytral umbo covered with tuft of black setae, elytral setae very densely distributed in elytral apical half. *Head* (Figures 7(a), 7(b), and 12(a)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 7(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 12(b)), laterolacinia present, terminal palpomere securiform; labium, ligula not deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch large; antenna (Figure 7(g)), capitate, capitulum lax, somewhat narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres somewhat narrow, antennomeres 9 and 10 triangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 7(c) and 7(d)), from subquadrate to longer than broad, disc indented with large setiferous punctations, side margins sinuous, rounded near middle, dorsolateral ridge spans posterior third of pronotal sides, ridge surface coarse, sclerotized

region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations in basal half, punctations minute in distal half, punctations seriate in basal half, not binodal, 1° setae always adjacent to asetiferous punctations, 2° setae densely distributed, not arranged serially, epipleural fold laterally positioned, extended to elytral apical three-fourths, anterior margin carinate; metathoracic wing (Figure 7(f)), wedge cell open; metendosternite (Figure 7(e)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, lobate distally, lobes fimbriate; phallic lateral plates narrow, phallic apex extended, spicular plates very narrow, spicular apodemes fused at extremity, intraspicular plate rod-shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: not studied. Male *mesodermal internal reproductive organs*: Not studied. *Female mesodermal internal reproductive organs* (Figure 7(h)): spermathecal capsule capitates, well sclerotized, spermathecal gland attached to base of spermathecal capsule, spermathecal duct very long, saccular bursa copulatrix present.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Blackburniella intricata (Blackburn) and one new species.

Notes. Kolibáč [6] provided drawings of the male genitalia and female mesodermal reproductive organs.

4.5.7. Curacavi Solervicens (Figures 3(a)–3(h), 6(a) and 6(b)). *Curacavi* Solervicens [20].*Type species: Curacavi dentatus* Solervicens [20]. By monotypy.

Synapotypic Characteristics. Pronotal punctations elongate, epipleuron in ventral position.

Diagnosis. The serrulated lateral margins of the pronotum will distinguish the members of this genus within Tarsostenninae.

Description. Size: length 4.0 mm; width 1.8 mm. *Form* (Figure 3(a)): oblong rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with dark setae, elytral disc vested with 1° setae and shorter sparsely distributed 2° setae. *Head* (Figure 6(a)): cranium quadrate, frons much wider than width of eye, indented with small setiferous punctations and lager asetiferous punctations; gula large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, medial incision shallow, mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere subsecuriform; labium, ligula not deeply incised, terminal palpomere securiform; eyes very small, coarsely faceted, ocular notch large; antenna (Figure 3(d)), capitate, capitulum lax and narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres narrow, antennomeres 9 and 10 near capitate, antennomere 11 ovoid. *Thorax*: pronotum (Figures 3(b), 3(c), and 6(b)), transverse, convex, lateral margins serrulated, sculptured with large round setiferous punctations at anterior of disc, punctations elongate in remainder of disc, prebasal fissure well developed, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations, latter seriate and binodal, 1° setae always adjacent to asetiferous punctations, 2° setae present, arranged serially, epipleural fold ventrally positioned, extended to elytral apex, margin minutely serrulated, anterior margin carinate; metathoracic wing with open wedge cell; metendosternite (Figure 3(e)), with furcal lamina, furcal anterior plate large, transverse; legs, tibial spur formula 2-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus as long as length of abdomen, phallobase lobate distally (Figure 3(g)), lobes acuminate, not fimbriate; phallic lateral plate not very broad (Figure 3(h)), acuminate, with preapical uncus, spicular plates slender, with lateral uncus, spicular apodemes not fused (Figure 3(f)), intraspicular plate rod-shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: stomodaeal valve comprised of four primary lobes, dorsal lobe very broad and half as long as other three lobes. *Male mesodermal internal reproductive organs*: Not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule well sclerotized, barrel shaped, spermathecal gland attached to apex of spermathecal capsule.

Distribution. The members of this genus have been found only in Chile.

Species Examined. This is a monotypic genus.

Notes. For illustrations see Solervicens [21].

4.5.8. Fallopylus gen. nov. (Figures 8(a)–8(j), 12(c), 12(d), 21(f), and 22(f)). Type species: Pylus pallipes MacLeay [22]. Kolibáč [6].

Synapotypic Characteristics. Bursal sclerites cyclic.

Diagnosis. Blackburniella Chapin, *Tarsostenodes* Blackburn, *Tarsostenus* Spinola, and *Fallopylus*, gen. nov., are all characterized by a 1-2-1 tarsal spur formula. However, in specimens of *Fallopylus* there is a distinct tubercle on the lateral margins of the pronotum, which is not the case in specimens of the other three aforementioned genera.

Description. Size: length 4.0–7.5 mm; width 1.4–3.2 mm. *Form* (Figure 22(f)): oblong short rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter 2° setae. *Head* (Figures 8(a) and 8(b)): cranium quadrate, frons slightly wider than width of eye, indented with very small setiferous punctations; gula (Figure 8(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible (Figure 8(g)), body long, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere securiform or subsecuriform; labium, ligula deeply incised, terminal palpomere securiform; eyes large, coarsely faceted, ocular notch small; antenna (Figure 8(e) and 21(f)), capitate, capitulum lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres expanded, antennomeres 9 and 10 capitate, antennomere 11 ovoid, rarely acuminate. *Thorax*: pronotum (Figures 8(c) and 8(d)), transverse, disc indented with small setiferous punctations, lateral margins with tubercle sinuous, dorsolateral ridge extends from posterior angle to anterior angle, not fractured by coarse punctations, prebasal fissure prominent, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with small or large bimodal asetiferous punctations, punctations seriate and extend to elytral apex, 1° setae always adjacent to asetiferous punctations, 2° setae sparsely distributed, arranged serially, epipleural fold laterally positioned, narrowed to elytral apex three, anterior margin carinate; metathoracic wing (Figure 8(h)), wedge cell closed; metendosternite (Figure 8(f)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figure 8(j)), shorter than length of abdomen, phallobase reduced, lobate and narrowed distally, lobes not fimbriate; phallobasic rod transverse, phallic lateral plates very spinous, phallic apex minute, spicular plates very narrow (Figure 8(i)), spicular apodemes not fused at extremity, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule ovoid, well sclerotized, spermathecal gland attached to base of spermathecal capsule, spermathecal duct long, saccular bursa copulatrix present, with cyclic basal sclerites.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Fallopylus pallipes (MacLeay) (new combination) and three new undescribed species.

Etymology. The generic name *Fallopylus* stems from the Latin *fallo* (=deceive) and the name of the genus *Pylus* Newman.

Notes. Kolibáč [6] provides drawings of the female mesodermal reproductive organs.

4.5.9. Globoclava gen. nov. (Figure 22(g)). Type species: Pilus (=Pylus) quadrimaculata Chevrolat [23]. Shifting *Pilus quadrimaculata* Chevrolat from synonymy to a valid species of *Globoclava* represents a *new status* and *new combination.*

Synapotypic Characteristics. Last antennomere very large.

Diagnosis. The extensively globose development of the last antennomere will distinguish the members of this genus from other genera.

Description. Size: length 4.0–5.5 mm; width 1.2–1.6 mm. *Form* (Figure 22(g)): oblong short rectangular, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested profusely with erect setae, elytral disc vested with tall erect setae and shorter decumbent setae. *Head*: cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia not studied, terminal palpomere subsecuriform; labium, ligula not incised, terminal palpomere subsecuriform; eyes large, finely faceted, ocular notch large; antenna, capitate, capitulum compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres expanded, antennomeres 9 and 10 expanded, antennomere 11 very large and ovoid. *Thorax*: pronotum quadrate, disc indented profusely with large setiferous punctations, lateral margins convex, lateral tubercle poorly developed, dorsolateral ridge extends from posterior angle to anterior angle, not fractured and with smooth surface, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process expanded distally; pronotal projections short; elytron sculptured with asetiferous punctations, punctations subseriate, epipleural fold laterally positioned, narrowed at elytral posterior two-thirds, anterior margin carinate; metathoracic wing not studied; metendosternite not studied; legs, tibial spur formula 0-1-1, tarsal pulvillar formula 3-3-3, pulvillus of metabasitarsomere very reduced, unguis with denticle. *Abdomen*: not examined. *Alimentary canal*: not studied. Male mesodermal internal reproductive *organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. Known only from South Africa.

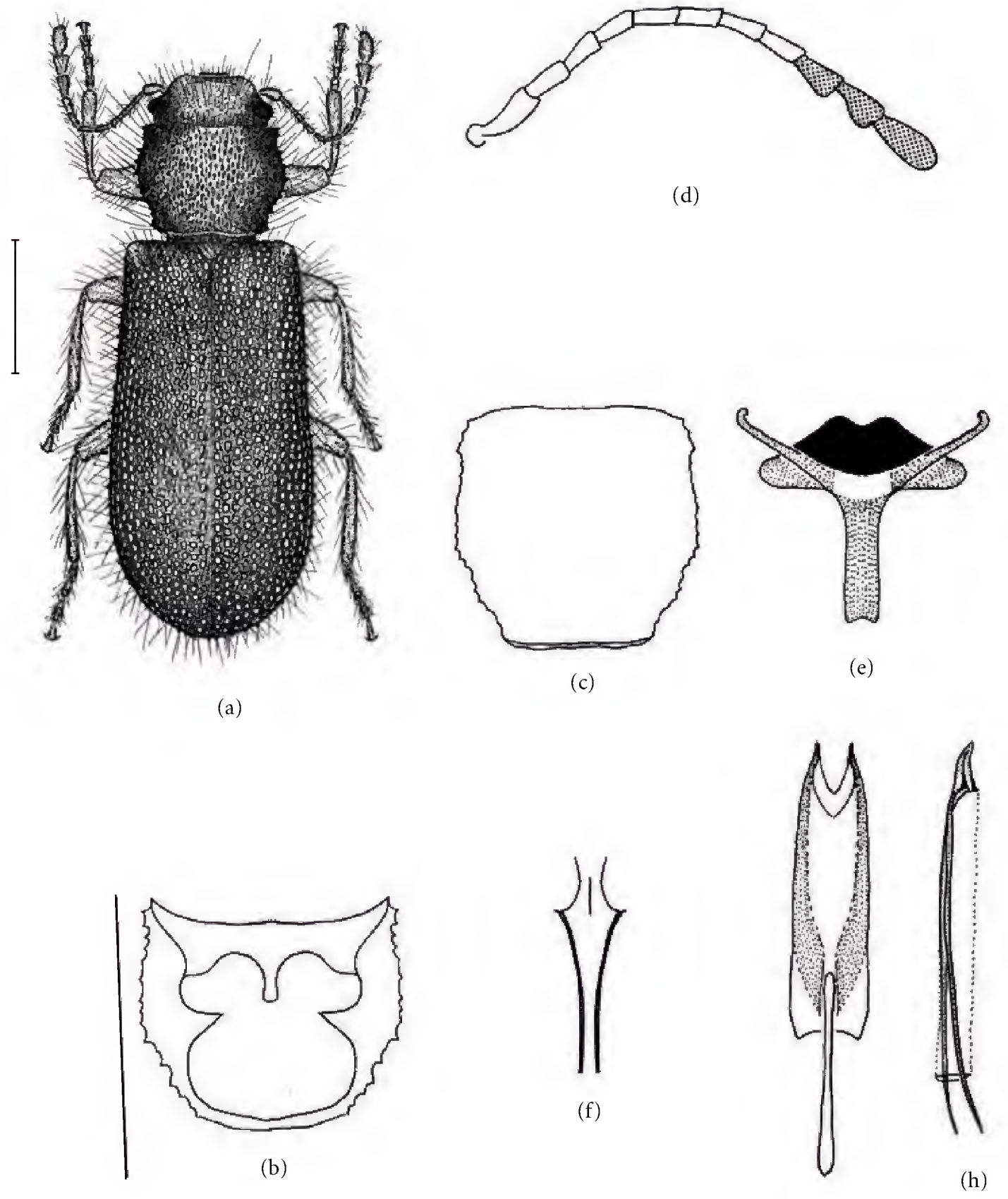


FIGURE 3: Various organs of *Curacavi dentatus*. (a) Habitus. (b, c) Thorax ((b) ventral, (c) dorsal). (d) Antenna. (e) Metendosternite. (f) Spiculum. (g) Tegmen. (h) Phallus.

Species Examined. Globoclava anthicides (Newman) (new combination).

Etymology. The generic name *Globoclava* stems from the Latin *globus* (=sphere) and the Latin *clavus* (= nail). I refer to the shape of the antenna in this beetle.

4.5.10. Neopylus Solervicens (Figures 9, 10, 12(e), and 22(h)). Neopylus Solervicens [24]. *Type species: Neopylus nahuelbutensis* Solervicens [24].

Synapotypic Characteristics. Pronotal and elytral disc vested with small white setal wisps.

Diagnosis. The small wisps of white setae on the dorsum of these beetles will distinguish them from superficially similar specimens of the Australian genus *Pylus* Newman.

Description. Size: length 9.0–10.0 mm; width 3.5–4.0 mm. *Form* (Figure 9): oblong long rectangulate, robust about 3 times longer than broad. *Vestiture*: disc of cranium vested profusely with white recumbent setae, pronotum and elytral disc vested with small wisps of white setae pale setae, elytral disc vested with 1° setae and shorter 2° setae. *Head* (Figures 10(a), 10(b), and 12(e)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 10(a)), large, trapezoidal, sutures oblique,

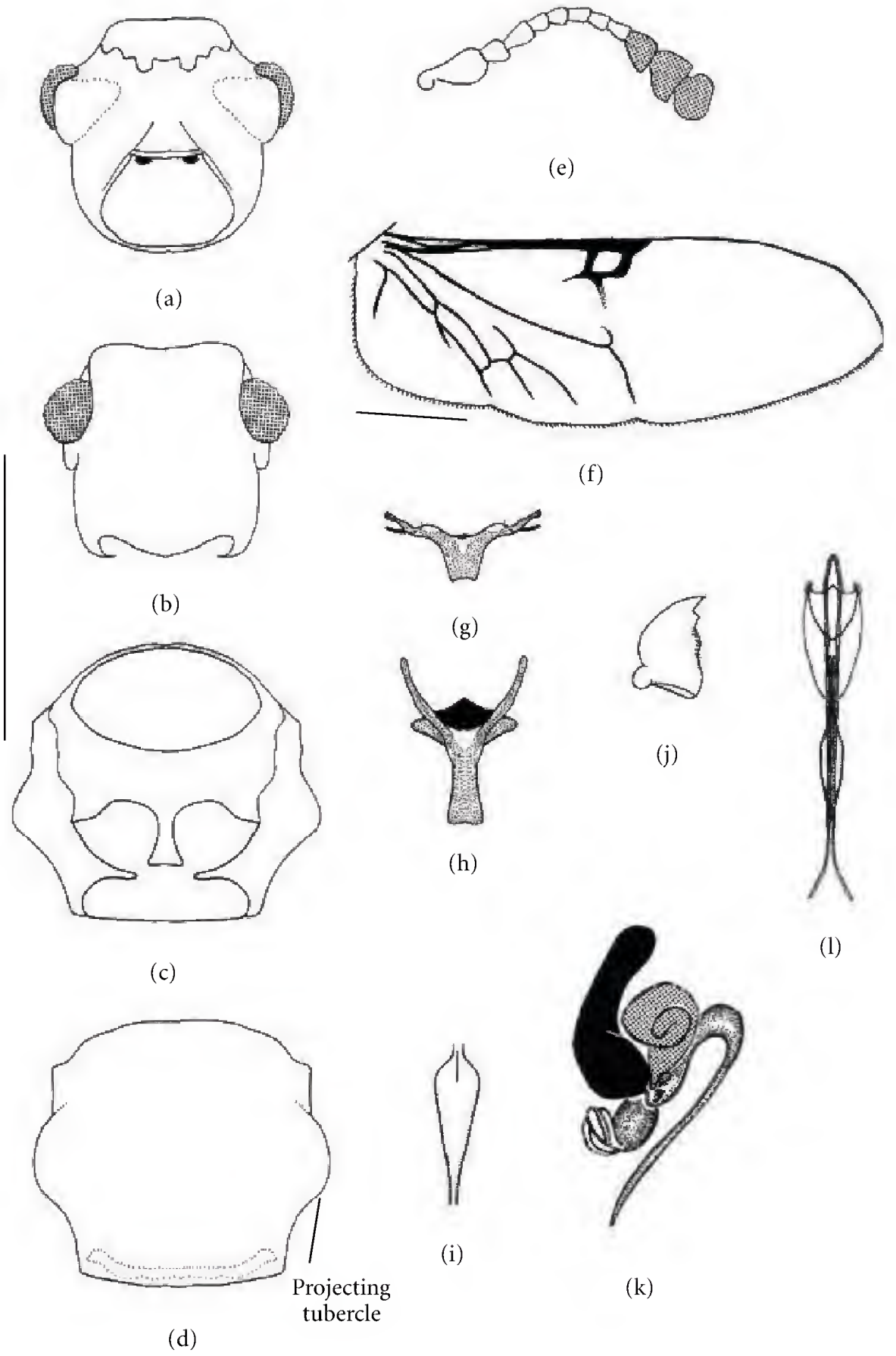


FIGURE 4: Various organs. (a)–(g) and (h)–(l) of *Pseudopylus okei*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metathoracic wing. (g) Metendosternite. (h) Metendosternite of *Apopylus unumgarensis*. (i) Spiculum. (j) Mandible. (k) Male mesodermal internal reproductive organs. (l) Aedeagus.

gular processes widely separated, processes in form of two setiferous tubercles; labrum (Figure 10(j)), short, not deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible (Figure 10(k)), body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 10(i)), laterolacinia present, terminal palpomere securiform; labium (Figure 10(g)), ligula not deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch large; antenna (Figure 10(f)), capitate, capitulum lax, somewhat narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres somewhat narrow, antennomeres 9 and 10 triangular, antennomere 11 somewhat truncate. *Thorax*: pronotum (Figures 10(c) and 10(d)), transverse, disc indented with large setiferous punctations, central lineal fissure, and with small elevations, side margins with distinct tubercle, dorsolateral ridge extends from posterior angle to anterior angle, not fractured but made coarse by coarse punctations, sclerotized region above pronotal projection with setiferous punctations at base, glabrous in remainder, prebasal fissure shallow, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with large spheroid asetiferous punctations, punctations smaller near elytral apex, punctations not seriate, 1° setae always adjacent to asetiferous punctations, 2° setae not

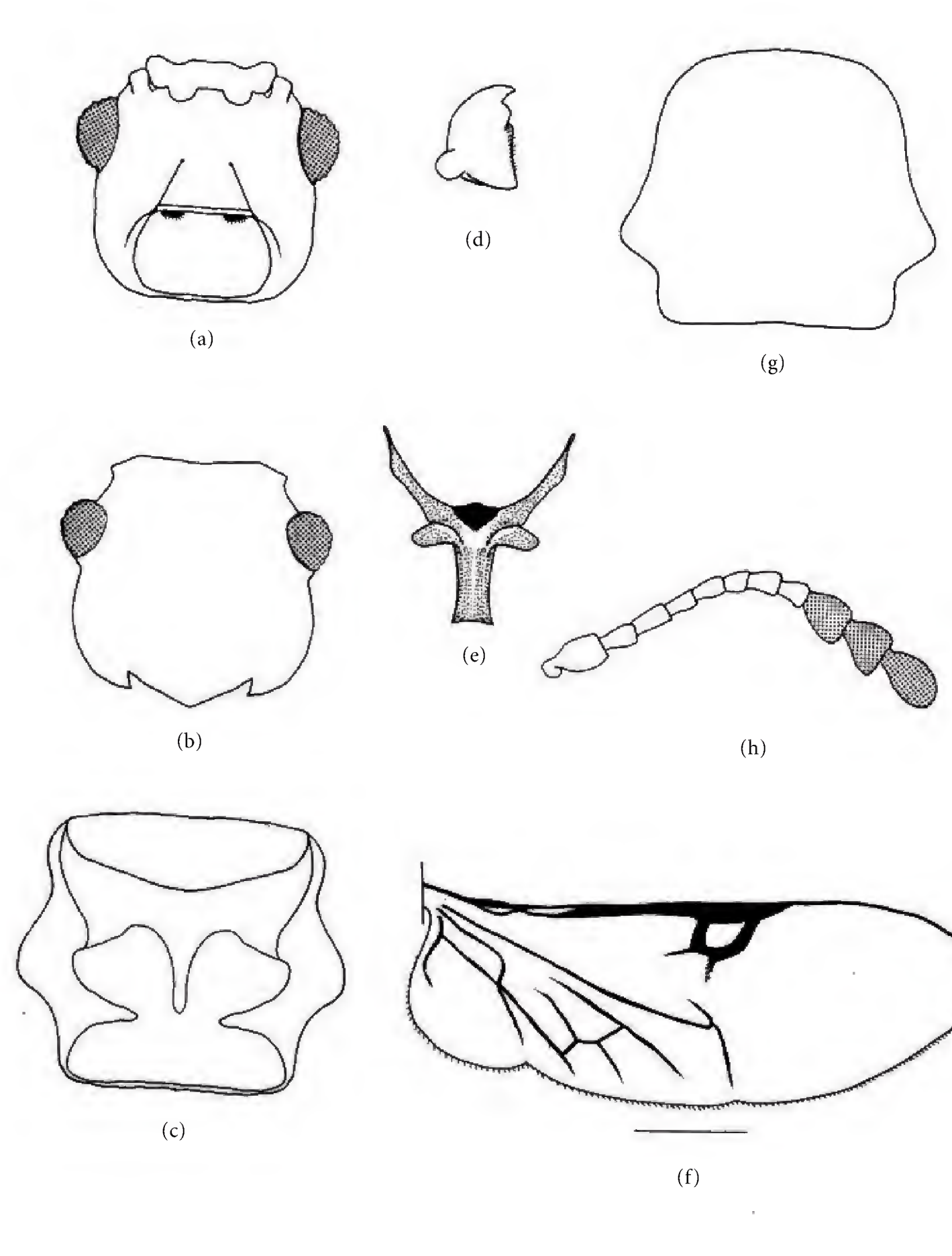


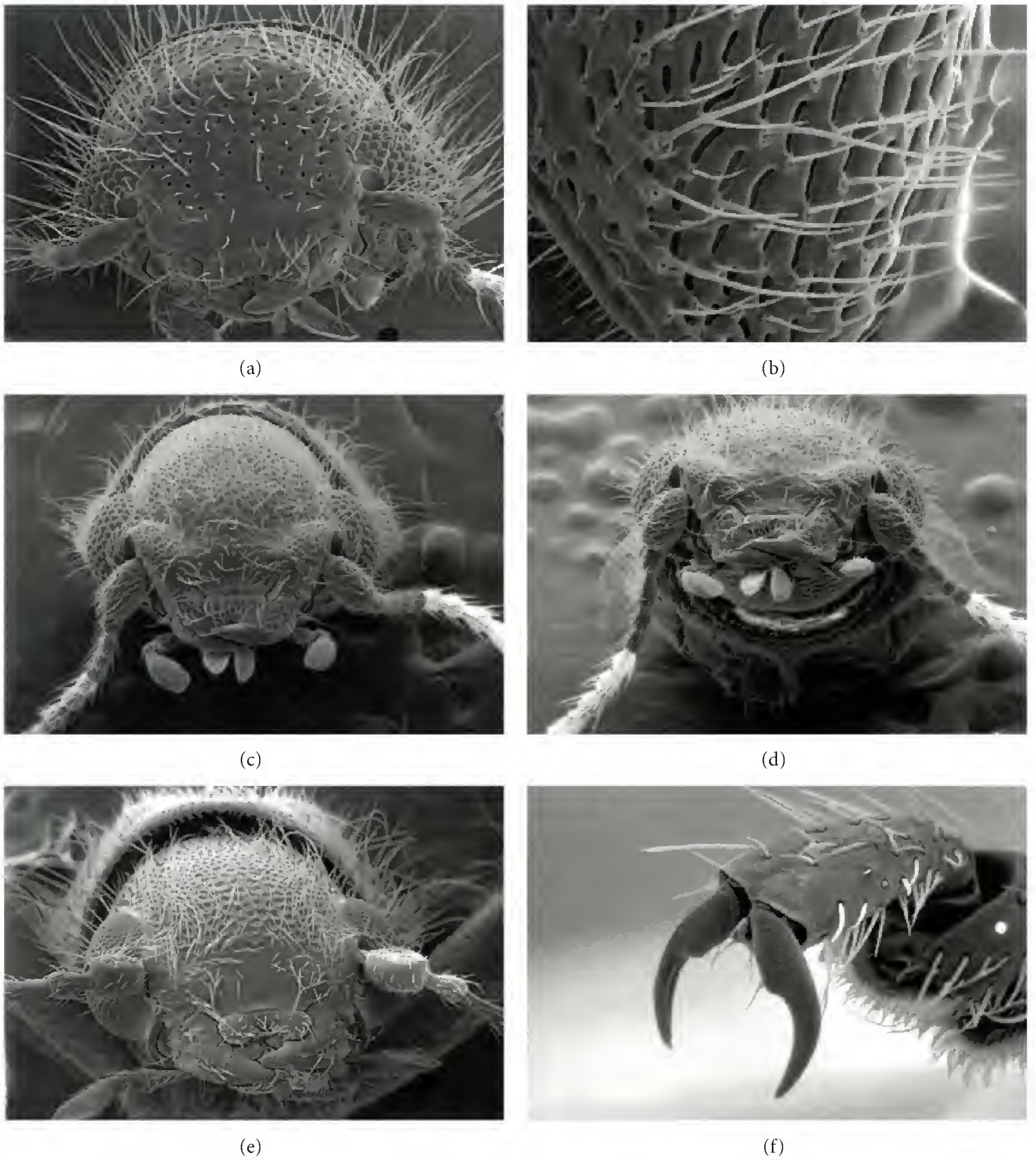
FIGURE 5: Various organs of *Apteropilo chrysocome*. (a, b) Head ((a) ventral, (b) dorsal). (c) Pronotum (ventral). (d) Mandible. (e) Metendosternite. (f) Metathoracic wing. (g) Pronotum (dorsal). (h) Antenna.

arranged serially, epipleural fold laterally positioned, narrow near elytral apex, anterior margin carinate; metathoracic wing (Figure 10(e)), wedge cell closed; metendosternite (Figure 10(h)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figures 10(m) and 10(n)), shorter than length of abdomen, phallobase not reduced, lobate distally, lobes not fimbriate; phallic lateral plates broad, phallic apex stout, spicular plates narrow (Figure 10(l)), slightly acuminate, spicular apodemes fused, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: saccular bursa copulatrix present, bursa with two basal sclerites.

Distribution. The members of this genus have been found only in Chile.

Species Examined. This is a monotypic genus.

4.5.11. Parapylus Blackburn (Figures 11(a)–11(l), 12(f), and 22(i)). Parapylus Blackburn [17]. *Type species: Parapylus bicinctus* Newman [25]. By monotypy.



(a) (b) (c) (d) (e) (f)

FIGURE 6: Various organs. (a, b) *Curacavi dentatus* ((a) head, (b) pronotal disc). (c, d) *Pseudopilus okei* ((c) head, (d) mouthparts). (e, f) *Apteropilo chrysocome* ((e) head, (f) protarsus).

Synapotypic Characteristics. Tarsal spur formula 2-2-2, elytral disc vested with transverse setal fascia, only apex of spermathecal capsule visibly sclerotized.

Diagnosis. Transverse setal fascia on the elytral disc and a tarsal formula of 2-2-2.

Description. Size: length 4.0–7.0 mm; width 1.8–3.8 mm. *Form* (Figure 22(i)): oblong short rectangulate, robust, about 2 times longer than broad. *Vestiture*: disc of cranium vested profusely with dark setae, pronotum and elytral vested profusely vested with dark setae, elytral disc vested with 1° setae and shorter 2° setae, elytral disc narrow fascia of white setae. *Head* (Figures 11(a), 11(b), 12(f)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 11(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate not discernible; mandible, body long, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 11(i)), laterolacinia present, terminal subdigitiform; labium (Figure 11(h)), ligula deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch large; antenna (Figure 11(e)), capitate, capitulum compact, wide, scape about as long as combined

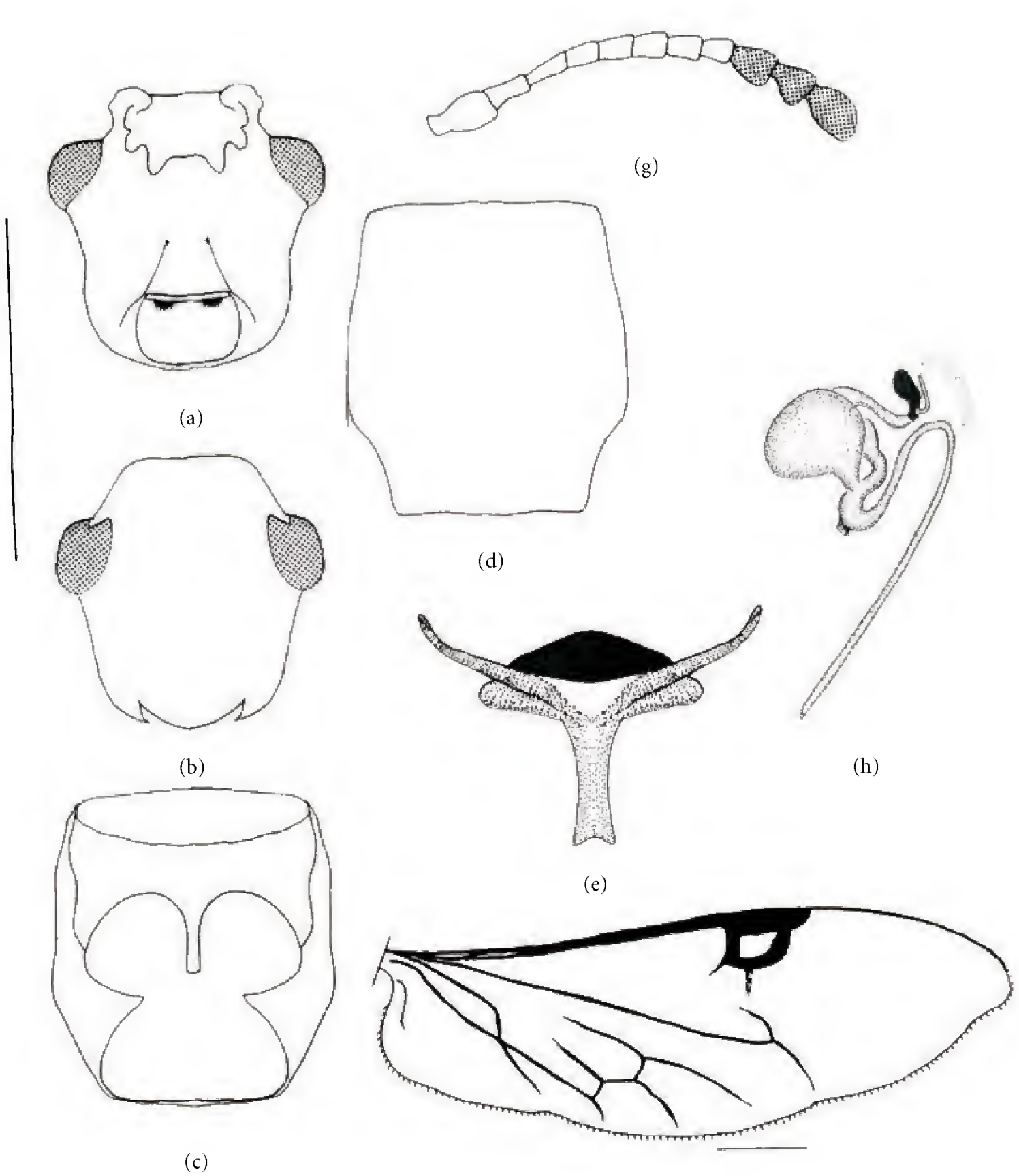


FIGURE 7: Various organs of *Blackburniella intricata*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Metendosternite. (f) Metathoracic wing. (g) Antenna. (h) Female mesodermal organs.

length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres wide, antennomeres 9 and 10 expanded laterally, antennomere 11 transverse. *Thorax*: pronotum (Figures 11(c) and 11(d)), transverse, disc indented with large setiferous punctations, with central linear fissure, and small paralateral elevations, side margins with distinct tubercle, dorsolateral ridge extends from posterior angle to anterior angle, not fractured but made coarse by coarse punctations, sclerotized region above pronotal projection setose, not glabrous, prebasal fissure well developed, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with large spheroid asetiferous punctations, punctations smaller in elytral apical half, punctations not seriate, 1° setae adjacent to asetiferous punctations, 2° setae not arranged serially, epipleural fold laterally positioned, narrows towards elytral apex, anterior margin carinate; metathoracic wing (Figure 11(f)), wedge cell closed; metendosternite (Figure 11(g)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 2-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase reduced, lobate distally, lobes fimbriate, phallobasic rod bifid distally; phallic lateral plates narrow, phallic apex minute, spicular plates triangular (Figure 11(j)), spicular apodemes not fused, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs* (Figure 11(k)): two pairs of accessory glands, lateral pair divided. *Female*

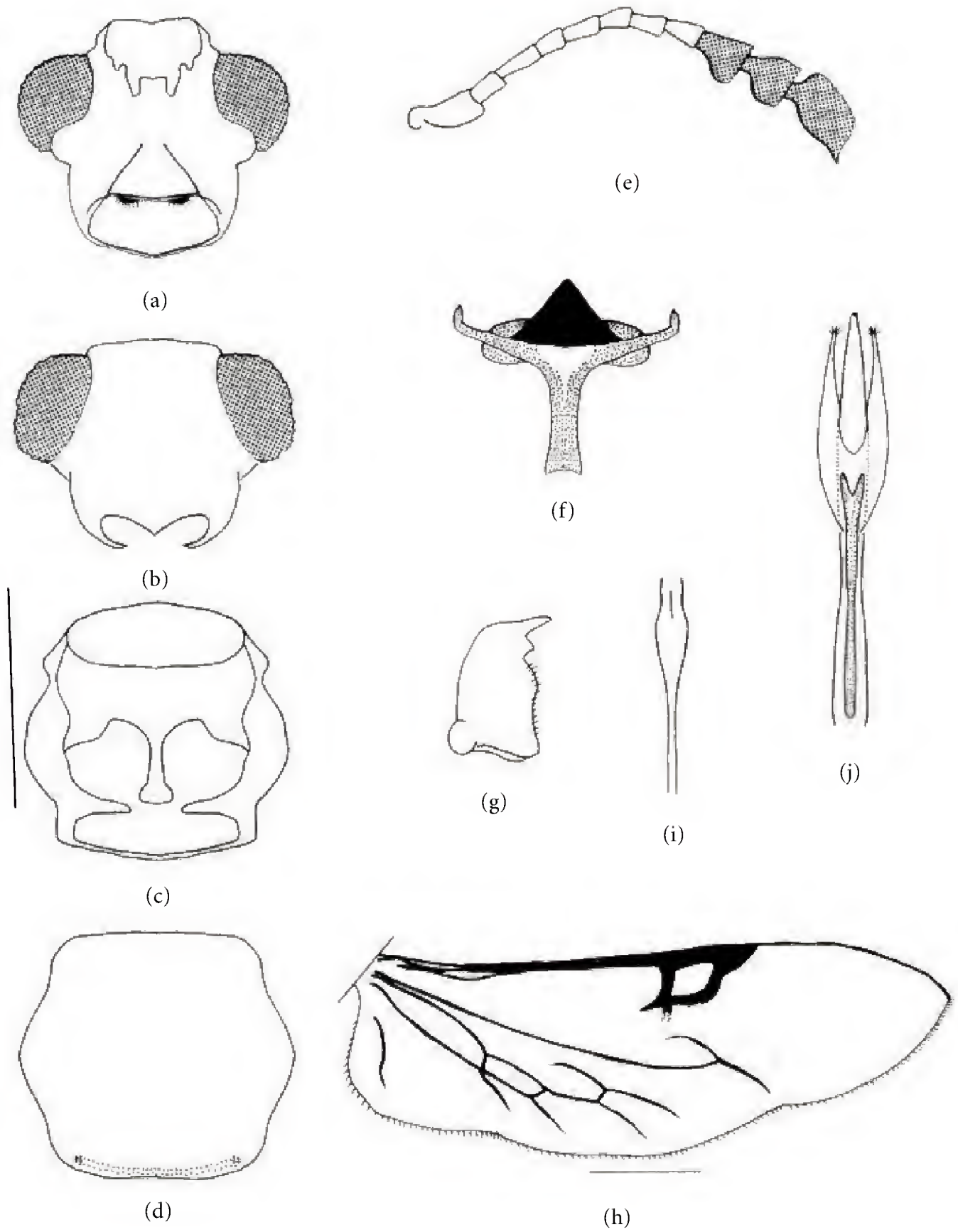


FIGURE 8: Various organs of *Fallopylus pallipes*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metendosternite. (g) Mandible. (h) Metathoracic wing. (i) Spiculum. (j) Aedeagus.

mesodermal internal reproductive organs (Figure 11(l)): saccular bursa copulatrix present, bursa with two basal sclerites, only apex of spermathecal capsule sclerotized, spermathecal gland attached to apex of spermatheca.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Parapylus bicinctus Newman and *P. sedlaceki* (Kolibáč) (new combination).

Notes. As *Parapylus* Blackburn Newman is characterized by having a tarsal spur formula of 2-2-2 its two species cannot be retained in *Pylus* Newman, which is characterized by a tarsal formula of 2-2-1. Other differences involve smaller ommatidia and the elytra of *Parapylus* have short longitudinal ridges on the posterior half of the elytral disc, the elytra are setose-fascia and show a basal umbo. Kolibáč [6] provides illustrations of various organs of *Parapylus bicinctus* Newman.

4.5.12. Pseudopylus, gen. nov. (Figures 4(a)–4(g), 4(i)–4(l), 6(c), 6(d), and 23(a)).

Type Species. Pylus okei Elston [26]. Kolibáč [6].

Synapotypic Characteristics. Tibial spur formula 0-0-0.

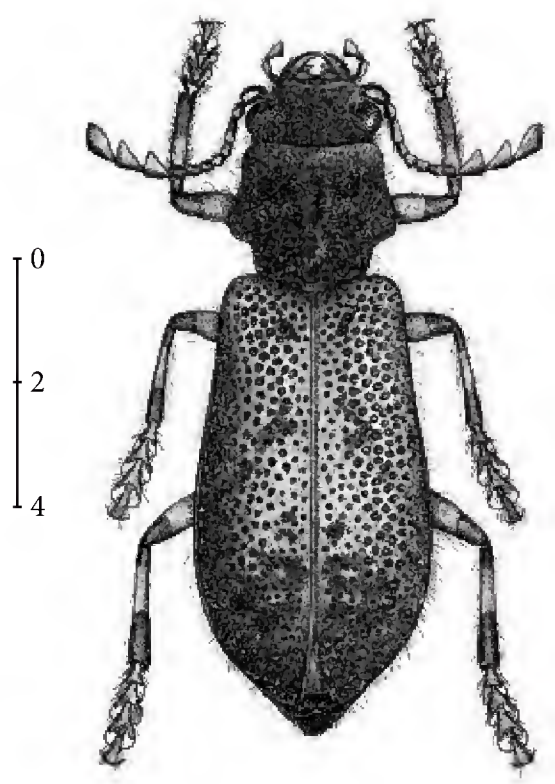


FIGURE 9: Habitus of *Neopylus nahuelbutensis*.

Diagnosis. The absence of glabrous tumescences on the pronotal disc of specimens of *Pseudopylus* will distinguish them from those of *Apteropilo*.

Size: length 3.8–7.0 mm; width 1.4–3.6 mm. *Form* (Figure 23(a)): oblong short rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 4(a), 4(b), and 6(c)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 4(a)) large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes not confluent, epipharyngeal plate very small; mandible (Figure 4(j)), body short, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 6(d)), laterolacinia present, terminal palpomere sub securiform; labium, ligula not deeply incised, terminal palpomere subsecuriform; eyes small, coarsely faceted, ocular notch small; antenna (Figure 4(e)), capitate, capitulum compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres wide, antennomeres 9 and 10 triangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 4(c), and 4(d)), transverse, side margins with distinct tubercles, disc with large oblong or round setiferous punctations, dorsolateral ridge extends from posterior angle to anterior angle, surface coarse and fractured by coarse punctations, prebasal fissure well developed, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with large spheroid asetiferous punctations, latter seriate, punctations bimodal, 1° setae always adjacent to asetiferous punctations, 2° setae arranged serially, epipleural fold laterally positioned, extended to elytral posterior four-fifths, anterior margin carinate; metathoracic wing (Figure 4(f)), rarely absent, when present wedge cell closed; metendosternite (Figure 4(g)), with furcal lamina, furcal lamina may be reduced in size, furcal anterior plate present or absent; legs, tibial spur formula 0-0-0, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figure 4(l)), as long as length of abdomen, phallobase reduced, lobate distally, lobes fimbriate; phallic lateral plates very narrow, particularly long, spicular plates very narrow, spicular apodemes not fused (Figure 4(i)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: six malpighian tubules. *Male mesodermal internal reproductive organs* (Figure 4(k)): two pairs of accessory glands, medial gland coiled, vesiculated at base, lateral glad digitiform, seminal vesicle bulbous, testis very small. *Female mesodermal internal reproductive organs*: spermathecal capsule well sclerotized, spermathecal gland attached near base of spermathecal capsule, saccular bursa copulatrix present, two denticulated basal bursal sclerites present.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Pseudopylus okei (Elston) (new combination) and three undescribed species.

Etymology. The generic name *Pseudopylus* stems from the Greek *pseudo* (=fallacy) and the name of the genus *Pylus* Newman.

Notes. Kolibáč [6] provides excellent drawings of the aedeagus and female mesodermal internal reproductive organs of *Pseudopylus okei* (Elston). Note the poor development of the metendosternite of *P. okei* (Elston) (Figure 4(g)) a species in which the metathoracic wings are absent. The metendosternite is well developed in *A. unumgarensis* Kolibáč (Figure 4(h)) whose specimens show well-developed membranous wings.

4.5.13. Pylus Newman (Figures 13(a)–13(j), 16(a), 16(b), and 23(b)). Pylus Newman [27]. *Type species: Pylus fatuus* Newman [27]. Corporaal [5].

Ylotis Spinola [28]. Synonymized by Corporaal, 1950.

Ylotis Spinola [12]. Synonymized by Corporaal, 1950.

Synapotypic Characteristics. Spermathecal gland very long (Figure 13(i)).

Diagnosis. Within Tarsosteninae elytral disc punctations are serially rowed to the elytral apex in specimens of *Apopylus* Kolibáč, *Fallopylus* gen. nov., and in *Pylus* Newman. However, the tibial spur formula is 2-2-2 in *Pylus* Newman, 0-0-0 in *Apopylus* Kolibáč, and 1-2-1 in *Fallopylus* gen. nov.

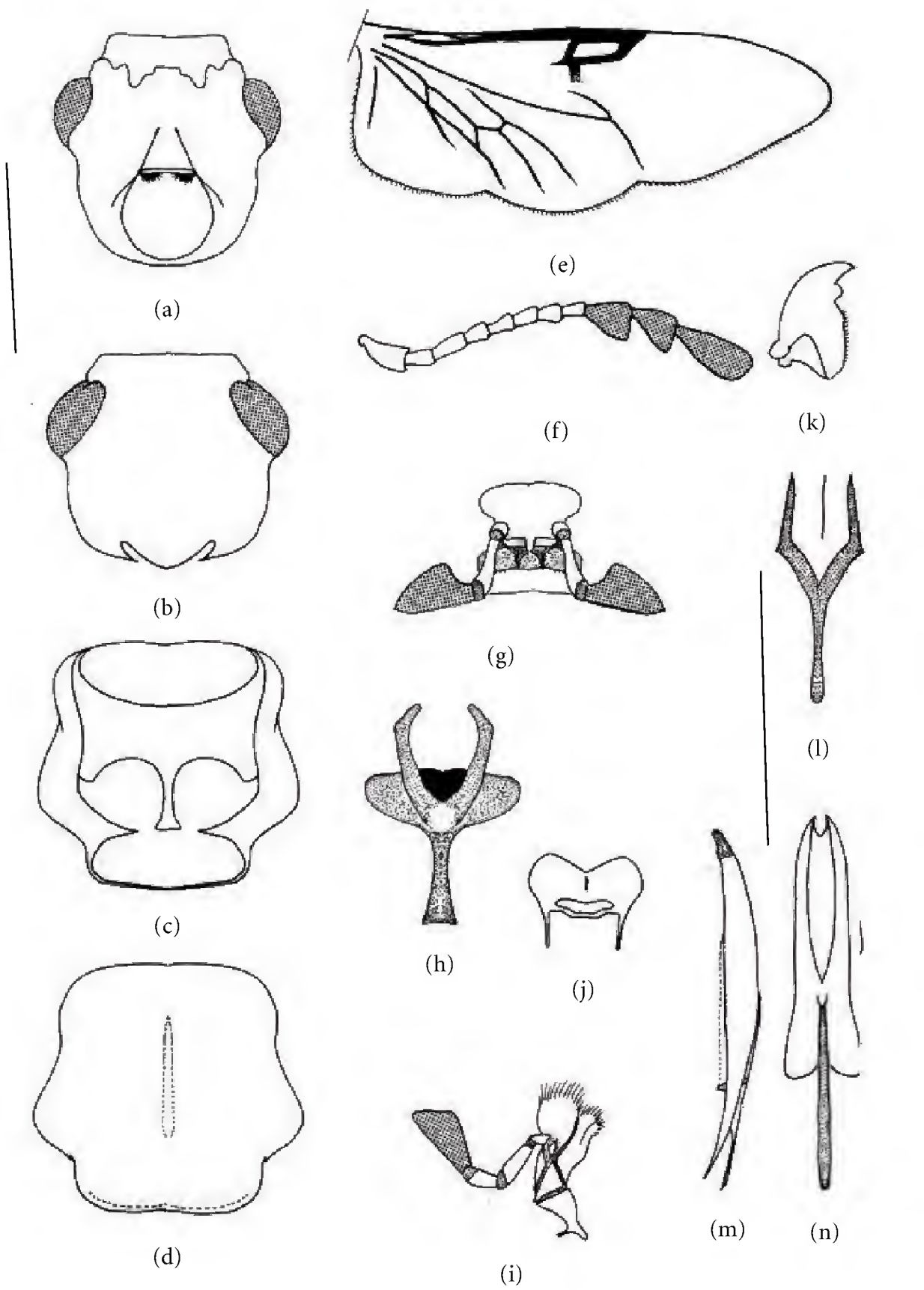


FIGURE 10: Various organs of *Neopylus nahuelbutensis*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Thorax ((c) ventral, (d) dorsal). (e) Metathoracic wing. (f) Antenna. (g) Labium. (h) Metendosternite. (i) Maxilla. (j) Labrum. (k) Mandible. (l) Spiculum. (m) Phallus. (n) Tegmen.

Description. *Size*: length 5.0–15.5 mm; width 2.0–5.2 mm. *Form* (Figure 23(b)): oblong long rectangulate, robust, about 3 times longer than broad. *Vestiture*: disc of cranium vested profusely with dark setae, pronotum and elytral vested profusely vested with dark setae, elytral disc vested with short 1° setae and shorter 2° setae. *Head* (Figures 13(a), 13(b), and 16(a)): cranium quadrate, frons wider than width of eye, indented with large setiferous punctations that give cranium rugose appearance; gula (Figure 13(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, not deeply incised, transverse tormal processes confluent, epipharyngeal plate small; mandible, body long, anterior, medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 16(b)), laterolacinia present, terminal subsecuriform; labium (Figure 16(b)), ligula deeply incised, terminal palpomere securiform; eyes large, coarsely faceted, ocular notch large; antenna (Figure 13(e)), capitate, capitulum compact, wide, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres wide, antennomeres 9 and 10 expanded laterally, antennomere 11 transverse. *Thorax*: pronotum (Figures 13(c) and 13(d)), transverse, disc indented with large setiferous punctations, with central fissure, and small paralateral elevations, side margins with distinct tubercle at middle and knob at

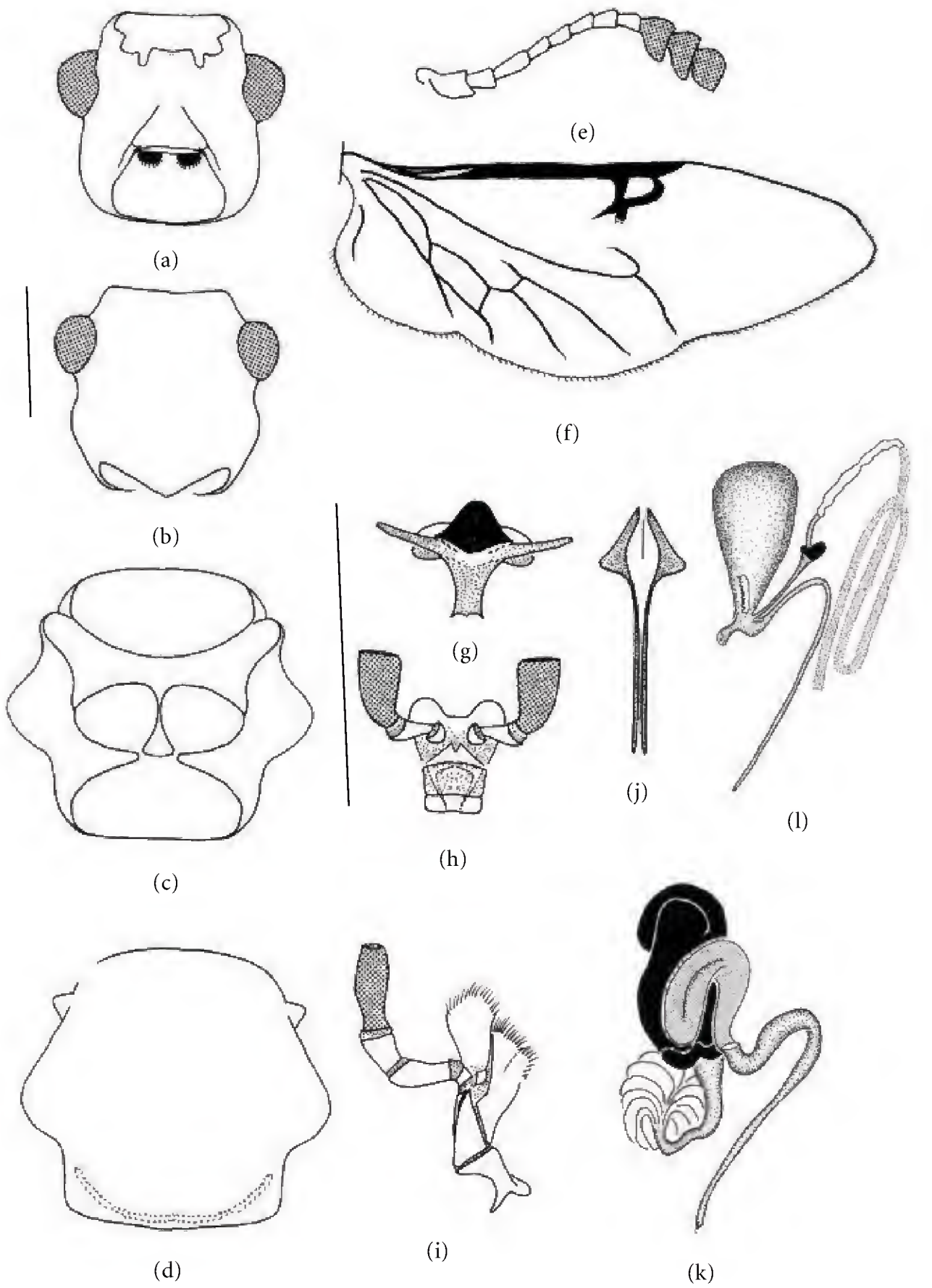


FIGURE 11: Various organs of *Parapylus bicinctus*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metathoracic wing. (g) Metendosternite. (h) Labium. (i) Maxilla. (j) Spiculum. (k) Male mesodermal reproductive organs. (l) Female mesodermal reproductive organs.

anterior angles, dorsolateral ridge extends from posterior angle to anterior angle, not fractured but made coarse by coarse punctations, sclerotized region above pronotal projection not glabrous, setose, prebasal fissure well developed, prointercoxal process expanded distally; pronotal projections long; elytron sculptured with large binoded asetiferous punctations, punctations seriate, extend to elytral apex seriate, 1° setae adjacent to asetiferous punctations, 2° setae serially arranged, epipleural fold laterally positioned, narrows towards elytral apex, anterior margin carinate; metathoracic wing (Figure 13(f)), wedge cell closed; metendosternite with furcal lamina, furcal anterior plate prominent; legs, first tarsomere may be slender, acuminate and extended towards second tarsomere, tibial spur formula 2-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figures 13(h) and 13(j)), shorter than length of abdomen, phallobase reduced, lobate distally, lobes fimbriate, phallobasic rod not bifid distally; phallic lateral plates narrow, phallic apex minute, spicular plates narrow, with small rounded lateral extension, spicular apodemes not fused (Figure 13(g)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive*

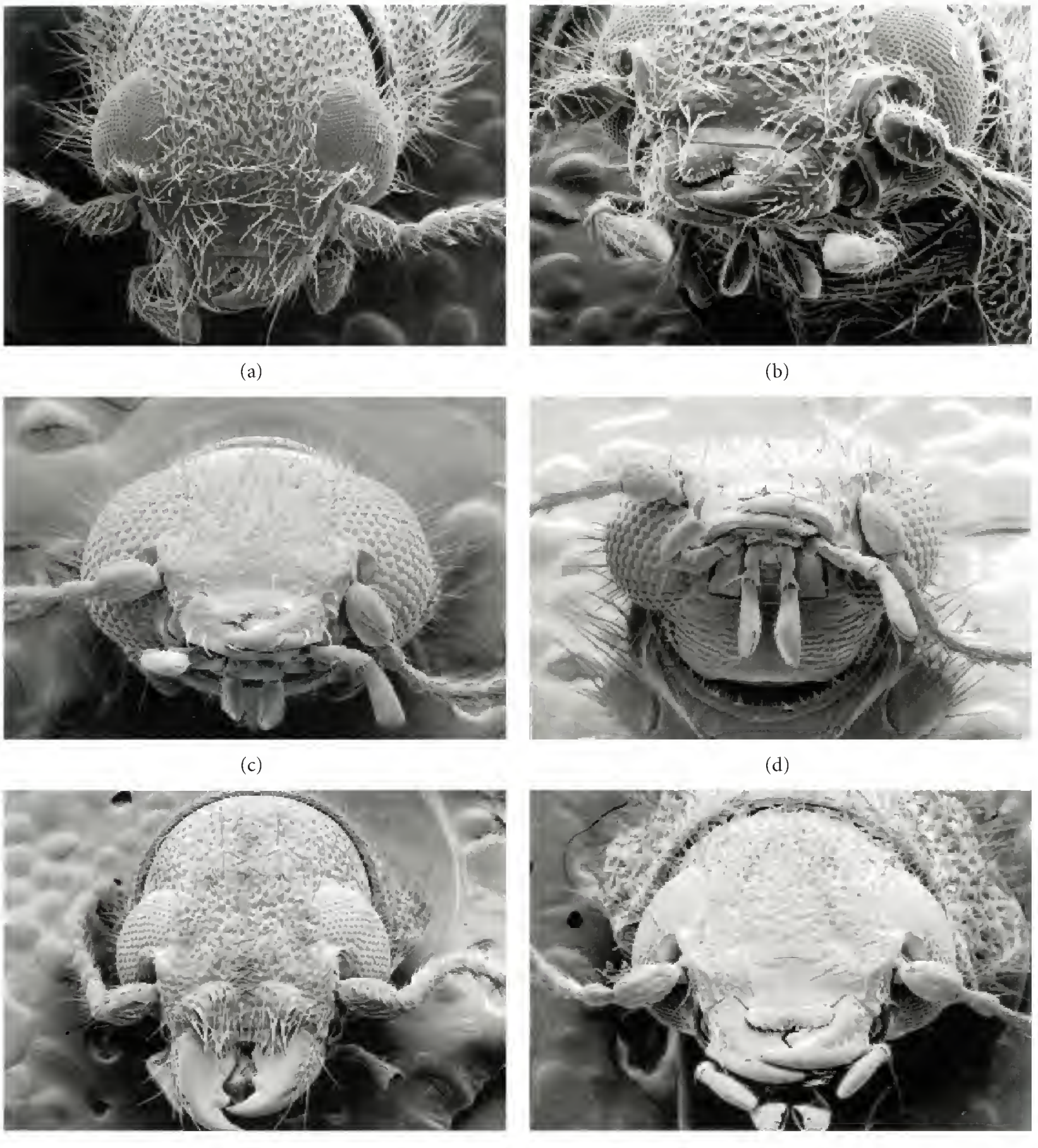


FIGURE 12: Various organs. (a, b) *Blackburniella intricata* ((a) head, (b) mouthparts). (c, d) *Fallopylus pallipes* ((c) head, (d) mouthparts). (e) *Neopylus nahuelbutensis* head. (f) *Parapylus bicinctus* head.

organs (Figure 13(i)): saccular bursa copulatrix present, bursa with two basal sclerites, spermathecal capsule well sclerotized, spermathecal gland very long, attached to base of spermatheca.

Distribution. The members of this genus have been found only in Australia.

Species Examined. Pylus fatuus Newman and one undescribed species.

Notes. Kolibáč [6] provides illustrations of various organs of *Pylus fatuus* Newman.

4.5.14. Rhophaloclerus Fairmaire (Figures 14(a)–14(g), 16(c), 16(d), and 23(c)). Rhophaloclerus Fairmaire [29, 30]. Type species: *Rhophaloclerus coquerelii* Fairmaire [29]. By monotypy. Kuwert [31]. Corporaal [5]. *Liostylus* Fairmaire [29]. *New Synonymy.*

Synapotypic Characteristics. Tibial spur formula 1-2-2.

Diagnosis. This is the only genus of Tarsosteninae with a tibial formula 1-2-2 and known to occur in Madagascar and the Comoros.

Description. Size: length 3.0–6.0 mm; width 0.7–3.0 mm. *Form* (Figure 23(c)): oblong rectangulate, most species

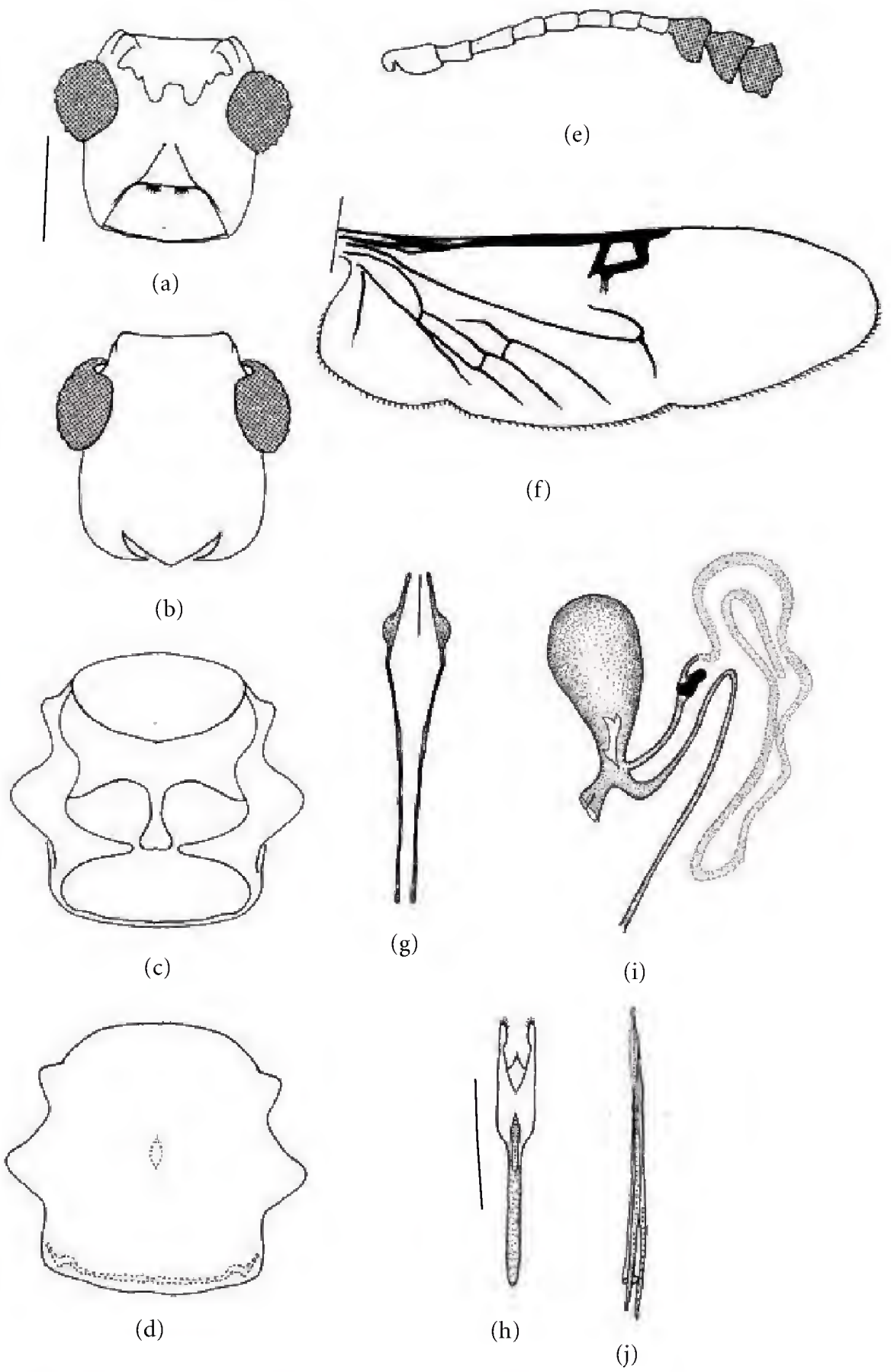
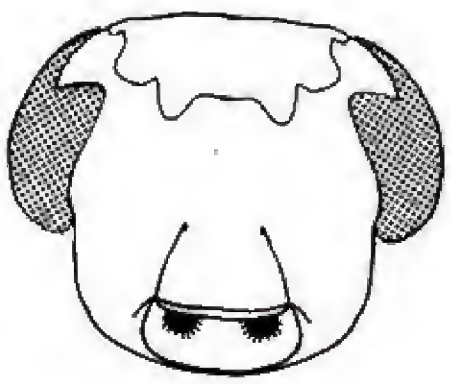
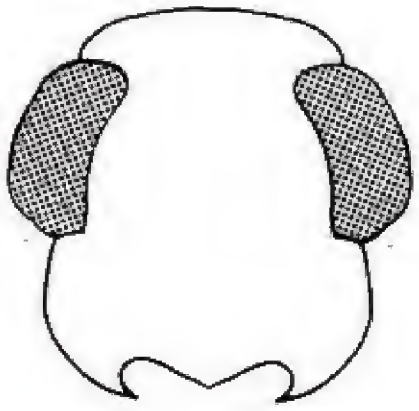


FIGURE 13: Various organs of *Pylus fatuus*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metathoracic wing. (g) Spiculum. (h) Tegmen. (i) Female mesodermal reproductive organs. (j) Phallus.

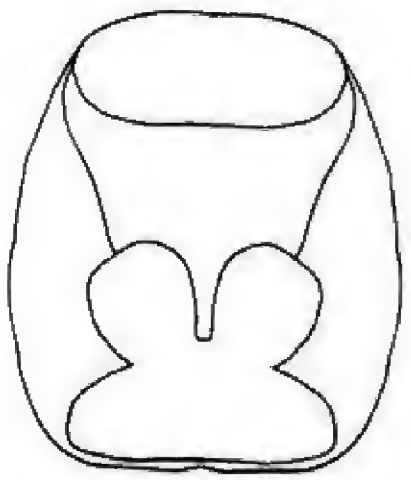
narrow, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 14(a), 14(d), and 16(c)): cranium quadrate, frons much wider than width of eye, indented with very small setiferous punctations; gula (Figure 14(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes not confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere securiform; labium, ligula not incised, terminal palpomere securiform; eyes large, finely facetted, ocular notch large; antenna (Figure 16(d)), capitate, capitulum usually lax, rarely compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres expanded, antennomeres 9 and 10 expanded, antennomere 11 ovoid. *Thorax*: pronotum (Figures 14(b), and 14(c)), usually elongate, rarely transverse-ovoid, disc indented with small setiferous punctations, lateral margins variously convex, dorsolateral ridge extends from posterior angle to anterior angle, not fractured and with smooth surface, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with shallow asetiferous punctations, punctations seriate and diminish in size to elytral apex, 1° setae always adjacent to asetiferous punctations, 2° setae densely distributed, epipleural



(a)

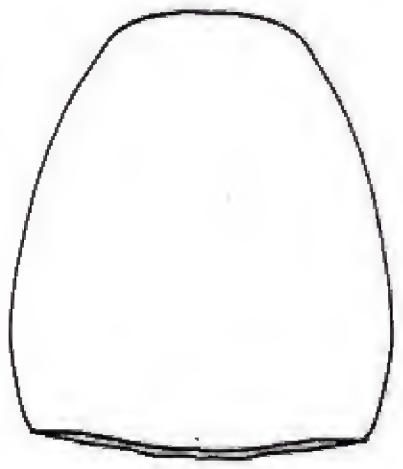


(d)



(b)

(e)



(c)

(f)

(g)

FIGURE 14: Various organs of *Rhophaloclerus coquerelii*. (a) Head (ventral). (b, c) Pronotum ((b) ventral, (c) dorsal). (d) Head (dorsal). (e) Metathoracic wing. (f) Metendosternite. (g) Spiculum.

fold laterally positioned, narrowed to elytral apex, anterior margin carinate; metathoracic wing (Figure 14(e)), wedge cell open; metendosternite (Figure 14(f)), with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-2, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, lobate and narrowed distally, lobes fimbriate; phallic lateral plates narrow, phallobasic rod linear or transverse, furcated distally or not furcated, phallic apex minute, spicular plates very narrow (Figure 14(g)), spicular apodemes not fused at extremity, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule subovoid, well sclerotized, spermathecal gland attached to middle of spermathecal capsule.

Distribution. Known only from Madagascar and Comoros.

Species Examined. Rhophaloclerus coquerelii Fairmaire, *R. pictus* (Fairmaire) (new combination), *R. vadoni* Pic, and five species that may or may not be described.

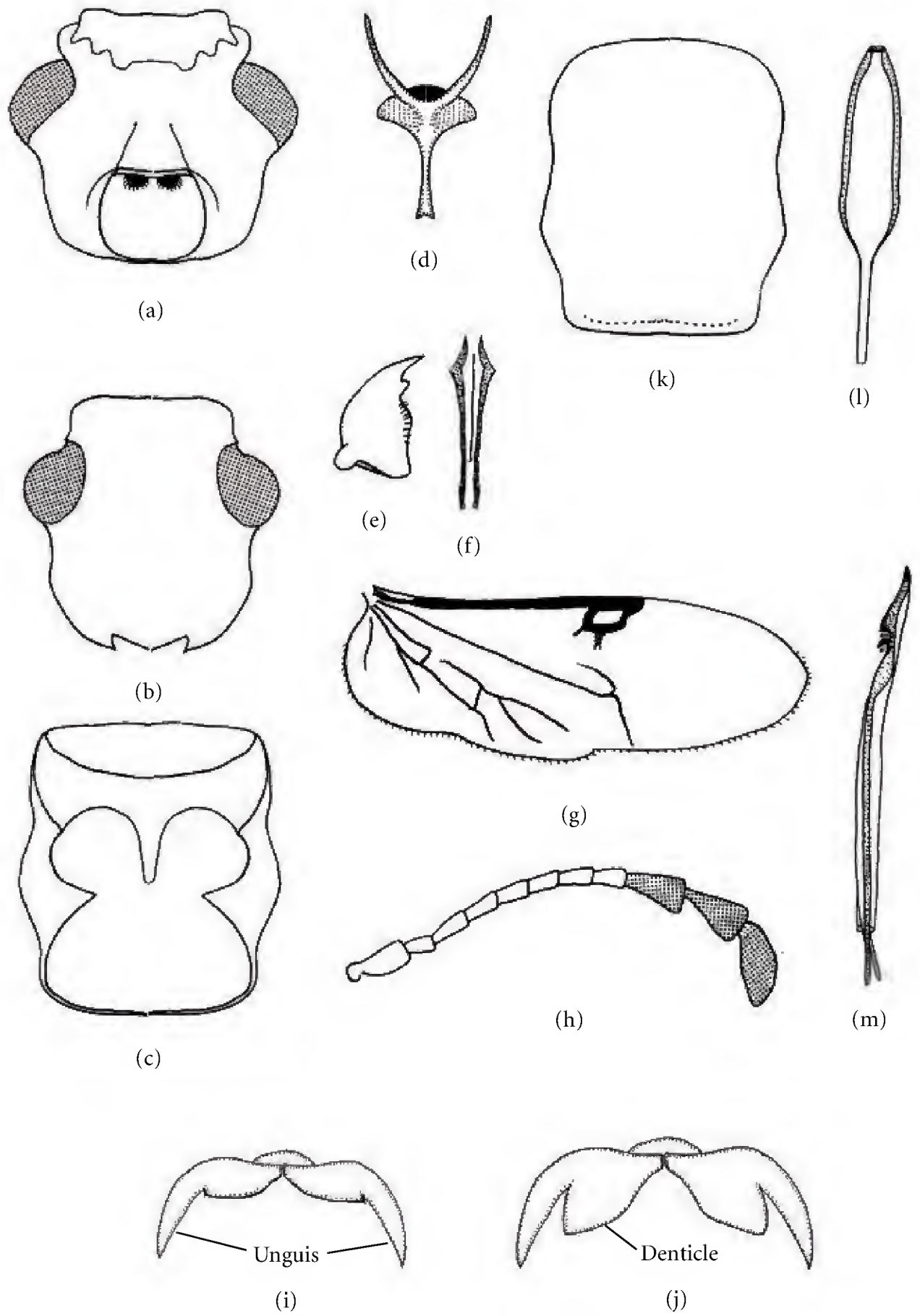


FIGURE 15: Various organs. (a)–(h), and (k)–(m) *Riotenerus fossipenne*. (a, b) Head ((a) ventral, (b) dorsal). (c) Pronotum (ventral), (d) Metendosternite. (e) Mandible. (f) Spiculum. (g) Metathoracic wing. (h) Antenna. (i) and (j) Generalized unguis ((i) without denticle, (j) with denticle). (k) Pronotum (dorsal). (l) Tegmen. (m) Phallus.

Notes. By placing the description of *Rhophaloclerus* Fairmaire amidst descriptions of other Tillinae genera Fairmaire [29, 32] gave the impression to subsequent authors [5, 33] that the genus in question belongs in Tillinae. The reduced 4th tarsomere and the characteristics of the antenna clearly indicate that *Rhophaloclerus* Fairmaire is appropriately classified in Tarsosteninae.

4.5.15. Riotenerus Pic (Figures 15(a)–15(h), 15(k)-15(m), 16(e), 16(f), 21(e), and 23(d)). Riotenerus Pic [34]. Type species: *Pelonium fossipenne* Schenkling [21]. By monotypy. Solevicens [35]. Opitz [1].

Synapotypic Characteristics. Elytral disc devoid of 2° setae; aedeagus with uncinate projections; interspicular plate very long (Figure 15(f)).

Diagnosis. Distinguishable from the superficially similar specimens of *Abiliella* Peracchi by the lack of elytral 2° setae.

Description. Size: length 5.0–8.5 mm; width 2.0–2.5 mm. *Form* (Figure 23(d)): oblong rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested with white setae, elytral disc vested with 1° setae, 2° setae absent. *Head* (Figures 15(a), 15(b), and 16(e)):

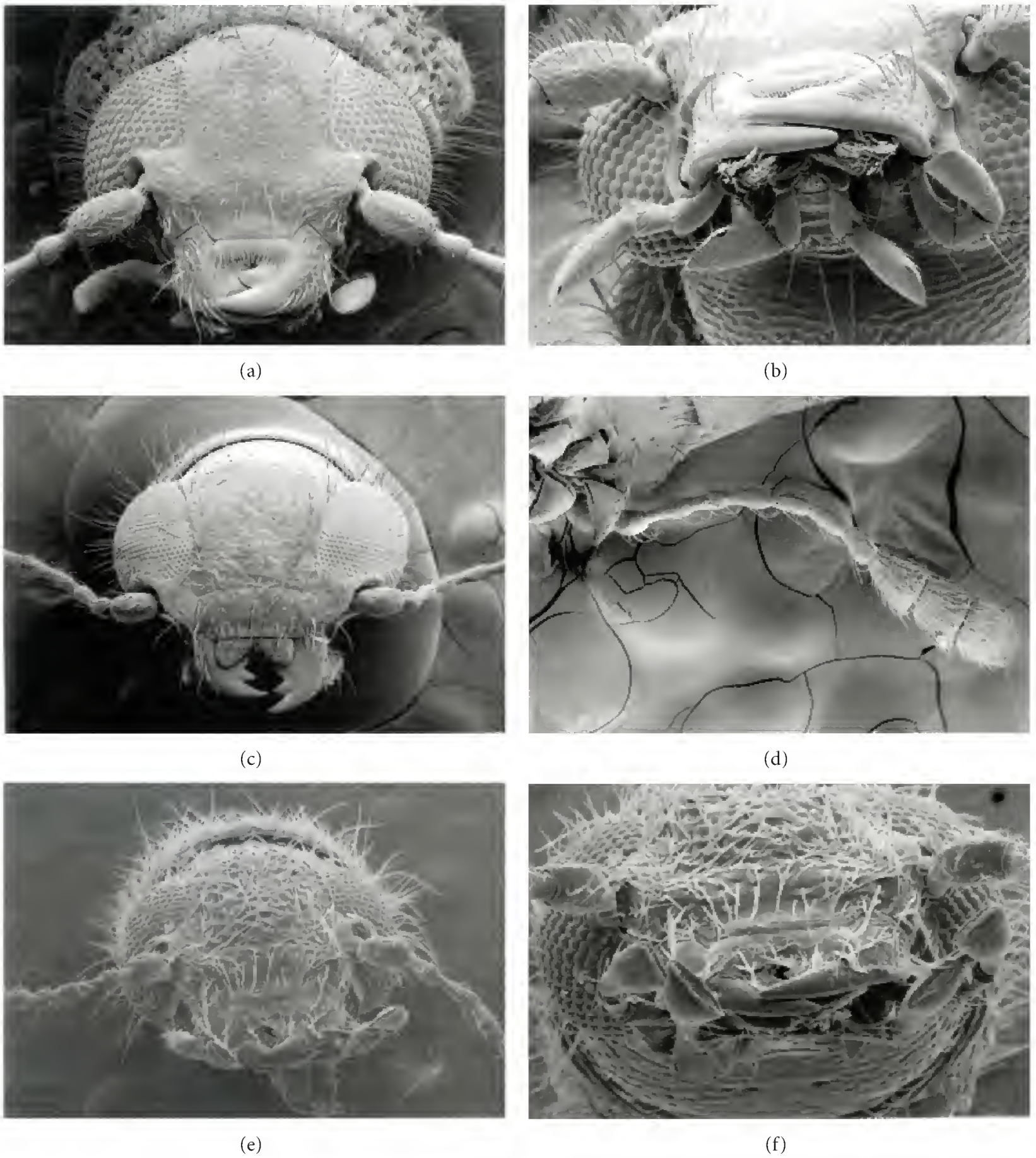


FIGURE 16: Various organs. (a, b) *Pylus fatuus* ((a) head, (b) mouthparts). (c, d) *Rhophaloclerus coquerelli* ((c) head, (d) antenna). (e, f) *Riotenerus fossipenneus* ((e) head, (f) mouthparts).

cranium quadrate, frons wider than width of eye, indented at middle and with large setiferous punctations that give cranium rugose appearance; gula (Figure 15(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, medial incision shallow, transverse tormal processes sinuous, confluent, epipharyngeal plate very small; mandible (Figure 15(e)), body stout, anterior, medial, and posterior dens well developed, penicillus well developed; maxilla (Figure 16(f)), laterolacinia present, terminal palpomere securiform; labium (Figure 16(f)), ligula not deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch small; antenna (Figures 15(h) and 21(e)), capitate, capitulum lax and narrow, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres narrow, antennomeres 9 and 10 subrectangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 15(c), and 15(k)), elongate, convex, side margins sinuous, sculptured with large round setiferous punctations, dorsolateral ridge extends from posterior angle to pronotal midline, surface coarsely punctated, prebasal fissure well developed, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with large spheroid asetiferous punctations, latter sub seriate, 1° setae always adjacent to asetiferous punctations, 2° setae absent, epipleural fold

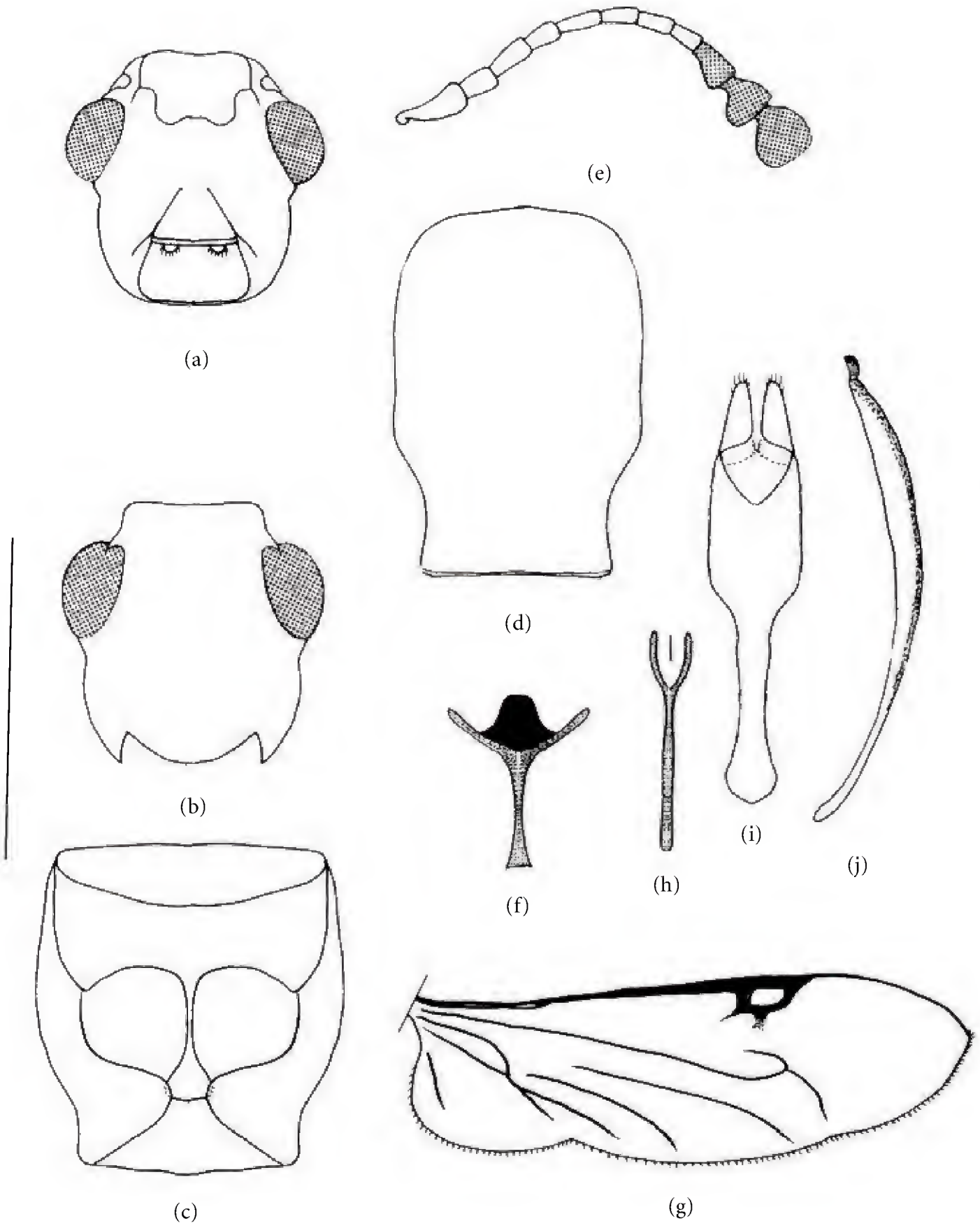


FIGURE 17: Various organs of *Tarsostenodes simulator*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Antenna. (f) Metendosternite. (g) Metathoracic wing. (h) Spiculum. (i) Tegmen. (j) Phallus.

laterally positioned, extended to elytral apex, anterior margin carinate; metathoracic wing (Figure 15(g)), wedge cell open; metendosternite (Figure 15(d)), with furcal lamina, furcal anterior plate diminutive, acuminate; legs, tibial spur formula 0-1-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figures 15(l), and 15(m)), shorter than length of abdomen, phallobase not lobate distally; phallic lateral plates very broad and uncinate distally; spicular plates small, triangular, acuminate, rarely, spicular apodemes not fused (Figure 15(f)), intraspicular plate rod shaped and very long; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. The members of this genus have been found only in Argentina.

Species Examined. Riotenerus fossipenne (Schenkling).

4.5.16. Tarsostenodes Blackburn (Figures 17(a)–17(j), 20(a), 20(b), and 23(f)). Tarsostenodes Blackburn [36]. Type species*: Tarsostenodes simulator* Blackburn [36]. By monotypy. Corporaal [5]. Kolibáč [6]. Bartlett [37].

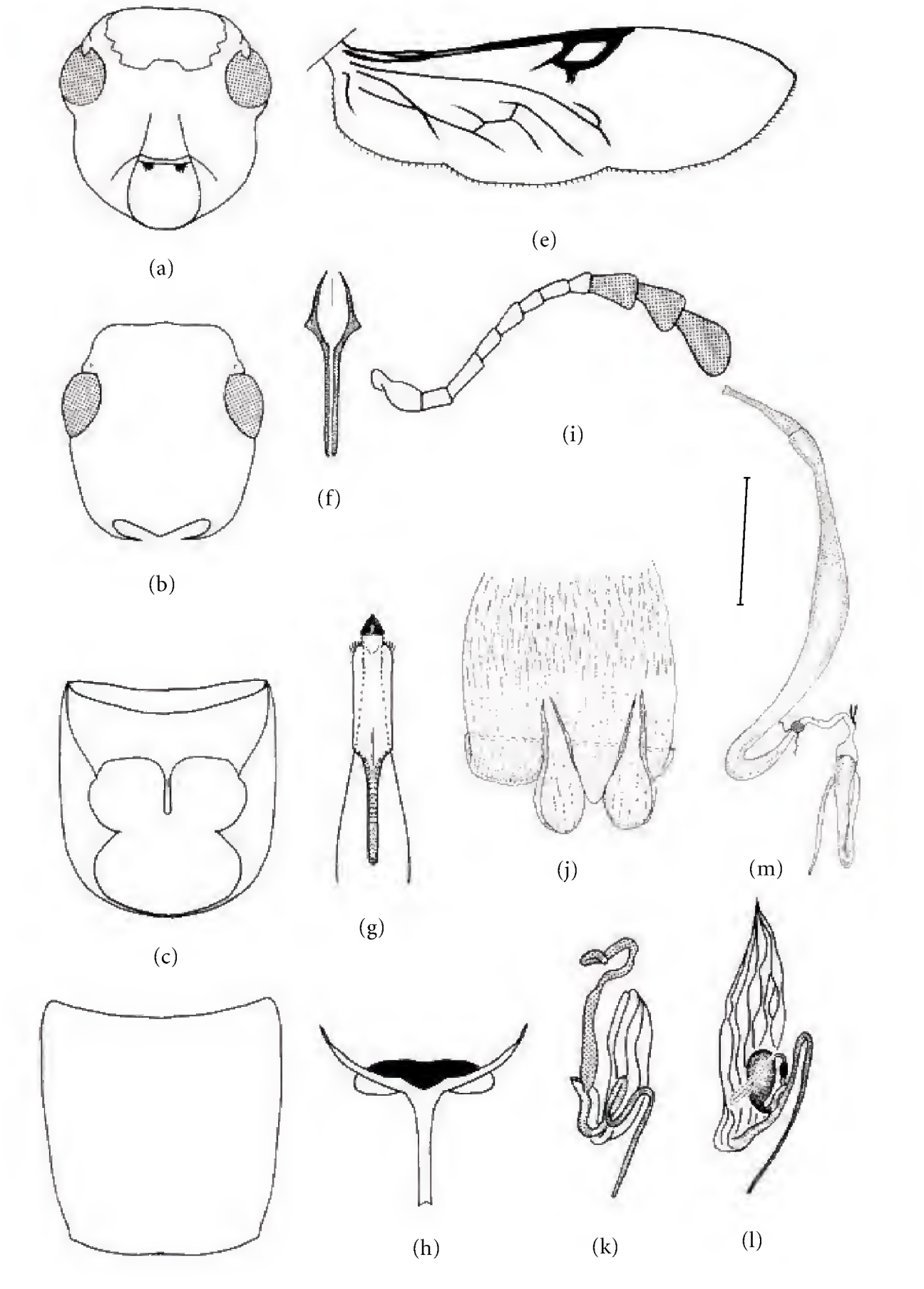


FIGURE 18: Various organs of *Tarsostenus univittatus*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Metathoracic wing. (f) Spiculum. (g) Aedeagus. (h) Metendosternite. (i) Antenna. (j) Stomodaeal valve (interior view). (k) Male mesodermal reproductive organs. (l) Female mesodermal reproductive organs. (m) Alimentary canal.

Synapotypic Characteristics. Procoxal cavity closed externally, pronotal projections wide, metathoracic wing cross veins rudimentary or missing, furcal lamina absent, epipleural fold narrow, elytra constricted at middle, phallobasic rod absent.

Diagnosis. The closure of the external aspects of the procoxal cavities (Figure 17(c)) will separate the members of this genus from any other within Tarsosteninae.

Description. Size: length 4.0–8.0 mm; width 1.0–2.0 mm. *Form* (Figure 23(f)): oblong, narrow rectangulate, about 4 times longer than broad. *Vestiture*: disc of cranium and pronotum densely vested with pale setae, elytral disc vested with 1° setae and shorter profusely distributed 2° setae. *Head* (Figures 17(a), 17(b), and 20(a)): cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula (Figure 17(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes not confluent, epipharyngeal plate very small; mandible, body long, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 20(b)), laterolacinia present, terminal palpomere securiform; labium, ligula

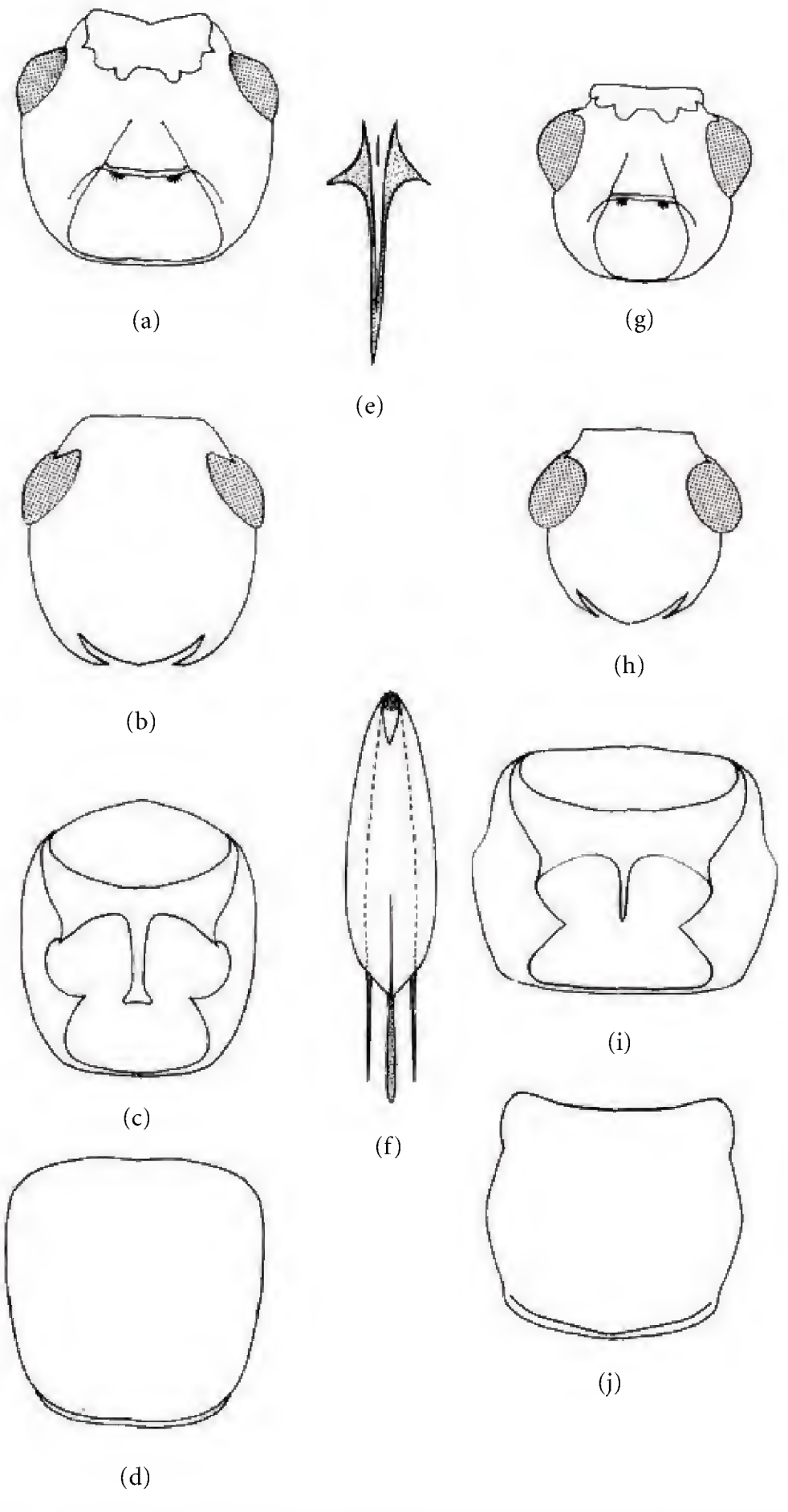


FIGURE 19: Various organs. (a)–(f) *Thriocera pectoralis*. (a, b) Head ((a) ventral, (b) dorsal). (c, d) Pronotum ((c) ventral, (d) dorsal). (e) Spiculum. (f) Aedeagus. (g)–(j) *Thriocerodes bifasciatus*. (g, h) Head ((g) ventral, (h) dorsal). (i) and (j) Pronotum ((i) ventral, (j) dorsal).

deeply incised, terminal palpomere securiform; eyes large, coarsely faceted, ocular notch large; antenna (Figure 17(e)), capitate, capitulum usually compact, rarely lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres expanded, antennomeres 9 and 10 expanded, antennomere 11 ovoid. *Thorax*: pronotum (Figures 17(c), and 17(d)), elongate, usually campaniform, rarely suboval, usually deeply constricted at base, disc indented with large setiferous punctations, interstitial spaces (spaces between punctations), very elevated, dorsolateral ridge extends from posterior angle to anterior midpoint, fractured by coarse punctations, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process very expanded distally, process confluent with pronotal projections (Figure 17(c)), latter particularly wide; elytron sculptured with deep asetiferous punctations, punctations seriate, rarely diminish in size to elytral apex, 1° setae always adjacent to asetiferous punctations, 2° setae densely distributed, interstitial spaces elevated, epipleural fold laterally positioned, very narrow to elytral apex, anterior margin not carinate; metathoracic wing (Figure 17(g)), cross veins rudimentary or missing; metendosternite (Figure 17(f)), without furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figures 17(i), and 17(j)), shorter

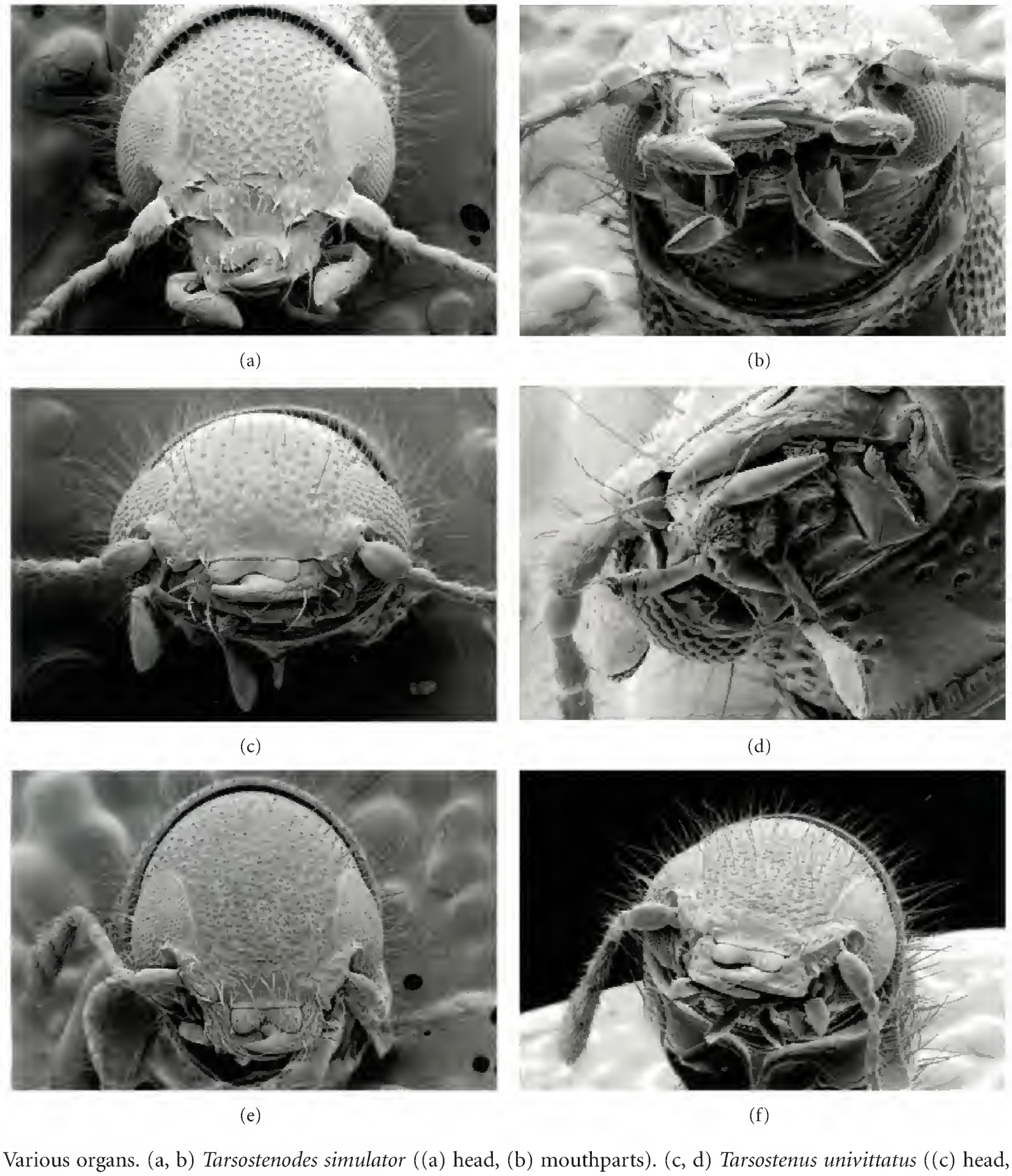


FIGURE 20: Various organs. (a, b) *Tarsostenodes simulator* ((a) head, (b) mouthparts). (c, d) *Tarsostenus univittatus* ((c) head, (d) mouthparts). (d, e) *Thriocera pectoralis* ((d) head, (e) mouthparts).

than length of abdomen, phallobase not reduced, lobate, lobes fimbriate; phallic lateral plates wide, phallobasic rod absent, phallic apex robust, spicular plates very narrow (Figure 17(h)), spicular apodemes fused, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male 6th sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule tubular, well sclerotized, spermathecal gland attached to middle of spermathecal capsule.

Distribution. Known only from continental Australia and Lord Howe Island.

Species Examined. Tarsostenodes albonotatus Pic, *T. cribripennis* Schenkling, *T. guttulus* (White), *T. howensis* Bartlett, *T. leucogramma* Elston, *T. simulator* Blackburn, and four undescribed species.

4.5.17. Tarsostenosis Heller (Figure 23(e)). *Tarsostenosis* Heller [38]. Type species: *Tarsostenosis tricolor* Heller [38]. By monotypy. Corporaal [5].

Synapotypic Characteristics. Pronotum with linear glabrous elevations.

Diagnosis. Specimens belong to this genus if their pronotum shows a poorly developed lateral tubercle, the pronotum has

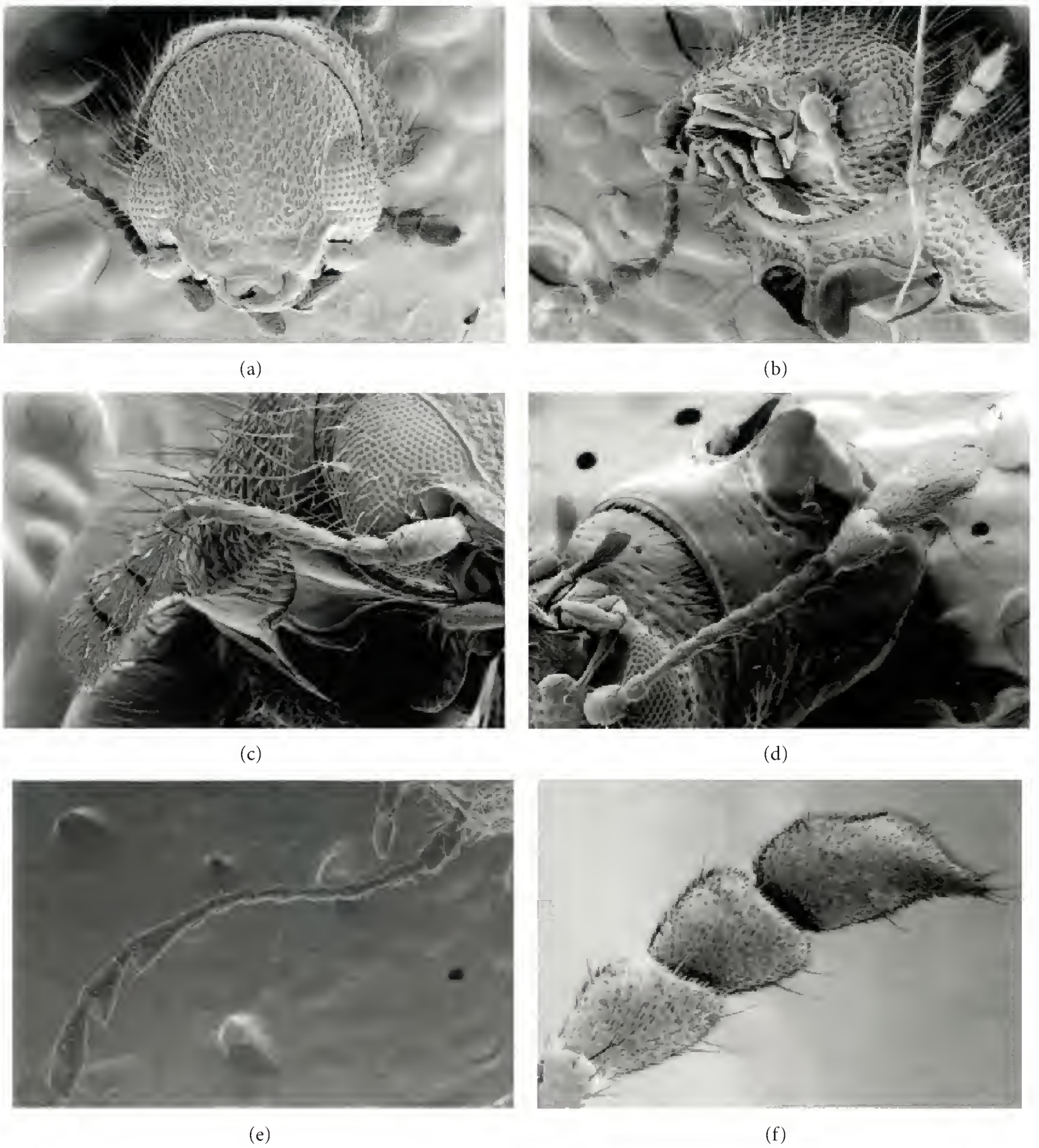


FIGURE 21: Various organs. (a, b) *Thriocerodes bifasciatus* ((a) head, (b) mouthparts). (c) *Thriocera pectoralis* antenna. (d) *Tarsostenus univttatus* antenna. (e) *Riotenerus fossipenneus* antenna. (f) *Fallopylus pallipes* capitulum.

shallow linear glabrous elevations, and the elytra are devoid of 2° setae.

Description. Size: length 3.5–5.0 mm; width 1.0–1.5 mm. *Form* (Figure 23(e)): oblong, narrow rectangulate, about 4 times longer than broad. *Vestiture*: disc of cranium and sides of pronotum densely vested with pale setae, pronotal middle sparsely setose, elytral disc vested with 1° setae, 2° setae absent. *Head*: cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla, laterolacinia present, terminal palpomere securiform; labium, ligula deeply incised, terminal palpomere securiform; eyes small, coarsely faceted, ocular notch small; antenna, capitate, capitulum lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres slightly expanded, antennomeres 9 and 10 subtriangular, antennomere 11 ovoid. *Thorax*: pronotum quadrate, disc at sides indented with large setiferous punctations, disc at middle sparsely punctate, with linear glabrous elevations, dorsolateral ridge extends from posterior angle to anterior midpoint, fractured by coarse punctations, sclerotized region above pronotal projection not glabrous, prebasal fissure shallow, prointercoxal process not expanded distally, pronotal projections

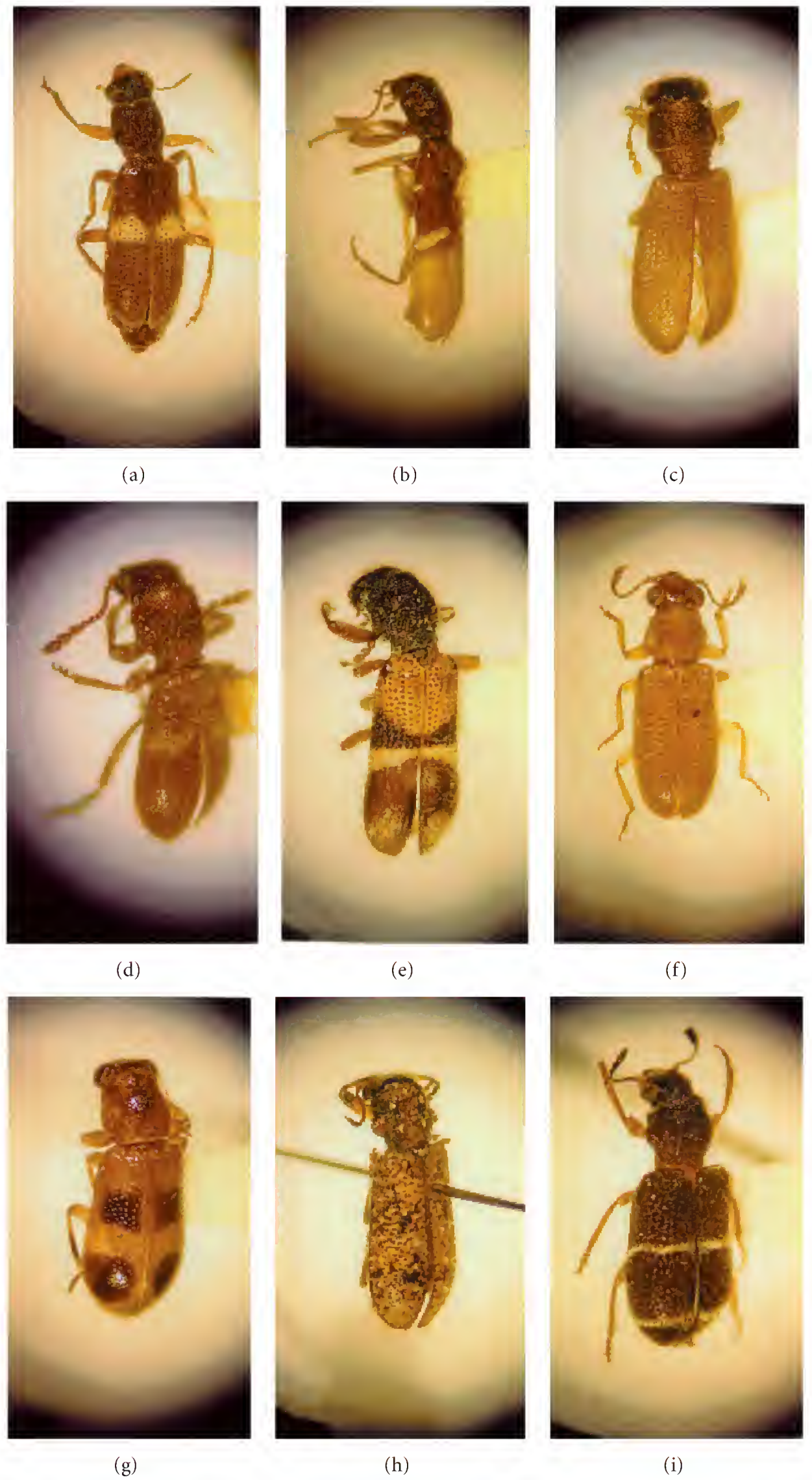


FIGURE 22: Habitus. (a) *Abiliella fasciata*. (b) *Agapetilus vietus*. (c) *Apopylus unumgarensis*. (d) *Apteropilo pictipes*. (e) *Blackburniella intricata*. (f) *Fallopylus pallipes*. (g) *Globoclava quadrimaculata*. (h) *Neopylus nahuelbutensis*. (i) *Parapylus bicinctus*.

FIGURE 23: Habitus. (a) *Pseudopylus okei*. (b) *Pylus fatuus*. (c) *Rhophaloclerus coquerelii*. (d) *Riotenerus fossipennes*. (e) *Tarsostenosis tricolor*. (f) *Tarsostenodes simulator*. (g) *Tarsostenus univttatus*. (h) *Thriocera pectoralis*. (i) *Thriocerodes bifasciatus*.

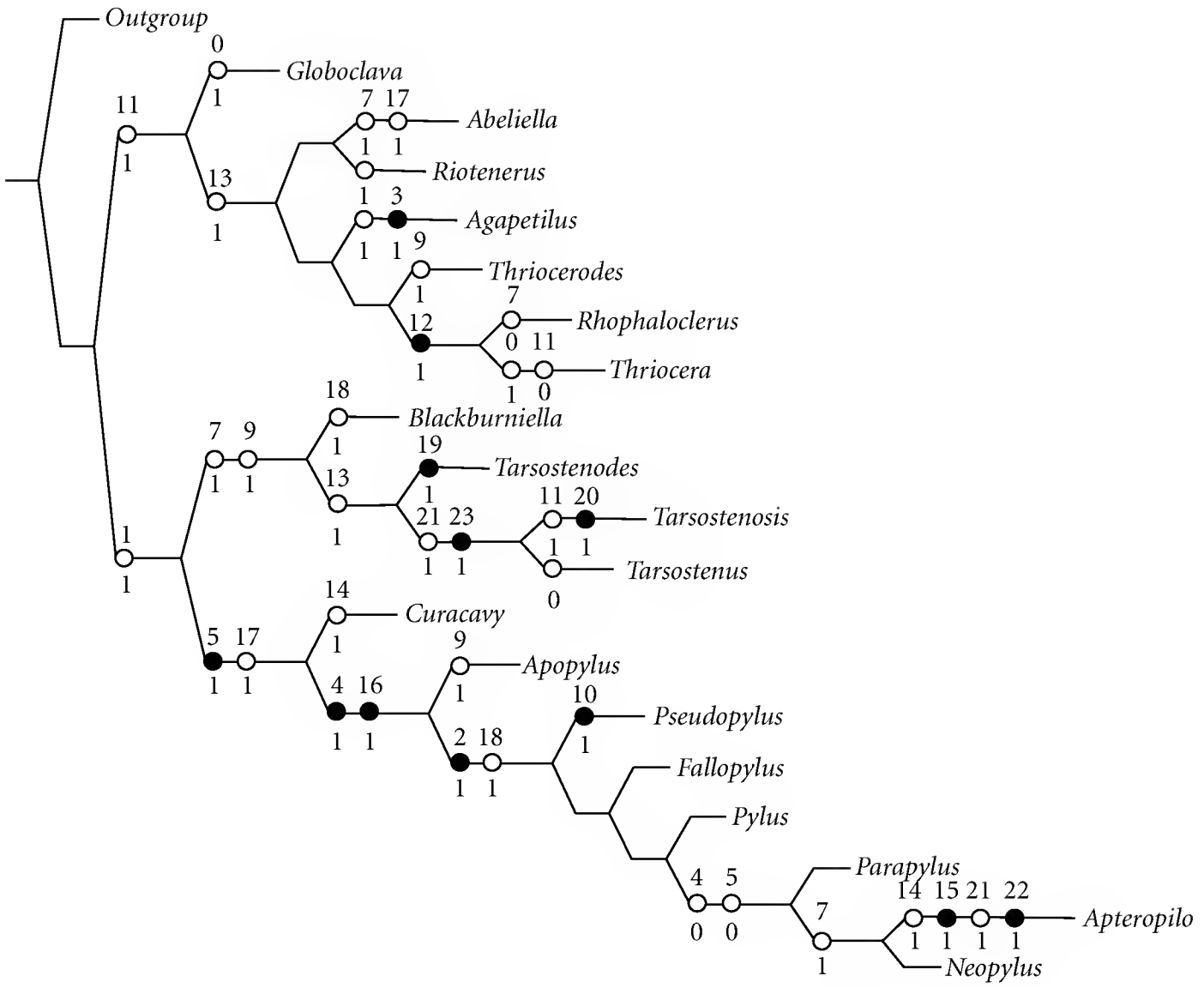


FIGURE 24: Phylogeny of genera of Tarsosteninae.

short; elytron sculptured with round asetiferous punctations, punctations seriate and extend to elytral distal two-thirds, 1° setae always adjacent to asetiferous punctations, 2° setae absent, interstitial spaces smooth, epipleural fold laterally positioned, very narrow to elytral apex, anterior margin carinate; metathoracic wing with open wedge cell; metendosternite with furcal lamina, furcal anterior plate shallow; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, not lobate but fimbriate; phallic lateral plates narrow, phallobasic rod linear, phallic apex robust, spicular plates narrow, slightly acuminate, spicular apodemes not fused, intraspicular plate rod shaped; ovipositor not examined. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studies. *Female mesodermal internal reproductive organs*: not studied.

Distribution. Species are known from Australia and New Caledonia.

Species Examined. Tarsostenosis hilaris (Westwood), (new combination), *T. tricolor* (Heller), and one undescribed species.

4.5.18. Tarsostenus Spinola (Figures 18(a)–18(m), 20(c), 20(d), 21(d), and 23(g)). Tarsostenus Spinola [12]. Type species: *Clerus univittatus* Rossi [39]. By monotypy. Corporaal [5]. Crowson [40]. Ekis and Gupta [41]. Matthews [22]. Opitz [42]. Kolibáč [6].

Synapotypic Characteristics. Aedeagus inverted, one pair of accessory gland.

Diagnosis. Specimens belong to this genus if their pronotum lacks a lateral tubercle, the dorsolateral ridge is present only in pronotal basal half, and the elytra are devoid of 2° setae.

Description. Size: length 3.5–8.0 mm; width 1.0–2.0 mm. *Form* (Figure 23(g)): oblong, narrow rectangulate, about 4 times longer than broad. *Vestiture*: disc of cranium and sides of pronotum densely vested with pale setae, pronotal middle sparsely setose, elytral disc vested with 1° setae, 2° setae absent. *Head* (Figures 18(a), 18(b), and 20(c)): cranium quadrate, frons much wider than width of eye, indented with large setiferous punctations; gula (Figure 18(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 20(d)), laterolacinia present, terminal palpomere subsecuriform; labium, ligula deeply incised, terminal palpomere subsecuriform; eyes small, coarsely faceted, ocular notch small;

antenna (Figure 18(i)), capitate, capitulum lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres slightly expanded, antennomeres 9 and 10 subtriangular, antennomere 11 ovoid. *Thorax*: pronotum (Figures 18(c) and 18(d)), quadrate, disc at sides indented with large setiferous punctations, disc at middle sparsely punctated, dorsolateral ridge extends from posterior angle to anterior midpoint, fractured by coarse punctations, sclerotized region above pronotal projection not glabrous, prebasal fissure shallow, prointercoxal process not expanded distally, pronotal projections short; elytron sculptured with round asetiferous punctations, punctations seriate and extend to elytral distal two-thirds, 1° setae always adjacent to asetiferous punctations, 2° setae absent, interstitial spaces smooth, epipleural fold laterally positioned, very narrow to elytral apex, anterior margin carinate; metathoracic wing (Figure 18(e)), wedge cell open; metendosternite (Figure 18(g)), with furcal lamina, furcal anterior plate shallow; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus (Figure 18(g)), shorter than length of abdomen, phallobase not reduced, lobate, lobes fimbriate; phallic lateral plates narrow, phallobasic rod linear, phallic apex robust, spicular plates narrow, slightly acuminate, spicular apodemes not fused (Figure 18(f)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal* (Figure 18(m)): no external evidence of ventricular crypts, four cryptonephridial malpighian tubules, stomodaeal valve comprised of four primary lobes, dorsal and ventral lobes reduced in size. *Male mesodermal internal reproductive organs* (Figure 18(k)): one pair of accessory gland. *Female mesodermal internal reproductive organs* (Figure 18(l)): spermathecal capsule ovoid, well sclerotized, spermathecal gland attached to apex of spermathecal capsule.

Distribution. Tarsostenus univittatus (Rossi) and *T. carus* (Newman) are cosmopolitan. Other species are known from Australia and New Caledonia.

Species Examined. Tarsostenus carus (Newman), *T. univittatus* (Rossi), and one undescribed species.

Notes. Kolibáč [43] presented a variety of illustrations about the organs of *T. univittatus* (Rossi).

4.5.19. Thriocera Gorham (Figures 19(a)–19(f), 20(e), 19(f), 21(c), and 23(h)). Thriocera Gorham [44]. Type species: *Corynetes pectoralis* Klug [45]. By original designation. Corporaal [5].

Synapotypic Characteristics. A uniquely derived characteristic has not been found.

Diagnosis. This is the only genus of Tarsosteninae which is oblong-short, lacks asetiferous punctations on the elytral disc, and has a 0-2-2 tibial spur formula.

Description. Size: length 3.6–7.0 mm; width 1.2–2.5 mm. *Form* (Figure 23(h)): oblong short rectangulate to narrow rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested profusely with erect setae, elytral disc vested profusely with tall erect setae and shorter decumbent setae. *Head* (Figures 19(a), and 19(b)): cranium quadrate, frons much wider than width of eye, indented with very small setiferous punctations; gula (Figure 19(a)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 20(f)), laterolacinia absent, terminal palpomere subsecuriform; labium, ligula not incised, terminal palpomere subsecuriform; eyes small, finely faceted, ocular notch large; antenna (Figure 21(c)), capitate, capitulum compact, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres filiform, capitular antennomeres expanded, antennomeres 9 and 10 expanded, antennomere 11 ovoid. *Thorax*: pronotum (Figures 19(c) and 19(d)), quadrate, disc indented profusely with small setiferous punctations, lateral margins variously convex, dorsolateral ridge extends from posterior angle to anterior angle, not fractured and with smooth surface, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process expanded distally; pronotal projections short; elytron sculptured with numerous setiferous punctations, punctations not seriate, basal uncus variously developed, with or without elongate setae, epipleural fold laterally positioned, narrowed abruptly narrowed at elytral posterior two-thirds, anterior margin carinate; metathoracic wing with wedge cell open; metendosternite with furcal lamina, furcal anterior plate not prominent; legs, tibial spur formula 0-2-2, tarsal pulvillar formula 3-3-3, unguis with denticle. *Abdomen*: aedeagus (Figure 19(f)), shorter than length of abdomen, phallobase not reduced, not lobate, not fimbriate; phallic lateral plates narrow, phallobasic rod linear, phallic apex globose, spicular plates triangular and acuminate, spicular apodemes fused in posterior third (Figure 19(e)), intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: not studied.

Distribution. Known only from Africa.

Species Examined. Thriocera pectoralis (Klug) and 12 other species which may or not be described.

4.5.20. Thriocerodes Wolcott & Dybas (Figures 19(g)–19(j), 21(a), 21(b), and 23(i)). Thriocerodes Wolcott & Dybas [46]. Type species: *Incorynetes bifasciatus* Pic [47]. Designation by Kolibáč [6]. Corporaal [5].

Synapotypic Characteristics. Phallic apex digitiform, CuA vein oblique.

Diagnosis. *Blackburniella* Chapin, *Fallopylus* gen. nov., *Tarsostenodes* Blackburn, *Tarsostenus* Spinola, and *Thriocerodes* Wolcott & Dybas are characterized by having a 1-2-1 tibial spur formula. Specimens of *Thriocerodes* may be distinguished from specimens of the other aforementioned genera by having a transverse pronotum.

Description. *Size*: length 3.0–6.0 mm; width 1.0–2.0 mm. *Form* (Figure 23(i)): oblong short rectangulate, about 3 times longer than broad. *Vestiture*: disc of cranium and pronotum vested profusely with pale setae, elytral disc with serially arranged asetose punctations and with 1° setae and shorter serially arranged 2° setae. *Head* (Figures 19(g), 19(h), and 21(a)): cranium quadrate, frons much wider than width of eye, indented with very small setiferous punctations; gula (Figure 19(g)), large, trapezoidal, sutures oblique, gular processes widely separated, processes in form of two setiferous tubercles; labrum short, not deeply incised, transverse tormal processes confluent, epipharyngeal plate very small; mandible, body short, anterior and medial dens well developed, posterior dens not well developed, penicillus well developed; maxilla (Figure 21(b)), laterolacinia present, terminal palpomere subsecuriform; labium, ligula incised, terminal palpomere subsecuriform; eyes large, coarsely faceted, ocular notch large; antenna, capitate, capitulum lax, scape about as long as combined length of pedicel and antennomere 3, funicular antennomeres subfiliform, capitular antennomeres slightly expanded, antennomeres 9 and 10 expanded, antennomere 11 subquadrate. *Thorax*: pronotum (Figures 19(i) and 19(j)), subquadrate, lateral margins with median tubercle, dorsolateral ridge extends from posterior angle to anterior angle, sclerotized region above pronotal projection glabrous, prebasal fissure shallow, prointercoxal process not expanded distally; pronotal projections short; elytron sculptured with serially arranged asetiferous punctations, epipleural fold laterally positioned, narrowed to elytral apex, anterior margin carinate; metathoracic wing with wedge cell open, CuA vein oblique; metendosternite with furcal lamina, furcal anterior plate prominent; legs, tibial spur formula 1-2-1, tarsal pulvillar formula 3-3-3, unguis without denticle. *Abdomen*: aedeagus shorter than length of abdomen, phallobase not reduced, lobate, not fimbriate; phallic lateral plates narrow, phallobasic rod linear, phallic apex digitiform, spicular plates triangular, not acuminate, spicular apodemes fused or not, intraspicular plate rod shaped; ovipositor, ventral and dorsal laminae unilobed, laminal rod present; distal margin of pygidium not incised, distal margin of male sixth sternite slightly incised. *Alimentary canal*: not studied. *Male mesodermal internal reproductive organs*: not studied. *Female mesodermal internal reproductive organs*: spermathecal capsule well sclerotized, spermatheca gland attached to subapex of spermathecal gland, saccular bursa copulatrix well developed.

Distribution. This genus is known from Australia.

Species Examined. *Thriocerodes bifasciatus* (Pic), *T. bipartitus* (Pic), *T. corporaali* Wolcott & Dybas, *T. pygmaeus* (Blackburn), *T. pyloides* Kolibáč, *T. rolciki* Kolibáč, and four undescribed species.

Notes. Kolibáč [6] provides illustrations of species of *Thriocerodes*.

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