Psyche

Hindawi Publishing Corporation
https://www.hindawi.com

Volume 2013

Psyche: A Journal of Entomology

Psyche: A Journal of Entomology

Volume 2013

ISSN: 0033-2615 (Print), ISSN: 1687-7438 (Online), DOI: 10.1155/6152

Contents

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 865102, 5 pages
http://dx.doi.org/10.1155/2013/865102

Research Article

Application of Bait Treated with the Entomopathogenic Fungus *Metarhizium anisopliae* (Metsch.) Sorokin for the Control of *Microcerotermes diversus* Silv.

Amir Cheraghi, Behzad Habibpour, and Mohammad Saied Mossadegh

Department of Plant Protection, College of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz 61351-831351, Iran

Correspondence should be addressed to Amir Cheraghi; amircheraghi2009@gmail.com

Received 21 April 2012; Revised 14 December 2012; Accepted 21 December 2012

Academic Editor: Brian Forschler

Microcerotermes diversus Silvestri (Isoptera, Termitidae) is considered to be the most destructive termite in Khuzestan province (Iran), and its control by conventional methods is often difficult. Biological control using entomopathogenic fungi could be an alternative management strategy. Performance of a bait matrix treated with the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin, Strain Saravan (DEMI 001), against *M. diversus* was evaluated in this paper. The highest rate of mortality occurred at concentrations of 3.7×10^7 and 3.5×10^8 (conidia per mL). There was no significant difference between treatments, in the rate of feeding on the bait. The fungal pathogen was not repellent to the target termite over the conidial concentrations used. The current results suggest potential of such bait system in controlling termite. However the effectiveness of *M. anisopliae* as a component of integrated pest management for *M. diversus* still needs to be proven under field conditions.

1. Introduction

Currently, species in the genera, *Amitermes* and *Microcerotermes* (Termitidae), *Anacanthotermes* (Hodotermitidae), and *Psammotermes* (Rhinotermitidae), are the most important termites in Iran [1]. Majority of termites in the Khuzestan province belong to the subterranean termite group [2]. Studies show *Microcerotermes diversus* is the most destructive termite in Khuzestan province. It has a wide foraging area and is able to form secondary colonies in walls, ceilings of buildings, and in trees. This termite is also prevalent in other parts of Iran and in Iraq, Kuwait, Oman, United Arab Emirates (UAE), and Saudi Arabia and is one of the most important pests of date palms (*Phoenix dactylifera* L.) in Iran, Iraq, and Saudi Arabia [3]. Current management of subterranean termites in Iran involves the application of soil insecticides [1]. However, continuous use of chemical pesticides in the environment is a concern [4–6], especially in areas with a high groundwater table, as in the city of Ahvaz [7]. Biological control has been suggested as an alternative strategy to the widespread application of chemical pesticides. Following this interest in the use of entomopathogenic fungi to combat insect pests has increased. Application of entomopathogenic fungi against termites has the minimum negative impact on the environment [8]. There have been a number of studies evaluating the efficacy of the hypocrealean Hyphomycete, *Beauveria bassiana* (Bals.) Vuillemin, against subterranean termites [9]. Similarly Ascomycete, *Metarhizium anisopliae* (Metsch.) Sorokin, present in the soil also acting as a causal agent for "green muscardine" of insects, is an important pathogen for the biological control of pests [10, 11]. This study investigates the efficiency of cellulose bait treated with conidia of *M. anisopliae* against *M. diversus*.

2. Materials and Methods

2.1. Collection of Termites. Termites were collected from blocks of beech wood (*Fagus orientalis* Lipsky) by embedding the blocks in soil adjacent to nests in the Ahvaz region. Collected termites were then transported to the laboratory. The termites were maintained in a dark incubator at temperature

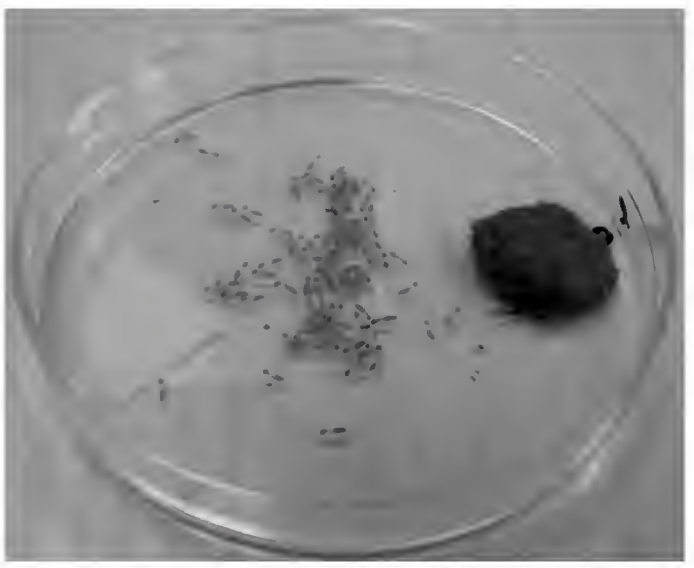


Figure 1: Petri dish-based test system to examine the response of *M. diversus* to *Metarhizium*-treated bait (BMet) versus UFP.

of 28 ± 1°C and 85 ± 5% relative humidity and kept on beech blocks (3 × 6 × 20 cm) before bioassays. Only mature worker termites were used for the test.

2.2. Fungal Isolate. *M. anisopliae* Strain Saravan (DEMI 001) from the collection maintained at Iranian Research Institute of Plant Protection was used. The fungus was cultured on Sabouraud Dextrose Agar with 1% yeast extract. Petri dishes were maintained in a dark incubator at a temperature of 28±1°C and 85±5% relative humidity. Two-to three-week-old fungal cultures were used for this experiment.

2.3. Preparation of Fungal Suspension. Conidial suspensions were prepared by lightly scraping the surface of fungal cultures with a sterile wooden spatula and suspending the conidia in 100 mL distilled sterile 0.01% of polysorbate monooleate (Tween 80). The conidial concentration of the suspensions was determined using a haemocytometer.

2.4. Bait Preparation. The bait was prepared the following way: 0.5 g of agar and 0.5 g of sugarcane molasses were poured into 25 mL of fungal conidial suspension and shaken for around 30 min until the mixture was uniform. Then 75 g of cellulose powder (SIGMA) was added and mixed well by hand. Concentrations of 1.1×10^5, 2.7×10^6, 3.7×10^7, and 3.5×10^8 conidia per mL were used, based upon preliminary experiments.

2.5. Bait Test

(A) Conidia-Treated Bait versus Untreated Filter Paper. In the first experiment, the test unit included a bait treated with *M. anisopliae* conidia (BMet) and untreated filter paper (UFP). Four grams of BMet was placed at one side of a 100 mm wide plastic Petri dish together with pieces of filter paper (Whatman No. 1001; 42 mm diameter, cut into two halves) at opposite sides of the dish (Figure 1). The filter paper was moistened with sterile distilled water. In the control, the same bait matrix treated with a solution of 0.01% Tween 80 (BCon) instead of the conidial suspension was offered. Each treatment was replicated four times. One hundred termite workers were added to each Petri dish. Units were then housed/placed in a dark incubator at 28 ± 1°C and 85 ± 5% relative humidity. Termite mortality was recorded daily for 14 days.

Table 1: LC_{50} and LC_{90} in both experiments.

Baits	LC_{50} (conidia per mL) (95% Fiducial limits)	LC_{90} (conidia per mL) (95% Fiducial limits)
BMet + UFP*	2.1×10^6 (7.3×10^5–6.1×10^6)	3.2×10^7 (1×10^7–3.2×10^8)
BMet + BCon**	3×10^6 (1.4×10^6–6.3×10^6)	7.3×10^7 (2.9×10^7–3.1×10^8)

*Bait with *Metarhizium* conidia and untreated filter paper.
**Bait with *Metarhizium* conidia and untreated bait.

(B) Bait with (BMet) and without (BCon) Metarhizium Conidia. The second experiment aimed to explore whether the presence of untreated bait (BCon) affected the consumption of bait treated with *Metarhizium* conidia (BMet). In this test, 4 g of BMet was placed on one side of a Petri dish and 4 g of BCon at the opposite side. Both baits were again placed on top of sections of filter paper as described above.

2.6. Statistical Analysis. Mortality data was subjected to angular transformation and analyzed using analysis of variance (ANOVA). PROC MIXED was used in the SAS software (SAS Institute, 2000). Mean was compared by the least significant difference (LSD) at $\alpha = 0.05$ after ANOVA (SAS Institute, 2000). Corrected mortality from fungal treatments was calculated using the formula by Abbott (1925). Graphs were plotted using Excel 2007 software.

3. Results

(A) Conidia-Treated Bait (BMet) versus UFP. In the experiment comparing treated bait (BMet) and untreated filter paper (UFP), there was a significant dose effect on *M. diversus* mortality (ANOVA $F = 29.75$, $df = 14$, $P < 0.0001$). The LC_{50} and LC_{90} values (Table 1) were 2.1×10^6 and 3.2×10^7 conidia per mL, respectively. Table 2 shows values of LT_{50} and LT_{90} for the same test. The highest and lowest levels of LT_{50} and LT_{90} were observed at the concentrations of 1.1×10^5 and 3.5×10^8 conidia per mL, respectively. At concentrations of 3.7×10^7 and 3.5×10^8 conidia per mL, the rate of mortality was highest with 100%. There was no significant difference between the two lower concentrations of 1.1×10^5 and 2.7×10^6 conidia per mL; both gave less than 40% mortality (Figure 2). However, the rate of mortality was significantly different from the mortality in the controls at all concentrations (ANOVA $F = 85.44$, $df = 4$, $P < 0.001$).

The feeding rate on untreated filter paper in the presence of BMet is shown in Figure 3. Only the rate of feeding on cellulose compound with a concentration of 3.5×10^8 conidia per mL was significantly less than that for the other treatments, except for the next lowest dose, 3.7×10^7 conidia per mL (ANOVA $F = 0.67$, $df = 4$, $P = 0.62$).

Table 2: LT_{50} and LT_{90} in both experiments.

Concentration (conidia per mL)	Baits	LT_{50} (day) (95% Fiducial limits)	LT_{90} (day) (95% Fiducial limits)
1.1×10^5	BMet + UFP*	—	—
	BMet + BCon**	—	—
2.7×10^6	BMet + UFP	11.12 (9.93–12.85)	—
	BMet + BCon	—	—
3.7×10^7	BMet + UFP	1.33 (1.28–1.39)	2.24 (2.14–2.36)
	BMet + BCon	4.22 (3.42–4.95)	12.71 (10.41–17.01)
3.5×10^8	BMet + UFP	1.01 (1–1.12)	1.54 (1.24–1.65)
	BMet + BCon	1.47 (0.99–1.91)	2.37 (1.83–4.08)

*Bait with *Metarhizium* conidia and untreated filter paper.
**Bait with *Metarhizium* conidia and untreated bait.
The high values of LT_{50} and LT_{90} are not reported.

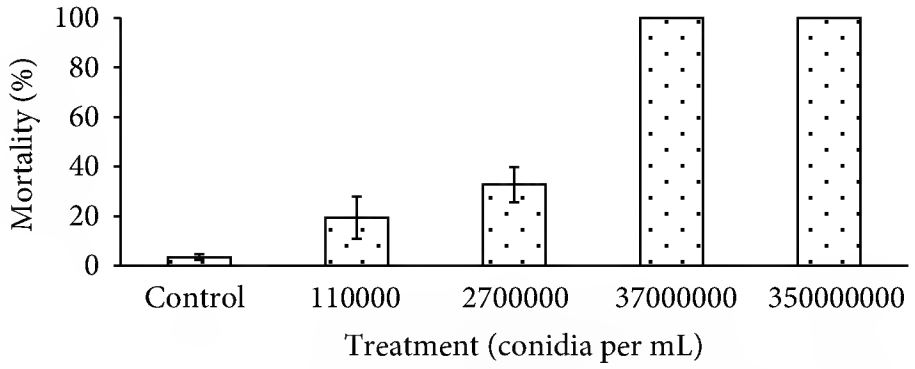


Figure 2: Effect of conidial concentration in the bait (BMet) on *M. diversus* mortality in the presence of UFP. Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

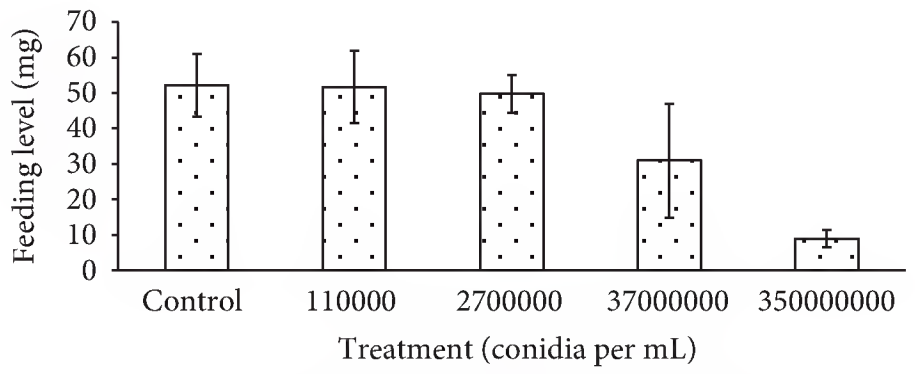


Figure 3: Effect of conidial concentration on mean *M. diversus* feeding rate (mg dry weight) on untreated filter paper in the presence of fungus-treated cellulose compound, as affected by conidial concentration. Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

Figure 4 shows the effect of conidial concentration on the mean feeding rate on BMet. Feeding on BMet was not significantly different from that of BCon and the same for all four conidial concentrations.

(B) Bait with (BMet) and without (BCon) Metarhizium Conidia. The values of LC_{50} and LC_{90} for BMet versus BCon against *M. diversus* is represented in Table 1. The rate of LC_{50} and LC_{90} was achieved at 3×10^6 and 7.3×10^7 conidia per mL respectively (ANOVA $F = 57.92$, $df = 14$, $P < 0.0001$). Table 2 shows the rate of LT_{50} and LT_{90} for the same test. The highest and the least level of LT_{50} and LT_{90} belonged to concentrations of 1.1×10^5 and 3.5×10^8 conidia per mL respectively.

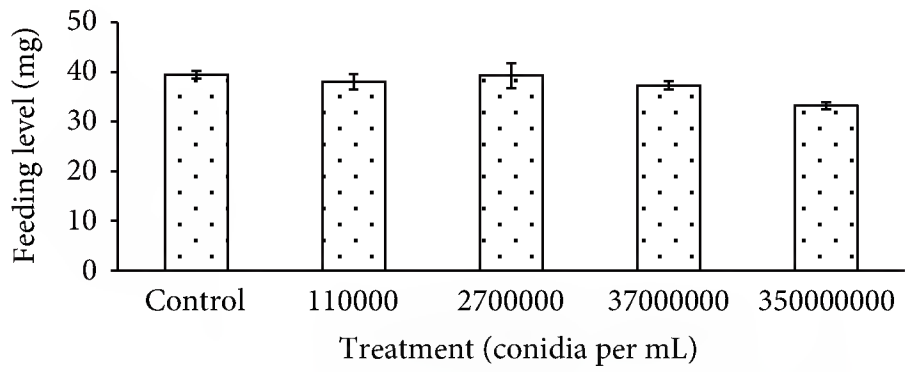


Figure 4: Effect of conidial concentration on the mean *M. diversus* feeding rate on *Metarhizium*-treated cellulose compound in the presence of UFP. Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

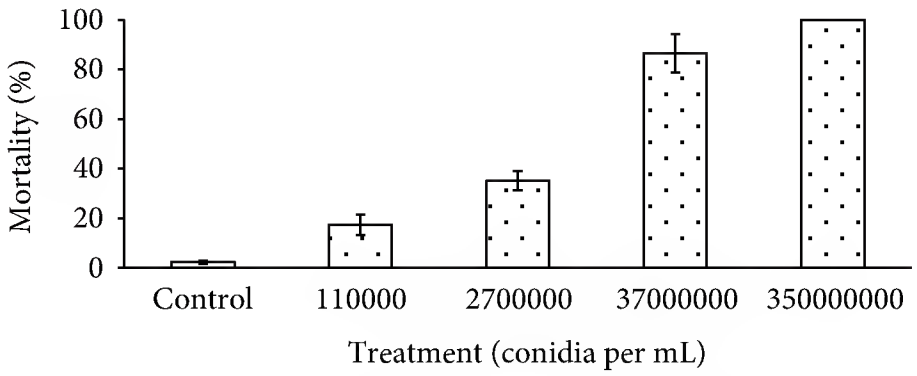


Figure 5: Effect of conidial concentration in bait (BMet) on mortality of *M. diversus* in the presence of untreated bait (BCon); same letter above the bars indicates absence of a significant difference at $P = 0.05$.

The comparison of mean mortality is shown in Figure 5. Overall, there was a significant difference in the rate of mortality between treatments. The maximum rate of mortality was observed at concentration of 3.5×10^8 conidia mL^{-1} (ANOVA $F = 99.76$, $df = 4$, $P < 0.0001$).

Figure 6 shows the comparison of mean consumption rates on BCon. The feeding rate did not differ between treatments (ANOVA $F = 2.08$, $df = 4$, $P = 0.3996$). Figure 7 shows the comparison of the mean feeding rate. The feeding rate did not show any significant difference across treatments (ANOVA $F = 0.41$, $df = 4$, $P = 0.7962$).

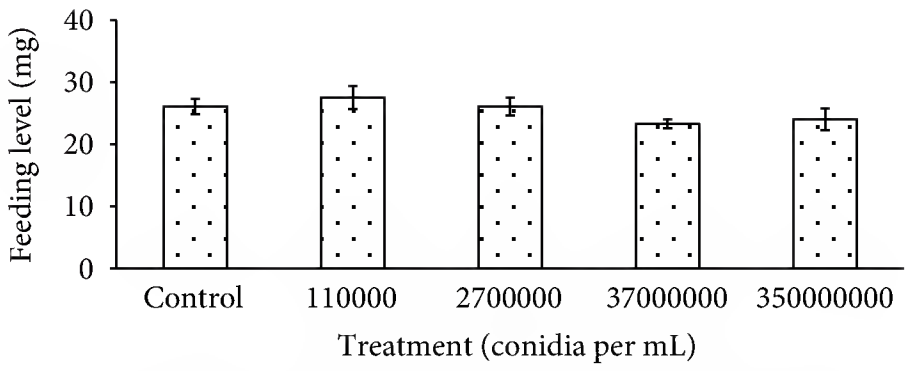


Figure 6: Effect of conidial concentration in bait (BMet) on *M. diversus* feeding on BMet in the presence of untreated bait (BCon). Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

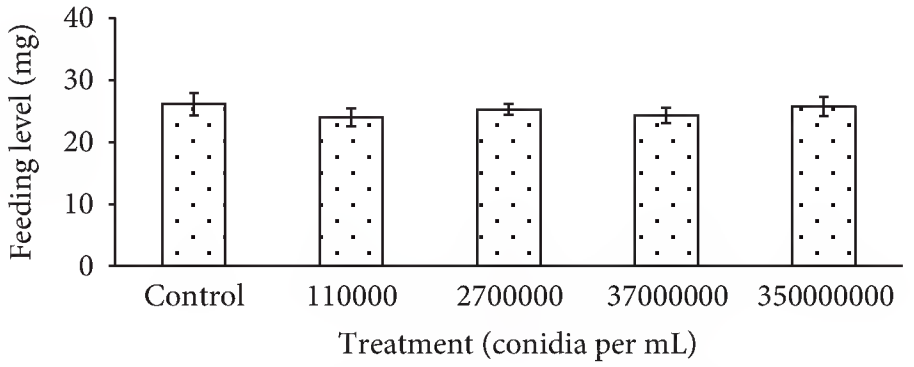


Figure 7: Effect of conidial concentration on the mean feeding rate of *M. diversus* on fungus-treated cellulose compound in the presence of untreated cellulose compound. Same letter above the bars indicates absence of a significant difference at $P = 0.05$.

4. Discussion

The results obtained in this experiment show best values of LC_{50} and LC_{90} were obtained when BMet was offered with UFP than when offered with BCon. The same was true for LT_{50} and LT_{90} values in both experiments. The type of untreated component in the chosen experiments has shown to have caused this difference. Filter paper was the least attractive food compared to the matrix of BMet, making them feed more on BMet and hence had higher exposure to conidia. But when offered with BMet and BCon at the same time, their overall exposure to conidia was reduced since they had chosen to feed on both substrates.

The overall mortality rate increased with higher concentrations of conidia. The means of bait consumption did not show any significant differences between treatments. Hence, the conidia of the *M. anisopliae* isolate used in our study were not repellent to *M. diversus*. Significantly reduced feeding on the bait matrix at the highest conidia dose (Figure 3) is due to high mortality of workers.

Bayon et al. also observed that conidia of *M. anisopliae* were not repellent for *Reticulitermes santonensis* Feytaud and hence could be added readily to baits [8]. Effective concentrations of *M. anisopliae* were also not repellent in cellulose powder baits that Wang and Powell offered to *Reticulitermes flavipes* Kollar and *Coptotermes formosanus* Shiraki [12]. Their baits with conidia eliminated groups of termite *in vitro*. In addition, it was stated that more attractive bait formulations may be required for increasing impact of *M. anisopliae* against their target species.

The results obtained from this study show good potential for using baits with entomopathogenic fungus as an active ingredient in controlling pest termites. Irrespective of many issues cited in the literature, methods are available to improve the efficiency of entomopathogenic fungi against termites. One of the avenues is to develop a suitable matrix as carrier of fungal pathogens that is readily acceptable and consumed by termites over other food items. Ramakrishnan et al. showed that a very targeted use of pesticides such as Imidacloprid in sublethal doses together with fungal pathogen can enhance performance of the fungi [13]. Also Hussain et al. used a pesticide formulation containing entomopathogenic fungi as well against termites [14]. The compatibility of an entomopathogenic fungus formulated for use with another toxicant must be tested in any effort to integrate control methodologies.

Acknowledgments

This research was supported by Shahid Chamran University of Ahvaz, Iran. The authors thank M. Lenz (Canberra, Australia) for comments on an earlier version of the paper.

References

[1] B. Habibpour, *Laboratory and field evaluation of bait-toxicants for suppression subterranean termite populations in Ahvaz (Iran) [Ph.D. thesis]*, Department of Plant Protection, College of Agriculture, Shahid Chamran University of Ahvaz, Khuzestan, Iran, 2006.

[2] M. J. Pearce, *Termites: Biology and Pest Management*, CAB International, New York, NY, USA, 1997.

[3] J. W. M. Logan and A. El Bakri, "Termite damage to date palms (*Phoenix dactylifera* L.) in Northern Sudan with particular reference to the dongle district," *Tropical Science*, vol. 30, pp. 95–108, 1990.

[4] N. Y. Su, "Evaluation of bait-toxicants for suppression of subterranean termite populations," *Sociobiology*, vol. 19, pp. 211–220, 1991.

[5] N. Y. Su, P. M. Ban, and R. F. Scheffrahn, "Suppression of foraging population of the Formosan subterranean termite (Isoptera: Rhinotermitidae) by field application of a slow-acting toxicant bait," *Journal of Economic Entomology*, vol. 84, no. 5, pp. 1525–1531, 1991.

[6] M. Verma, S. Sharma, and R. Prasad, "Biological alternatives for termite control: a review," *International Biodeterioration and Biodegradation*, vol. 63, no. 8, pp. 959–972, 2009.

[7] B. Habibpour, M. S. Mossadegh, G. Henderson, and S. Moharramipour, "Laboratory evaluation of two insect growth regulators (IGRs) on *Microcerotermes diversus* (Silvestri) (Isoptera: Termitidae) in Southwest Iran," *Sociobiology*, vol. 50, no. 3, pp. 1199–1209, 2007.

[8] I. L. Bayon, D. Ansard, C. Brunet, S. Girardi, and I. Paulmier, "Biocontrol of Reticulitermes santonensis by entomopathogenic fungi improvement of the contamination process," IRG/WP/DOC 00-10359, The International Research Group on Wood Protection, 2000.

[9] J. E. M. Almeida, S. B. Alves, and R. M. Pereira, "Selection of *Beauveria* spp. isolates for control of the termite Heterotermes

tenuis (Hagen, 1858)," *Journal of Applied Entomology*, vol. 121, no. 9-10, pp. 539–543, 1997.

[10] D. W. Roberts and R. J. St. Leger, "*Metarhizium* spp., cosmopolitan insect-pathogenic fungi: mycological aspect," *Advances in Applied Microbiology*, vol. 54, pp. 1–70, 2004.

[11] M. A. Tajick Ghanbary, A. Asgharzadeh, A. R. Hadizadeh, and M. Mohammadi Sharif, "A quick method for *Metarhizium anisopliae* isolation from cultural soils," *American Journal of Agricultural and Biological Science*, vol. 4, no. 2, pp. 152–155, 2009.

[12] C. Wang and J. E. Powell, "Cellulose bait improves the effectiveness of *Metarhizium anisopliae* as a microbial control of termites (Isoptera: Rhinotermitidae)," *Biological Control*, vol. 30, no. 2, pp. 523–529, 2004.

[13] R. Ramakrishnan, D. R. Suiter, C. H. Nakatsu, R. A. Humber, and G. W. Bennett, "Imidacloprid-enhanced *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) susceptibility to the entomopathogen *Metarhizium anisopliae*," *Journal of Economic Entomology*, vol. 92, no. 5, pp. 1125–1132, 1999.

[14] A. Hussain, S. Ahmed, and M. Shahid, "Laboratory and field evaluation of *Metarhizium anisopliae* var. anisopliae for controlling subterranean termites," *Neotropical Entomology*, vol. 40, no. 2, pp. 244–250, 2011.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 568536, 5 pages
http://dx.doi.org/10.1155/2013/568536

Research Article

Nonintegrated Host Association of *Myrmecophilus tetramorii*, a Specialist Myrmecophilous Ant Cricket (Orthoptera: Myrmecophilidae)

Takashi Komatsu,[1] Munetoshi Maruyama,[2] and Takao Itino[1,3]

[1] *Department of Biology, Faculty of Science, Shinshu University, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan*
[2] *Kyushu University Museum, Hakozaki 6-10-1, Fukuoka 812-8581, Japan*
[3] *Institute of Mountain Science, Shinshu University, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan*

Correspondence should be addressed to Takashi Komatsu; corocoro1232000@yahoo.co.jp

Received 10 January 2013; Accepted 19 February 2013

Academic Editor: Alain Lenoir

Myrmecophilus ant crickets (Orthoptera: Myrmecophilidae) are typical ant guests. In Japan, about 10 species are recognized on the basis of morphological and molecular phylogenetic frameworks. Some of these species have restricted host ranges and behave intimately toward their host ant species (i.e., they are host specialist). We focused on one species, *M. tetramorii*, which uses the myrmicine ant *Tetramorium tsushimae* as its main host. All but one *M. tetramorii* individuals were collected specifically from nests of *T. tsushimae* in the field. However, behavioral observation showed that all individuals used in the experiment received hostile reactions from the host ants. There were no signs of intimate behaviors such as grooming of hosts or receipt of mouth-to-mouth feeding from hosts, which are seen in some host-specialist *Myrmecophilus* species among obligate host-ant species. Therefore, it may be that *M. tetramorii* is the species that is specialized to exploit the host by means other than chemical integration.

1. Introduction

Myrmecophilus (Orthoptera: Myrmecophilidae) is the only genus of orthopteran myrmecophilous insect [1]. About 60 species are described, and all of them are myrmecophilous species. These inquiline crickets live in ant nests and exploit food resources in diverse ways (i.e., eating ant eggs, larvae, and nest debris; licking the surfaces of the ants' bodies; disrupting ant trophallaxis; or feeding via direct mouth-to-mouth transfer) [2–8]. Some *Myrmecophilus* species mimic the ant colony's chemicals by acquiring cuticular hydrocarbons from the ants via physical contact to establish a "chemical mimicry" [5–7].

In Japan, at least 10 species of *Myrmecophilus* are recognized on the basis of differences in the surface structure of the body and are collected from the nests of specific ant species [9]. By using molecular phylogenetic methods, we previously found [10] that Japanese *Myrmecophilus* crickets can be grouped into at least two types on the basis of their host specificity: one is commensally associated with a few ant species (specialist) and the other with many ant species or genera (generalist). This interesting differentiation of host specificities among congeneric species raises the question of whether behavioral differentiation also occurs.

The host ranges of some parasitic organisms are associated with the organisms' degree of behavioral specialization in relation to exploitation of food resources [11–14]. We observed the parasitic behaviors of two types of *Myrmecophilus* species, one of which used only a few ant species, the other, several ant species [8, 15]. From these observations, we hypothesized that all specialist *Myrmecophilus* species always show intimate behavior toward their host ant species.

The Japanese species *Myrmecophilus tetramorii* Ichikawa, which is distributed on the Japanese mainland islands of Honshu, Shikoku, and Kyushu, uses a few ant species as hosts [16]. The main host species is the myrmicine ant *Tetramorium*

tsushimae [16], but the details of the cricket's interaction with its host ant are unknown. If *M. tetramorii* is a specialist of *T. tsushimae*, like other specialist *Myrmecophilus* species [8, 15], it may show some intimate behaviors toward this ant.

We conducted exhaustive sampling across Japan to count the individuals of *M. tetramorii* collected from *T. tsushimae* nests. In addition, we observed the crickets' feeding behaviors and their interaction with ants in the laboratory.

2. Materials and Methods

2.1. Field Survey. Sampling was conducted from 2004 to 2008 in or around hardwood tree stands ranging from Honshu to Kyushu (total 88 sites), Japan. This sampling was conducted as part of our work about molecular phylogeny of Japanese *Myrmecophilus* crickets. Adult or nymph crickets were collected from host-ant nests. At each sampling site, we located all ant nests within 20 study plots, each 2 m × 5 m per randomly selected unit area (30 m × 30 m). Once a nest was located, we collected as many crickets as possible by excavating the nest if it was subterranean or spraying an insect repellent (to keep mosquitoes out) into the nest if it was arboreal. Most of ant species tend to avoid insect repellent (Komatsu and Maruyama's personal observations). So when repellent was sprayed into the entrance of ant nest, a lot of ant workers cause panic and escape out of nest, together with some individuals of myrmecophilous insects that contain *Myrmecophilus* crickets. The crickets were immediately preserved in 100% ethanol. We sorted individuals of *M. tetramorii* from all of the samples to count them and determine their host ant species. Generally, identification of *Myrmecophilus* by eye is difficult. However, *M. tetramorii* is easily distinguished from other species because of the specific shape of its body hair [9].

We also collected live *M. tetramorii* ($n = 20$) and a colony of *T. tsushimae* (about 200 workers and some dozens of larvae) to use them in experiments. All cricket individuals were collected from the same colony. Prior to the observation on cricket-ant interactions, ants and crickets were reared together for at least 3 days in a small plastic container (10 cm × 10 cm × 10 cm).

2.2. Cricket-Ant Interactions. Behavioral observations were performed by the same method we used previously [8, 15]. Four crickets and 20 to 30 *T. tsushimae* ant workers were released into a small plastic container (10 cm × 10 cm × 10 cm); they were supplied only with water and left undisturbed for 24 h. The next day, we placed 5 ant larvae from collected colony of *T. tsushimae* into the container, as well as a dead mealworm and 50% sugar water; these items closely approximated the foods of ant crickets and ants in the wild [1]. The ant larvae and the dead mealworm were placed on the floor of the container, and the sugar water was absorbed into a ball of cotton and placed on a 1 cm high stand that only the ants could climb and the crickets could not feed upon directly. We then recorded the number of times in 1 h that each cricket (a) was attacked by ants (i.e., the ants opened their mandibles and pursued or bit the cricket) and immediately escaped from the ant; (b) fed directly on the items provided; (c) groomed an ant body; (d) disrupted trophallaxis between ants; and (e) fed via direct mouth-to-mouth transfer from the ants. Each cricket individual was distinguishable by subtle disparity of body size or body color. We repeated these observations 5 times with different sets of crickets and ants. These results were compared with those from our previous study of one clade within *M. kubotai* [10, 15] that lives sympatrically with *M. tetramorii* and also uses *T. tsushimae* frequently as a main host.

Table 1: Host ant species investigated and numbers of *Myrmecophilus* spp. and *M. tetramorii* crickets collected.

Host subfamily	Host genus	Host species	Total no. of crickets	No. of *M. tetramorii*
Formicinae	*Camponotus*	*japonicus*	8	0
		obscuripes	1	0
	Formica	*hayashi*	4	0
		japonica	17	1
		sanguinea	1	0
		yessensis	1	0
	Lasius	*capitatus*	1	0
		flavus	5	0
		fuji	3	0
		japonicus	40	0
		nipponensis	7	0
		sakagamii	2	0
		spathepus	5	0
		orientalis	2	0
		umbratus	1	0
	Polyrhachis	*lamellidens*	1	0
	Polyergus	*samurai*	2	0
Myrmicinae	*Aphaenogaster*	*japonica*	1	0
	Myrmica	*jessensis*	1	0
		kotokui	1	0
	Pristomyrmex	*punctatus*	1	0
	Tetramorium	*tsushimae*	79	33
Termites	*Reticulitermes*	*speratus*	1	0
Outside ant nest			2	0
Total			187	34

2.3. Statistical Analyses. Behavioral differences between the two cricket species in the host colony were compared by using Wilcoxon's rank-sum test based on the averages for 20 individuals of each species. Statistical analysis was performed with the R software package [17].

3. Results

3.1. Field Survey. We collected a total of 200 *Myrmecophilus* ant crickets from the nests of 22 ant species. In addition, one cricket was collected from a termite nest and two from

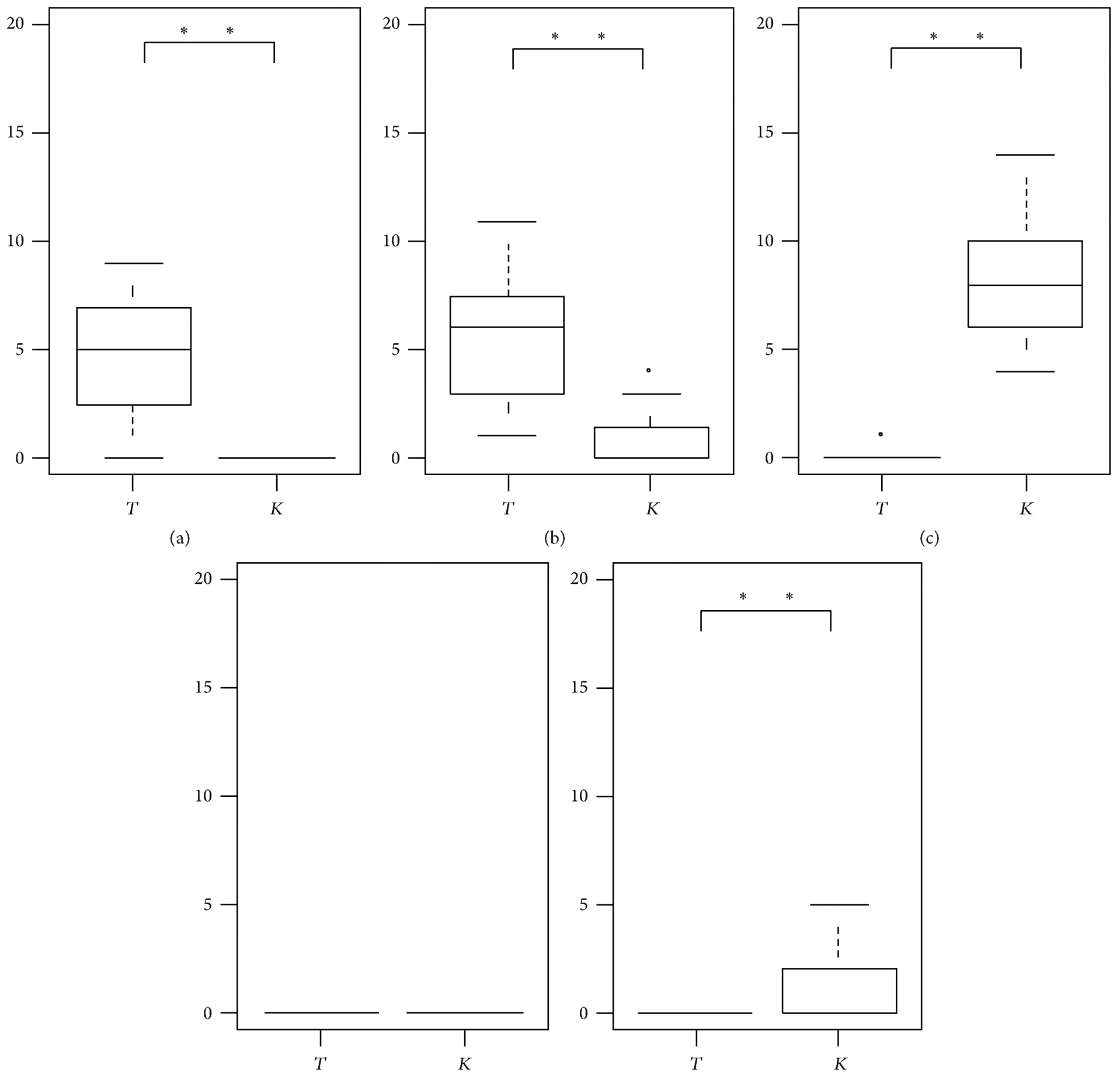


FIGURE 1: Behavior recognized in *M. tetramorii* (T) and in *M. kubotai* (K) in colonies of *T. tsushimae*. (a) Being attacked by ants and escaped from them immediately, (b) feed foods for themselves, (c) groom ant body, (d) muscle in trophallaxis between ants, (e) be done a feeding by direct mouth-to-mouth transfer by ants. Results of each behavior were based on averages of all individuals of each species ($N = 20$) observed. The box plot represents 25th, 50th, and 75th percentiles. The top and bottom whiskers represent largest and smallest nonoutlier observations, respectively. Dots represent outliners that are any value greater than 1.5 times the spread outside the closest hinge. $^{*}P < 0.05$, $^{**}P < 0.01$ by Wilcoxon rank-sum test.

outside an ant nest (Table 1). Thirty-four of the crickets were *M. tetramorii*; 33 came from *Tetramorium tsushimae* nests and 1 from a *Formica japonica* nest. All individuals of *M. tetramorii* were collected from Honshu to the west.

3.2. Cricket-Ant Interactions. Aggressive reactions by the ants to *M. tetramorii* crickets were significantly higher than those to *M. kubotai* (*M. tetramorii* versus *M. kubotai*, mean ± SD: 5.2 ± 2.8 versus 0 events/h, $P < 0.001$) (Figure 1). Both species of crickets fed directly on the items available, but feeding by *M. tetramorii* was significantly more frequent (6.1 ± 2.9 versus 0.8 ± 1.2 events/h, $P < 0.001$). *Myrmecophilus tetramorii* always ate the solid foods (ant larvae and dead insects). *Myrmecophilus kubotai* licked the surface of the ants' bodies significantly more frequently (0.2 ± 0.4 versus 8.4 ± 2.6 events/h, $P < 0.001$). Disruption of trophallaxis between

ants was not observed in either cricket species (0 versus 0 events/h). *Myrmecophilus tetramorii* showed no begging behavior toward its hosts, whereas *M. kubotai* did, especially just after fresh foods had been introduced; the cricket was fed by the ant via direct mouth-to-mouth transfer (0 versus 0.9 ± 1.5 events/h, $P < 0.001$).

4. Discussion

All but one individual of *M. tetramorii* were collected from nests of *T. tsushimae* in several regions of Japan. Therefore, this species should be classified as a specialist in terms of its host species range. Nevertheless, it ate only solid foods while it did not show any intimate behaviors toward *T. tsushimae*, like eating liquid food via direct mouth-to-mouth transfer. This means that our hypothesis that all specialist *Myrmecophilus* species always show intimate behaviors is not valid. In Japan, two other specialist species, *M. albicinctus* and one clade within *M. kubotai* [10, 15], have been collected from the nests of specific ant species and have comparatively specialized parasitic behaviors [8, 15]. They train or habituate clusters of ants and groom the bodies of the ants insistently; they even receive direct feeding. By contrast, *M. tetramorii* did not show any obvious integrated behaviors toward its host ants. Its series of behaviors, such as eating only solid foods and receiving hostile reactions from ants, resembled those of *M. formosanus*, a generalist species that can use several ant subfamilies as hosts [8]. Previous studies by using several parasite taxa suggested that parasitic behaviors of specialist species are more adapted to exploit specific host. However, at least for *Myrmecophilus*, the tendency is not always applicable.

It is unclear why *M. tetramorii* did not behave intimately toward the host ants. However, competition for food resources among *Myrmecophilus* species could be one reason. In mainland Japan, some *Myrmecophilus* species show a distinct preference for either a shaded or an open habitat [10]. In addition, some species that share the same habitat tend to differentiate host ant taxa [10]. However, *M. tetramorii* and one clade within *M. kubotai* occur exceptionally in the same open habitat and share the same ant species as their main host [10, 15]. It is possible that the trend we found here reflects the differentiation of food resources and feeding habits between two cricket species to avoid interspecific competition related to microhabitat.

Various degrees of host range or specificity, or both, are recognized in *Myrmecophilus* crickets. We showed that specialization does not necessarily correlate with intimate behavior of the ants in this genus. Nevertheless *M. tetramorii* is obviously adapted to *T. tsushimae* without sophisticated integration cues. This is surprising because congeneric species (e.g., *M. kubotai*) show such a high grade of integration. Moreover, within the genus, there are specialists and generalists and *M. tetramorii* is a specialist that is not as much integrated as a generalist. In laboratory observation, *M. tetramorii* quickly robbed food resources, such as ant larvae and dead insects, from ants. Several species of *Tetramorium* are known as the slow-moving ants [18, 19], and so is *T. tsushimae* [10]. One can argue that *M. tetramorii* is specialist species that did not develop behavioral intimacy toward host ants but that developed foraging behavior without physical contact with ants.

Acknowledgments

The authors thank S. Ueda for his comments on the paper, and they thank T. Befu, Y. Hagiwara, K. Harukawa, A. Ichikawa, S. Nomura, K. Kinomura, Y. Koshiyama, T. Kurihara, K. Maruyama, Y. Mori, T. Ito, T. Takagi, S. Takaishi, and Y. Tsuneoka for sampling. This work was supported by a Grant-in-Aid to Fellows of the Japan Society for the Promotion of Science (no. 19-6495).

References

[1] D. H. Kistner, "The social insects' bestiary," in *In Social Insects*, H. R. Hermann, Ed., vol. III, pp. 1–244, Academic Press, New York, NY, USA, 1982.

[2] E. Wasmann, "Zur Lebensweise der Ameisengrillen (Myrmecophila)," *Natur und Offenbarung*, vol. 47, pp. 129–152, 1901.

[3] K. Hölldobler, "Studien uber die Ameisengrille (*Myrmecophila acervorum* Panzer) im mittleren Maingebiet," *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, vol. 20, no. 7, pp. 607–648, 1947.

[4] W. M. Wheeler, "The habits of *Myrmecophila nebrascensis* Bruner," *Psyche*, vol. 9(1900), no. 294, pp. 111–115, 1900.

[5] G. Henderson and R. D. Akre, "Biology of the myrmecophilous cricket, *Myrmecophila manni*, Orthoptera: Gryllidae," *Journal of the Kansas Entomological Society*, vol. 59, no. 3, pp. 454–467, 1986.

[6] H. Sakai and M. Terayama, "Host records and some ecological information of the ant cricket *Myrmecophilus sapporensis* Matsumura," *Ari*, vol. 19, pp. 2–5, 1995 (Japanese).

[7] T. Akino, R. Mochizuki, M. Morimoto, and R. Yamaoka, "Chemical camouflage of myrmecophilous cricket *Myrmecophilus* sp. to be integrated with several ant species," *Japanese Journal of Applied Entomology and Zoology*, vol. 40, no. 1, pp. 39–46, 1996.

[8] T. Komatsu, M. Maruyama, and T. Itino, "Behavioral differences between two ant cricket species in nansei islands: host-specialist versus host-generalist," *Insectes Sociaux*, vol. 56, no. 4, pp. 389–396, 2009.

[9] M. Maruyama, "Four new species of Myrmecophilus (Orthoptera, Myrmecophilidae) from Japan," *Bulletin of the National Science Museum Series A*, vol. 30, no. 1, pp. 37–44, 2004.

[10] T. Komatsu, M. Maruyama, S. Ueda, and T. Itino, "mtDNA phylogeny of japanese ant crickets (Orthoptera: Myrmecophilidae): diversification in host specificity and habitat use," *Sociobiology*, vol. 52, no. 3, pp. 553–565, 2008.

[11] W. Sheehan, "Response by generalist and specialist natural enemies to agroecosystem diversification: a selective review," *Environmental Entomology*, vol. 15, no. 3, pp. 456–461, 1986.

[12] L. E. M. Vet and M. Dicke, "Ecology of infochemical use by natural enemies in a tritrophic context," *Annual Review of Entomology*, vol. 37, pp. 141–172, 1992.

[13] D. E. Dussourd, "Plant exudates trigger leaf-trenching by cabbage loopers, *Trichoplusia ni* (Noctuidae)," *Oecologia*, vol. 112, no. 3, pp. 362–369, 1997.

[14] E. A. Bernays, T. Hartmann, and R. F. Chapman, "Gustatory responsiveness to pyrrolizidine alkaloids in the *Senecio specialist*, *Tyria jacobaeae* (Lepidoptera, Arctiidae)," *Physiological Entomology*, vol. 29, no. 1, pp. 67–72, 2004.

[15] T. Komatsu, M. Maruyama, and T. Itino, "Differences in host specificity and behavior of two ant cricket species (Orthoptera: Myrmecophilidae) in Honshu, Japan," *Journal of Entomological Science*, vol. 45, no. 3, pp. 227–238, 2010.

[16] M. Maruyama, "Family Myrmecophilidae Saussure," in *Orthoptera of the Japanese Archipelago in Color*, Orthopterological Society of Japan, Ed., pp. 490–492, Hokkaido University Press, Sapporo, Japan, 1870.

[17] R Development Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for statistical computing, Vienna, Austria, 2005, http://www.r-project.org/.

[18] J. Retana and X. Cerdá, "Agonistic relationships among sympatric Mediterranean ant species (Hymenoptera: Formicidae)," *Journal of Insect Behavior*, vol. 8, no. 3, pp. 365–380, 1995.

[19] K. Fiedler, "Effects of larval diet on myrmecophilous qualities of *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenidae)," *Oecologia*, vol. 83, no. 2, pp. 284–287, 1990.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 564528, 38 pages
http://dx.doi.org/10.1155/2013/564528

Research Article

Climatic, Regional Land-Use Intensity, Landscape, and Local Variables Predicting Best the Occurrence and Distribution of Bee Community Diversity in Various Farmland Habitats in Uganda

M. B. Théodore Munyuli[1,2,3,4]

[1] *Academic Affairs and Research Program, Cinquantenaire University (UNI-50/Lwiro), D.S. Bukavu, South-Kivu Province, Democratic Republic of Congo*
[2] *Department of Biological, Agricultural and Environment Sciences, National Center for Research in Natural Sciences (CRSN/Lwiro), D.S. Bukavu, South-Kivu Province, Democratic Republic of Congo*
[3] *Centre of research for health promotion (CRPS), Department of Nutrition and Dietetics, Bukavu Higher Institute of Medical Techniques (ISTM/Bukavu), P.O. Box 3036, Bukavu, South-Kivu Province, Democratic Republic of Congo*
[4] *Department of Environmental and Natural Resource Economics, Faculty of Natural Resources and Environmental Sciences, Namasagali Campus, Busitema University, P.O. Box 236, Tororo, Uganda*

Correspondence should be addressed to M. B. Théodore Munyuli; tmunyuli@gmail.com tmunyuli@yahoo.com

Received 8 September 2012; Revised 14 December 2012; Accepted 17 December 2012

Academic Editor: Fernando B. Noll

This study was conducted in 2006 in central Uganda to provide baseline data on relationships between bee community variables and local, climatic, landscape and regional drivers affecting bee community abundance and diversity in agricultural landscapes. Bee abundance and species richness increased significantly ($P < 0.05$) with increase in percent cover of semi-natural habitats and the abundance of wild and cultivated floral resources in the landscape. There were strong linear declines ($P < 0.001$) in bee species richness and abundance with cultivation intensity. Bee species richness declined very steeply with forest distance. Bee species richness and abundance were negatively affected by land-use intensity ($P < 0.01$). Bee species richness and abundance were strongly negatively correlated ($P < 0.001$) with increase in mean annual temperatures in the previous years than in current years indicating potential vulnerability of local bee species to future climate changes. The percent cover of semi-natural habitats and natural in the farmland predicted best the occurrence and distribution in central Uganda. It is therefore recommended to policy-makers and to farmers to invest in the protection of forest fragments (and related semi-natural habitats) acting as buffer in the mitigation of negative effects of climate change on bee biodiversity and pollination services delivery.

1. Introduction

Pollinators provide a crucial ecosystem service through their role in the sexual reproduction of both wild plants and crops [1–3]. Pioneering works highlighted the fact that wild bees are by far the most important providers of vital pollination services in the world [4–7]. Their ongoing decline and potential ecological and economic consequences are therefore of major concern [8–11]. Long-term losses of certain pollinator species may threaten future ability of rural landscapes to maintain current levels of crop production.

There exist multitude factors (pressures), but currently suspected drivers (working alone or in synergy to produce negative or positive impacts) with potential effects (e.g., likely causing decline) in bees include land use change, use of pesticides (pesticide exposure) and reductions in population genetic diversity, farming and farm management practices changes, habitat loss and fragmentation, introduction of non

native invasive species, species competition for resources, parasites and pathogen spread, heavy metal pollution, and climate change [9, 11–13]. Interactions between these multiple factors and various other factors are likely; for example, nutritional stress, due to a lack of floral resources or their poor quality, may lower the tolerance of pollinators to pesticides and diseases [14].

With an estimated 20,000–30,000 species worldwide [15, 16], bees are a useful group for the study of biodiversity and ecosystem services delivery in farmland habitats [17–21]. Bees are therefore important bioindicators of ecosystem health [19, 22–24] and environmental quality in different land-uses and ecosystems [24] since they reflect ecological changes by their richness and related parameters [25] and are sensitive to environmental alterations.

Animal pollinators contribute to approximately one-third of global food production [1], and pollination by bees and other insects is responsible for as much as 70%–84% of the 264 crop species grown in the world. Globally, the economics gains from crop pollination by free ecological services provided by wild bees, honeybees, and other insect pollinators are high and have been estimated to be worth several billions US dollars per year [2, 6]. The value of pollination to agricultural production worldwide is currently estimated to be worth US$226 billion (€153 billion) per year or approximately 39% of the world crop production value (€625 billion) from the total value of 46 insect pollinated direct crop species [25, 26]. Although one-third of the world's food production relies on animals for pollination, it is, however, projected that insect pollinators (bees) may be responsible of more than one-third US$1 trillion in annual sales of agricultural products worldwide [27–30]. Challenges related to the conservation of bee faunas in tropical agricultural landscapes include the absence of basic knowledge about their biology, natural history, vulnerability to climate change and spatio-temporal distribution of communities (abundance, richness, diversity) in agricultural landscapes. In addition, factors driving the distribution of different bee species in different localities in farmlands of Uganda are not documented.

There exist several land-use pressures and land degradation problems in central Uganda; and agroforestry systems were developed and disseminated in rural areas of central Uganda as a way of maximizing yields on small scale farms [30]. In the coffee-banana agroforestry system of central Uganda, small-scale farmers grow and rely (for their livelihoods) on several foods (e.g., banana) and food crops (e.g., coffee) that highly require animal pollination to set fruit/seeds [27]. Due to negative effects of various drivers, decline in solitary and social wild bees (that are important pollinators of wild plant species and many insect-pollinated crops) has been predicted worldwide. Decline in pollinator availability may be caused to a greater extent by variation in the abundance of generalist rather than specialist bee pollinators [31, 32]. Decline in growth rates of bees may be likely affected by both abiotic factors and biotic factors.

Although pollination is a critical ecosystem service and that bees are the most important pollinators, they are endangered by intensive agricultural practices. Knowledge, on the relationships between insect pollinators and landscape structure/composition, land use change and habitat quality in Uganda, is still limited. While landscape context and habitat quality are known to influence species richness and abundance of bees [17], there is lack of information of the influences of climate factors on bee species richness and abundance. Climate change and variability may be contributing in boosting declining bee populations including afrotropical bees. Worldwide, empirical studies that take climatic variables into account are rare. However, there exist scantly speculative literature on potential effects climate change on plant-pollinator interactions and the consequence for pollination services delivery [33]. No previous studies have examined experimentally the combined (simultaneous) effects of micro, local, landscape, land-use intensity and regional and climatic factors on abundance and species richness bees in agricultural landscapes in Uganda and in Sub-Saharan Africa. However such studies are important to help in developing strategies to prevent future decline in bee species and guaranteeing future stability of pollinator-dependent crop yields and for food security of human communities depending on these crops. It is still not clear how in the future local, landscape and climatic factors will simultaneously affect the pattern of bee species richness and abundance in agricultural landscapes in Uganda.

Climate warming may interact to disrupt this crucial mutualism by altering plant chemistry in ways that alter floral attractiveness or even nutritional rewards for bees [33]. One possible effect of climate change is the generation of a mismatch in the seasonal timing of interacting organisms, owing to species-specific shifts in phenology. Local environmental conditions are the primary determinants of emergence phenology in bees and their food plants. In other words, phenology of plants and bees is regulated in similar ways by temperature, but that plants are more likely than bees to advance phenology in response to springtime warming. Different responses of bees towards climate change may lead to an increasing asynchrony in the life cycles of bees and flowering plants [29]. With the predicted climate change in interaction with land-use change and habitat alteration, bee species richness and abundance are expected to change patterns in occurrence with the resultant negative effect on pollination services delivery. Understanding how landscape characteristics (composition, structure, and configuration) affect bee biodiversity patterns and ecological processes at local and landscape scales will be critical for mitigating future negative effects of global environmental change [33] on bee biodiversity.

Climate change is only starting to shape the pollination service research agenda. The complexity of the impact of this phenomenon on bee biodiversity and pollination services delivery remains largely unveiled particularly in Sub-Saharan Africa. Future climate change is expected to have different scenarios (rate of shifts in distribution range, or rate of extirpation, rate of decline or disappearance in the landscape) upon bees. Responses of different species to change in climatic conditions are predicted to range from thriving (i.e., species capable of living under the new set of conditions) to adapting (i.e., species capable of surviving a change in global

conditions by changing their ecology, physiology, and/or distribution) and going extinct.

Responses of different bee species to climate change are not experimentally documented in Uganda and in East Africa. It may be important to identify how different bee species will adapt to future climatic change as well predicting how bee species will disappear. However, global environmental changes (driven by multiple interacting drivers/pressures) are expected to have manifold effects (and unanticipated outcomes) on bee species richness and populations and on pollination services [28]. Global warming or climate change (changes in temperature and rainfall) is likely to have significant impacts on bee species richness and populations across different locations in eastern and central Africa. Specialized species may be vulnerable and reach high levels of risk of extinction in the landscapes.

Likely impacts of climate change on bee species richness may be linked to life history traits of different bee species (phenology, sociality, and bee-host plant synchronization). Trait-based approaches to predict and analyze the effects of climate change in interaction with other local and landscape drivers have been suggested by scientists. Responses of bees may vary among taxa. Life-history traits are related to the specialization of the bee species (nesting guide and feeding habit) and to sensitivity lower/higher risk of harm from various threats.

Changing flowering phenologies under climate change is well documented in temperature regions. The impact of climate change on plant-pollinator mutualisms is little understood or well predicted in Africa. Despite the enormous economic and ecological importance of bees as pollinators, currently there are no studies investigating the interaction between bee species and abundance and historical/current climatic factors (rainfall and temperature) in Uganda and in eastern and central Africa. However, such studies are also important to monitor and prevent decline in species richness and in pollination services delivery. Now that climate change is a reality in Africa, there is an urgent need to investigate its potential impact on bee species richness and abundance to foster to speculation on potential consequences of climate change on bee richness and pollination service delivery for food security and livelihoods of people.

Overall, there is a need to know the degree at which different environmental factors may affect bee communities to better plan conservation strategies of these pollinating service agents and prevent their decline in face of future climate changes, thus guaranteeing yield stability of pollinator-dependent crops while improving small-scale farmers' food security and livelihoods. Speculating on bee species vulnerability to future changes in environmental conditions in which they are found, and modeling (predicting/forecasting) future changes in species populations may be an approach to inform conservation policy.

Factors influencing patterns of occurrence [30, 34] of bee communities in relationship to climate factors have not yet been studied in Uganda. Understanding how land-use intensity, climatic, landscape and local level-factors influence the presence/absence of different bee faunal species in different localities of an agricultural landscape can be very useful in influencing rural development policies about defining strategies to prevent future decline in face global environmental change threat.

Current bee abundance and richness may be the result of the simultaneous cumulative effects of local, landscape composition and regional/climatic factors over the recent years. Thus accounting for the recent of weather variability may be relevant in predicting future response of bee communities to climatic changes.

The general objective of this study was therefore to determine the relationships between bee community variables (abundance, species richness) and climatic, regional, landscape and local variables. The overall goal was to determine the degree to which these different variables can powerfully predict future patterns of bee communities in farmlands of central Uganda. It was hypothesized that bee abundance and species richness on agricultural landscapes in central Uganda are related to local and landscape variables, but not to regional climatic variables. The sub-hypothesis tested also whether precipitations/temperatures (climatic factors) in the current and previous year were associated with current bee abundance and richness, since precipitation is a primary driver of plant population dynamics and of bee emergence dynamics in most agricultural and natural regions [17, 18].

2. Material and Methods

2.1. Study Area. This study was conducted in the banana-coffee system of Lake Victoria Arc covering several districts of central Uganda (Figure 1). The study zone (average latitude: 0.5°31′22″; longitude: 31°11′71″; altitude: 1080–1325 m) is characterized by ferrisoils with high to medium fertility level and receives on average 1000–1800 mm of rainfall per annum on a bimodal pattern (rainy seasons: March-May, September-November; semi-dry to dry seasons: June-August, December-February) with 28.7 ± 2.77°C and 68.65 ± 8.91% of mean annual temperature and relative humidity respectively [29, 35]. But the rainfall amounts and patterns are unpredictable. The study zone belong to the Lake Victoria phytochorion [19–21] with shrubs of *Acacia* spp., legume trees, melliferous plant species, *Papyrus* and palms ranging from 2 to 15 m high dominating the remnant secondary vegetation [28]. In this study region, coffee (*Coffea canephora* Pierre ex Froehner) is the main cash crop and banana the main staple food crop. Several pollinator-dependent food and cash crops are grown in small-scale monoculture and/or polyculture fields that are integrated into this coffee-banana agroforestry system including home-gardens. There were no standard crops per study sites but most crops were found grown in almost all study sites. Crops grown as sole or in association with coffee and or banana include cassava (*Manihot esculentum* L.), sweet-potato, (*Ipomoea batatus* L.), maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogea* L.), tomato (*Lycopersicon esculentum* L.), watermelon (*Citrullus lanatus* L.), pumpkin (*Cucurbita moschata* L.), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), chilies (*Capsicum* spp.); and several other fruits, vegetables and horticultural crops (cabbage, onion, etc., egg plants,

sim-sim, etc.). The majority of these crops are grown in small-scale monoculture and or polyculture fields that are integrated into the coffee-banana agroforest production systems. The agroforestry system is also dominated by several native/indigenous, fruit and agroforestry tree species [27–30, 35].

Rural central Uganda is mosaic landscape where "islands" of patches of natural habitats (forest fragments, forest reserves, wetlands, woodlands) and linear (e.g., hedgerows) and non-linear (fallow fields, grasslands, woodlots, cattle pastures or rangelands) features of semi-natural habitats [27, 28] that serve as "field boundaries" of the variety of small-scale fields; are found scattered within agricultural matrices. Compared to other regions (districts) of the country, the study area (central Uganda) is also characterized by high demographic pressure, limited access to arable lands, continuous cultivation and over-exploited lands under unrevised land policies [18].

2.2. Study Sites. In this study region, data was collected in 26 different study sites (1 km^2 size each) with different environmental characteristics (Figure 1). The 26 study sites were chosen distant one from another to reduce on confounding factors. Prior to the selection of different study sites, a study tour of different sites was made, and sites characteristics were noted. Thus, the 26 study sites were selected along contrasting environmental gradients, farm management systems, agroecological, semi-natural habitats, vegetation characteristics, and land-use intensity gradients. The study was designed to minimize spatial autocorrelation between local and landscape-scale variables measured within study sites by maintaining a minimum distance between study sites and clusters (each cluster was composed of 2 or 4 sites located in the same zone with similar general vegetation and environmental characteristics). The minimum distance between two study sites selected within a cluster was of 2–25 km (which is beyond the normal foraging range of most pollinator species), and the minimum distance between clusters was of 50–250 km. All 26 agricultural field sites had also some forest remnant tree species retained within them, ranging from 1 to 175 trees/ha found both in crop fields as well inside remnant natural vegetations scattered inside the forest. The distance between a study site and the nearest forest fragment/wetland varied from 2 to >2000 m and the size of the forest fragments found in the vicinity of crop fields varied from very small fragments of 0.1 ha to large forests greater than 850 ha in size. Shade cover within the coffee-banana agroforests ranged from 10% to 92%, and shade tree density was of more than 5–500 trees (all species combined) per hectare, excluding the density of other agroforestry and fruit tree species. Overall, the species richness of typical coffee shade trees, agroforestry trees, forest remnant trees, and fruit trees varied from 1.23 to 15.45 species per hectare.

There exist in this study region some large monoculture plantations (sugar cane plantations, coffee plantations, tea plantations, etc.). However, compared to large scale plantations, study sites that were covered by typical banana-coffee agroforests mixed with semi-natural habitats (e.g., young fallows) and related small-scale farms were of 95%, whereas study sites located within or in the proximity of large scale plantations covered approximately 5% of the farm-landscape studied. Most study sites were less similar in terms of altitude (altitude: 1080–1325 m) and in terms of type of semi-natural habitats surrounding all 1 km^2 study sites selected. However, few study sites (16 sites) had in their immediate vicinity natural forests and/or large wetlands.

2.3. Sampling Design and Bee Sampling Methods. Bees were sampled in each of the 26 study sites. Each study site was divided into five linear transects of 1000 × 200 m each. Transects were used as basic units for bee sampling. They were also used as basic units for measuring all land-uses, habitats, and vegetation data. In each round of data collection, one transect per site was used. Thus, bees were sampled on one central transect (200 m wide × 1000 m long) per study site. A sampling belt (20 m wide × 1000 m long) was selected in the middle of each central transect as recommended [17, 18, 30] to reduce bias in bee sampling. Using a tape measure, the sampling belt of each selected central transect was divided into 10 sections (sampling plots) of the same size (20 m wide × 100 m long each). The 10 sampling plots of each sampling belt were visited at each sampling date.

Bee sampling with each of the three sampling methods was concentrated in the sampling belt. Bees were sampled using three complementary sampling methods: hand-netting, transect walk-and-counts, and pan-trapping. Multiple trapping methods are recommended to be used to catch a wide array of bees as each sampling method has their own biases [17].

Transects walks-and-counts (standardized transect walks) method was used to sample foraging bees in the sampling belt by two observers per site as recommended [29, 30, 36]. Transects walks-and-counts are one of the standard protocols for visual observations and presence recording of bees in the fields. Observations of foraging activities and bee counts were done on flowering patches found within different habitats and land-uses while walking along transects at a slow speed (<0.25 km per hour). Observations were made under conditions favorable for bee flights: sunny or cool weather and weak wind. Hand-netting ("sweep-net" sampling) of bees with a hand-net (30 cm diameter) was conducted in patches of fresh flowers in each plot immediately after visual censuses. Transects were sampled from 08 h00 to 17 h00 local time to capture bees on wild blooming plants as recommended [11, 17, 19, 34, 37, 38]. Only hand-net bee samples were transferred to zip lock plastic bags and placed in a portable cooler.

Pan traps have been used to sample (bees and other insects) for almost 2 decades [9, 11, 17]. In the pan-trapping method, a single trapping point was established within each plot. Pan traps were constructed from 236.5 mL white, blue and yellow plastic bowls [1, 9, 10, 39, 40]. The bowls (brand "Solo" supplied by Prof Simon Potts from the University of Reading, UK) were sprayed with ultra-violet bright paint colors (blue, yellow and white). Pantraps were filled with water and a small amount of detergent to reduce surface

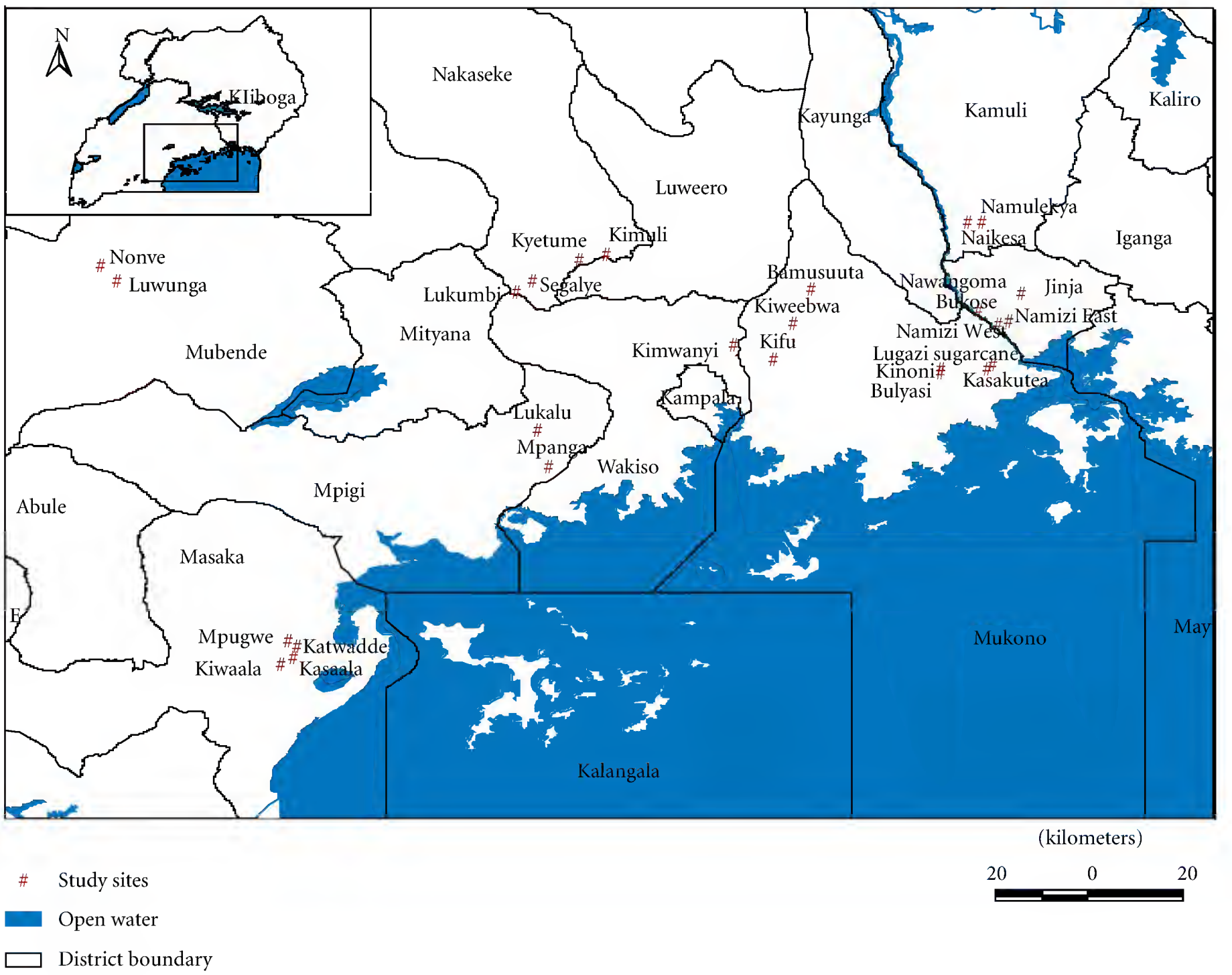


FIGURE 1: Location of study sites in which bee fauna survey was conducted in the banana-coffee growing area around Lake Victoria in Uganda in 2006.

tension to help insect sink and increase UV-light reflection [28, 29], thereby attracting bees and other insects, which fly into the water and drown.

At each sampling point, three pan traps (blue, yellow and white) were spaced 5 m apart on the ground as recommended [35]. Pan traps were placed elevated in the plant canopy at the height of flowers easily visible by flying insects. Bowls were hung on plant branches or on stakes fixed in the soil. In each pan trap, unscented, biodegradable liquid detergent was poured into pan traps (2.5 mL detergent/liter water). Pan-traps were left in place for about two days before collecting samples to reduce bias in the sampling procedure [11, 40]. Most pans used were generally placed far from tracks to reduce disturbance by curious un-informed villagers or school children. A limited number of traps were found disturbed (i.e., empty pan traps, pan-traps taken away, etc.) during the second and the fourth rounds of data collection, resulting in a few missing data points. During the rainy seasons, holes were added in the upper zone of each pan-trap to allow excess water to drain without washing away bee samples.

For each of the three sampling methods, transect walk- and counts, hand-netting, and pan-trapping [10, 18], bees were sampled during five consecutive rounds from January 2006 to December 2006 (Round 1: January-April, Round 2: May-June, Round 3: July-August, Round 4: September-October and Round 5: November-December). Sampling was conducted consistently across crop growing seasons in order to compare sample yields (bee species richness and individuals estimates) and between rainy (wet) and dry seasons. Bee fauna surveys were conducted across months of highest (September-May) and lowest (July-August) bee abundance and species richness [17]. Each round of data collection lasted 5 to 7 weeks. Across sampling methods, bees were sampled for 30-min period per sampling plot between 8 h00–17 h00. Although being are that some bees start foraging [22, 30] even before sunrise and stop soon afterwards (example: crepuscular bee species representing less than 0.1% of bee species found in central Uganda and that may be lost during bee sampling), bees were sampled between 8 h00 and 17 h00 due to time constraint and depending on local light conditions and ambient temperature. Bee specimens from

each sampling method (hand-nets, pan-traps) were strained into plastic vials containing alcohol (70% ethanol) and taken to the laboratory for identification to the highest possible taxonomic level. Bee samples were sorted out at Makerere University (Zoology department museums). The majority (95% of bee samples) were identified up to species levels at bio-systematic division of the plant protection research institute (Pretoria- South Africa). Other minor identifications were conducted at Natural History Museums-London (UK), Smithsonian Tropical Research Institute-Panama, and University of Reading (UK). The established reference collection of bees from central Uganda is deposited at Makerere University Zoology Museum.

Surveys on bee food plants and bee nest trees were conducted parallel to bee faunistic surveys. Data on different types and size of semi-natural habitats/land-uses was also collected parallel to bee surveys. During bee surveys, the number of nests and nesting sites for most bee species were counted in these different habitats/land-use types. Bee food plants and Bee nest were identified in the field with author experience. However, when in doubt, bee food plant specimens were collected and identifications to species-level were confirmed at Makerere University Herbarium.

2.4. Measurement of Correlative (Factors) Variables. In this study, different metrics (local, landscape, regional factors) affecting pollinator populations and species richness in farm-landscapes of central Uganda were measured. In this study, local variables (farm-level variables) referred to factors affecting bee communities at a local scale (1–500 m radius). Local factors are limited at an individual plot/land-use level [30]. Landscape-scale factors were referred to factors operating at a large scale level (>0.5–1 km radius) covering flight range of various typical farmland native bee species [29]. Regional variables referred to broad scaled factors that may have an indirect effect on bee activities at local level from very far. These are factors operating at a larger scale level (>1–100 km radius). Thus, few local, landscape, regional and climatic factors of importance for bees were measured at different scale levels.

Local and landscape variables were measured following approaches by previous workers [41–45]. Measured local variables included the amount of floral resources [29]. These included the percent cover of wild flowering plants (trees, shrubs and herbs) or the mass flowering of wild plants, the number of wild blooming plant species, and the percent cover of cultivated floral resources (percent of cultivated pollinator-dependent and non-pollinator dependent crops) per 1 km^2 area (site). Data on herbs were collected from ten quadrats measuring 5 m × 5 m (25 m^2) while those on shrub and trees were collected for in twenty quadrats measuring 10 m × 50 m (500 m^2). All trees with stems greater than 10 cm diameter at breast height (dbh) were recorded. Data on the number of fresh flowers were also recorded in "plots" of 5 × 5 m (25 m^2) dimension [46]. The quadrats (plots) were randomly established in each study site as recommended [22, 23] to determine the number of plant species. Measurement of wild floral resources focused only on plant in bloom. Wild blooming floral resources were measured in five samples (five rounds) as the bee data.

The percentage cover (area covered in hectares) of cultivated floral resources was determined based on land-use data collected about the proportion cover of each type of crops cultivated [1] in a 1 km^2 site area. Later, all cultivated crops were grouped in two subcategories [17, 18, 29] and their respective proportion determined based on area (ha) covered by (i) pollinator-dependent crops and (ii) by non-pollinator-dependent crops. Therefore, crops were categorized into two subgroups based on the dependence ratios used by Klein et al. [1].

Landscape-level land-use data were collected within a 1 km^2 site. Each square kilometre was delineated using a global positioning system (GPS). Because there were no previously published data on small-scale land-use patterns in the study region, to facilitate basic measurements about different land uses, the km^2 area was divided into five transects of 200 m × 1000 m [18, 28]. Here, the areas with different land-use types were measured using GPS or a tape in case of small fields (50 m × 50 m and less). Land-use types were grouped into major land-use types based on their size (0.06 ha–1 ha to 9.95–16.45 ha) and frequency of occurrence in order to calculate the area covered by semi-natural habitats, the area covered by crops and the cover of dependent and nondependent cultivated crops per km^2 area [22, 27, 30, 35]. The term semi-natural habitats included all linear (hedgerows, field margins, roadsides, track-sides, stream edges, etc.) and non-linear (fallow fields, grasslands, woodlands, woodlots, etc.) semi-natural habitats [47–49] of ecological importance for pollinators living in farm-landscapes [27, 28, 35]. These semi-natural habitats play an important role in the maintenance of many bee species in rural landscapes in Uganda [27, 29]. Semi-natural habitats have been shown to be important in structuring bee communities in agricultural landscapes [22]. Young fallow fields play a particular role as foraging habitats and bee refugia and as reservoir of other ecosystem services delivery agents in rural landscapes [22, 23, 27–30, 35]. Pollinator-dependent crops are those that require a visit to its flowers by a pollinator to set fruits/seeds [1, 29].

Three landscape variables of ecological importance [50] for bee biodiversity and pollination studies [29] in agricultural matrices were then calculated for each 1 km^2 study site: (i) the percent of semi-natural habitats; (ii) the cultivation intensity, that is, the percentage of the total land area cropped; (iii) the distance from a given study site to the nearest potential natural pollinators' source (forest and wetlands)—the distance from a large (defined) forest fragment/natural wetland [22, 30]. Distances up to 100 m were measured with a tape, otherwise with GPS (corrected to ±1 m accuracy with Pathfinder v 2.0).

Regional land-use categories (gradients) were obtained from the Makerere University Geographic Information Services [23, 28]. Broad land uses classified as low-intensity use include areas where at least three-quarters of the land is uncultivated. Medium are managed habitat types where there is an almost equal distribution of cultivated and uncultivated land. High are areas dominated by crops or livestock (such

as only one-quarter of the total land is used for other purposes rather crops and livestock). Very high represents large monoculture estates of tea, sugar, coffee, and so on [22, 23, 27–30].

Preliminary analysis of relationships between butterfly community variables (species richness and abundance) and weather/climatic factors (rainfalls, temperature, etc.) in previous years (10 years, 2 years before) and current years revealed potential influences of climate change on the distribution, occurrence, and activities of different butterfly species [17, 18]. Thus, this study aimed at verifying if this trend was the same for all farmland bee species. Therefore, data on regional climatic factors were obtained from meteorological stations located in the study area including Kamenyamigo meteorological station covering Masaka cluster (sites: Kasaala, Katwadde, Kiwaala, and Mpugwe) in Masaka district; Entebbe meteorological station covering Kalagi (sites: Bamusuuta, Kifu, Kimwanyi, and Kiweebwa), Lugazi (sites: Kasaku and Sugar), Mpigi (sites: Lukalu and Mpanga) and Mabira (sites: Bulyasi and Kinoni) clusters in Mukono district; Jinja meteorological station covering Kamuli (sites: Naikesa and Namulekya), and Bujjagali (sites: Bukose, Namizi-East, Namizi West, and Nawangoma) clusters in Kamuli district and Kiige meteorological stations covering Nakaseke (sites: Kimuli, Kyetume, Lukumbi, and Segalye) and Kaweri coffee plantation (sites: Nomve and Luwunga) clusters in Mubende district. From the raw data obtained from the different meteorological stations, a monthly mean for 10 years (1998 to 2007) of temperatures and rainfalls was calculated to see the trends in the rainfall patterns and temperature since such oscillations can affect the patterns of pollinator communities in rural landscapes. Other variables (helping in detecting current patterns of bees in relationship to past climatic events) calculated included (i) the overall mean rains (means/month/10 years); (ii) the overall daily mean minimum temperature (mean of 10 years); (iii) the overall daily mean maximum temperature (mean 10 years); (iv) the mean monthly rainfalls (2007); (v) the mean monthly maximum temperature (2005); the mean monthly maximum temperature (2006); (vi) the mean monthly maximum temperature (2007); (vii) the mean monthly minimum temperature (2005); (iix) the mean monthly minimum temperature (2006); (ix) the mean monthly minimum temperature (2007); (x) the mean monthly rainfalls (2005) and (xi) the mean monthly rainfalls (2006).

2.5. Data Analysis. Although collections were not similar, data from the three sources (transect walk-and-counts, pan-traps, and hand-nets) were pooled as recommended [18, 36] to provide total bee abundance and species richness estimates per transect/study site/sampling round. In fact, bee abundances (χ^2 2 df = 26.78, $P < 0.001$) and species richness (χ^2 2 df = 12.56, $P < 0.01$) were significantly different among the three sampling methods, but pooling data from the three different sampling methods was still conducted and motivated by the fact the interests was estimates of overall abundance and specie richness and not on comparing the efficiency of the three different sampling methods. In addition, each of three sampling methods applied can be associated with bias towards number of species and individuals detectable by the sampling method [18].

Genera-tribe richness, species richness, abundance and dominance were calculated to highlight (indicate) the structure/characteristic of bee communities studied; they were expected to be driven by various local, landscape, regional and climatic factors. Thus, species richness of some taxa (e.g., genera, tribe) was calculated as the number of species belonging to that taxa.

Bee abundance was calculated as the total number of individuals recorded per transect each sampling day. The species richness was calculated as the total numbers of bee species recorded per transect per study site each sampling day. Species dominance (D) was calculated according to Munyuli [18] and Magurran [51]: D = (abundance of a species/total abundances recorded) × 100. If $D > 5\%$, the species was termed a dominant species. Species accumulation and estimation curves were constructed/generated using the Jacknife-1 estimator [51, 52].

To determine "indicator species" or "characteristic species" of pollinator communities from farmland habitats of central Uganda, indicator (*IndVal*) method of Dufrêne and Legendre was adopted and used in this study in a modified form as recommended by Munyuli [22] to identify indicator. Indicator species are ecological "characteristic species" or ubiquitous/common species of bee communities inhabiting certain type of habitats of a given landscape [22]. Indicator species are potentially effective pollinator species delivering pollination services to wild and cultivated crops in the landscape [18, 30]. Knowledge of indicator species is important to acquire since it gives an idea on reliable spatio-temporal pollination services delivery agent species in the rural landscapes. Such knowledge may help in predicting/speculating on responses of different bee species to various drivers.

The correlation of independents variables (e.g., meteorological variables, local and landscape variables) characterizing the 26 study sites with dependent variables (bee species richness, bee abundance) were tested using Pearson correlation. The derived correlation coefficients and P-values associated with the paired variables were presented in matrix of correlation. Correlation analysis was used to determine the suite of variables most closely related to bee species richness and abundances measured. The different independents variables were checked to prevent collinearity following approaches previously described by Munyuli [30]. Based on the correlation matrix of all variables measured, only independents variables that were significantly ($P < 0.05$) related to dependent variables (bee communities) were chosen for further analyses in simple regression analyses. These illustrated the trends and magnitude of the effects of independent factors expected to affect bee communities (abundance and or species richness).

Prior for conducting analyses, Kolmogorov-Smirnov test was used to check if variables were normally distributed. Data was transformed if found necessary to meet the assumption of normality and homogeneity of variances. Variable data expressed as percentages were arcsine square-root (+0.5)

transformed. The number of species and of individuals were square root + 1 transformed and log-transformed using ln $(x + 1)$, respectively. Back-transformed data are reported [22, 23, 30].

Simple regression methods were mainly applied to explore relationships between local (abundance and richness of wild and cultivated floral resources) and landscape (cultivation intensity, amount of semi-natural habitats, and forest distance) variables and the abundance and species richness of bees. Scatter plots were used to illustrate scale dependency of bees on different local and landscape variables measured. Therefore, for all simple regression models obtained, the coefficient of determinations (R^2) (that measures the proportion of the total variance of observed data explained by predicted data) was calculated to demonstrate the level of influence of the type of variables that correlate with the abundance and species richness of bees. In other words, the coefficient of determination (R^2) was used for determining the proportion (%) of influence of all different variables on the abundance and species richness of bees. Relationship between the density of nests and bee abundance/species was explored using simple regression analysis in Minitab15. All simple regression analyses were conducted in Minitab version-15 and the results plotted.

When interested in distinguishing/exploring the combined (simultaneous) effects of multiple predictor variables (local, landscape, and climatic variables) on bee communities (species richness and abundance), generalized linear models (GLMs) were performed. Models were computed including (i) local, landscape, and climatic variables as predictor variables; (ii) bee species richness or abundance as continuous/response variable. Generalized linear models (GLMs), with normal error distribution and log-link function, followed by a likelihood ratio test, and with three iteration levels, were fitted given the type of measured response (which changes may change scale from discrete counts to continuous frequency). The generalized linear modelling (GLM) framework was constructed in STATA version 8 for windows. Models were simplified using the Akaike's Information Criterion (AIC) and a drop function of variables for collinearity reasons (if any). The significance of the simultaneous effects of different variables was tested using Z test.

The effects of categorical predictors (land-use intensity gradient categories) on bee species richness and abundance were analyzed by applying a general linear model (GLM). Analysis of variance (ANOVA) in Minitab statistical software version-15 was conducted with bee community variables (abundance and species richness) as the dependent variables, and the categorical variables (low, medium, high, and very high) as fixed factors. The Tukey tests were used as post hoc tests at $P < 0.05$. Differences between means were inspected using Tukey's honestly significant difference (HSD).

3. Results

3.1. Characteristics of Bee Assemblages. In total, 652 species, (Table 8) representing 76 genera were recorded, and these comprised a total of 80883 individuals recorded. For data collection, three bee sampling methods of different efficiency and accuracy levels were employed in this study. Most bees Tables 8 and 9 were recorded through transect counts (85% of total individuals), and very few individuals were captured by hand-net (8%) and pan-traps (7%). Although hand-net had significantly high bee species than pan-traps, the two methods recorded almost equal number of bee individuals. Total of 59, 314, and 559 bee species were recorded in transect counts, pan-traps, and hand-nets, respectively. Thus, transect count was not accurate in estimating species richness as compared to hand-net and pan-trap. The majority of bee species registered were native. There was no managed bee species. Population of honey bees encountering foraging during field is a population from wild established colonies and not from colonies established in hives.

Overall, bee species richness assessment was incomplete as taxon accumulation curves did not achieve asymptotes. Species richness was still increasing at the end of the sampling period and never reached the asymptote. The number of observed species was 652 species, whereas the average projected true species richness estimated was of 931 species according to Jacknife-1 estimator. Estimates of the expected richness indicated that 70% of the species present at the sampling sites during the period of study were found.

The most species-rich genera were *Megachile* (12.5% of total species recorded), *Lasioglossum* (8.5%), *Lipotriches* (6.2%), *Patellapis* (5.8%), *Scrapter* (5.7%), *Nomia* (5.4%) and *Ceratina* (5.1%). Similarly, Megachilini (14.42%), Halictini (11.7%), Anthophorni (8.2%), Allodapini (7.94%), Ceratini (7.18%), Anthidiini (6.35%), Eucerini (5.05%), and *Xylocopini* (5.05%) tribes were the most species-rich and abundant taxa. The abundant and most widespread (>5% of total individuals recorded) bee species were *Apis mellifera adansonii* Linnaeus (23.20%), *Hypotrigona gribodoi* Magretti (18.89%), *Meliponula ferruginea* Lepeletier (12.54%), *Lasioglossum ugandicum* Cockerell 6.90%), *Apis mellifera scutellata* Latreille (5.92%), *Allodapula acutigera* Cockerell (5.89%), *Ceratina rufigastra* Cockerell (5.60%), *Braunsapis angolensis* Cockerell (5.29%), and *Seladonia jucundus* Smith (5.02%). Most bee species observed were polylectic; that is, they forage for pollen on a diverse array of plant species, and few of them were oligolectic bees. Most species were short-tongued species. Moreover, the community was dominated by ground-nesting species, whereas above-ground nesting species were rare.

Approximately 17 bee species were identified as ubiquitous and "ecologically characteristic" of the coffee-banana agroforests of central Uganda. These included *Apis mellifera adansonii, Hypotrigona gribodoi, Meliponula ferruginea, Lasioglossum trichardti* Cockerell, *Apis mellifera scutellata, Lipotriches dentipes* Friese, *Lasioglossum ugandicum, Braunsapis angolensis, Heriades speculiferus* Cockerell, *Seladonia jucundus* Smith, *Meliponula nebulata* Smith, *Ceratina rufigastra* Cockerell, *Ceratina tanganyicensis* Strand, *Allodapula acutigera*, *Nomia atripes* Friese, *Allodape microsticta* Cockerell, and *Halictus frontalis* Smith.

Although the different bee sampling methods used prevent direct comparison, the data indicate that the study area

harbours one of the most diverse bee faunas in central and East Africa. Factors likely favouring the high bee diversity in this area include moderated climate and diversity of land use and related semi-natural habitats within agricultural matrix.

3.2. Individual and Combined Effects of Various Factors on Bee Community Parameters. Climatic factors (rainfall, minimum and maximum temperatures) were cross-correlated with bee community parameters (abundance and species richness) and with several local and landscape variables measured. The correlation between rainfalls/temperatures of years 2004, 2005 and 2006 with current abundance and species richness was determined since it has previously been observed that, most often, current afrotropical pollinator communities structures are also the reflect of variability in previous climatic events [17, 18, 53].

Thus, bee community was strongly correlated with some climatic variables. In fact, rainfalls of year 2005 was found to be negatively correlated with both species richness ($r = -0.61$, $P < 0.001$, $n = 26$) and abundance ($r = -0.44$, $P < 0.05$, $n = 26$) of bees. Thus, bee species richness and abundance were significantly and negatively associated with precipitation in previous (not the current) year. Previous year is likely to be the stronger predictor of bee species richness (not bee abundance) in central Uganda. In other words, cumulative precipitation in the previous year is likely to be a good predictor of bee richness in the current year. Also, the mean rainfall of year 2006 was negatively correlated to species richness ($r = -0.50$, $P < 0.001$, $n = 26$) abundance ($r = -0.46$, $P < 0.05$, $n = 26$) of bees. By the contrast, the abundance and species richness of bees were not significantly ($P > 0.05$) associated with the mean rainfall of year 2007 and with the overall mean rainfall of 10 years (Table 1). In addition bee abundance was negatively correlated with both the mean maximum temperature of 10 years ($r = -0.51$, $P < 0.05$, $n = 26$), and mean maximum temperature of year 2005 ($r = -0.52$, $P < 0.05$, $n = 26$); but positively correlated with both the mean minimum temperature of 10 years ($r = -0.49$, $P < 0.05$, $n = 26$), and with the mean minimum temperature of year 2006 ($r = -055$, $P < 0.001$, $n = 26$; Table 1).

Results of the generalized linear model (GLM), applied to explore the simultaneous of multiple factors, revealed few significant ($P < 0.05$) predictor variables with combined and or interactive effects on bee species richness. Variables with combined negative/positive effects included the distance to forest, overall 10-years monthly mean rainfalls, mean monthly rainfalls of year 2006 and mass flowering wild plant resources. Variables with significant combined negative/positive effects on bee abundance included (i) overall daily mean minimum temperature mean of 10 years, (ii) mean monthly rainfalls 2007, (iii) mean monthly rainfalls 2006, (iv) number of flowering plant species, (v) mass blooming wild plant species, (vi) overall daily mean minimum temperature mean of 10 years (vii) proportion cultivation intensity, (iix) mean monthly maximum temperature, (ix) cultivated floral resources with positive, (x) mean monthly minimum temperature of year 2006 with, the percent cover of semi-natural habitats, and so forth (Table 2). These GLMs also indicated that current trend in occurrence of bee species richness and abundance is the consequence of various interacting factors operating at different scale levels but with simultaneous negative/positive effects.

Simple linear regression analysis revealed that floral resources exhibited positive and significant relationships with bee abundance and species richness. Bee species richness and abundance were related to richness and abundance of wild blooming plants. Bee species richness was also related to the abundance cultivated floral resources. The list of pollinator-dependent and non-pollinator dependent crops grown in Uganda is presented in Table 6. Several wild blooming plant species were registered during the course of the study (Table 7). In addition, there was a seasonal variability in richness and abundance of wild blooming plant species (Figure 2). Across the five rounds of data collection, there was variability in cultivated and noncultivated floral resources following different environmental conditions found in the different study sites.

Species richness (not the abundance) of cultivated crops decreased linearly ($R^2 = 0.226$, $n = 26$, $P < 0.001$) with cultivation intensity. A reverse trend ($R^2 = 0.198$, $n = 26$, $P < 0.05$) was observed for wild blooming plants (herbs, weeds, etc.). Species richness (not the abundance) of wild blooming plants increased linearly with increase in % cover of semi-natural habitats ($R^2 = 0.293$, $n = 26$, $P < 0.001$) but declined linearly with forest distance ($R^2 = 0.455$, $n = 26$, $P < 0.001$). These results indicated that isolated sites or overcultivated sites were associated with low species richness in flowering plant species although, at some times, abundant mass blooming crops could be observed in overcultivated areas or in areas located very far from forests. On the contrast, areas that were covered by a high proportion of semi-naturals were also associated with high species richness of wild blooming plants and not necessarily with abundant blooming plant populations.

The results of simple linear and non-linear regressions (quadratic regressions) indicated that percent of wild floral resources (blossom cover) was significant and positively related to both species richness (Figure 3(a)) and abundance (Figure 3(b)) of bees. This result suggested that the amount of wild floral resources played a critical role in shaping flower-visiting bee communities in agricultural landscapes of central Uganda. Overall, wild blossoms (from wild blooming plants) cover explained 86% and 61% of variation in bee species richness and in bee abundance, respectively. Interestingly, flowering wild plant species richness was significantly ($P < 0.05$) related to bee species richness (Figure 3(c)) but was not significantly ($P > 0.05$) related to bee abundance (Figure 3(d)). The number of wild blooming plant species accounted for 19.6% of variation in bee species richness. These results indicated that increase in the diversity of flowering plant species can attract a high number of bee species; however, beyond 30 plant species, bee species richness can start to drop. The drop in bee species richness was unexpected and may be difficult to explain since at some points there is an expected plateau due to carrying capacity. In other words, bee

Table 1: Cross-correlation matrix showing naïve multiple correlations of Apoidea (bee) variables with environmental, local, and landscape variables (*different levels of significance of Spearman rank of correlation coefficients:* $^{*}P < 0.05$; $^{**}P < 0.001$; *otherwise not significant when no value given*).

	O	P	Q	R	S	T	U	V	W	X
B		0.49^{*}			0.49^{*}					
C					-0.51^{*}			-0.56^{**}		
E					-0.52^{*}					
J	-0.40^{*}		0.51^{**}		0.55^{**}	0.39^{*}	0.59^{**}	0.41^{*}	-0.58^{**}	-0.67^{**}
L		0.51^{**}	0.46^{*}	-0.61^{**}	-0.44^{*}	-0.56^{**}	0.52^{**}			
M		0.44^{*}	0.51^{**}	-0.50^{**}	-0.46^{*}	-0.57^{**}	0.57^{**}			
N	0.43^{*}	-0.65^{**}	-0.62^{**}	0.426^{*}		-0.70^{**}			0.525^{*}	
O		-0.60^{**}	-0.68^{**}	0.93^{**}	0.78^{**}	-0.66^{**}			0.57^{**}	0.55^{**}
P			0.84^{**}	-0.58^{**}	-0.61^{**}	0.88^{**}			-0.68^{**}	-0.71^{**}
Q				-0.62^{**}	-0.78^{**}	0.94^{**}		0.44^{*}	-0.95^{**}	-0.98^{**}
R					0.82^{**}	-0.76^{**}	0.51^{*}		0.56^{**}	0.52^{*}
S							0.68^{*}		0.71^{**}	0.78^{**}

Legend: A = overall mean rainfalls (means/month/10 years); B = overall daily mean minimum temperature (mean 10 years); C = overall daily mean maximum temperature (mean 10 years); D = mean monthly rainfalls (2007); E = mean monthly maximum temperature (2005); F = mean monthly maximum temperature (2006); H = mean monthly maximum temperature (2007); I = mean monthly minimum temperature (2005); J = mean monthly minimum temperature (2006); K = mean monthly minimum temperature (2007); L = mean monthly rainfalls (2005); M = mean monthly rainfalls (2006); N = wild flowering plant species richness; O = density of plant species; P = human population density; Q = proportion cultivation intensity; R = mean bee species richness; S = mean bee abundances; T = forest distance (m); U = cultivated with floral resources (pollinator-dependent crops); V = cultivated without resources (pollinator nondependent crops); W = % seminatural habitats/site; X = % young fallows per site.

species richness response to richness of wild floral resources richness is not necessarily linear under local conditions in Uganda.

As expected, the cover of cultivated non-pollinator-dependent floral resources was not significantly ($R^2 = 0.122$, $n = 26$, $P > 0.05$) related to either bee species richness or to bee abundance. Yet, the percentage cover of cultivated non-pollinator-dependent crops decreased as bee species richness and/or abundance increased. Conversely, the number of species of cultivated non-pollinator-dependent floral resources was not related ($P > 0.05$) to bee species richness (Figure 3(e)) and/or abundance (Figure 3(f)) under local conditions in central Uganda. Practically, cultivated pollinator-dependent floral resources were significantly ($P < 0.05$) and positively related to bee species richness (Figure 3(e)) and bee abundance (Figure 3(f)). The proportion of cultivated pollinator-dependent crops explained 26% and 47% of the variation in bee species and in bee abundance, respectively (Figure 3). This indicates that cultivated pollinator-dependent crops species provide sufficient pollen resources to bee communities visiting crop fields in the agricultural mosaics of central Uganda. In other worlds, species richness and population density of bees in the agricultural landscapes are also influenced by the abundance of cultivated pollinator-dependent crop floral resources. Although no clear relationships between cover of cultivated and cover of noncultivated pollinator-dependent crops in the local farm-landscape were detected, when farmers increased the land area dedicated to pollinator-dependent crops, cover of non-pollinator-dependent crops reduced in terms of area coverage. Overall, species richness and population density of bees in the agricultural landscapes were found to be largely influenced (were more predicted) by the percent cover of bloom of wild plant species than by the proportion of cultivated crops that are pollinator dependent.

The regression analysis revealed that some landscape factors showed highly significant ($P < 0.05$) associations with bee community parameters. Cultivation intensity was significantly ($P < 0.05$) and negatively related to both bee species richness (Figure 4(a)) and population density (Figure 4(b)). The proportion cultivation intensity explained 38% and 62% of variation in bee species richness and in bee abundance, respectively. These results suggested a "steep" (abrupt) "decline" (reduction) in bee species richness and abundance with cultivation intensity. The proportion of semi-natural habitats in a 1 km^2 area was significantly ($P < 0.05$) and positively related to both species richness (Figure 4(c)) and abundance (Figure 4(d)) of bees. The proportion of semi-natural habitats accounted for 31% and 50% of variation in bee species richness and in bee abundance respectively. This result suggested that any increase in amount of semi-natural habitats in the landscape was likely to lead to an increase in the number of bee species and individuals in the agricultural matrices. Surprisingly, forest distance was significantly ($P < 0.05$) and negatively related to bee species richness (Figure 4(e)) but was not significantly ($P > 0.05$) related to bee abundance (Figure 4(f)). Forest distance explained 58% of the variation in bee species (Figure 4). This result suggested also that there was a strong decline in bee species with forest distance. In other words, study sites that were riparian of forest reserves harbored high species richness than did sites that were isolated or located far way from forest reserves.

3.3. Effects of Regional Land-Use Intensity Factors. There were significant ($P < 0.01$) effects of the different land-use categories on bee species richness (Figure 5(a)) and population density (Figure 5(b)). Bee species richness was on average significantly ($P < 0.05$) greater in study sites of low to

Table 2: Generalized linear models (GLMs) testing the effects of local, landscape, and climatic factors on bee abundance and species richness in farmlands of central Uganda.

	Bee species richness	Coef.	Std. Err.	z	$P > \|z\|$	95% Conf. Interval	
Landscape variables	Forest distance	−0.3040771	0.0372303	−8.17	**0.000**	−0.3770472	−0.2311071
	Proportion cultivation intensity	−0.0417633	0.0184095	−2.27	**0.023**	−0.0778453	−0.0568113
	% seminatural habitats	0.0260201	0.0098786	2.63	**0.008**	0.0066585	0.0453817
	Human population density (inhabitants/Km^2)	0.0136742	0.0417017	0.33	0.743	−0.0680597	0.0954081
Climatic variables	Overall daily mean minimum temperature (mean 10 years)	−2.36636	2.509198	−0.94	0.346	−7.284298	2.551578
	Overall daily mean maximum temperature (mean 10 years)	−0.8994122	0.3635115	−2.47	**0.013**	−1.611882	−0.1869429
	Mean monthly maximum temperature (2005)	−5.517305	5.026883	−1.1	0.272	−15.36982	4.335206
	Mean monthly maximum temperature (2006)	−0.0751644	0.1294036	0.561	0.561	−0.3287908	0.178462
	Mean monthly minimum temperature (2006)	0.0237176	0.0198609	1.19	0.232	−0.015209	0.0626443
	Overall mean rainfall (means/month/10 years)	0.8934966	0.4196737	2.13	**0.033**	0.0709512	1.716042
	Mean monthly rainfalls (2007)	−5.14455	5.405548	−0.95	0.341	−15.73923	5.450129
	Mean monthly rainfalls (2006)	−0.9998961	0.3946438	−2.53	**0.011**	−1.773384	−0.2264084
Local variables	Density of wild plants (weeds/herbs/trees)	0.6940608	0.3129488	2.22	**0.027**	0.0806924	1.307429
	Number of flowering wild plant species (weeds/herbs/trees)	−2.874384	0.8531344	−3.37	**0.001**	−4.546496	−1.202271
	% of cultivated floral resources (pollinator-dependent crops)	0.4345841	2.249894	0.19	0.847	−3.975128	4.844296
	% cultivated floral resources (nonpollinator-dependent crops)	0.8652652	2.129935	0.41	0.685	−3.309331	5.039861
	Constant	548.8108	500.5592	1.1	0.273	−432.2671	1529.889
Other statistics: Log likelihood = −124.727761; AIC (Akaike's Information Criterion) = 12.70252; BIC (Schwarz's Bayesian Criterion) = −21.62718472							
	Bee abundance						
Landscape variables	Forest distance	0.0039313	0.0054335	0.72	0.4669	−716.9601	0.0145808
	Proportion cultivation intensity	−613.0046	180.3214	−3.4	**0.001**	−966.4281	−259.5811
	% of seminatural habitats	0.001616	0.0001599	10.10	**0.000**	0.0013026	0.0019295
	Overall monthly mean rainfalls (means of 10 years)	26.26025	2.734129	9.6	**0.000**	−19.34479	31.61905
Climatic variables	Overall daily mean minimum temperature (mean of 10 years)	−377.1344	18.25537	−20.66	**0.000**	−9.023715	−341.3545
	Overall daily mean maximum temperature (mean of 10 years)	−383.902	169.9307	−2.26	**0.024**	−716.9601	−50.84403
	Mean monthly minimum temperature (2006)	−263.8625	35.75006	−7.38	**0.000**	−333.9313	−193.7937
	Overall daily mean minimum temperature mean 10 years	5.595728	0.3103008	18.03	**0.000**	4.98755	6.203907
	Mean monthly maximum temperature (2005)	−66.88049	32.75358	−2.04	**0.041**	−131.0763	−2.684648
	Mean monthly rainfalls (2007)	−14.75942	2.339518	−6.31	**0.000**	−19.34479	−10.17404
	Mean monthly rainfalls (2006)	−5.413767	1.841844	−2.94	**0.003**	−9.023715	−1.803819
	Mean number of flowering wild plant species	−4.652631	4.882926	−0.95	0.341	−14.22299	4.917729
Local variables	Mean density of wild flowering plants	1.686491	0.1404906	12	**0.000**	1.411134	1.961847
	Cultivated floral resources (pollinator dependent crops)	−192.0184	16.07023	−11.95	**0.000**	−223.5154	−160.5213
	Cultivated—without resources (nonpollinator-dependent crops)	0.1851433	0.1925751	0.9611	0.336	−0.192297	0.5625835
	Constant	36034.16	3331.619	10.82	0.0000	29504.3	42564.01
Other statistics: Log likelihood = −390.1545969; AIC (Akaike's Information Criterion) = 36.83224; BIC (Schwarz's Bayesian Criterion) = 568.065439							

Table 3: Richness and abundance of bees attracted to different habitats (land uses) frequently observed in the coffee-banana farming systems of central Uganda. *Most attractive habitats were those with > 20% of weeds/crops/grass/wild plant species blooming at the time of visit.*

Foraging habitats: seminatural habitat types/crop-field habitat types	Habitat frequency	Habitat size range	*N*	Bee species ($X \pm$ SE)	Bee abundance ($X \pm$ SE)
Undisturbed field margins associated with or without termite mounds and shrubs/trees/grass species	125	0.05–0.5	159	**22.45±3.45a**	223.80 ± 35.67 d
Field boundaries/hedgerows/track-sides	100	0.01–0.05	150	**24.32±5.23a**	256.56 ± 25.78 d
Forest remnants/forest patches	35	0.5–40	45	16.54 ± 2.41 b	169.65 ± 19.21 d
Woodlots (pines, etc.)	20	0.5–40	30	14.22 ± 2.17 b	87.981 ± 11.23 f
Woodlots (*Eucalyptus* blooming)	15	0.5–30	22	5.291 ± 1.45 d	876.12 ± 125.7 b
Agroforestry tree species (*Moringa* sp., *Sesbania* sp., *Ricinus communis*,*Leucaena leucocephala*,*Calliandra* sp, *Cassia* sp., *Sena spectabilis*, etc.)	35	0.05–2	45	14.12 ± 2.31 b	259.54 ± 35.78 c
Grazed fields (Pasturelands), grasslands	115	0.05–10	190	13.76 ± 1.21 b	47.891 ± 7.782 f
Swampy (marshland) habitats, and (streams-sides)	54	0.3–5	89	**21.45±2.31a**	211.45 ± 34.51 d
Abandoned gardens	45	0.02–1	258	14.23 ± 1.13 c	233.67 ± 15.67 d
Fallows (weedy, grassy, bushy, woody, grassy-bushy, bush-woody, scrubby)	122	0.03–3	356	15.21 ± 1.16 b	176.56 ± 13.76 d
Herbaceous crop-habitats (unweeded fields) and weedy harvested fields	135	0.02–3	367	**19.23±1.27a**	284.56 ± 22.67 c
Perennial crops blooming (coffee)	106	0.05–12	600	**19.54±1.61a**	876.23 ± 68.98 b
Perennial crops blooming (banana)	112	0.05–15	789	11.12 ± 1.93 c	251.12 ± 31.76 c
Perennial fruit crops blooming (avocado, mango, citrus, orange, lemon, tangerines, guava, papaya, etc.)	62	0.03–10	98	4.783 ± 1.32 e	**1986.5±398.6a**
Perennial fruit crops blooming (passion fruits)	15	0.01–3	22	12.76 ± 1.93 c	76.456 ± 10.43 f
Home gardens of annual vegetable crop species blooming (pumpkin, watermelon, *Amaranthus*, *Cleome*, bitter *Solanum*, onion, cucumber, garlic, lettuce, etc.)	59	0.01–0.5	79	12.56 ± 1.35 c	367.65 ± 51.73 c
Annual commercial/cash vegetable crops (tomato, eggplant, chiles, pepper)	88	0.02–6	121	8.514 ± 1.25 d	34.125 ± 8.911 f
Annual commercial/cash crops (sim-sim, sun flower)	25	0.02–10	48	11.34 ± 2.11 c	113.32 ± 11.74 e
Annual cereals (maize) mixed with pulse crops (beans, cowpea, greengram, soybean, groundnut): maize blooming	91	0.04–7	421	2.125 ± 2.11 f	198.23 ± 23.56 d
Annual cereals (maize, sorghum, millet) mixed with pulse crops (beans, cowpea, greengram, soybean, groundnut): sorghum-millet blooming mixed crops	33	0.03–6	259	5.126 ± 1.45 d	59.546 ± 14.65 f
Annual cereals (maize, sorghum, millet) mixed with pulse crops (beans, soybean, groundnut): beans-cowpea blooming mixed crops	45	0.02–5	342	4.211 ± 1.13 e	19.176 ± 1.542 f
Annual cereals (maize, sorghum, millet) mixed with pulse crops (beans, soybean, groundnut): groundnut blooming	65	0.02–6	403	3.214 ± 0.91 e	14.548 ± 3.541 f
Bi-annual crops (cassava blooming)	71	0.02–8	198	1.897 ± 0.25 f	57.459 ± 15.21 f
Annual crops (sweet-potato blooming)	78	0.02–3	400	5.128 ± 1.27 d	157.67 ± 12.67 d
Annual cereals (rice blooming)	38	0.05–15	55	3.459 ± 0.87 e	21.235 ± 3.563 f
Annual crops (Irish potato blooming)		0.05–12	43	7.126 ± 1.41 d	28.659 ± 6.124 f

Habitat frequency = number of observation cases or number of times the habitat type was encountered across all 26 study sites and all sampling rounds.
Habitat size range (ha) = the data show the minimum and the maximum size of the type of habitat encountered during butterfly faunistic surveys.
N = number of samples (bees species and individuals) recorded in five sampling rounds across the 26 study sites in 2006.
Within columns, different letters show significant differences of the means at $P = 0.05$ according to Tukey test performed after Kruskall-Walis ANOVA test indicating that the habitat type was significant ($P < 0.01$) for the number of species and individuals attracted.

Table 4: Density ($x \pm$ sd) of bee nests (number of individual bee nests counted during transects walks and counts different farmland habitats/land-uses) per nesting type.

Types of seminatural habitats/land-uses (bee reservoirs/refugia)	Habitat size (ha)	Bee hives	Foliage nests	Ground-nests	Termite mounds	House-wall nests	Wood/tree nests
Roadsides/track-sides					12.21 ± 4.5 (n^{**} = 125)	125.7 ± 78 (n^{***} = 15)	2.57 ± 0.78 (n^{*} = 5)
Hedgerows, field boundaries (field margins)	0.21 ± 0.05	1.00 ± 0.00 (n = 2)		12.7 ± 7.8 (n^{****} = 45)	125.7 ± 87 (n = 115)		2.5 ± 1.8 (n = 6)
Small tropical forest remnants/forest patches	7.22 ± 21.6	5.7 ± 3.4 (n = 4)	1.5 ± 1.1 (n = 5)	18.1 ± 4.6 (n = 25)	9.1 ± 4.6 (n = 25)		15.7 ± 3.1 (n = 24)
Ecotones/edge of forest reserves	1.84 ± 0.21		2.2 ± 1.4 (n = 6)	2.2 ± 1.4 (n = 6)	16.2 ± 1.4 (n = 6)	725.7 ± 278 (n = 25)	35.7 ± 13.1 (n = 11)
Woodlots (pines/eucalyptus) and woodlands	9.45 ± 34.78	3.2 ± 1.4 (n = 8)		43.2 ± 21.4 (n = 58)	23.2 ± 11.6 (n = 38)	25.3 ± 2.8 (n = 5)	53.2 ± 31.4 (n = 88)
Edge of wetlands/streams	0.91 ± 2.12		1.00 ± 0.0 (n = 3)	19.34 ± 12.5 (n = 23)	2.2 ± 1.1 (n = 5)		3.2 ± 1.1 (n = 8)
Abandoned gardens	0.06 ± 0.04	1.2 ± 0.7 (n = 4)		9.4 ± 5.5 (n = 16)	4.2 ± 1.2 (n = 10)	421.7 ± 367 (n = 6)	11.57 ± 1.78 (n = 15)
Fenced cattle keeping fields, large pasturelands	9.43 ± 4.91			13.1 ± 3.5 (n = 13)	10.2 ± 7.2 (n = 13)		27.7 ± 12.7 (n = 17)
Lantanacamara/Erlangea tomentosa fallows	0.45 ± 1.29			53.1 ± 13.5 (n = 63)	17.2 ± 4.2 (n = 11)		7.2 ± 2.1 (n = 7)
Swampy fallows (different ages)	4.45 ± 1.29		4.2 ± 1.4 (n = 7)	3.1 ± 1.5 (n = 6)	3.1 ± 1.2 (n = 5)		17.2 ± 12.1 (n = 7)
Forest fallows (>5–7 years)	4.32 ± 24.31	11.2 ± 3.4 (n = 17)	1.00 ± 0.00 (n = 1)	45.1 ± 21.5 (n = 12)	13.1 ± 7.1 (n = 15)		47.2 ± 32.1 (n = 17)
Young fallow (<1-2 years aged)	0.06 ± 0.67			39.3 ± 11.5 (n = 78)	4.1 ± 2.1 (n = 45)		11.2 ± 2.1 (n = 65)
Small and large grasslands	0.43 ± 4.91			25.3 ± 5.6 (n = 18)	2.1 ± 1.1 (n = 15)		2.2 ± 1.1 (n = 15)
Small grazing fields (for small ruminants)	0.37 ± 3.51			5.3 ± 2.6 (n = 8)	1.7 ± 0.86 (n = 4)		1.2 ± 0.5 (n = 5)
Simple agroforestry systems (agroforestry trees + fruits)	0.32 ± 1.13	3.8 ± 1.4 (n = 7)		2.3 ± 1.2 (n = 45)	1.1 ± 0.46 (n = 25)	321.7 ± 167 (n = 16)	21.2 ± 10.5 (n = 15)
Complex agroforests (agroforest trees + fruits + native trees)	0.72 ± 1.35	5.2 ± 2.4 (n = 10)		3.1 ± 1.3 (n = 48)	2.5 ± 1.2 (n = 25)	821.7 ± 461 (n = 9)	29.1 ± 20.5 (n = 19)
Perennial crops associated headed by coffee + banana	0.87 ± 5.62	3.2 ± 1.1 (n = 5)		12.3 ± 5.6 (n = 38)	1.8 ± 1.2 (n = 24)	456.6 ± 121 (n = 7)	21.2 ± 11.5 (n = 25)
Homegardens with annual vegetable crop species	0.34 ± 0.291			1.0 ± 0.0 (n = 1)	1.0 ± 0.0 (n = 1)	399.7 ± 111 (n = 11)	1.2 ± 0.5 (n = 4)
Marshland habitats and reclaimed wetlands	0.99 ± 6.97			4.1 ± 1.2 (n = 5)	1.0 ± 0.0 (n = 1)		1.9 ± 0.7 (n = 6)
Bi-annual root crops (Cassava) fields	0.79 ± 5.32			1.1 ± 0.1 (n = 5)	2.00 ± 0.0 (n = 2)	323.1 ± 145 (n = 7)	1.00 ± 0.00 (n = 1)
Annual root/tuber crops (sweet potato) fields	0.24 ± 1.97			7.1 ± 2.1 (n = 15)	2.00 ± 0.0 (n = 2)	723.1 ± 345 (n = 5)	3.9 ± 1.7 (n = 6)
Annual cereal (maize, sorghum, rice) + legume (bean, groundnut) crops	0.41 ± 2.765			1.6 ± 0.6 (n = 11)	1.0 ± 0.0 (n = 1)	412.1 ± 156 (n = 8)	2.2 ± 1.2 (n = 16)

The density is the number of nests counted per nesting site per 5 ha-transect. The data reflect the number of observations or number of time this nest was recorded in five rounds of data collection conducted during 2006 in central Uganda. n = the number of time the individual bee nest was recorded in a particular land-use/habitat type during five rounds of data collection across 26 study sites. Number of individual bee nests included all type of solitary and social bee species and all type of bee nests location combined.

n^{****} = number of active ground nets recorded in that habitat; n^{**} = number of active termite mounds recorded in that habitat; n^{***} = number of active nests counted on house wall (old houses, nests, livestock houses, abandoned or not) established near the habitat described; n^{*} = number of active wood/tree nests seen and counted during transect walks.

Table 5: Density (number of trees/5 ha) of nest tree species recorded in farmlands of central Uganda during bee faunistic surveys conducted in 2006. *Data are means of 26 study sites and five sampling rounds conducted in 2006.*

Family	Species name	Density of trees (Mean ± SD)
Caesalpiniaceae	*Senna occidentalis*	236.92 ± 34.76
Bignoniaceae	*Markhamia lutea*	188.82 ± 4.11
Myrsinaceae	*Maesa lanceolata*	146.222 ± 34.67
Asteraceae	*Vernonia amygdalina*	133.23 ± 76.1
Myrtaceae	*Psidium guajava*	109.06 ± 45.7
Moraceae	*Ficus saussureana*	87.31 ± 5.87
Lauraceae	*Persea americana*	87.31 ± 56.91
Anacardiaceae	*Mangifera indica*	77.34 ± 6.76
Myrtaceae	*Eucalyptus grandis*	67.97 ± 5.67
Tiliaceae	*Theobroma cacao*	67.97 ± 45.12
Papilionaceae	*Erythrina abyssinica*	24.47 ± 10.11
Proteaceae	*Grevillea robusta*	19.33 ± 8.98
Solanaceae	*Solanum wrightii*	19.33 ± 5.67
Caesalpiniaceae	*Cassia spectabilis*	14.80 ± 65.2
Cupressaceae	*Cupressus lusitanica*	14.80 ± 9.56
Moraceae	*Ficus mucuso*	14.80 ± 7.54
Mimosaceae	*Leucaena leucocephala*	10.87 ± 13.89
Bignoniaceae	*Spathodea campanulata*	10.87 ± 8.98
Rutaceae	*Citrus lemon*	7.55 ± 7.65
Apocynaceae	*Funtumia africana*	7.55 ± 5.43
Mimosaceae	*Calliandra calothyrsus*	4.83 ± 3.45
Bignoniaceae	*Jacaranda mimosifolia*	4.83 ± 3.12
Euphorbiaceae	*Bridelia micrantha*	2.71 ± 1.12
Moraceae	*Ficus thonningii*	2.72 ± 2.34
Bignoniaceae	*Kigelia africana*	2.71 ± 5.12
Euphorbiaceae	*Macaranga schweinfurthii*	2.71 ± 5.12
Papilionaceae	*Sesbania sesban*	2.71 ± 3.45
Mimosaceae	*Albizia chinensis*	1.21 ± 1.23
Mimosaceae	*Albizia coriaria*	1.20 ± 4.54
Rubiaceae?	*Coffeacanephora*	1.20 ± 5.65
Caesalpiniaceae	*Senna spectabilis*	1.20 ± 1.23
Mimosaceae	*Acrocarpus fraxinifolius*	1.33 ± 5.12
Mimosaceae	*Albizia glaberrima*	1.50 ± 2.54
Mimosaceae	*Albizia grandibracteata*	1.90 ± 4.12
Mimosaceae	*Albizia gummifera*	1.30 ± 5.32
Moraceae	*Artocarpus heterophyllus*	1.30 ± 2.12
Meliaceae	*Azadirachta indica*	1.30 ± 3.45
Caesalpiniaceae	*Cassia siamea*	1.30 ± 3.23
Ulmaceae	*Celtis africana*	0.90 ± 2.12
Ulmaceae	*Celtis mildbraedii*	0.83 ± 3.12
Rutaceae	*Citrus sinensis*	0.81 ± 3.12
Palmae	*Elaeis guineensis*	0.70 ± 2.43
Moraceae	*Ficus asperifolia*	0.68 ± 3.55
Moraceae	*Ficus barteri*	0.67 ± 3.23
Moraceae	*Ficus benjamina*	0.62 ± 1.56
Moraceae	*Ficus brachypoda*	0.57 ± 2.32
Moraceae	*Ficus cyathistipula*	0.50 ± 1.14
Moraceae	*Ficus dicronystilla*	0.46 ± 1.23
Moraceae	*Ficus exasperata*	0.40 ± 1.54
Moraceae	*Ficus natalensis*	0.36 ± 1.45
Moraceae	*Ficus ottoniifolia*	0.35 ± 0.97
Moraceae	*Ficus polita*	0.34 ± 0.76
Moraceae	*Ficus pseudomangifera*	0.33 ± 6.71
Moraceae	*Ficus stipulifera*	0.31 ± 4.56
Moraceae	*Ficus sur*	0.30 ± 2.78
Moraceae	*Ficus sycomorus*	0.30 ± 4.56
Moraceae	*Ficus valifolia*	0.29 ± 5.76
Moraceae	*Ficus vasta*	0.28 ± 3.61
Mimosaceae	*Grilicia sepium*	0.27 ± 3.11
Ulmaceae	*Holopteria grandis*	0.26 ± 2.71
Rhamnaceae	*Maesopsis eminii*	0.24 ± 1.51
Moringaceae	*Moringa oleifera*	0.21 ± 3.19
Pinaceae	*Pinus carribaea*	0.20 ± 1.12
Podocarpaceae	*Podocarpus milinjuanus*	0.19 ± 3.13
Rosaceae	*Prunus africana*	0.18 ± 2.13
Myrtaceae	*Syzygium cuminii*	0.16 ± 0.91
Combretaceae	*Terminalia sperba*	0.15 ± 0.45
Ulmaceae	*Trema orientalis*	0.13 ± 0.30

medium land-use intensity categories compared to study sites under high to very high land-use categories (Figure 5(a)). Bee species richness and abundance were significantly higher in low land-use intensity than in all the other land-use categories. Similarly, bee population density was on average significantly ($P < 0.05$) two to three times greater in study sites with low to medium land-use intensity gradients compared to study sites with high to very high land-use intensity gradients (Figure 5(b)).

3.4. Nest Density and Bee Attraction to Various Types of Habitats/Land-Uses. Few environmental factors (land-uses/habitats) were observed to be significantly ($P < 0.05$) associated with high species richness and abundance of bees (Table 3). They were of high value for bees. Across study sites and sampling rounds, some habitats attracted a high number of bee species and individuals both during rainy and dry seasons: fallows, forest plantations, and woodlands. Count of nesting sites was conducted concurrently to bee surveys. Across study sites and sampling rounds, nest density (mean number of nests/0.5 ha transect/site) was significantly and positively related to bee species richness ($R^2 = 0.381$, $F_{(1,28)} = 8.97$, $P < 0.001$) and to bee abundance ($R^2 = 0.211$, $F_{(1,28)} = 5.67$, $P < 0.05$). There was a variation in the density of nests per nesting type per land-use/habitat type (Table 4).

Table 6: List of pollinator-dependent crops and nonpollinator crops grown in Uganda (*the crop species are presented per dependency status, crop category, common names, and scientific names*).

Dependency status	Crop category	Common names	Scientific name
Nonpollinator-dependent crop	Banana	Plantains	*Musa sp.*
Nonpollinator-dependent crop	Cereal crops	Finger millet	*Eleusine sp.*
Nonpollinator-dependent crop	Cereal crops	Maize	*Zea maize*
Nonpollinator-dependent crop	Cereal crops	Sorghum	*Sorgum bicolor*
Nonpollinator-dependent crop	Cereal crops	Rice	*Oryza sativa*
Nonpollinator-dependent crop	Cereal crops	Wheat	*Triticum sp.*
Nonpollinator-dependent crop	Root/tuber crops	Sweet potatoes	*Ipomoea batatus*
Nonpollinator-dependent crop	Root/tuber crops	Potatoes	*Solanum tuberusum*
Nonpollinator-dependent crop	Root/tuber crops	Cassava	*Manihot esculentum*
Pollinator-dependent crop	Pulse crops	Beans	*Phaseolus vulgarus*
Pollinator-dependent crop	Pulse crops	Field peas	*Pisum arvense*
Pollinator-dependent crop	Pulse crops	Cowpeas	*Vigna unguiculata*
Pollinator-dependent crop	Pulse crops	Greengram seeds	*Vigna radiata*
Pollinator-dependent crop	Pulse crops	Peageon peas	*Cajanus cajan*
Pollinator-dependent crop	Pulse crops	Bambaranut	*Vigna subterranea*
Pollinator-dependent crop	Pulse crops	Groundnut	*Arachis hypogea*
Pollinator-dependent crop	Industrial crops/edible oils	Soy beans	*Glycine max*
Pollinator-dependent crop	Industrial crops/edible oils	Sim-Sim seeds	*Sesamum indicum*
Pollinator-dependent crop	Industrial crops/edible oils	Sun-flower seeds	*Helianthus annus*
Pollinator-dependent crop	Industrial crops/edible oils	Coffee beans	*Coffea canephora/arabica*
Pollinator-dependent crop	Industrial crops/edible oils	Cotton seed	*Gossypium sp.*
Pollinator-dependent crop	Industrial crops/edible oils	Tobacco seed	*Nicotiana tabacum*
Pollinator-dependent crop	Industrial crops/edible oils	Tea	*Camelia sinsensis*
Pollinator-dependent crop	Industrial crops/edible oils	Sugar	*Sugar cane*
Pollinator-dependent crop	Industrial crops/edible oils	Cocoa	*Theobroma cacao*
Pollinator-dependent crop	Industrial crops/edible oils	Coconut	*Cocos nucifera*
Pollinator-dependent crop	Fruit crops	Avocado	*Persea americana*
Pollinator-dependent crop	Fruit crops	Mangos	*Mangifera indica*
Pollinator-dependent crop	Fruit crops	Orange and tangerine	*Citrus myrtifolia/reticulata*
Pollinator-dependent crop	Fruit crops	Grapefruits	*Citrus grandis*
Pollinator-dependent crop	Fruit crops	Passion fruits	*Passiflora edulis*
Pollinator-dependent crop	Fruit crops	Papaw	*Carica papaya*
Pollinator-dependent crop	Fruit crops	Guavas	*Psidium guajava*
Pollinator-dependent crop	Fruit crops	Apples	*Malus domestica*
Pollinator-dependent crop	Fruit crops	Jackfruit	*Artocarpus heterophyllus*
Pollinator-dependent crop	Vegetable crops	Tomato fruits	*Lycopersicon esculentum*
Pollinator-dependent crop	Vegetable crops	Eggplants	*Solanum melongena*
Pollinator-dependent crop	Vegetable crops	Pepper fruits	*Capsicum fruitescens*
Pollinator-dependent crop	Vegetable crops	Mustard seeds	*Brassica alba*
Pollinator-dependent crop	Vegetable crops	Okra, Gumbo	*Abelmoschus esculentus*
Pollinator-dependent crop	Vegetable crops	Pumpkin	*Cucurbita maxima/moschata*
Pollinator-dependent crop	Vegetable crops	Squash	*Cucurbitamixta*
Pollinator-dependent crop	Vegetable crops	Gourde	*Lagenaria siceraria*
Pollinator-dependent crop	Vegetable crops	Watermelon	*Citrullus lunatus*
Pollinator-dependent crop	Vegetable crops	Cucumber	*Cucumis sativus*
Pollinator-dependent crop	Spices and condiments	Vanilla	*Vanilla planifolia*
Pollinator-dependent crop	Medicinal plants	Moringa	*Moringa oleifera*

Table 7: List of common wild flowering plant species visited by bees during floral resources collection in the coffee-banana farming system of central Uganda. *Species are arranged per family, life cycle type, and type of flower colour/shape. These are plant species met in blooming periods and that were observed being visited by different bees species during transect surveys.*

Family	Species	Lifecycle	Flower color/shape
Mimosaceae	*Acacia hockii* De Wild	Tree	Yellow
Mimosaceae	*Acacia gerradii* Benth	Tree	Dirty/white
Mimosaceae	*Acacia zanziberica*	Shrub	Yellow
Euphorbiaceae	*Acalypha bipartita* Muell. Arg.	Shrub	Green
Euphorbiaeae	*Acalypha ornata* A.Rich.	Shrub	Red
Acanthaceae	*Acanthus pubescens* Engl	Shrub	Pink
Asteraceae	*Acmella caulirhiza* Delile	Herb	Yellow
Asteraceae	*Ageratum conyzoides* L.	Herb	White
Mimosaceae	*Albizzi grandibracteata* Taub	Tree	Pale/green
Mimosaceae	*Albizzia glaberrima* (Schumach. and Thonn.) Benth	Tree	White
Mimosaceae	*Albizzia adianthifolia* (Schumach.) W.F. Wight	Tree	White
Mimosaceae	*Albizzia coriaria* Oliv.	Tree	White
Scrophulariaceae	*Alectra sessiliflora* (Vahl) Kuntze	Herb	Yellow
Amaranthaceae	*Amaranthus dubius* Thell	Herb	Green
Amaranthaceae	*Amaranthus hybridus* L.subsp.h*ybridus*	Herb	Green
Aristolochiaceae	*Aristolochia elegans* Mast.	Herb	Reddish/purple
Asteraceae	*Aspilia africana* (Pers) C.D.Adams	Herb	Yellow
Acanthaceae	*Asystasia gangetica* (L.) T.Andersson	Herb	White
Acanthaceae	*Asystasia mysorensis* (Roth) T.Anderson	Herb	White
Acanthaceae	*Barleria spinisepale*	Herb	Purple
Asteraceae	*Berkheya spekeana* Oliv.	Herb	Yellow
Asteraceae	*Bidens pilosa* L.	Herb	White/yellow
Oxalidaceae	*Biophytum abyssinicum* A.Rich.	Herb	Yellow
Euphorbiaceae	*Bridelia micrantha* (Hochst) Baill	Tree	Greenish/yellow
Caesalpiniaceae	*Caesalpinia decapetala* (Roth) Alston	Shrub	Yellow
Papilionaceae	*Cajanus cajan* (L.) Millsp	Shrub	Yellow
Mimosaceae	*Calliandra calothyrsus* Meissner	Shrub	Red
Myrtaceae	*Callistemon lanceolatus* DC.	Tree	Red
Theaceae	*Camellia sinensis* (L.) O.Ktze.	Tree	White/cream
Pailionaceae	*Canavalia africana* Dunn	Herb	Purple
Pailionaceae	*Canavalia virosa* (Roxb.) Wight	Herb	Purple
Solanaceae	*Capsicum annum* L.	Herb	Green
Sapindaceae	*Cardiospermum halicacabum* L.	Herb	White
Caesalpiniaceae	*Cassia hirsuta* L.	Herb	Yellow
Caesalpiniaceae	*Cassia kirkii* Oliv.	Herb	Yellow
Meliaceae	*Cedrella odorata* L.	Tree	Yellow/greenish
Rutaceae	*Citrus aurantifolia* Swingle	Tree	White
Rutaceae	*Citrus lemon* (L.) Burm.f.	Tree	White
Rutaceae	*Citrus reticulata* Blanco	Tree	White
Rutaceae	*Citrus sinensis* (L.) Osbeck	Tree	White
Capparaceae	*Cleome gynandra* (L.) Briq.	Herb	White
Capparaceae	*Cleome monophylla* L.	Herb	Purple
Verbenaceae	*Clerodendrum myricoides* (Hochct.) Vodke	Shrub	Blue
Verbenaceae	*Clerodendrum rotundifolium* Oliv.	Herb	White
Palmae	*Cocos nucifera* L.	Tree	Orange/yellow
Commelinaceae	*Commelina benghalensis* L.	Herb	Yellow
Commelinaceae	*Commelina africana* L.	Herb	Yellow
Asteraceae	*Crassocephalum montuosum* (S. Moore) Milne-Redh.	Herb	Red
Asteraceae	*Crassocephalum vitellinum* (Benth.) S. moore	Herb	Yellow

Table 7: Continued.

Family	Species	Lifecycle	Flower color/shape
Papilionaceae	*Crotalaria incana* L.	Herb	Yellow
Papilionaceae	*Crotalaria brevidens* Benth.var. *Intermedia* (Kotschy) Polh.	Herb	Yellow
Papilionaceae	*Crotalaria laburnifolia* L.	Herb	Yellow
Papilionaceae	*Crotalaria natalica* Meisn	Herb	Yellow
Vitaceae	*Cyphostemma adenocaule* (A.Rich.) Willd. and Drummond	Herb	Red
Papilionaceae	*Desmodium Salicifolium* (Poir.) DC.	Herb	Pink/purple
Papilionaceae	*Desmodium tortusum* (Sw.) DC.	Herb	Pink
Asteraceae	*Dichrocephala integrifolia* (L.f.) O.Ktze	Herb	Greenish/whitish
Acanthaceae	*Dyschoriste radicans* Nees	Herb	Yellow
Palmae	*Elaeis huineensis* Jacq	Tree	Yellow
Asteraceae	*Emilia javanica* (Burm.f.) C.B. Rob.	Herb	Red
Papilionaceae	*Eriosema psoraleoides* (Lam.) G.Don	Herb	Yellow
Asteraceae	*Erlangea cordifolia* (Oliv) S. Moore	Herb	Purple
Asteraceae	*Erlangea tomentosa* S. Moore	Herb	Light purple
Asteraceae	*Erlangea ugandensis* S. Moore	Shurb	Blue
Cruciferae	*Erucastrum arabicum* Fisch and Mey	Herb	Yellow
Papilionaceae	*Erythrina abyssinica* DC	Tree	Red
Myrtaceae	*Eucalyptus grandis* Marden	Tree	Pale
Myrtaceae	*Eucalyptus camaldulensis*	Tree	Cream
Euphorbiaceae	*Euphorbia heterophylla* L.	Herb	Cream
Euphorbiaceae	*Euphorbia hirta* L.	Herb	Green/purple
Asteraceae	*Galisonga parviflora* Cav.	Herb	White/yellow
Papilionaceae	*Gliricidia sepium* (Jacq.) Walp.	Tree	NIL
Papilionaceae	*Glycine wightii* (Wight and Arn.) Verdc. *var.longicanda* (Scheinf.) Verdc.	Herb	Pale/grey
Asteraceae	*Guizotia scabra (*Vis.) Chiov.	Herb	Yellow
Asteraceae	*Helianthus annua* L.	Herb	Yellow
Convulvulaceae	*Hewittia sublobata* (L.f.) O.Ktze.	Herb	Yellow
Malvaceae	*Hibiscus surrantensis* L.	Herb	Yellow
Malvaceae	*Hibiscus diversifolius* Jacq	Shrub	Yellow
Malvaceae	*Hibiscus ludwigii* Eckl. and Zeyh.	Shrub	Yellow
Lamiaceae	*Hoslundia opposita* Vahl	Herb	Yellowish
Papilionaceae	*Indiofera spicata* Forssk.	Herb	Red
Convulvulaceae	*Ipomoea cairica* (L.) Sweet	Herb	Purple
Convulvulaceae	*Ipomoea hederifolia* L.	Herb	Red
Convulvulaceae	*Ipomoea obscura* (L.) Ker-Gawl	Herb	Yellow
Convulvulaceae	*Ipomoea purpurea* (L.) Roth	Herb	Purple
Convulvulaceae	*Ipomoea wightii* (Wall.) Choisy	Herb	Purple
Acanthaceae	*Justicia flava* (Forsk) Vahl	Herb	Yellow
Acanthaceae	*Justicia heterocarpa* T.Andersson	Herb	Pink
Cyperaceae	*Kyllinga bulbosa* P.Beauv.	Sedge	White
Verbenaceae	*Lantana camara* L.	Shrub	Pink
Verbenaceae	*Lantana trifolia* L.	Shrub	Light purple
Lamiaceae	*Leonotis nepetifolia* (L.) Ait.f.	Herb	Red
Mimosaceae	*Leucaena leucocephala* (Lam.) De Wit	Tree	Red
Lamiaceae	*Leucas deflexa* Hook.f.	Herb	White
Verbenaceae	*Lippia abyssinica* (Otto and Diebr) Cuf.	Herb	White/yellow
Lamiaceae	*Luecas martinicensis* (Jacq) Ait.f.	Herb	White
Myrsinaceae	*Maesa lanceolata* Forsk	Tree	Green
Anacardiaceae	*Mangifera indica* L.	Tree	Cream
Bignoniaceae	*Markhamia lutea* K.Schum.	Tree	Yellow

Table 7: Continued.

Family	Species	Lifecycle	Flower color/shape
Asteraceae	*Melanthera scandens* (Schumach and Thonn) Roberty	Herb	Yellow
Asteraceae	*Microglossa pyrifolia* (Lam.) O.Ktze.	Shurb	Pale/yellow
Moraceae	*Milicia excelsa* (Welw.) C.C. Berg.	Tree	Greenish
Mimosaceae	*Mimosa pigra* L.	Shrub	Pink
Mimosaceae	*Mimosa pudica* L.	Herb	Purple
Rubiaceae	*Mitracarpus villosus* (S.W.) DC	Herb	White
Cucurbitaceae	*Momordica foetida* Schumach.	Herb	Yellow
Moringaceae	*Moringa oleifera* Lam.	Tree	White/cream
Moraceae	*Morus alba* L.	Tree	Nil
Musaceae	*Musa paradisiaca* L.	Tree	Red/brown/purple
Musaceae	*Musa sapienthum*	Tree	Red/brown/purple
Lamiaceae	*Ocimum gratissimum* (L.) O.Suave	Herb	White
Lamiaceae	*Ocimum gratissimum var.rutshuruensis* De Wild	Herb	White
Lamiaceae	*Ocimum suave* Willd.	Herb	White
Oxalidaceae	*Oxalis corniculata* L.	Herb	Yellow
Oxalidaceae	*Oxalis latifolia* L.	Herb	Pink
Polygonaceae	*Oxygonum snuatum* (Meisn.) Dammer	Herb	Pale/pink
Passifloraceae	*Passiflora edulis* Sims	Tree	Purple
Lauraceae	*Persea americana* Mill.	Tree	Cream
Solanaceae	*Physalis peruviana* L.	Herb	Yellow
Phytolacaceae	*Phytolacca dodecandra* L'Herit	Herb	Greenish/white
Lamiaceae	*Plastostoma africanum* P. Beauv.	Herb	White
Lamiaceae	*Plectranthus barbatus* Andr.	Herb	Purple
Rubiaceae	*Pnetas parvifolia*	Herb	Red/marron
Polygalaceae	*Polygala pygmaea* Gurke	Herb	Yellow
Polygonaceae	*Polygonum setosulum* A.Rich.	Herb	Pink
Papilionaceae	*Pseudarthria hookeri* Wight and Arn.	Herb	Pink
Myrtaceae	*Psidium guajava* L.	Tree	White
Euphorbiceae	*Ricinus communis* L.	Tree	Yellowish
Polygonaceae	*Rumex abyssinicus* Jacq	Herb	Greenish
Polygonaceae	*Rumex bequaertii* De Wild	Herb	Greenish
Asteraceae	*Senecio discifolius* Oliv.	Herb	Yellow
Caesalpiniaceae	*Senna didimobotrya* L.	Shrub	Yellow
Pedaliaceae	*Sesamum angolense* Welw	Herb	Purple
Malvaceae	*Sida acuta* Burm.f.	Herb	Cream/pale
Malvaceae	*Sida cordifolia* L.	Herb	Yellow
Malvaceae	Sida cuneifolia Roxb.	Herb	Yellow
Malvaceae	*Sida rhombifolia* L.	Herb	Whitish/yellowish
Asteraceae	*Siegesbeckia abyssinica* (Ch. Bip.) Oliv. and Hiern	Herb	Yellow
Asteraceae	*Siegesbeckia orientalis* L.	Herb	Yellow
Solanaceae	*Solanum nigrum* L.	Herb	White/yellow
Solanaceae	*Solanum aculeastrum* Dunal	Shrub	White
Solanaceae	*Solanum anguivii* Lam.	Herb	White
Solanaceae	*Solanum florulentum* Bitt.	Herb	White
Solanaceae	*Solanum incanum* L.	Herb	Purple
Solanaceae	*Solanum macrocarpon* L.	Herb	Purple
Solanaceae	*Solanum mauritianum* Scop	Shrub	Purple
Bignoniaceae	*Spathodea nilotica* Seem	Herb	Red
Rubiaceae	*Spermacoce princeae* (K. Schum.)	Herb	White
Verbenaceae	*Stachytarpheta cayennensis* (Rich.) Vahl	Herb	Blue

Table 7: Continued.

Family	Species	Lifecycle	Flower color/shape
Papilionaceae	*Tephrosia rhodesia* Bak.f.	Herb	Purple
Papilionaceae	*Tephrosia vogelii* Hook.f.	Shrub	Purple/white
Apocynaceae	*Thevetia pereviana* (Pob) K.Schum.	Shrub	Yellow
Acanthaceae	*Thumbergia holstii*	Herb	Purple
Acanthaceae	*Thunbergia alata* Sims	Herb	Yellow
Asteraceae	T*ridax procumbens*	Herb	Yellow
Tiliaceae	*Triumfetta tomenosa* Boj.	Shrub	Yellow
Tiliaceae	*Triumfetta rhomboidea* Jacq	Herb	Yellow
Tiliaceae	*Triumfetta trichocarpa* A.Rich.	Herb	Cream
Typhaceae	*Typha domingensis* Pers	Sedge	Brown
Malvaceae	*Urena lobata* L.	Herb	Pink
Asteraceae	*Vernonia cinerea* (L.) Less.	Herb	Purple
Asteraceae	*Vernonia amygdalina* Del.	Tree	White/cream
Asteraceae	*Vernonia auriculifera* Hiern	Tree	Purple
Asteraceae	*Vernonia auriculifera* Hiern	Tree	Cream/white
Asteraceae	*Vernonia campanea* S. moore	Herb	Purple
Asteraceae	*Vernonia kirungei*	Shrub	Purple
Asteraceae	*Vernonia lasiopus* O.Haffm.	Herb	Purple
Papilionaceae	*Vigna vexillata* (L.) A.Rich.	Herb	Purple

Different bee species used a variety of tree species (fruit and agroforestry tree species) as nest trees (Table 5). In other words, apart from establishing nests in various semi-natural habitats surrounding fields, some bee species (e.g., eusocial bees) managed to establish their nests in hallows of living trees, meaning that one should know these tree species and maintain them in the farm-landscape to increase nesting sites opportunities of good pollinators living within agricultural matrices.

4. Discussion

4.1. Effects of Climatic Factors on Bee Communities. Results from this study indicated that bee species richness and abundance were affected by climatic factors. In fact, species richness and abundance of bees were correlated with 10 years average temperature, as well as with temperature in the current year and two years prior to the study. Thus, temperature was found to be a very good and significant predictor of bee species in previous and current years. In climatic regions with strong wet-dry seasonality and low cold-hot seasonality, the main factor influencing occurrence, temporal distribution of different foraging bee species is temperature [18]. Even when there is a great variability in humidity and solar radiation (light intensity) along the year and the day, their influences on bee foraging seem to be small at the regional level although the influence at the microlevel may be higher. Temperature plays therefore a crucial role in occurrence and emergence of different adult bee species. Temperature is a key determinant of phenology of insect pollinated plants in natural and agricultural landscapes. Temperature is expected to be of primary importance in regulating phenology of pollinators, with other factors playing a secondary role [32, 54, 55]. The temperature seems to be responsible for adult appearance of bees in the environment, and this strong dependence may be expected because the average daily temperatures matters in the foraging behavior of many bee species. Multi-years monitoring data showed that climatic fluctuations are primarily responsible for the interannual variability in appearance phenology of bee species belonging to several functional groups [18].

While for some bee species (such as *Apis mellifera*), occurrence (presence/absence) may be related to increasing temperature, for most bee species, some factors (microhabitats, farming practices, and land-use intensity) may influence the occurrence/appearance and phenology. Although bee species richness and abundance were observed to be negatively and significantly correlated to maximum temperature and minimum temperature one to two years prior to the study, at the moment there exists no clear explanation for such pattern. The fact that the species richness and abundance of bees were strongly correlated with mean annual temperature in the previous years than in current years indicated potential vulnerability of local/native bee species to global environment and climatic changes [18]. Consequently, various bee species may be at risk of disappearance (extinction) in face of future climate change and variability in central Uganda. Such patterns were previously predicted for other pollinating species such as butterflies, flies, and beetles [18] in central Uganda.

Strong associations between previous (not current) year precipitation and the abundance/richness of bees were found in this study.

These results are consistent with the observation that previous year precipitation cues bee emergence in agricultural regions of Sub-Saharan Africa including Uganda [18].

Table 8: List of bee species collected in coffee-banana agroforestry systems in central Uganda in 2006.

Family	Species	Family	Species
Andrenidae	*Andrena africana* (Friese, 1909)	Halictidae	*Lasioglossum somereni* (Cockerell, 1945)
Andrenidae	*Andrena notophila* (Cockerell)	Halictidae	*Lasioglossum stellatifrons* (Cockerell, 1945)
Andrenidae	*Melitturga penrithorum* (Eardley, 1991)	Halictidae	*Lasioglossum trichardti* (Cockerell)
Andrenidae	*Meliturgula braunsi* (Friese, 1903)	Halictidae	*Lasioglossum ugandicum* (Cockerell, 1937)
Andrenidae	*Meliturgula eardleyana* (Patiny, 2000)	Halictidae	*Lasioglossum zonaturum* (Cockerell)
Andrenidae	*Meliturgula flavida* (Friese, 1913)	Halictidae	*Lassioglossum simulator* (Cockerell, 1935)
Andrenidae	*Meliturgula rozeni* (Eardley, 1991)	Halictidae	*Lipotriches ablusa* (Cockerell)
Andrenidae	*Meliturgula scriptifrons* (Walker, 1871)	Halictidae	*Lipotriches amatha* (Cockerell, 1935)
Andrenidae	*Meliturgula wilmattae* (Cockerell, 1932)	Halictidae	*Lipotriches angustifrons* (Cockerell)
Apidae	*Afromelecta bicuspis* (Stadelmann, 1898)	Halictidae	*Lipotriches armatipes* (Friese, 1930)
Apidae	*Allodape armatipes* (Friese, 1924)	Halictidae	*Lipotriches aureotecta* (Cockerell, 1931)
Apidae	*Allodape brachycephala* (Michener, 1971)	Halictidae	*Lipotriches aurifrons* (Smith, 1853)
Apidae	*Allodape ceratinoides* (Gribodo, 1884)	Halictidae	*Lipotriches brevipennis* (Friese, 1915)
Apidae	*Allodape collaris* (Vachal, 1903)	Halictidae	*Lipotriches clavata* (Cockerell)
Apidae	*Allodape exoloma* (Strand, 1915)	Halictidae	*Lipotriches collaris* (Vachal)
Apidae	*Allodape friesei* (Strand, 1915)	Halictidae	*Lipotriches cubitalis* (Vachal)
Apidae	*Allodape interrupta* (Vachal, 1903)	Halictidae	*Lipotriches dentipes* (Friese, 1930)
Apidae	*Allodape macula* (Strand, 1912)	Halictidae	*Lipotriches digitata* (Friese, 1909)
Apidae	*Allodape microsticta* (Cockerell, 1934)	Halictidae	*Lipotriches ethioparca* (Cockerell, 1935)
Apidae	*Allodape punctata* (Lepeletier and Audinet-Serville, 1825)	Halictidae	*Lipotriches flavitarsis* (Friese)
Apidae	*Allodape quadrilineata* (Cameron, 1905)	Halictidae	*Lipotriches friesei* (Magretti, 1899)
Apidae	*Allodape rufogastra* (Lepeletier and Audinet-Serville, 1825)	Halictidae	*Lipotriches gratiosa* (Strand)
Apidae	*Allodape tridentipes* (Cockerell, 1933)	Halictidae	*Lipotriches guluensis* (Cockerell)
Apidae	*Allodapula acutigera* (Cockerell, 1936)	Halictidae	*Lipotriches hirsutula* (Cockerell)
Apidae	*Allodapula hessei* (Michener)	Halictidae	*Lipotriches inaequalis* (Cockerell)
Apidae	*Allodapula jucunda* (Smith, 1879)	Halictidae	*Lipotriches kampalana* (Cockerell, 1935)
Apidae	*Allodapula maculithorax* (Michener, 1971)	Halictidae	*Lipotriches longipes* (Strand)
Apidae	*Allodapula melanopus* (Cameron, 1905)	Halictidae	*Lipotriches macropus* (Friese)
Apidae	*Allodapula monticola* (Cockerell, 1933)	Halictidae	*Lipotriches meadewaldoi* (Brauns, 1912)
Apidae	*Allodapula palliceps* (Friese, 1924)	Halictidae	*Lipotriches natalensis* (Cockerell, 1916)
Apidae	*Allodapula rozeni* (Michener, 1975)	Halictidae	*Lipotriches notabilis* (Schletterer)
Apidae	*Allodapula variegata* (Smith, 1854)	Halictidae	*Lipotriches nubecula* (Smith, 1875)
Apidae	*Amegilla acraensis* (Fabricius, 1793)	Halictidae	*Lipotriches oberthurella* (Saussure)
Apidae	*Amegilla africana* (Friese, 1905)	Halictidae	*Lipotriches orientalis* (Friese, 1909)
Apidae	*Amegilla albocaudata* (Dours, 1869)	Halictidae	*Lipotriches patellifera* (Westwood, 1875)
Apidae	*Amegilla atrocincta* (Lepeletier, 1841)	Halictidae	*Lipotriches picardi* (Gribodo)
Apidae	*Amegilla bothai* (Friese)	Halictidae	*Lipotriches reichardia* (Strand, 1911)
Apidae	*Amegilla calens* (Lepeletier, 1841)	Halictidae	*Lipotriches rubella* (Smith)
Apidae	*Amegilla capensis* (Friese)	Halictidae	*Lipoiriches rufipes* (Smith, 1875)
Apidae	*Amegilla eritrina* (Friese, 1915)	Halictidae	*Lipotriches ruwenzorica* (Cockerell, 1935)
Apidae	*Amegilla fallax* (Smith, 1879)	Halictidae	*Lipotriches sessensis* (Cockerell)
Apidae	*Amegilla madecassa* (Saussure)	Halictidae	*Lipotriches sjoestedti* (Friese, 1909)
Apidae	*Amegilla mimadvena* (Cockerell, 1916)	Halictidae	*Lipotriches speculina* (Cockerell, 1942)
Apidae	*Amegilla natalensis* (Friese, 1922)	Halictidae	*Lipotriches spinulifera* (Cockerell)
Apidae	*Amegilla nila* (Eardley, 1994)	Halictidae	*Lipotriches tanganyicensis* (Strand, 1913)
Apidae	*Amegilla niveata* (Friese, 1905)	Halictidae	*Lipotriches viciniformis* (Cockerell, 1939)
Apidae	*Amegilla nubica* (Lepeletier, 1841)	Halictidae	*Lipotriches vulpina* (Gerstäcker, 1857)

Table 8: Continued.

Family	Species	Family	Species
Apidae	*Amegilla obscuriceps* (Friese, 1905)	Halictidae	*Lipotriches welwitschi* (Cockerell, 1908)
Apidae	*Amegilla penicula* (Eardley, 1994)	Halictidae	*Lipotriches whitfieldi* (Cockerell, 1942)
Apidae	*Amegilla punctifrons* (Walker, 1871)	Halictidae	*Nomia amabilis* (Cockerell, 1908)
Apidae	*Amegilla rapida* (Smith, 1879)	Halictidae	*Nomia atripes* (Friese, 1909)
Apidae	*Amegilla regalis* (Cockerell, 1946)	Halictidae	*Nomia bouyssoui* (Vachal, 1903)
Apidae	*Amegilla rufipes* (Lepeletier, 1841)	Halictidae	*Nomia brevipes* (Friese, 1914)
Apidae	*Amegilla sierra* (Eardley, 1994)	Halictidae	*Nomia candida* (Smith, 1875)
Apidae	*Amegilla somalica* (Magretti)	Halictidae	*Nomia chandleri* (Ashmead, 1899)
Apidae	*Amegilla terminata* (Smith, 1879)	Halictidae	*Nomia clavicauda* (Cockerell)
Apidae	*Anthophora vestita* (Smith, 1854)	Halictidae	*Nomia elephas* (Strand, 1911)
Apidae	*Anthophora armata* (Friese, 1905)	Halictidae	*Nomia ethiopica* (Pauly, 2000)
Apidae	*Anthophora basalis* (Smith)	Halictidae	*Nomia felina* (Cockerell)
Apidae	*Anthophora braunsiana* (Friese, 1905)	Halictidae	*Nomia forbesii* (W. F. Kirby, 1900)
Apidae	*Anthophora diversipes* (Friese, 1922)	Halictidae	*Nomia garambensis* (Pauly, 2000)
Apidae	*Anthophora glaucopis* (Friese, 1905)	Halictidae	*Nomia granulata* (Vachal, 1903)
Apidae	*Anthophora rufolanata* (Dours)	Halictidae	*Nomia lutea* (Warncke, 1976)
Apidae	*Anthophora rufovestita* (Cockerell)	Halictidae	*Nomia maculata* (Friese)
Apidae	*Anthophora schultzei* (Friese, 1909)	Halictidae	*Nomia marginata* (Pauly, 1990)
Apidae	*Anthophora strucki* (Eardley and Brooks, 1989)	Halictidae	*Nomia nigrociliata* (Cockerell, 1932)
Apidae	*Anthophora wartmanni* (Friese, 1905)	Halictidae	*Nomia politula* (Cockerell)
Apidae	*Anthophora xanthostoma* (Cockerell, 1932)	Halictidae	*Nomia postscutellaris* (Strand, 1914)
Apidae	*Apis mellifera adansonii* (Linnaeus, 1758)	Halictidae	*Nomia pretoriensis* (Cockerell, 1946)
Apidae	*Apis mellifera scutellata* (Latreille, 1804)	Halictidae	*Nomia rozeni* (Pauly, 2000)
Apidae	*Braunsapis facialis* (Gerstäcker, 1857)	Halictidae	*Nomia rufosuffusa* (Cockerell, 1935)
Apidae	*Braunsapis albipennis* (Friese, 1909)	Halictidae	*Nomia senticosa* (Vachal, 1897)
Apidae	*Braunsapis albitarsis* (Friese, 1924)	Halictidae	*Nomia somalica* (Friese, 1908)
Apidae	*Braunsapis angolensis* (Cockerell, 1933)	Halictidae	*Nomia stageri* (Pauly, 2000)
Apidae	*Braunsapis bouyssoui* (Vachal, 1903)	Halictidae	*Nomia theryi* (Gribodo, 1894)
Apidae	*Braunsapis flavitarsis* (Gerstaecker)	Halictidae	*Nomia viridicincta* (Meade-Waldo)
Apidae	*Braunsapis foveata* (Smith, 1854)	Halictidae	*Nomia whiteana* (Cameron, 1905)
Apidae	*Braunsapis gorillarum* (Cockerell, 1936)	Halictidae	*Nomia zonaria* (Walker, 1871)
Apidae	*Braunsapis leptozonia* (Vachal)	Halictidae	*Nomioides micheneri* Pesenko and Pauly
Apidae	*Braunsapis minutula* (Friese, 1914)	Halictidae	*Patellapis aberdarica* (Cockerell, 1945)
Apidae	*Braunsapis natalica* (Michener, 1970)	Halictidae	*Patellapis albofasciata* (Smith, 1879)
Apidae	*Braunsapis neavei* (Vachal, 1910)	Halictidae	*Patellapis benoiti* (Pauly)
Apidae	*Braunsapis rhodesi* (Cockerell, 1936)	Halictidae	*Patellapis bilineata* (Friese)
Apidae	*Braunsapis strandi* (Masi, 1930)	Halictidae	*Patellapis communis* (Smith, 1879)
Apidae	*Braunsapis vitrea* (Vachal, 1903)	Halictidae	*Patellapis disposita* (Cameron, 1905)
Apidae	*Ceratina aliceae* (Cockerell, 1937)	Halictidae	*Patellapis flavofasciata* (Friese, 1915)
Apidae	*Ceratina armata* (Smith, 1854)	Halictidae	*Patellapis flavorufa* (Cockerell, 1937)
Apidae	*Ceratina braunsi* (Eardley and Daly, 2007)	Halictidae	*Patellapis glabra* (Pauly, 1989)
Apidae	*Ceratina excavata* (Cockerell)	Halictidae	*Patellapis gowdeyi* (Cockerell, 1937)
Apidae	*Ceratina labrosa* (Friese, 1905)	Halictidae	*Patellapis hargreavesi* (Cockerell)
Apidae	*Ceratina lineola* (Vachal, 1903)	Halictidae	*Patellapis harunganae* (Pauly, 1989)
Apidae	*Ceratina lunata* (Friese, 1905)	Halictidae	*Patellapis kivuensis* (Pauly, 1989)
Apidae	*Ceratina minuta* (Friese, 1905)	Halictidae	*Patellapis macrozonia* (Cockerell)
Apidae	*Ceratina moerenhouti* (Vachal)	Halictidae	*Patellapis minima* (Friese, 1909)

Table 8: Continued.

Family	Species	Family	Species
Apidae	*Ceratina nasalis* (Friese, 1905)	Halictidae	*Patellapis minutior* (Friese, 1909)
Apidae	*Ceratina nigriceps* (Friese, 1905)	Halictidae	*Patellapis mosselina* (Cockerell)
Apidae	*Ceratina nilotica* (Cockerell, 1937)	Halictidae	*Patellapis neavei* (Cockerell, 1946)
Apidae	*Ceratina paulyi* (Daly, 1988)	Halictidae	*Patellapis nomioides* (Friese, 1909)
Apidae	*Ceratina penicillata* (Friese, 1905)	Halictidae	*Patellapis obscurescens* (Cockerell)
Apidae	*Ceratina rufigastra* (Cockerell, 1937)	Halictidae	*Patellapis partita* (Cockerell, 1933)
Apidae	*Ceratina ruwenzorica* (Cockerell)	Halictidae	*Patellapis patriciformis* (Cockerell, 1933)
Apidae	*Ceratina speculifrons* (Cockerell, 1920)	Halictidae	*Patellapis perineti* (Benoist, 1954)
Apidae	*Ceratina tanganyicensis* (Strand, 1911)	Halictidae	*Patellapis perpansa* (Cockerell, 1933)
Apidae	*Ceratina viridifrons* (Cockerell, 1934)	Halictidae	*Patellapis pondoensis* (Cockerell)
Apidae	*Ceratina viridis* (Guérin-Méneville, 1844)	Halictidae	*Patellapis retigera* (Cockerell)
Apidae	*Ceratina whiteheadi* (Eardley and Daly, 2007)	Halictidae	*Patellapis ruwensorensis* (Strand, 1911)
Apidae	*Cleptotrigona cubiceps* (Friese, 1912)	Halictidae	*Patellapis schultzei* (Friese, 1909)
Apidae	*Compsomelissa nigrinervis* (Cameron, 1905)	Halictidae	*Patellapis spinulosa* (Cockerell)
Apidae	*Compsomelissa nigrinervis* (Cameron, 1905)	Halictidae	*Patellapis terminalis* (Smith, 1853)
Apidae	*Ctenoplectra albolimbata* (Magretti)	Halictidae	*Patellapis tshibindica* (Cockerell)
Apidae	*Ctenoplectra antinorii* (Gribodo, 1884)	Halictidae	*Patellapis vittata* (Smith, 1853)
Apidae	*Ctenoplectra armata* (Magretti, 1895)	Halictidae	*Pseudapis aliceae* (Cockerell, 1935)
Apidae	*Ctenoplectra polita* (Strand, 1912)	Halictidae	*Pseudapis anomala* (W. F. Kirby, 1900)
Apidae	*Ctenoplectra terminalis* (Smith, 1879)	Halictidae	Pseudapis anthidioides (Gerstäcker, 1857)
Apidae	*Ctenoplectra ugandica* (Cockerell, 1944)	Halictidae	*Pseudapis armata* (Olivier, 1812)
Apidae	*Ctenoplectrina politula* (Cockerell, 1930)	Halictidae	*Pseudapis flavicarpa* (Vachal)
Apidae	*Dactylurina schmidti* (Stadelmann, 1895)	Halictidae	*Pseudapis kenyensis* (Pauly, 1990)
Apidae	*Dactylurina staudingeri* (Gribodo)	Halictidae	*Pseudapis patellata* (Magretti, 1884)
Apidae	*Epeolus amabilis* (Gerstäcker, 1869)	Halictidae	*Pseudapis rhodocantha* (Cockerell)
Apidae	*Epeolus corniculatus* (Bischoff)	Halictidae	*Pseudapis rugiventris* (Friese, 1930)
Apidae	*Epeolus friesei* (Brauns, 1903)	Halictidae	*Pseudapis schubotzi* (Strand)
Apidae	*Epeolus natalensis* (Smith, 1879)	Halictidae	*Seladonia africana* (Friese)
Apidae	*Hypotrigona gribodoi* (Magretti, 1884)	Halictidae	*Seladonia jucundus* (Smith)
Apidae	*Liotrigona bottegoi* (Magretti, 1895)	Halictidae	*Seladonia jucundus* (Smith, 1853)
Apidae	*Macrogalea candida* (Smith, 1879)	Halictidae	*Seladonia valligensis* (Cockerell, 1937)
Apidae	*Meliponula bocandei* (Spinola, 1853)	Halictidae	*Spatunomia filifera* (Cockerell)
Apidae	*Meliponula ferruginea* (Lepeletier, 1836)	Halictidae	*Sphecodes abyssinicus* (Sichel)
Apidae	*Meliponula lendliana* (Friese, 1900)	Halictidae	*Sphecodes braunsi* (Blüthgen)
Apidae	*Meliponula nebulata* (Smith, 1854)	Halictidae	*Sphecodes centralis* (Cockerell)
Apidae	*Nomada africana* (Friese, 1911)	Halictidae	*Sphecodes dilutus* (Cockerell)
Apidae	*Nomada aurantifascia* (Eardley and Schwarz, 1991)	Halictidae	*Sphecodes fimbriatus* (Blüthgen)
Apidae	*Nomada eximia* (Eardley and Schwarz, 1991)	Halictidae	*Sphecodes hagensi* (Ritsema)
Apidae	*Nomada gigas* (Friese, 1905)	Halictidae	*Sphecodes luteiventris* (Friese)
Apidae	*Nomada whiteheadi* (Eardley and Schwarz, 1991)	Halictidae	*Sphecodes punctatus* (Sichel, 1865)
Apidae	*Pachymelus abessinicus* (Friese, 1913)	Halictidae	*Sphecodes punctiscutum* (Eardley and Urban)
Apidae	*Pachymelus bettoni* (Cockerell)	Halictidae	*Sphecodes ugandae* (Blüthgen, 1928)
Apidae	*Pachymelus ciliatus* (Friese, 1922)	Halictidae	*Sphecodes woodi* (Cockerell)
Apidae	*Pachymelus claviger* (Benoist, 1962)	Halictidae	*Thrinchostoma bequaerti* (Blüthgen)

Table 8: Continued.

Family	Species	Family	Species
Apidae	*Pachymelus conspicuus* (Smith, 1879)	Halictidae	*Thrinchostoma emini* (Blüthgen, 1930)
Apidae	*Pachymelus festivus* (Dours, 1869)	Halictidae	*Thrinchostoma mwangai* (Blüthgen)
Apidae	*Pachymelus reichardti* (Stadelmann, 1898)	Halictidae	*Thrinchostoma sjoestedti* (Friese, 1909)
Apidae	*Pasites appletoni* (Cockerell, 1910)	Halictidae	*Thrinchostoma torridum* (Smith)
Apidae	*Pasites barkeri* (Cockerell, 1919)	Halictidae	*Thrinchostoma ugandae* (Blüthgen, 1930)
Apidae	*Pasites braunsi* (Bischoff, 1923)	Halictidae	*Thrinchostoma umtaliellus* (Cockerell, 1937)
Apidae	*Pasites carnifex* (Gerstäcker, 1869)	Halictidae	*Thrinchostoma wissmanni* (Blüthgen, 1930)
Apidae	*Pasites dichroa* (Smith, 1854)	Halictidae	*Systropha ugandensis* (Cockerell)
Apidae	*Pasites friesei* (Cockerell, 1910)	Megachilidae	*Afranthidium braunsi* (Friese, 1904)
Apidae	*Pasites humecta* (Eardley, 1997)	Megachilidae	*Afranthidium junodi* (Friese, 1904)
Apidae	*Pasites jenseni* (Friese, 1915)	Megachilidae	*Afranthidium sjoestedti* (Friese, 1909)
Apidae	*Pasites jonesi* (Cockerell, 1921)	Megachilidae	*Afranthidium tanganyicola* (Strand, 1911)
Apidae	*Pasites rotundiceps* (Bischoff, 1923)	Megachilidae	*Afroheriades larvatus* (Friese, 1909)
Apidae	*Pasites rufipes* (Friese, 1915)	Megachilidae	*Afroheriades reicherti* (Brauns, 1929)
Apidae	*Pasites somalicus* (Eardley, 1997)	Megachilidae	*Anthidiellum bipectinatum* (Pasteels, 1984)
Apidae	*Plebeina hildebrandti* (Friese, 1900)	Megachilidae	*Anthidiellum eritrinum* (Friese, 1915)
Apidae	*Sphecodopsis aculeata* (Friese, 1922)	Megachilidae	*Anthidiellum rubellum* (Friese, 1917)
Apidae	*Sphecodopsis capensis* (Friese, 1915)	Megachilidae	*Anthidium abjunctum* (Cockerell, 1936)
Apidae	*Sphecodopsis capicola* (Strand, 1911)	Megachilidae	*Anthidium basale* (Pasteels, 1984)
Apidae	*Sphecodopsis minutissima* (Cockerell, 1933)	Megachilidae	*Anthidium cordiforme* (Friese, 1922)
Apidae	*Sphecodopsis vespericena* (Eardley, 1997)	Megachilidae	*Anthidium niveocinctum* (Gerstäcker, 1857)
Apidae	*Tetralonia boharti* (Eardley, 1989).	Megachilidae	*Anthidium pontis* (Cockerell, 1933)
Apidae	*Tetralonia caudata* (Friese, 1905)	Megachilidae	*Anthidium severini* (Vachal, 1903)
Apidae	*Tetralonia macrognatha* (Gerstäcker, 1870)	Megachilidae	*Coelioxys aurifrons* (Smith)
Apidae	*Tetralonia obscuriceps* (Friese, 1916)	Megachilidae	*Coelioxys caffra* (Friese)
Apidae	*Tetralonia penicillata* (Friese, 1905)	Megachilidae	*Coelioxys cherenensis* (Friese)
Apidae	*Tetralonia ruficollis* (Friese, 1911)	Megachilidae	*Coelioxys foveolata* (Smith)
Apidae	*Tetralonia trichardti* (Cockerell, 1933)	Megachilidae	*Coelioxys nasuta* (Friese)
Apidae	*Tetraloniella apicalis* (Friese, 1905)	Megachilidae	*Coelioxys natalensis* (Cockerell, 1920)
Apidae	*Tetraloniella aurantiflava* (Eardley, 1989)	Megachilidae	*Coelioxys odin* (Strand, 1912)
Apidae	*Tetraloniella braunsiana* (Friese, 1905)	Megachilidae	*Coelioxys recusata* (Schulz)
Apidae	*Tetraloniella brevikeraia* (Eardley, 1989)	Megachilidae	*Coelioxys torrida* (Smith)
Apidae	*Tetraloniella capensis* (Lepeletier, 1841)	Megachilidae	*Coelioxys ultima* (Pasteels)
Apidae	*Tetraloniella elsei* (Eardley, 1989)	Megachilidae	*Coelioxys verticalis* (Smith, 1854)
Apidae	*Tetraloniella friesei* (Meade-Waldo, 1914)	Megachilidae	*Eoanthidium rothschildi* (Vachal)
Apidae	*Tetraloniella junodi* (Friese, 1909)	Megachilidae	*Euaspis abdominalis* (Fabricius)
Apidae	*Tetraloniella katangensis* (Cockerell, 1930)	Megachilidae	*Euaspis abdominalis* (Fabricius, 1773)
Apidae	*Tetraloniella michaelseni* (Friese, 1916)	Megachilidae	*Heriades arnoldi* (Friese)
Apidae	*Tetraloniella minuta* (Friese, 1905)	Megachilidae	*Heriades bequerti* (Cockerell)
Apidae	*Tetraloniella nanula* (Cockerell, 1932)	Megachilidae	*Heriades bouyssoui* (Vachal, 1903)
Apidae	*Tetraloniella paulyi* (Eardley, 2001)	Megachilidae	*Heriades capicola* (Strand, 1912)
Apidae	*Tetraloniella sierranila* (Eardley, 1989)	Megachilidae	*Heriades eximius* (Friese)
Apidae	*Tetraloniella simpsoni* (Meade-Waldo, 1914)	Megachilidae	*Heriades fumipennis* (Cockerell)
Apidae	*Tetraloniella sjoestedti* (Friese, 1909)	Megachilidae	*Heriades humilis* (Cockerell)
Apidae	*Tetraloniella whiteheadi* (Eardley, 1989)	Megachilidae	*Heriades rufifrons* (Cockerell, 1932)

Table 8: Continued.

Family	Species	Family	Species
Apidae	*Thyreus abyssinicus* (Radoszkowski, 1873)	Megachilidae	*Heriades scutellatus* (Friese, 1922)
Apidae	*Thyreus albomaculatus* (DeGeer, 1778)	Megachilidae	*Heriades speculiferus* (Cockerell)
Apidae	*Thyreus axillaris* (Vachal, 1903)	Megachilidae	*Hoplitis infrapicta* (Cockerell, 1916)
Apidae	*Thyreus bouyssoui* (Vachal, 1903)	Megachilidae	*Lithurgus pullatus* (Vachal, 1903)
Apidae	*Thyreus calceatus* (Vachal, 1903)	Megachilidae	*Lithurgus spiniferus* (Cameron)
Apidae	*Thyreus delumbatus* (Vachal, 1903)	Megachilidae	*Lithurgus spiniferus* (Cameron, 1905)
Apidae	*Thyreus interruptus* (Vachal, 1903)	Megachilidae	*Megachile abessinica* (Friese, 1915)
Apidae	*Thyreus meripes* (Vachal)	Megachilidae	*Megachile accraensis* (Friese, 1903)
Apidae	*Thyreus neavei* (Cockerell, 1933)	Megachilidae	*Megachile aculeata* (Vachal, 1910)
Apidae	*Thyreus niloticus* (Cockerell, 1937)	Megachilidae	*Megachile admixta* (Cockerell, 1931)
Apidae	*Thyreus oxaspis* (Cockerell, 1936)	Megachilidae	*Megachile afra* (Pasteels, 1965)
Apidae	*Thyreus pretextus* (Vachal)	Megachilidae	*Megachile albocincta* (Radoszkowski, 1874)
Apidae	*Thyreus scotaspis* (Vachal, 1903)	Megachilidae	*Megachile aliceae* (Cockerell, 1932)
Apidae	*Thyreus somalicus* (Strand, 1911)	Megachilidae	*Megachile altera* (Vachal)
Apidae	*Thyreus stellifera* (Cockerell)	Megachilidae	*Megachile apiformis* (Smith, 1853)
Apidae	*Xylocopa africana* (Fabricius, 1781)	Megachilidae	*Megachile attenuata* (Vachal, 1910)
Apidae	*Xylocopa albiceps* (Fabricius, 1804)	Megachilidae	*Megachile aurifera* (Cockerell)
Apidae	*Xylocopa apicalis* (Smith, 1854)	Megachilidae	*Megachile basalis* (Smith, 1853)
Apidae	*Xylocopa braunsi* (Dusmet and Y Alonso, 1924)	Megachilidae	*Megachile battorensis* (Meade-Waldo, 1912)
Apidae	*Xylocopa caffra* (Linnaeus, 1767)	Megachilidae	*Megachile beniticola* (Strand, 1912)
Apidae	*Xylocopa calcarata* (Le Veque, 1928)	Megachilidae	*Megachile bilobata* (Friese, 1915)
Apidae	*Xylocopa calens* (Lepeletier, 1841)	Megachilidae	*Megachile boswendica* (Cockerell)
Apidae	*Xylocopa erythrina* (Gribodo, 1894)	Megachilidae	*Megachile burungana* (Cockerell)
Apidae	*Xylocopa flavicollis* (DeGeer, 1778)	Megachilidae	*Megachile capitata* (Smith, 1853)
Apidae	*Xylocopa flavorufa* (DeGeer, 1778)	Megachilidae	*Megachile chrysopogon* (Vachal)
Apidae	*Xylocopa gaullei* (Vachal, 1898)	Megachilidae	*Megachile cincta* (Fabricius)
Apidae	*Xylocopa gribodoi* (Magretti, 1892)	Megachilidae	*Megachile cognata* (Smith, 1853)
Apidae	*Xylocopa hottentota* (Smith, 1854)	Megachilidae	*Megachile congruens* (Friese)
Apidae	*Xylocopa imitator* (Smith, 1854)	Megachilidae	*Megachile coniformis* (Friese, 1922)
Apidae	*Xylocopa inconstans* (Smith, 1874)	Megachilidae	*Megachile cornigera* (Friese, 1904)
Apidae	*Xylocopa lateritia* (Smith, 1854)	Megachilidae	*Megachile crassitarsis* (Cockerell, 1920)
Apidae	*Xylocopa mixta* (Radoszkowski, 1881)	Megachilidae	*Megachile curtula* (Gerstaecker, 1857)
Apidae	*Xylocopa modesta* (Smith, 1854)	Megachilidae	*Megachile devexa* (Vachal, 1903)
Apidae	Xylocopa nigrita (Fabricius, 1775)	Megachilidae	*Megachile digiticauda* (Cockerell, 1937)
Apidae	*Xylocopa olivacea* (Fabricius, 1778)	Megachilidae	*Megachile discolor* (Smith)
Apidae	*Xylocopa praeusta* (Smith, 1854)	Megachilidae	*Megachile dolichognatha* (Cockerell)
Apidae	*Xylocopa pubescens* (Spinola, 1838)	Megachilidae	*Megachile dorsata* (Smith, 1853)
Apidae	*Xylocopa senior* (Vachal, 1899)	Megachilidae	*Megachile edwardsiana* (Friese, 1925)
Apidae	*Xylocopa torrida* (Westwood, 1838)	Megachilidae	*Megachile ekuivella* (Cockerell, 1909)
Apidae	*Xylocopa ustulata* (Smith, 1854)	Megachilidae	*Megachile erythrura* (Pasteels, 1970)
Apidae	*Xylocopa varipes* (Smith, 1854)	Megachilidae	*Megachile eupyrrha* (Cockerell, 1937)
Apidae	*Xylocopa villosa* (Friese, 1909)	Megachilidae	*Megachile eurymera* (Smith, 1864)
Apidae	*Xylocopa wellmani* (Cockerell, 1906)	Megachilidae	*Megachile excavata* (Cockerell)
Colletidae	*Colletes eardleyi* (Kuhlmann)	Megachilidae	*Megachile fastigiata* (Vachal)
Colletidae	*Colletes opacicollis* (Friese)	Megachilidae	Megachile felina (Gerstäcker, 1857)
Colletidae	*Colletes reginae* (Cockerell)	Megachilidae	*Megachile fimbriata* (Smith, 1853)
Colletidae	*Colletes rothschildi* (Vachal)	Megachilidae	*Megachile flavipennis* (Smith, 1853)

Table 8: Continued.

Family	Species	Family	Species
Colletidae	*Colletes rufitarsis* (Friese)	Megachilidae	*Megachile fulva* (Smith, 1853)
Colletidae	*Colletes schultzei* (Friese)	Megachilidae	*Megachile fulvitarsis* (Friese, 1910)
Colletidae	*Colletes somereni* (Cockerell)	Megachilidae	*Megachile fulvohirta* (Friese, 1904)
Colletidae	*Hyaeus tinctulus* (Cockerell)	Megachilidae	*Megachile funebris* (Radoszkowski, 1874)
Colletidae	*Hylaeus alfkeni* (Friese, 1913)	Megachilidae	*Megachile garambana* (Pasteels)
Colletidae	*Hylaeus braunsi* (Alfken, 1905)	Megachilidae	*Megachile gastracantha* (Cockerell)
Colletidae	*Hylaeus fortis* (Cockerell)	Megachilidae	*Megachile globiceps* (Pasteels)
Colletidae	*Hylaeus heraldicus* (Smith, 1853)	Megachilidae	*Megachile gowdeyi* (Cockerell, 1931)
Colletidae	Hylaeus lineaticeps (Friese, 1913)	Megachilidae	Megachile gratiosa (Gerstäcker, 1857)
Colletidae	*Hylaeus magrettii* (Vachal)	Megachilidae	*Megachile griseola* (Cockerell)
Colletidae	*Hylaeus neave*i (Cockerell, 1942)	Megachilidae	*Megachile hecate* (Vachal)
Colletidae	*Hylaeus scutispinus* (Alfken)	Megachilidae	*Megachile hirticauda* (Cockerell)
Colletidae	*Hylaeus subfortis* (Cockerell)	Megachilidae	*Megachile hopilitis* (Vachal, 1903)
Colletidae	*Hylaeus ugandicus* (Cockerell, 1939)	Megachilidae	*Megachile ikuthaensis* (Friese)
Colletidae	*Scrapter albitarsis* (Friese, 1909)	Megachilidae	*Megachile invenita* (Pasteels)
Colletidae	*Scrapter algoensis* (Friese, 1925)	Megachilidae	*Megachile junodi* (Friese, 1904)
Colletidae	*Scrapter amplispinatus* (Eardley, 1996)	Megachilidae	*Megachile laminata* (Friese)
Colletidae	*Scrapter amplitarsus* (Eardley, 1996)	Megachilidae	*Megachile leucospila* (Cockerell, 1933)
Colletidae	*Scrapter armatipes* (Friese, 1913)	Megachilidae	*Megachile lineofasciata* (Pasteels, 1965)
Colletidae	*Scrapter aureiferus* (Cockerell, 1932)	Megacliilidae	*Megachile luteociliata* (Pasteels)
Colletidae	*Scrapter avius* (Eardley, 1996)	Megachilidae	*Megachile mabirensis* (Cockerell)
Colletidae	*Scrapter basutorum* (Cockerell, 1915)	Megachilidae	*Megachile mackieae* (Cockerell, 1937)
Colletidae	*Scrapter bicolor* (Lepeletier and Audinet-Serville, 1825)	Megachilidae	*Megachile maculosella* (Pasteels, 1965)
Colletidae	*Scrapter caesariatus* (Eardley, 1996)	Megachilidae	*Megachile manyara* (Eardley and Urban)
Colletidae	*Scrapter calx* (Eardley, 1996)	Megachilidae	*Megachile masaiella* (Cockerell, 1930)
Colletidae	*Scrapter capensis* (Friese, 1909)	Megachilidae	*Megachile meadewaldoi* (Brauns, 1912)
Colletidae	*Scrapter catoxys* (Davies, 2005)	Megachilidae	*Megachile mimetica* (Cockerell)
Colletidae	*Scrapter chloris* (Eardley, 1996)	Megachilidae	*Megachile mixtura* (Eardley and R. P. Urban, 2005)
Colletidae	*Scrapter chrysomastes* (Davies, 2005)	Megachilidae	*Megachile nasalis* (Smith, 1879)
Colletidae	*Scrapter erubescens* (Friese, 1925)	Megachilidae	*Megachile natalica* (Cockerell, 1920)
Colletidae	*Scrapter flavipes* (Friese, 1925)	Megachilidae	*Megachile neave*i (Vachal, 1910)
Colletidae	*Scrapter flavostictus* (Cockerell, 1934)	Megachilidae	*Megachile nigroaurea* (Pasteels)
Colletidae	*Scrapter glarea* (Davies, 2005)	Megachilidae	*Megachile niveicauda* (Cockerell, 1920)
Colletidae	*Scrapter heterodoxus* (Cockerell, 1921)	Megachilidae	*Megachile niveofasciata* (Friese, 1904)
Colletidae	*Scrapter leonis* (Cockerell, 1934)	Megachilidae	*Megachile panda* (Cockerell)
Colletidae	*Scrapter luridus* (Eardley, 1996)	Megachilidae	*Megachile paupera* (Pasteels, 1965)
Colletidae	*Scrapter niger* (Lepeletier and Audinet-Serville, 1825)	Megachilidae	*Megachile perfimbriata* (Cockerell, 1920)
Colletidae	*Scrapter nitidus* (Friese, 1909)	Megachilidae	*Megachile postnigra* (Cockerell)
Colletidae	*Scrapter pallidipennis* (Cockerell, 1920)	Megachilidae	*Megachile pulvinata* (Vachal)
Colletidae	*Scrapter pruinosus* (Davies, 2006)	Megachilidae	*Megachile pyrrhothorax* (Schletterer, 1891)
Colletidae	*Scrapter pyretus* (Davies, 2006)	Megachilidae	*Megachile rosarum* (Cockerell)
Colletidae	*Scrapter rufescens* (Friese, 1913)	Megachilidae	*Megachile rufa* (Friese, 1903)
Colletidae	*Scrapter ruficornis* (Cockerell, 1916)	Megachilidae	*Megachile rufigaster* (Cockerell, 1945)
Colletidae	*Scrapter striatus* (Smith, 1853)	Megachilidae	*Megachile rufipennis* (Fabricius, 1793)
Colletidae	*Scrapter viciniger* (Davies, 2006)	Megachilidae	*Megachile rufipes* (Fabricius, 1781)
Colletidae	*Scrapter whiteheadi* (Eardley, 1996)	Megachilidae	*Megachile scindularia* (du Buysson)
Halictidae	*Ceylalictus muiri* (Cockerell)	Megachilidae	*Megachile selenostoma* (Cockerell)

Table 8: Continued.

Family	Species	Family	Species
Halictidae	*Eupetersia similis* (Benoist)	Megachilidae	*Megachile semiflava* (Cockerell, 1937)
Halictidae	*Evylaeus kampalensis* (Cockerell)	Megachilidae	*Megachile silverlocki* (Meade-Waldo)
Halictidae	*Evylaeus latesellatus* (Cockerell)	Megachilidae	*Megachile simulator* (Cockerell)
Halictidae	*Evylaeus microsellatus* (Cockerell)	Megachilidae	*Megachile sinuata* (Friese, 1903)
Halictidae	*Evylaeus nigritulinus* (Cockerell)	Megachilidae	*Megachile striatula* (Cockerell, 1931)
Halictidae	*Evylaeus semilucidus* (Cockerell)	Megachilidae	*Megachile torrida* (Smith, 1853)
Halictidae	*Halictus bidens* (Cameron)	Megachilidae	*Megachile truncaticeps* (Friese)
Halictidae	*Halictus chalybaeus* (Friese, 1908)	Megachilidae	*Megachile ungulata* (Smith, 1853)
Halictidae	*Halictus fascialis* (Smith)	Megachilidae	*Megachile utra* (Vachal)
Halictidae	*Halictus frontalis* (Smith, 1853)	Megachilidae	*Megachile venustella* (Cockerell)
Halictidae	*Halictus harveyi* (Cockerell)	Megachilidae	*Megachile vittatula* (Cockerell, 1920)
Halictidae	*Halictus jonesi* (Cockerell)	Megachilidae	*Megachile wahlbergi* (Friese, 1901)
Halictidae	*Halictus obscurifrons* (Cockerell, 1945)	Megachilidae	*Megachile waterbergensis* (Strand, 1911)
Halictidae	*Halictus picaninus* (Cockerell)	Megachilidae	*Noteriades tricarinatus* (Bingham)
Halictidae	*Halictus placatus* (Cockerell)	Megachilidae	*Othinosmia braunsiana* (Friese)
Halictidae	*Halictus rugicollis* (Friese)	Megachilidae	*Othinosmia globicola* (Stadelmann, 1892)
Halictidae	*Halictus zonatus* (Friese)	Megachilidae	*Othinosmia nitidula* (Cockerell)
Halictidae	*Lasioglossum aethiopicum* (Cameron, 1905)	Megachilidae	*Pachyanthidium apicatum* (Smith)
Halictidae	*Lasioglossum bouyssoui* (Vachal)	Megachilidae	*Pachyanthidium benguelense* (Vachal, 1903)
Halictidae	*Lasioglossum candidicinctum* (Cockerell, 1945)	Megachilidae	*Pachyanthidium bicolor* (Lepeletier, 1841)
Halictidae	*Lasioglossum choronotum* (Cockerell)	Megachilidae	*Pachyanthidium bouyssoui* (Vachal, 1903)
Halictidae	*Lasioglossum cinctulum* (Cockerell)	Megachilidae	*Pachyanthidium cordatum* (Smith, 1854)
Halictidae	*Lasioglossum claripenne* (Cockerell)	Megachilidae	*Pachyanthidium micheneri* (Pasteels)
Halictidae	*Lasioglossum duponti* (Vachal, 1903)	Megachilidae	*Pachyanthidium obscurum* (Pasteels)
Halictidae	*Lasioglossum entebbianum* (Cockerell, 1945)	Megachilidae	*Pachyanthidium paulinieri* (Guérin-Méneville)
Halictidae	*Lasioglossum flavolineatum* (Cockerell)	Megachilidae	*Pachyanthidium rufescens* (Friese, 1915)
Halictidae	*Lasioglossum geteinum* (Cockerell, 1945)	Megachilidae	*Pseudoanthidium lanificum* (Smith, 1879)
Halictidae	*Lasioglossum gossypiellum* (Cockerell)	Megachilidae	*Pseudoanthidium truncatum* (Smith, 1854)
Halictidae	*Lasioglossum griseocinctum* (Cockerell)	Megachilidae	*Pseudoanthidium tuberculiferum* (Brauns, 1905)
Halictidae	*Lasioglossum hancocki* (Cockerell)	Megachilidae	*Pseudoheriades moricei* (Friese, 1897)
Halictidae	*Lasioglossum macrurops* (Cockerell, 1937)	Megachilidae	*Pseudoheriades pellucidus* (Cockerell)
Halictidae	*Lasioglossum masaiense* (Cockerell)	Megachilidae	*Serapista denticulata* (Smith, 1854)
Halictidae	*Lasioglossum michaelseni* (Friese, 1916)	Megachilidae	*Serapista rufipes* (Friese, 1904)
Halictidae	*Lasioglossum moderatum* (Benoist, 1962)	Megachilidae	*Stenoheriades braunsi* (Cockerell, 1932)
Halictidae	*Lasioglossum modestum* (Benoist)	Megachilidae	*Stenoheriades mackieae* (Cockerell, 1936)
Halictidae	*Lasioglossum nairobicum* (Cockerell, 1945)	Megachilidae	*Stenoheriades truncaticeps* (Friese, 1922)
Halictidae	*Lasioglossum nairobiense* (Cockerell, 1945)	Melittidae	*Capicola braunsiana* (Friese, 1911)
Halictidae	*Lasioglossum namaense* (Friese, 1909)	Melittidae	*Capicola micheneri* (Michez, 2007)
Halictidae	*Lasioglossum natense* (Cockerell, 1935)	Melittidae	*Haplomelitta atra* (Michener, 1981)
Halictidae	*Lasioglossum nudatum* (Benoist, 1962)	Melittidae	*Meganomia andersoni* (Meade-Waldo, 1916)
Halictidae	*Lasioglossum nyasense* (Cockerell, 1945)	Melittidae	*Meganomia binghami* (Cockerell, 1909)
Halictidae	*Lasioglossum pachyacanthum* (Cockerell, 1937)	Melittidae	*Melitta albida* (Cockerell, 1935)
Halictidae	*Lasioglossum pellitosum* (Cockerell, 1934)	Melittidae	*Melitta arrogans* (Smith, 1879)
Halictidae	*Lasioglossum pernotescens* (Cockerell, 1934)	Melittidae	*Melitta danae* (Eardley, 2006)
Halictidae	*Lasioglossum plicatinum* (Cockerell)	Melittidae	*Melitta katherinae* (Eardley, 2006)
Halictidae	*Lasioglossum radiatulum* (Cockerell, 1937)	Melittidae	*Melitta schultzei* (Friese, 1909)
Halictidae	*Lasioglossum rubricaude* (Cameron, 1905)	Melittidae	*Melitta whiteheadi* (Eardley, 2006)
Halictidae	*Lasioglossum rubritarse* (Cockerell)	Melittidae	*Rediviva colorata* (Michener, 1981)
Halictidae	*Lasioglossum rufomarginatum* (Smith, 1853)	Melittidae	*Rediviva emdeorum* (Vogel and Michener, 1985)
Halictidae	*Lasioglossum semidiversum* (Cockerell, 1940)	Melittidae	*Redivivoides simulans* (Michener, 1981)

Table 9: Morpho species and doubtful identification.

Family	Morpho species	Family	Morpho species
Andrenidae	*Andrena* sp.1	Halictidae	*Nomia* sp.2
Andrenidae	*Andrena* sp.2	Halictidae	*Nomia* (*Leuconomia*) sp.
Andrenidae	*Melitturga* sp.2	Halictidae	*Nomia* (*Acunomia*) sp.
Andrenidae	*Melitturga* sp.1	Halictidae	*Nomia* (*Crocisapidia*) sp.
Andrenidae	*Melitturgula* sp.1	Halictidae	*Nomia* sp.1
Andrenidae	*Meliturgula* sp.2	Halictidae	*Nomioides* sp.
Apidae	*Afromelecta* sp.	Halictidae	*Patellapis* sp.3
Apidae	*Allodape* sp.1	Halictidae	*Patellapis* (*Archihalictus*) sp.
Apidae	*Allodape* sp.2	Halictidae	*Patellapis* (*Chaetalictus*) sp.
Apidae	*Allodapula* sp.1	Halictidae	*Patellapis* (*Lomatalictus*) sp.
Apidae	*Amegilla* sp.1	Halictidae	*Patellapis* (*Zonalictus*) sp.1
Apidae	*Ammobates* sp.	Halictidae	*Patellapis* (*Zonalictus*) sp.2
Apidae	*Anthophora* sp.1	Halictidae	*Patellapis* sp.1
Apidae	*Anthophora* sp.2	Halictidae	*Patellapis* sp.2
Apidae	*Braunsapis* sp.	Halictidae	*Patellapis* sp.4
Apidae	*Ceratina* (*Ctenoceratina*) sp.1	Halictidae	*Pseudapis* sp.1
Apidae	*Ceratina* (*Ctenoceratina*) sp.2	Halictidae	*Pseudapis* sp.2
Apidae	*Ceratina* (*Neoceratina*) sp.	Halictidae	*Sphecodes* sp.
Apidae	*Ceratina* (*Pithitis*) sp.	Halictidae	*Thrinchostoma* sp.
Apidae	*Ceratina* sp.1	Halictinae	*Halictus* sp.1
Apidae	*Ceratina* sp.2	Halictinae	*Halictus* sp.2
Apidae	*Ceratina* sp.3	Megachilidae	*Afranthidium* sp.
Apidae	*Cleptotrigona* sp.	Megachilidae	*Afroheriades* sp.1
Apidae	*Compsomelissa* sp.1	Megachilidae	*Anthidiellum* sp.1.
Apidae	*Ctenoplectra* sp.2	Megachilidae	*Anthidiellum* sp.2.
Apidae	*Ctenoplectra* sp.1	Megachilidae	*Anthidium* (*Severanthidium*) sp.1
Apidae	*Ctenoplectrina* sp.	Megachilidae	*Anthidium* sp.
Apidae	*Dactylurina* sp.	Megachilidae	*Dianthidium* sp.1
Apidae	*Epeolus* sp.	Megachilidae	*Euasapis* sp
Apidae	*Liotrigona* sp.	Megachilidae	*Fidelia* sp
Apidae	*Melecta* sp.	Megachilidae	*Heriades* sp.1
Apidae	*Nomada* sp.	Megachilidae	*Heriades* (*Amboheriades*) sp.
Apidae	*Pachymelus* sp.1	Megachilidae	*Heriades* (*Pachyheriades*) sp.
Apidae	*Pachymelus* sp.2	Megachilidae	*Heriades* sp.2
Apidae	*Pachymelus* sp.2	Megachilidae	*Hoplitis* sp.1
Apidae	*Pasites* sp.2	Megachilidae	*Hoplitis* sp.2
Apidae	*Pasites* sp.1	Megachilidae	*Lithurge* sp
Apidae	*Sphecodopsis* sp.	Megachilidae	*Lithurgus* sp
Apidae	*Tetralonia* sp.1	Megachilidae	*Megachile* (*Amegachile*) sp.
Apidae	*Tetralonia* sp.2	Megachilidae	*Megachile* (*Creightonella*) sp.1
Apidae	*Tetralonia* (*Eucara*) sp.1	Megachilidae	*Megachile* (*Creightonella*) sp.2
Apidae	*Tetralonia* (Eucara) sp.2	Megachilidae	*Megachile* (*Creightonella*) sp.3
Apidae	*Tetralonia* sp.3	Megachilidae	*Megachile* (*Eutricharaea*) sp.1
Apidae	*Tetraloniella* sp.4	Megachilidae	*Megachile* (*Eutricharaea*) sp.2
Apidae	*Tetraloniella* sp.1	Megachilidae	*Megachile* (*Paracella*) sp.
Apidae	*Tetraloniella* sp.2	Megachilidae	*Megachile* (*Xeromegachile*) sp.
Apidae	*Tetraloniella* sp.3	Megachilidae	*Megachile* sp.1
Colletidae	*Colletes* sp.1	Megachilidae	*Megachile* sp.2
Colletidae	*Colletes* sp.2	Megachilidae	*Noteriades* sp.
Colletidae	*Hylaeus* sp.1	Megachilidae	*Osmia* sp.1
Colletidae	*Hylaeus* sp.2	Megachilidae	*Osmi*a sp.2
Colletidae	*Scrapter* sp.	Megachilidae	*Pachyanthidium* sp.

Table 9: Continued.

Family	Morpho species	Family	Morpho species
Colletidae	*Scrapter* sp.1	Megachilidae	*Pseudoanthidium* sp.
Colletidae	*Scrapter* sp.2	Megachilidae	*Pseudoheriades* sp.
Halictidae	*Cellariella* sp.1	Megachilidae	*Serapista* sp.1
Halictidae	*Cellariella* sp.2	Megachilidae	*Serapista* sp.2
Halictidae	*Ceylalictus* sp	Megachilidae	*Stenoheriades* sp.
Halictidae	*Lasioglossum* sp.1	Melittidae	*Capicola* sp
Halictidae	*Lasioglossum* sp.2	Melittidae	*Capicola* sp
Halictidae	*Lasioglossum* sp.3	Melittidae	*Haplomelitta* sp.
Halictidae	*Lipotriches* sp.1	Melittidae	*Meganomia* sp.
Halictidae	*Lipotriches* (*Afronomia*) sp	Melittidae	*Melitta* sp.2
Halictidae	*Lipotriches* (*Macronomia*) sp.	Melittidae	*Melitta* sp.1
Halictidae	*Lipotriches* (Trinomia) sp.	Melittidae	*Rediviva* sp.1
Halictidae	*Lipotriches* sp.2	Melittidae	*Rediviva* sp.2
		Melittidae	*Redivivoides* sp.

In West Indies, the effects of climate on the plant-pollinator communities were studied, and it was found that rainfall and temperature affected richness and importance of the different pollinator functional groups and species. It was found in that study that rainfall explained most of the variation in pollinator richness and relative importance. However, effect of climate on other insect pollinator groups was more obscure [55]. Bees were strongly negatively affected by rainfall. Bee variation along the climate gradient could therefore be largely explained by their physiological capabilities to respond to rainfall and temperature [55].

While comparing historical pollination rates to present rates of visitations by pollinators to an orchid plant species in RSA, a decline in the pollination and in pollinator species richness and abundance was found [32]. Similarly, in a recent study in the USA, climate data suggested that patterns of precipitation in the current and previous year climate change drove variation in bee abundance because of its effects on cues for bee emergence in the current year and on the abundance of floral resources in the previous year [32, 55]. Thus, it is likely that accounting for the cumulative effects of climatic variables may be relevant for studying and predicting the future of bee communities in rural landscapes under climate change scenarios in Uganda.

Temporal and spatial fluctuations in the frequency of occurrence of different bee species can be associated with local climatic variation. In particular, bee abundance appeared in this study to be influenced positively by precipitation during previous years. The effects of the previous-year precipitation is likely indirect; greater precipitation tends to increase flower production in different bee food plants, which in turn may increase the food supply and reproductive success of generalist and specialist bees. This has consequence on the reproduction, emergence, phenology, and seasonality of bees, particularly for univoltine specialist solitary bees. Population dynamic in oligolectic bees (as opposed to polylectic bees) in response to year-to-year variation in floral resources may be also linked to previous climatic factors than to current ones [17]. Time lag in the population dynamics of bees in response to their food plants may also help in explaining in part dramatic fluctuations that can be observed in the visitation rates of different bee species to crop and wild blooming plant species.

In this study, it was also observed that rains two years prior to sampling had an effect on current bee populations. This was an indication of a potential long-term factor effects in shaping bee communities from rural landscapes of central Uganda. Although the average lifetime of the bee is much less than 1-2 years, it may appear controversial that past rainfall and temperature correlate with current/existing bee richness and abundance. In addition, such significant correlations may provide an indication of what has been happening as well as enabling speculative prediction of the future of species richness and abundance of bees in face of variability in climatic factors and the consequence on food security and livelihoods of people. Thus, studying separately (alone) the effects of current (specific period) weather factors on bee species richness and abundance may not provide an idea on the vulnerability of different bee species to future climate change. However, correlating weather data collected 2 to ≥10 years prior to the current period of the study is preliminary proven to be indicative in terms of predicting the vulnerability and risk of extension of various bee species to climate change and global environmental change.

Results of this study indicated variability in 10-year average temperature and rainfall. This variability was due to fact that the study area is located around the Equator. Previous observations of weather data in Uganda indicated a high variability in micro- and macroclimatic factors at a small-scaled scale [18]. Thus, this may also explain variability in response of bee species and individuals to variability in rainfall and temperature among study sites when these study sites cover a relatively small geographic region in Uganda (12% of the national land area).

In this study, it was also observed that bee species richness was correlated with previous climatic factors than current.

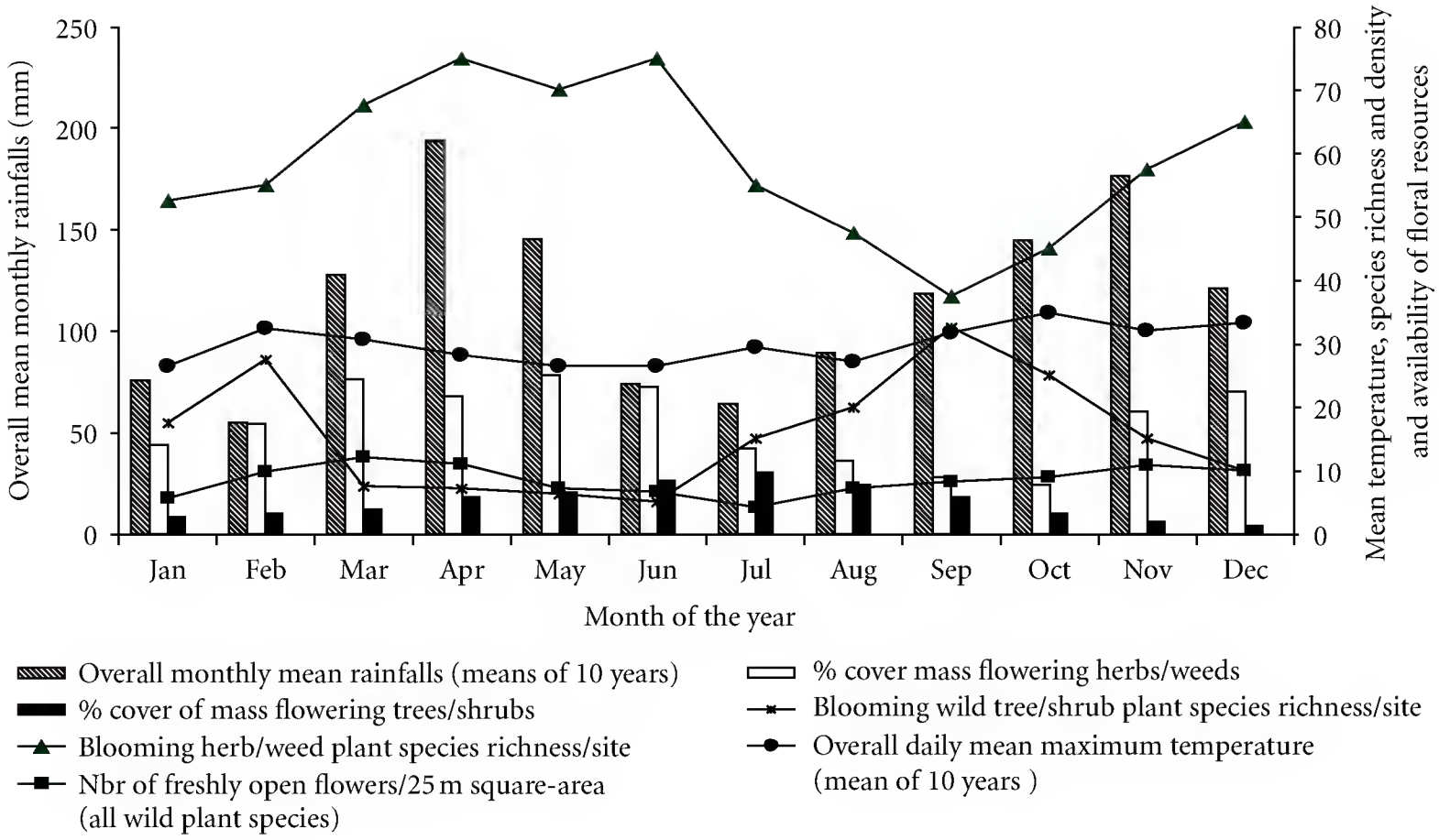


FIGURE 2: Trends in blooming wild plant species richness and abundance in relationships to rainfall and maximum temperature patterns in central Uganda. Blooming plant species richness and abundance had a significant correlation ($r = 0.35$, $P < 0.05$) with rainfall. The minor wet season (September–November) to early dry season (December-January) had more diverse blooming plant species with greater availability of herbs/weeds than trees and shrubs. However, both trees/shrubs and herbs/weeds blooming groups were least in June during the major wet season. Blooming tree/shrub richness showed a highly significant inverse correlation ($r = 0.36$, $P < 0.05$) with rainfall patterns. They declined a month ago after the start of the minor and major rainy seasons (September–March) and increased quickly two months later. This indicated that the two wild plant groups were not all in bloom at the same moment, and this is interesting for bees that need cover of floral resources around the year. Most (90%) blooming herbs and weeds were in full bloom towards the end of the major wet season and peaked in June-July when the maximum temperature was not high. The number of fresh flowers per 25 m square was not correlated to neither rainfall ($r = 0.14$, $P > 0.05$) nor to maximum temperature ($r = 0.16$, $P > 0.05$).

Variability in climatic factors is expected over large areas (separated by more than 50 Km) as the data from the current study indicated. This variability in climatic factors has got consequences on occurrence/disappearance, seasonality, and voltinism of certain bee species found in central Uganda. Although there are almost no studies from East Africa and from Uganda predicting future impacts of climate change and variability on bee species richness and abundance, this study yielded preliminary results indicating potential vulnerability of bee biodiversity and its future consequence on yield stability and food security in the country.

Much remains to be learned about how bee species richness and abundance will respond to future climate change in different landscapes in Sub-Saharan Africa and in Uganda. There may be various mechanism responses of different bee species to climatic variability: delaying foraging dates and times and phenology shifts. Variability in terms of extinction or disappearance of certain functional groups may be catastrophic for certain groups of crops/plant species. The climatic variability may lead to increased pollen limitation in crop species [18]. Such situation may in turn lead to crop yield failure and food and livelihood insecurities of people, particularly rural communities where high reliance to pollination services is observed in most Sub-Saharan African countries. Hegland et al. [31] found that species phenological responses to climate warming often seem to occur at parallel magnitudes in plants and pollinators, sometimes resulting in temporal mismatches among these mutualistic partners. It has been also found that climate change-induced phenological shifts may reduce the floral resources available to different bee species by 17%–50%, and this situation increases thereby extinction risks and disrupting crop/plant-pollinator interactions.

Central Uganda is the second African hotspot for bee biodiversity [22]. Future climate change is likely to particularly affect negatively and increase the risk of extinction of a number of bee species of local and unique Apoidea fauna found in central Uganda. Rising temperatures and altered rainfall regimes potentially have a huge impact on bee functional groups and life history traits like sociality in halictid bees [56] and host plant synchronization and, thus in turn, on pollination services and crop reproductive success.

In Uganda, future potential reductions in number of bee species and abundance were also predicted in this study. Novel mitigation mechanisms are highly needed to prevent decline in bee species and population in face of future climate change that may also alter crop/plant-pollinator mutualisms. For example, maintaining the spatio-temporal stability of bees (enhancing the persistence of populations) and their pollination services in the landscapes may require the establishment of observatories in rural landscapes.

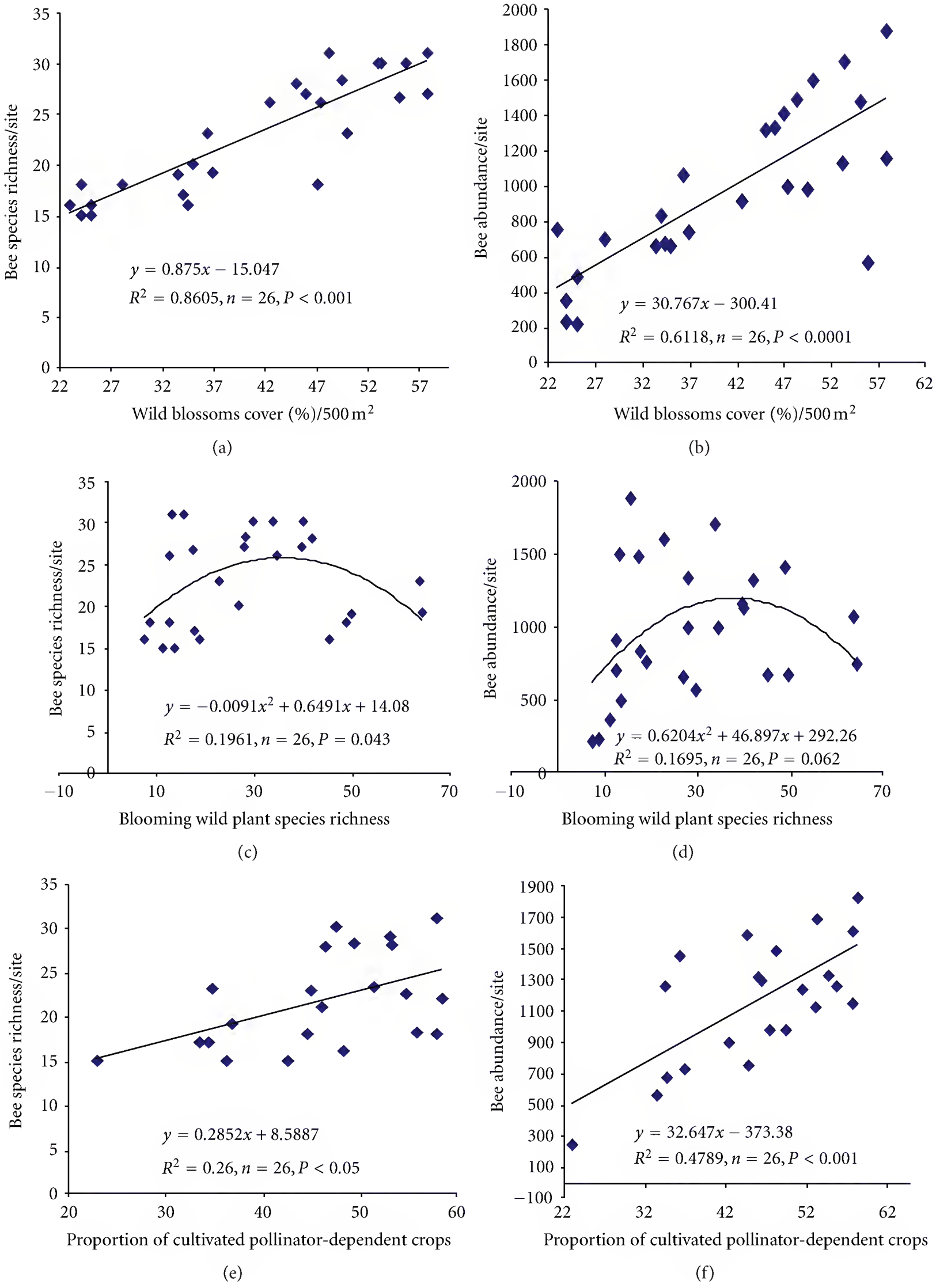


FIGURE 3: Relationship between (i) the abundance of wild floral resources (% blossoms cover), (ii) the abundance of cultivated floral resources (% of cultivated pollinator-dependent crops: all annual, bi-annual, perennial entomophilous crop species potentially offering pollen-nectar to bees), (iii) the number of flowering plant species (weeds, herbs, trees, and shrubs), and the number of bee species (a, c, and e) and bee abundance (b, d, and e) per study site.

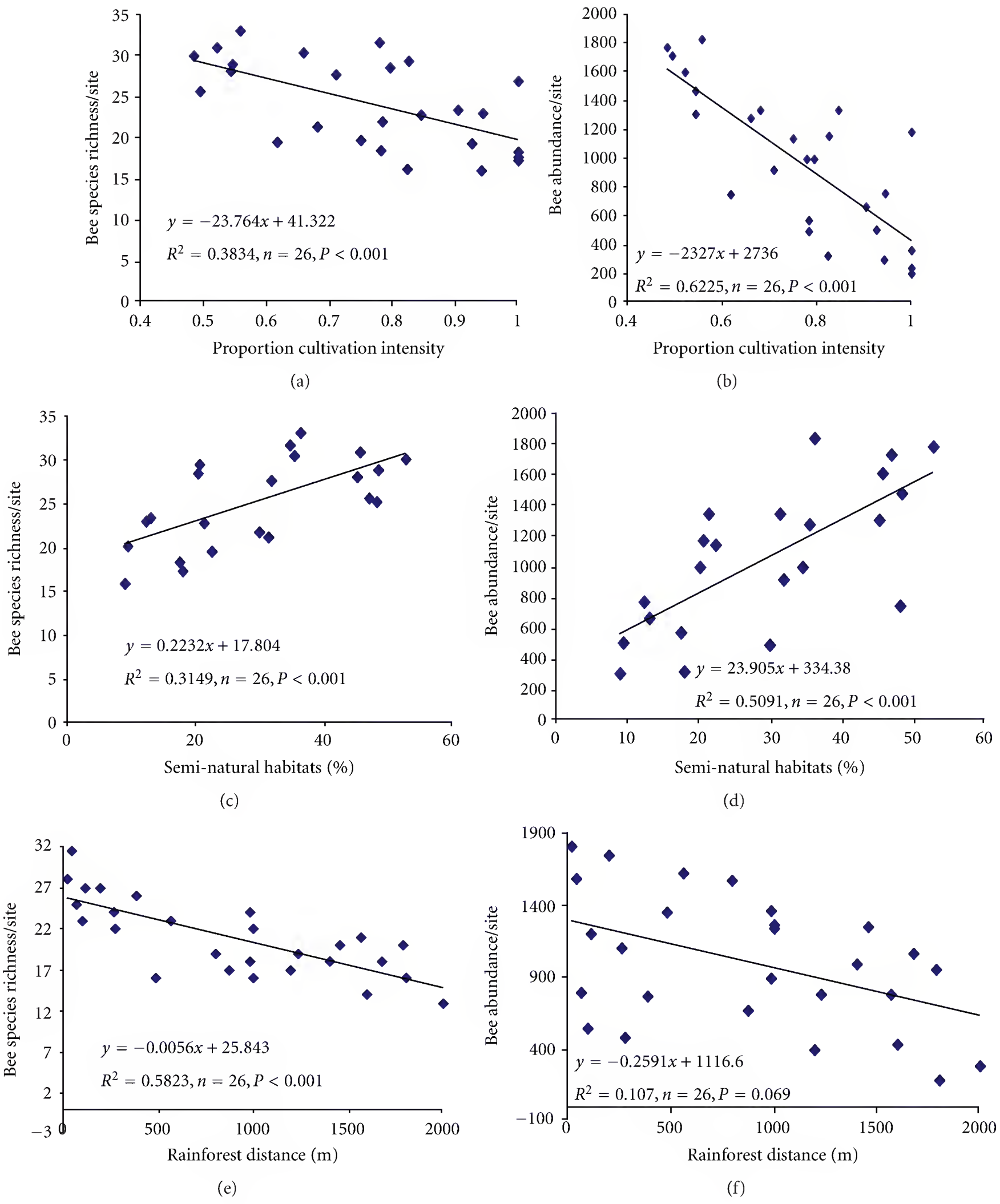


FIGURE 4: Relationship between landscape context variables (cultivation intensity, distance to forest margins, and % of semi-natural habitats) per Km^2-area and bee species richness (a, c, and e) and population density (b, d, and f) per study site.

For successful conservation of bee biodiversity under climate change-induced habitat fragmentation/loss and availability of migration corridors and reserves, dispersal rates and colonization ability may be crucial factors to enhance in rural landscapes of central Uganda. Understanding and monitoring the effects of rising temperatures and changing precipitation regimes on bee species richness and population dynamics may be vital for the development of efficient landscape and habitat conservation strategies in rural landscapes of Uganda to enhance the delivery of pollination services to various pollinator-dependent crops/wild plants.

Given the relevance of crop/plant-pollinator mutualisms for biodiversity and ecosystem integrity and crop productivity in farmland of central Uganda, it is crucial that future in-deep studies/investigations on the impact of climate change (in interaction with various abiotic/biotic drivers) are conducted on crop-animal interactions in different ecological zones of Uganda, with a particular focus on vital pollination function and service stability enhancement.

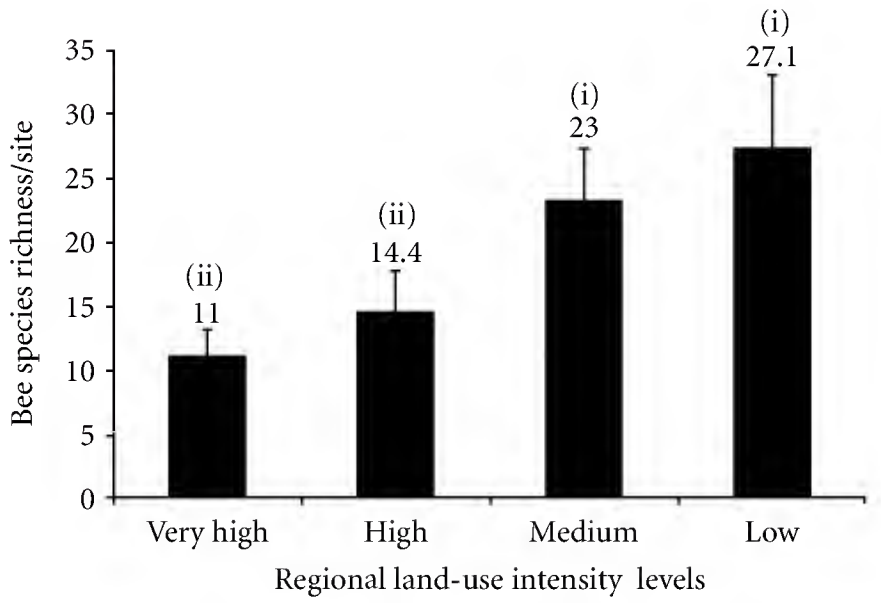
Anthropogenic, environmental, and climatic changes and the introduction of alien species have been predicted to affect plant-pollinator interactions [57] and delivery of pollination services to crops at the global level. In addition prediction in parallel declines in bee species richness and insect-pollinated plants indicated a potential reduction in pollination services and/or in available flower resources for flower-visiting insects. Bees are important plant pollinators, but they are among biota that are very sensitive to disturbance; particularly to anthropogenic activities, intensification in land-use systems and change in farming practices. Any decline in numbers or species or functional groups of bees, due to anthropogenic disturbances in interaction with variability in climatic factors, has consequence for crop productivity in Uganda since 75% of crops grown by farmers are pollinator-dependent crops [18]. Thus, the predicted population declines and species extinctions constitutes a significant threat both to biological diversity and their ecosystem services and to whole agricultural economics. The impacts of climate change on pollination services delivery may be more destructive in sub-Saharan Africa and in Uganda where there is a high livelihood dependency of human being to pollination services [18]. Thus the need to set and implement in advance climate-friendly conservation strategies before pollinators can disappear.

4.2. Effects of Regional Land-Use Intensity Factors on Bee Species Richness and Abundance. In this study, bee species richness and abundance were also found to be affected by regional land-use intensity variables as it was found in studies conducted elsewhere [1–3]. Results obtained from this study and those from other studies confirmed that the intensification of farmland management poses a threat to bee diversity [44, 58] and thus may reduce pollination services delivery to crops in rural landscapes. As human land-use intensifies, wild bees are exposed to habitat degradation/loss; exotic species (e.g., viruses, mites, and parasitoids) and spatial dissociation of food and nesting resources [1, 23, 33], including native bees [58, 59]. In rural landscapes of Uganda, bee communities are generally contingent on land-use, with solitary being more sensitive to anthropogenic activities than social bees [18]. Less anthropized areas generally harbor a greater richness and number of rare (singletons, doubletons) bee species while more intensively managed land-use types harbor higher population densities.

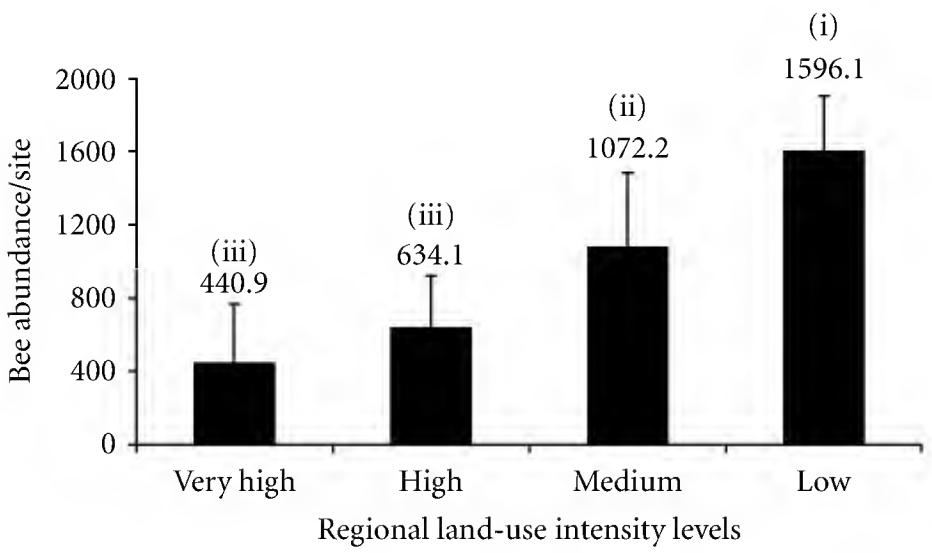
Land-use intensity may have indirect (reducing the diversity and cover of insect-pollinated plants, and thereby floral resources) negative effects on bee communities in rural landscapes. In fact, less intensively managed crop fields are expected to support more stable pollinator communities as a result of the higher availability of food resources. Higher stability of pollinators in rural landscapes has considerable effects on pollination success (e.g., reduced pollination limitation) and plant reproduction. Because bees are responsible for the pollination of many cultivated crops and wild plants, the decreased stability of bee communities (abundance and species richness), as well as the consequent decreased pollination services as a result of the negative impacts of land-use intensity (crop cultivation intensification) on bees, could have serious ecological and economical consequences [1, 58] in pollinator-dependent crop production systems of Uganda. Chronic pollen limitation caused by reduced pollinator availability is expected to result in strong crop yield failure/reduction and increase the likelihood of food insecurity of human communities.

From an ecological point of view, habitat loss, fragmentation (disruptions of habitat configuration and modification) are the major drivers of species extinction in the Anthropocene [60, 61] in the tropics. Their effects are exacerbated when interaction with climatic factors-stress related. Landscape disturbance primarily influences three components of pollination interactions: pollinator density, movement, and plant demography. The effects of habitat loss on each of these components are likely to differ substantially from the effects of fragmentation, which is likely to be more complex and may influence each pollination component in contrasting ways [60].

4.3. Effects of Local and Landscape Variables on Bee Communities. In this study, variations in bee abundance and species richness among different study locations were hypothesized to be related to landscape variables, but not to climatic and local variables. The results indicated the opposite. In fact, bee species richness and abundance were predicted by local factors (abundance and richness of floral resources) as well as by landscape and climatic factors. Thus, flowering (wild and cultivated) plants play significant role in structuring bee communities, particularly as sources of pollen and other floral resources needed for their survival in rural landscapes of central Uganda. In fact more than 24 flowering crop species (Table 6) and more than 50 wild blooming plant species (Table 7) were recorded during transect walk-and-counts in this study. Obviously, floral and nesting resources are critical for the occurrence/survival of diverse bee communities in natural and man-made landscapes [18]. Resource availability is a critical factor determining the dynamics of populations over space and time [62]. Several other studies have demonstrated that species richness of wild floral resources and



(a) [GLM-ANOVA, $F = 5.22$, $P = 0.007$, DF = (3, 100)]



(b) [GLM-ANOVA, $F = 7.24$, $P = 0.001$, DF = (3, 100)]

FIGURE 5: Effects of regional land-use intensity on the species richness (a) and abundances (b) of bees recorded per study site in farmlands of central Uganda. Error bars are ±SE. Means ($x \pm$ se) followed by different letters are significantly different at $P < 0.05$ according to Tukey test.

mass flowering of both wild plants and crop species actually influence both bee density and diversity that occur in farmed landscape [63–67].

Landscape variables (% cultivation intensity, % semi-natural habitats, and forest distance) were observed to be powerful predictive variables of the variation in abundance, richness, and diversity of bees; few farm-scaled local variables (% of wild floral resources) were equally powerful, whereas most of the local variables were significant but had weaker to nonsignificant effects.

In other words, landscape variables explained more variation in population density and species richness of bees than did local factors. In fact, bee species richness and abundance declined with forest distance. Geographic scales at which landscape effects on bee faunas are most pronounced remain largely unsurveyed in Sub-Saharan Africa. However, bee communities were observed to be affected by food resources within 0–200 m of their nesting sites [18]. As previously mentioned, forging habitat and nectar/pollen sources (located at <2000 m far from bee refugia) are critical ecological resources for maintaining pollination services by native bees in agricultural landscapes [7, 59]. Recently, in coffee-agroforest systems in India, it was found that the abundance of *Apis dorsata* decreased with increasing distance from a neighbouring forest patch, but this distance effect was reduced with an increase in size of the nearby forest [66], and this result indicated that justifying the conservation of large forest remnants may be problematic unless more studies are conducted to account for the direct effect of forest on crop fruit/seed set in most agricultural landscapes of the world [66].

Bee species and abundance were found to be linearly related to cover of semi-natural habitats although some studies [22–24, 27–30, 35] have found that bee species richness may be positively but non necessarily being linearly related to semi-natural habitat area. Studies conducted elsewhere stressed that landscape factors (% cover of semi-natural habitats, forest distance, and habitat fragmentation/isolation) are more important in determining the rule of occurrence [34] of Apoidea communities in rural landscapes [65–69]. Also, while investigating the effect of the quantity of surrounding natural habitat, organic management, and strips of semi-natural vegetation on flower visitation frequency of wild and managed bees in intensive agricultural landscapes in USA, it was realized that wild bee species visited almond flowers but only in orchards with adjacent semi-natural habitat or vegetation strips [1].

Linear remnants of native vegetation and related semi-natural habitats are known to contribute to bee assemblage heterogeneity by adding unique species to the regional pool [70]. In fact, semi-natural habitats and related noncrop habitats provide dispersal corridors and "habitat islands" required by many bee species as refuges and feeding areas [71]. Thus, an increase in amount of semi-natural habitats may lead to a more diverse flora in fields, providing valuable nesting and foraging resources for bees and other pollinators.

Although some farmland habitats may provide sufficient floral resources over the year, they may not be good nesting sites for bees. Short-distance to bee refugia (forest, wetlands, and remnant vegetation) and the percentage cover of semi-natural habitats are attributes that contribute to bee diversity in rural landscapes. Bee species richness and diversity are expected to increase with increase in the amount of semi-natural habitats in the landscapes [72, 73] because of availability of floral/nesting opportunities in such habitats [22, 27]. Semi-natural habitats are known to positively affect pollinators in the surrounding agricultural landscape presumably through contributing both nest sites and forage resources. Other non-crop areas such as field margins may also be beneficial provided that they are rich in flower resources. Non-crop and semi-natural areas add heterogeneity to the farm-landscape. These non-crop habitats often provide a continuous supply of nectar and pollen which bees can utilize. They can provide suitable habitats for bees to nest and have been shown to contain higher densities of bee nests in this study. Hence, they may promote pollinator abundance and species richness in agricultural landscapes of Uganda, even if measures promoting pollinators may not necessarily

benefit pollination of wild plants, because species may vary in their effectiveness as pollinators. Therefore, supporting wild pollinators and crop production in agricultural landscape requires the maintenance of mosaics of natural/semi-natural features and remnant vegetation in agricultural landscapes [70]. Networks of natural and semi-natural areas (hedgerows, grasslands, fallows, woodlands, riparian corridors, and roadsides) in the farmland can therefore be beneficial to agricultural production [9–11] although the effects of increasing semi-natural in the landscape may be taxon dependent [74] since some species richness may found beneficial while other many find these habitats hostile [74]. Because bees are among the important pollinator guilds in rural landscape, protection of the remaining natural habitat and vegetation in close proximity to farmland habitats is an imperative conservation strategy in host sport bee biodiversity regions.

Natural habitats, semi-natural habitats, and vegetation structure/composition generally explain most of the variance for the species richness and abundance of bees in agricultural landscapes [18]. There are a number of semi-natural habitats in the farmlands, but old fallows are generally associated with the highest species richness of bees. Other semi-natural habitats may harbor bee communities of similar species richness and composition [18]. In farm-landscapes with 20%–30% of habitats kept uncultivated as reservoirs for bees, crop yields are expected to be stable over time and space [22]. Thus, conservation programs aiming at enhancing pollination services in the farm-landscape should concentrate on strategies to maintain/improve/increase the amount of semi-natural habitats in the surrounding of crop fields to guarantee spatio-temporal availability of floral resources for beneficial insects including bees.

4.4. Contrasting Effects of Local and Landscape Factors on Bee Abundance, and Richness in Rural Landscapes. Occurrence, prevalence, abundance and richness of bees in rural landscapes may be linked to various local and landscapes. The degree at which different environmental characteristics influence bee communities is still subject of debate by scientists. Some studies have shown a relative high importance of landscape-scaled variables [16, 19] as compared to local-scale variables [18]. Overall, some studies stress that landscape variables are more determinants than local variables. Other studies indicate the importance of local variables in shaping bee communities variables. Within tropical regions, some studies often find that species richness and abundance are determined by local drivers, whereas studies from temperate regions report that landscape drivers are more determinants for bees in agricultural landscapes. For example, recently, Féon et al. [10] found that the abundance of bees (solitary bees) in fields increased positively with the increase in the proportion cover of semi-natural habitats (grasslands) than with increasing amount of flowering resources in an agricultural area of western France.

While in central Uganda both landscape and local factors had significant effect of bee abundance and diversity, in Mexico [75], it was recently found that local habitat factors, managed within agroforestry systems, had strong impacts on local bee abundance and species richness, more than landscape-level factors (e.g., % cover of forests). Both the local and landscape factors affected bee communities in Uganda and this is different from what is commonly reported from elsewhere. For instance, there is no clear explanation for such pattern and difference. Explanations may be linked to the bee community composition found in central Uganda. The community composition plays a significant role in explaining this pattern since different bee species may respond differently at different local and landscape scaled factors [23, 30]. In addition, in central Uganda, there coexist a diversity of bees with different nesting affinities and foraging ranges. There are evidences for existence of a high diversity of nesting resources and floral resources on which bees may depend on for pollen and nectar in farmlands of central Uganda [18]. Central Uganda had a variety of floral resources to support rich and diverse bee fauna. Some few common species may thus be affected (positively/negatively) by local factors (e.g., species flying at around 500 m from their nests), whereas others may only be affected by landscape factors (example species foraging up to 2000 m beyond their nests). Also, the difference in results between Uganda, and Mexico may be explained by the difference in local management strategies of fields. The diversity of floral resources combined to the diversity of nesting sites and to habitat heterogeneity was found to be likely explaining the survival and coexistence of great number of bee species with various ecological affinities in farmlands of central Uganda.

In brief, there is a need to conserve biodiversity to ensure the provision of ecosystem services in rural landscapes [75, 76] of Uganda and Sub-Saharan Africa. The conservation of pollinators and pollination services will play a significant role in a long-term viability of food supplies, in the livelihood security/improvement of smallholder farmers, in commercial agricultural enterprises, in generation of household and national revenues, and in production of diverse products to satisfy food, fibre, and fuel demands of expanding rural-urban populations.

5. Conclusion and Recommendations

The overall goal of this work was to understand how environmental characteristics operating at different scales affect the occurrence, distribution, and diversity of bee communities in farmlands of central Uganda. Understanding the response of species to various drivers is essential to designing conservation management, especially in mosaic agricultural landscapes. Aspects of the farm, surrounding landscape, and regional and climatic factors were found to be potentially useful predictors of bee abundance and species richness.

Overall, conservation, management, and policy efforts aiming at increasing ecological intensification of agricultural production systems and stabilizing food productivity in central Uganda should (i) first preserve and prevent degradation of remaining forest fragments, forest fallows, and wetlands: reducing natural and semi-natural vegetation clearance (retaining and maintaining the current status of natural forest patches and wetlands is important since these

ecosystems increase the bee species richness in riparian agricultural matrices); (ii) secondly, strongly encouraging small-scale farmers to maintain higher cover of multipurpose agroforestry tree species and good proportion of linear and non-linear features of semi-natural habitats; (iii) mimicking natural vegetation or natural ecosystems through promoting establishment of related natural habitats (woodlots of eucalyptus/pines) and community village forestry in the rural landscapes.

The most critical point and exciting finding from this study was to find out that species richness/abundance correlated with mean annual temperatures in previous years. It was also found in this study that availability of semi-natural habitats, abundance of wild and cultivated floral resources in the landscape, and distance to the closest forest also influence critically the bee communities. Thus, changes in these landscape variables along the years are expected to affect bee communities as the change in average temperature/rainfall does. Hence, if landscape and land-use change data is found available (registered) in the region, further research should focus on potential influence of landscape changes on occurrence, distribution, and current community structure of bees. In fact, it is expected that the change in landscape variables may influence the change (variability) in climatic factors, and the lack of stability in climatic factors may have strong negative effects [18] on the spatio-temporal occurrence and distribution and distribution of bee communities in the agricultural landscape.

There is a need for future research to be conducted in many parts of the world to get more evidence of the role played by forest fragments, wetlands and related semi-natural habitats (e.g., forest fallows) in agricultural landscapes to provide a variety of ecosystem services [66]. There is also a need to increase scientific ability to define and experimentally measure pollination resilience, determine under which conditions there will be pollinator population stability, as well as increase the understanding of the factors shaping this parameter to be able to support efforts to forecast the impact of climate change on the delivery of pollination services to pollinator-dependent crops [77, 78]. There is also a scientific need to estimate cost-benefits of conserving bee biodiversity and pollination services in natural and agricultural landscapes in [79] Uganda and in Sub-Saharan Africa. Findings from such studies may enable the development of opportunities to use semi-natural features in adaptation and mitigation activities related to future climate change.

Conflict of Interests

I declare having no conflict of interest with MINITAB Company. TheStatistical software used for data analysis (MINITAB-15, Englishversion) was officially purchased as single license use for onecomputer by the leader of the Darwin project (Dr Phil Atkinson fromBritish trust for ornithology) while in UK attending a training indata analysis between March and May 2007.

Acknowledgments

The author is very grateful to Darwin Initiative (Defra, UK; project reference: 14–032; project title: *Conserving biodiversity in modernized farm landscapes in Uganda*) for funding this study. Mr. Maurice Mutabazi (research assistant) is acknowledged for for his assistance in the field. He is also very grateful to Professor Simon Potts and Professor Philip Nyeko (scientific supervisors), as well as Dr. Juliet Vickery, Dr. Phil Atkinson, and Professor Derek Pomeroy (project scientific advisors) for all kind of help given. He is also very grateful to all bee taxonomists (Dr. Connal Eardley fromf ARC-RSA, Dr. David Roubik from Smithsonian Tropical Research Institute, Dr David Notton from the department of Entomology of Landon History Museums, UK, and Robert Stuart from the University of Reading, UK) who helped with proper identification and validation of names of different bee species collected during the course of this study.

References

[1] A. M. Klein, B. E. Vaissière, J. H. Cane et al., "Importance of pollinators in changing landscapes for world crops," *Proceedings of Royal society of London B*, vol. 274, no. 1608, pp. 303–313, 2007.

[2] C. Kremen, N. M. Williams, M. A. Aizen et al., "Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change," *Ecology Letters*, vol. 10, no. 4, pp. 299–314, 2007.

[3] J. C. Watson, A. T. Wolf, and J. S. Ascher, "Forested landscapes promote richness and abundance of native bees (Hymenoptera: Apoidea: Anthophila) in Wisconsin apple orchards," *Environmental Entomology*, vol. 40, no. 3, pp. 621–632, 2011.

[4] D. W. Roubik, *Pollination of Cultivated Plants in the Tropics*, FAO, Rome, Italy, 1995.

[5] T. H. Ricketts, J. Regetz, I. Steffan-Dewenter et al., "Landscape effects on crop pollination services: are there general patterns?" *Ecology Letters*, vol. 11, no. 5, pp. 499–515, 2008.

[6] R. Winfree, D. Cariveau, and I. Bartomeus, "Native pollinators in anthropogenic habitats," *Annual Review of Ecology, Evolution, and Systematics*, vol. 42, pp. 1–22, 2011.

[7] R. Winfree, B. J. Gross, and C. Kremen, "Valuing pollination services to agriculture," *Ecological Economics*, vol. 71, pp. 80–88, 2011.

[8] J. C. Biesmeijer, S. P. M. Roberts, M. Reemer et al., "Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands," *Science*, vol. 313, no. 5785, pp. 351–354, 2006.

[9] S. G. Potts, P. G. Kevan, and J. W. Boone, "Conservation in Pollination: collecting, surveying and monitoring," in *Pollination Ecology: A Practical Approach*, A. Dafni and P. Kevan, Eds., Enviroquest, Cambridge, UK, 2005.

[10] V. L. Féon, F. Burel, R. Chifflet et al., "Solitary bee abundance and species richness in dynamic agricultural landscapes," *Agriculture, Ecosystems and Environment*. In press.

[11] S. G. Potts, J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin, "Global pollinator declines: trends, impacts and drivers," *Trends in Ecology and Evolution*, vol. 25, no. 6, pp. 345–353, 2010.

[12] D. Moroń, I. M. Grzes, P. Skórka et al., "Abundance and diversity of wild bees along gradients of heavy metal pollution," *Journal of Applied Ecology*, vol. 49, no. 1, pp. 118–125, 2012.

[13] A.-M. Klein, "Plant-pollinator interactions in changing environments," *Basic and Applied Ecology*, vol. 12, no. 4, pp. 279–281, 2011.

[14] R. Winfree, R. Aguilar, D. P. Vázquez, G. Lebuhn, and M. A. Aizen, "A meta-analysis of bees' responses to anthropogenic disturbance," *Ecology*, vol. 90, no. 8, pp. 2068–2076, 2009.

[15] C. D. Michener, *The Bees of the World*, The John Hopkins University Press, Baltimore, Md, USA, 2007.

[16] C. D. Eardley, M. Gikungu, and M. P. Schwarz, "Bee conservation in Sub-Saharan Africa and Madagascar: diversity, status and threats," *Apidologie*, vol. 40, no. 3, pp. 355–366, 2009.

[17] P. Hoehn, I. Steffan-Dewenter, and T. Tscharntke, "Relative contribution of agroforestry, rainforest and openland to local and regional bee diversity," *Biodiversity and Conservation*, vol. 19, no. 8, pp. 2189–2200, 2010.

[18] T. M. B. Munyuli, *Pollinator biodiversity and economic value of pollination services in Uganda [Ph.D. dissertation]*, Makerere University, Kampala, Uganda, 2010.

[19] T. M. B. Munyuli, "Factors governing flower-visitation patterns and quality of pollination services delivered by social and solitary bee species to coffee in central Uganda," *African Journal of Ecology*, vol. 49, pp. 501–509, 2011.

[20] T. M. B. Munyuli, "Pollinator biodiversity in Uganda and in Sub-Sahara Africa: landscape and habitat management strategies for its conservation," *International Journal of Biodiversity and Conservation*, vol. 3, pp. 551–609, 2011.

[21] T. M. B. Munyuli, "Farmers' perceptions of pollinators' importance in coffee production in Uganda," *Agricultural Sciences*, vol. 2, pp. 318–333, 2011.

[22] A. S. Persson and H. G. Smith, "Bumblebee colonies produce larger foragers in complex landscapes," *Basic and Applied Ecology*, vol. 12, pp. 695–702, 2011.

[23] T. Tscharntke, A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies, "Landscape perspectives on agricultural intensification and biodiversity— ecosystem service management," *Ecology Letters*, vol. 8, no. 8, pp. 857–874, 2005.

[24] R. Bommarco, J. C. Biesmeijer, B. Meyer et al., "Dispersal capacity and diet breadth modify the response of wild bees to habitat loss," *Proceedings of the Royal Society B*, vol. 277, no. 1690, pp. 2075–2082, 2010.

[25] J. M. Tylianakis, A. M. Klein, and T. Tscharntke, "Spatiotemporal variation in the diversity of hymenoptera across a tropical habitat gradient," *Ecology*, vol. 86, no. 12, pp. 3296–3302, 2005.

[26] N. Gallai, J. M. Salles, J. Settele, and B. E. Vaissière, "Economic valuation of the vulnerability of world agriculture confronted with pollinator decline," *Ecological Economics*, vol. 68, no. 3, pp. 810–821, 2009.

[27] T. M. B. Munyuli, "Assessment of indicator species of butterfly assemblages in coffee-banana farming system in central Uganda," *African Journal of Ecology*, vol. 50, pp. 77–89, 2012.

[28] T. M. B. Munyuli, "Diversity of life-history traits, functional groups and indicator species of bee communities from farmlands of central Uganda," *Jordan Journal of Biological Sciences*, vol. 5, no. 1, pp. 1–14, 2012.

[29] T. M. B. Munyuli, "Micro, local, landscape and regional drivers of bee biodiversity and pollination services delivery to coffee (*Coffea canephora*) in Uganda," *International Journal of Biodiversity, Ecosystem Services and Management*, vol. 8, no. 3, pp. 190–203, 2012.

[30] T. M. B. Munyuli, "Is pan-trapping the most reliable sampling method for measuring and monitoring bee biodiversity in agroforestry systems in sub-Saharan Africa?" *International Journal of Tropical Insect Science*, vol. 33, no. 11, pp. 14–37, 2013.

[31] S. J. Hegland, A. Nielsen, A. Lázaro, A. L. Bjerknes, and Ø. Totland, "How does climate warming affect plant-pollinator interactions?" *Ecology Letters*, vol. 12, no. 2, pp. 184–195, 2009.

[32] D. A. Moeller, M. A. Geber, V. M. Eckhart, and P. Tiffin, "Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant," *Ecology*, vol. 93, no. 5, pp. 1036–1048, 2012.

[33] T. Tscharntke, Y. Clough, T. C. Wanger et al., "Global food security, biodiversity conservation and the future of agricultural intensification," *Biological Conservation*, vol. 151, no. 1, pp. 53–59, 2012.

[34] J. Banaszak and T. Manole, "Diversity and density of pollinating insects (Apoidea) in the agricultural landscape of Romania," *Polskie Pismo Entomologiczne*, vol. 57, pp. 747–766, 1987.

[35] T. M. B. Munyuli, "Pollination services delivery and food security in Sub-Sahara Africa are highly threatened by future climate changes: preliminary evidences," in *Proceedings of Climate Change Network Team Conference (CCNT '11)*, London, UK, September 2011.

[36] C. Westphal, R. Bommarco, G. Carré et al., "Measuring bee diversity in different European habitats and biogeographical regions," *Ecological Monographs*, vol. 78, no. 4, pp. 653–671, 2008.

[37] M. W. Gikungu, *Bee diversity and some aspects of their ecological interactions with plants in a successional tropical community [Ph.D. dissertation]*, University of Bonn, Bonn, Germany, 2006.

[38] R. Kajobe, *Foraging behaviour of equatorial Afro-tropical stingless bees: habitat selection and competition for resources [Ph.D. thesis]*, University of Utrecht, Utrecht, The Netherlands, 2008.

[39] R. Rader, B. G. Howlett, S. A. Cunningham, D. A. Westcott, and W. Edwards, "Spatial and temporal variation in pollinator effectiveness: do unmanaged insects provide consistent pollination services to mass flowering crops?" *Journal of Applied Ecology*, vol. 49, pp. 126–134, 2012.

[40] A.-M. Klein, C. Brittain, S. D. Hendrix, R. Thorp, N. Williams, and C. Kremen, "Wild pollination services to California almond rely on semi-natural habitat," *Journal of Applied Ecology*, vol. 49, pp. 723–732, 2012.

[41] A.-M. Klein, I. Steffan-Dewenter, D. Buchori, and T. Tscharntke, "Effects of land-use intensity in tropical agroforestry systems on coffee flower-visiting and trap-nesting bees and wasps," *Conservation Biology*, vol. 16, no. 4, pp. 1003–1014, 2002.

[42] R. Grundel, R. P. Jean, K. J. Frohnapple, G. A. Glowacki, P. E. Scott, and N. B. Pavlovic, "Floral and nesting resources, habitat structure, and fire influence bee distribution across an open-forest gradient," *Ecological Applications*, vol. 20, no. 6, pp. 1678–1692, 2010.

[43] E. Lonsdorf, C. Kremen, T. Ricketts, R. Winfree, N. Williams, and S. Greenleaf, "Modelling pollination services across agricultural landscapes.," *Annals of Botany*, vol. 103, no. 9, pp. 1589–1600, 2009.

[44] A. Kovács-Hostyánszki, P. Batáry, and A. Báldi, "Local and landscape effects on bee communities of Hungarian winter cereal fields," *Agricultural and Forest Entomology*, vol. 13, pp. 59–66, 2011.

[45] A. Holzschuh, I. Steffan-Dewenter, D. Kleijn, and T. Tscharntke, "Diversity of flower-visiting bees in cereal fields: effects of farming system, landscape composition and regional context," *Journal of Applied Ecology*, vol. 44, no. 1, pp. 41–49, 2007.

[46] M. C. B. Matos, L. Sousa-Souto, R. S. Almeida, and A. V. Teodoro, "Contrasting patterns of species richness and composition of solitary wasps and bees (Insecta: Hymenoptera) according to land-use," *Biotropica*, vol. 45, no. 1, pp. 73–79, 2013.

[47] C. Carvell, W. R. Meek, R. F. Pywell, D. Goulson, and M. Nowakowski, "Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins," *Journal of Applied Ecology*, vol. 44, no. 1, pp. 29–40, 2007.

[48] L. E. Hannon and T. D. Sisk, "Hedgerows in an agri-natural landscape: potential habitat value for native bees," *Biological Conservation*, vol. 142, no. 10, pp. 2140–2154, 2009.

[49] S. G. Potts, B. A. Woodcock, S. P. M. Roberts et al., "Enhancing pollinator biodiversity in intensive grasslands," *Journal of Applied Ecology*, vol. 46, no. 2, pp. 369–379, 2009.

[50] A. Pauw and J. A. Hawkins, "Reconstruction of historical pollination rates reveals linked declines of pollinators and plants," *Oikos*, vol. 120, no. 3, pp. 344–349, 2011.

[51] A. E. Magurran, *Measuring Biology Diversity*, Blackwell Publishing, Oxford, UK, 2004.

[52] B. J. Brosi, P. R. Armsworth, and G. C. Daily, "Optimal design of agricultural landscapes for pollination services," *Conservation Letters*, vol. 1, pp. 27–36, 2008.

[53] J. Memmott, P. G. Craze, N. M. Waser, and M. V. Price, "Global warming and the disruption of plant-pollinator interactions," *Ecology Letters*, vol. 10, no. 8, pp. 710–717, 2007.

[54] O. Gordo and J. J. Sanz, "Temporal trends in phenology of the honey bee *Apis mellifera* (L.) and the small white *Pieris rapae* (L.) in the Iberian Peninsula (1952–2004)," *Ecological Entomology*, vol. 31, no. 3, pp. 261–268, 2006.

[55] A. M. M. González, B. Dalsgaard, J. Ollerton et al., "Effects of climate on pollination networks in the West Indies," *Journal of Tropical Ecology*, vol. 25, no. 5, pp. 493–506, 2009.

[56] M. Kuhlmann, D. Guo, R. Veldtman, and J. Donaldson, "Consequences of warming up a hotspot: species range shifts within a centre of bee diversity," *Diversity and Distributions*, vol. 18, no. 9, pp. 885–897, 2012.

[57] O. Schweiger, J. C. Biesmeijer, R. Bommarco et al., "Multiple stressors on biotic interactions: how climate change and alien species interact to affect pollination," *Biological Reviews*, vol. 85, no. 4, pp. 777–795, 2010.

[58] A. Hudewenz, A.-M. Klein, C. Scherber et al., "Herbivore and pollinator responses to grassland management intensity along experimental changes in plant species richness," *Biological Conservation*, vol. 150, no. 1, pp. 42–52, 2012.

[59] R. L. Callum, J. J. Bennie, D. T. Chris, J. A. Hodgson, and J. R. Wilson, "Local and landscape management of an expanding range margin under climate change," *Journal of Applied Ecology*, vol. 49, pp. 552–561, 2012.

[60] S. Krishnan, C. G. Kushalappa, R. U. Shaanker, and J. Ghazoul, "Status of pollinators and their efficiency in coffee fruit set in a fragmented landscape mosaic in South India," *Basic and Applied Ecology*, vol. 13, no. 3, pp. 277–285, 2012.

[61] A. S. Hadley and M. G. Betts, "The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence," *Biological Reviews*, vol. 87, no. 3, pp. 526–544, 2012.

[62] N. M. Williams, J. Regetz, and C. Kremen, "Landscape-scale resources promote colony growth but not reproductive performance of bumble bees," *Ecology*, vol. 93, no. 5, pp. 1049–1058, 2012.

[63] A. Sárospataki, A. Báldi, P. Batáry, Z. Józan, S. Erdõs, and T. Rédei, "Factors affecting the structure of bee assemblages in extensively and intensively grazed grasslands in Hungary," *Community Ecology*, vol. 10, no. 2, pp. 182–188, 2009.

[64] F. Kohler, J. Verhulst, R. van Klink, and D. Kleijn, "At what spatial scale do high-quality habitats enhance the diversity of forbs and pollinators in intensively farmed landscapes?" *Journal of Applied Ecology*, vol. 45, no. 3, pp. 753–762, 2008.

[65] M. Otieno, B. A. Woodcock, A. Wilby et al., "Local management and landscape drivers of pollination and biological control services in a Kenyan agro-ecosystem," *Biological Conservation*, vol. 144, no. 10, pp. 2424–2431, 2011.

[66] S. E. R. Hoover, J. J. Ladley, A. A. Shchepetkina, M. Tisch, S. P. Gieseg, and J. M. Tylianakis, "Warming, CO_2, and nitrogen deposition interactively affect a plant-pollinator mutualism," *Ecology Letters*, vol. 15, no. 3, pp. 227–234, 2012.

[67] G. Carré, P. Roche, R. Chifflet et al., "Landscape context and habitat type as drivers of bee diversity in European annual crops," *Agriculture, Ecosystems and Environment*, vol. 133, no. 1-2, pp. 40–47, 2009.

[68] F. Jauker, T. Diekötter, F. Schwarzbach, and V. Wolters, "Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat," *Landscape Ecology*, vol. 24, no. 4, pp. 547–555, 2009.

[69] L. G. Carvalheiro, C. L. Seymour, R. Veldtman, and S. W. Nicolson, "Pollination services decline with distance from natural habitat even in biodiversity-rich areas," *Journal of Applied Ecology*, vol. 47, no. 4, pp. 810–820, 2010.

[70] P. E. Lentini, T. G. Martin, P. Gibbons, J. Fischer, and S. A. Cunningham, "Supporting wild pollinators in a temperate agricultural landscape: maintaining mosaics of natural features and production," *Biological Conservation*, vol. 149, pp. 84–92, 2012.

[71] E. Ockinger and H. G. Smith, "Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes," *Journal of Applied Ecology*, vol. 44, pp. 50–59, 2007.

[72] I. Steffan-Dewenter and C. Westphal, "The interplay of pollinator diversity, pollination services and landscape change," *Journal of Applied Ecology*, vol. 45, no. 3, pp. 737–741, 2008.

[73] N. E. Sjödin, J. Bengtsson, and B. Ekbom, "The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects," *Journal of Applied Ecology*, vol. 45, no. 3, pp. 763–772, 2008.

[74] T. Kehinde and M. J. Samways, "Endemic pollinator response to organic vs. conventional farming and landscape context in the Cape Floristic Region biodiversity hotspot," *Agriculture, Ecosystems and Environment*, vol. 146, pp. 162–167, 2012.

[75] S. Jha and J. H. Vandermeer, "Impacts of coffee agroforestry management on tropical bee communities," *Biological Conservation*, vol. 143, no. 6, pp. 1423–1431, 2010.

[76] C. Brittain and S. G. Potts, "The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination," *Basic and Applied Ecology*, vol. 12, no. 4, pp. 321–331, 2011.

[77] R. T. Gilman, N. S. Fabina, K. C. Abbott, and N. E. Rafferty, "Evolution of plant-pollinator mutualisms in response to climate change," *Evolutionary Applications*, vol. 5, no. 1, pp. 2–16, 2012.

[78] N. Pettorelli, "Climate changes as a main driver of ecological research," *Journal of Applied Ecology*, vol. 49, pp. 542–545, 2012.

[79] L. A. Garibaldi, I. Steffan-Dewenter, R. Winfree et al., "Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance," *Science*. 2013.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 320590, 11 pages
http://dx.doi.org/10.1155/2013/320590

Review Article

Diapriinae Wasps (Hymenoptera: Diaprioidea: Diapriidae) Associated with Ants (Hymenoptera: Formicidae) in Argentina

Marta S. Loiácono,[1] Cecilia B. Margaría,[1,2] and Daniel A. Aquino[1]

[1] *División Entomología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, 1900 La Plata, Buenos Aires, Argentina*
[2] *Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, 1900 La Plata, Buenos Aires, Argentina*

Correspondence should be addressed to Marta S. Loiácono; loiacono@fcnym.unlp.edu.ar

Received 19 December 2012; Revised 15 February 2013; Accepted 20 February 2013

Academic Editor: Jean-Paul Lachaud

We provide an overview of diapriid wasps associated with ants in Argentina and the diversity of interactions they have developed with their hosts. As a result, we report 16 species of nine genera of Diapriinae, two new geographic distributions, three new association records, illustrations, and photographs. We highlight myrmecophile symphylic species, with a high degree of integration with the host ants, adaptation being morphological and behavioral. A table with diapriid species and ant hosts is given.

1. Introduction

Diapriids are primary endoparasitoids of larvae-pupae or pupae, principally of dipterans, but a number of species are closely associated with ant nests. However, there are few behavioral data on host-diapriid myrmecophile interactions. Huggert and Masner [1] hypothesized that the ancestors of diapriines guests changed from Diptera to Formicidae. The intermediates in the presumed sequence of hosts seem to be the numerous synoeketic Diptera living in the refuse depot and bivouacs of various army ants of the subfamily Ecitoninae. Diapriines females, in the search for potential hosts, would have progressively integrated with formicids. According to Masner (personal communication) this change would have occurred more frequently in the Neotropical region where these ants have high distribution. The guests switch mechanism has determined morphological and behavioral specialization, manifested by the degree of integration of diapriines to ant colonies. These symphyles are often highly adapted to their hosts, exhibiting morphological and behavioral adaptations to living with ants (extensive morphological mimicry of the host ants coloration, ocellus regression, similar sculpture, presence of appeasement substances in specialized structures and trichomes, trophallaxis, etc.), which aid them in avoiding detection and/or aggression by host ants. Ants seem to have preference to lick certain parts of diapriid body to get exudates [2]. The adaptations include secondary apterism in which the wings of wasps are bitten off by either the parasite itself or its host. During the alate phase, the adults probably disperse, as the alate individuals, caught by sweeping, in Malaise traps and significantly by light traps indicating also the nocturnal activity in this phase of life [2]. The secondary apterism occurs in several species of diapriines, for example, *Asolenopsia rufa* Kieffer, *Bruchopria pentatoma* Kieffer, *Bruchopria hexatoma* Kieffer, *Notoxoides pronotalis* (Borgmeier), herein studied.

The current knowledge indicates that only a few diapriids are parasitoids of ant brood, attacking as solitary or gregarious koinobiont endoparasitoids of the host larvae, and worker and/or reproductive immature stages can be parasitized. From 121 diapriine species in 34 genera that had been collected in association with ants, development of immature stages as parasitoids of ant larvae has been demonstrated for only 26 species in seven genera, most of which are only known at the level of morphospecies [3]. There are only two species and one morphospecies recorded in Argentina as ant parasitoids [4].

A large number of diapriine wasps became associated with various groups of ants in Central and South America.

The associations are especially well developed with army ants (Ecitonini) and leaf cutting ants (Attini) with some 20 genera of Diapriinae already involved [5]. The vast majority of these species belong to Diapriini, although there are some exceptions like *Bruchopria* species that belong to the tribe Spilomicrini [6].

The New World fungus-growing ants (Hymenoptera: Formicidae: Attini) are especially diverse in the tropics. As true for the most social insects, they accumulate significant stores of resources within their nests, attracting a diverse array of predators, microbial pathogens, and parasites [7]. We studied aspects of the intensity and prevalence of these little-known diapriine wasps that attack the larvae of the fungus-growing ant, *Acromyrmex lobicornis* Emery, and noted a remarkably diverse community of parasitoids within host population from four localities of La Pampa, Argentina [4, 8]. In some cases, the rates of parasitoidism can reach high levels. Loiácono et al. [4] collected 1560 wasps (adults and immatures) from 430 parasitized larvae from three partial colonies of *Acromyrmex*, which shows how prevalent these wasps can be in attacking the ants. Fernández-Marin et al. [9] found that between 27% and 70% of the colonies of two species of *Cyphomyrmex* Mayr were parasitized by one species in Puerto Rico and by up to four concurrent morphospecies of diapriids in Panama. Similarly, Pérez-Ortega et al. [7] reported that another fungus-growing ant, *Trachymyrmex* cf. *zeteki*, was attacked by a diverse community of diapriids in Panama, with a mean intensity of larval parasitism per ant colony of 33.9%, and prevalence across all ant populations of 27.2%. Lachaud and Pérez Lachaud [3], based on the abundance and success in attacking ants, considered that diapriids and another group of microhymenopterans, the eucharitids, seem excellent potential models to explore how parasitoids impact ant colony demography, population biology, and ant community structure [3].

In Argentina, the study of myrmecophiles has attracted the attention of several scientists in the last two centuries. Carlos Bruch (1869–1943), a German naturalist selected by F. Moreno—first Director of Museo de La Plata—to organize its collections, was a pioneer of the entomological studies; it is important to remark his ability as a photographer and scientific illustrator, and his observations regarding special associations and behaviors of ants and beetles: termitophily and myrmecophily [10, 11]. Jean-Jacques Kieffer (1857–1925), a French entomologist who specialized in the study of parasitoids of insects, based his studies on Bruch's material and published articles about diapriines associated with ants [12, 13]. Alejandro Ogloblin (1891–1967), a Russian entomologist researcher at "Estación Experimental de Loreto" (Misiones, Argentina), collected there numerous diapriid wasps associated specially with myrmicine ants [14, 15]. Luis De Santis (1914–2000) catalogued associations between diapriids and ants [16, 17] and reported new geographic distributions [18]. Marta Loiácono and colleagues studied Neotropical myrmecophiles diapriids and their interactions with ants [4, 7, 8, 15, 19–29].

In this paper, we provide an overview of the diversity of diapriid wasps associated with ants in Argentina and the diversity of interactions they have developed with their hosts.

2. Material and Methods

Specimens for this study were reared in laboratory [4] or collected from ant nests, killed in alcohol, and mounted on cards or microscopic slides for further studies. Observations of the specimens were made through a stereomicroscope Leica S8APO. The photographs were taken by Daniel A. Aquino with a Leica DFC295 camera attached to the stereomicroscope. Digital images were mounted using open software CombineZM [30] and enhanced using Photoshop. Scanning micrographs were taken with a JEOL JSMT100 at Museo de La Plata operating at 15 KV.

Sharkey [31] was followed for the higher-level phylogeny of the Hymenoptera order, Bolton for ant valid names [32], Masner and García [5] for diapriid systematics, and Yoder et al. web site [33] for interactive keys and links.

Diapriid and ant specimens examined in this study are deposited at Museo de La Plata (Buenos Aires, Argentina). Most of them were collected and determined by Bruch and Ogloblin in Argentina. Type material of *Szelenyiopria reinchenspergeri* (Ferrière) was loan by Hungarian Natural History Museum.

Biology Section includes "hosts" wasps emerged from ant larvae or "associated" wasps found in or near nests or emigration columns of army ants.

3. Results

3.1. Tribe Diapriini Ashmead, 1893 [34]

3.1.1. Asolenopsia Kieffer, 1921 [12]. Asolenopsia Kieffer, 1921: 36 [12].

Euplacopria Ferrière, 1929: 157 [35].

Distribution. Tropical lowlands of Central and South America [5].

Biology. Associated with ecitonini ants of genus *Eciton Latreille*, *Labidus* Jurine and *Neivamyrmex* Borgmeier [5].

Remarks. Members of *Asolenopsia* are moderately to highly specialized associates to ecitonine ants [20]. Their wings are primarily developed but subsequently bitten off by ants or cast off spontaneously (alectomy). Winged adults are also collected in light traps [5].

3.1.2. Asolenopsia rufa Kieffer, 1921 [12] (Figure 1(a)). *Asolenopsia rufa* Kieffer, 1921: 37 [12].

Distribution. Argentina (Córdoba, Entre Ríos, and Santa Fe) [12, 17].

Biology. Associated with *Neivamyrmex carettei* (Forel) [12] (Figure 1(b)).

Material Studied. Syntype, female, dealated, with *Neivamyrmex carettei* worker, Argentina, Córdoba, Alta Gracia, La Granja, 1-8-IV-1920, Bruch coll.; one female, without date,

(a)

(b)

FIGURE 1: (a) *Asolenopsia rufa* female dealate in dorsal view. (b) *Neivamyrmex carettei*. Scale: 1 mm.

Santa Fe, Vera y Pintado (Fives Lille), Weiser coll.; female alated, Argentina, Misiones, Loreto, without date, Ogloblin coll.

3.1.3. Basalys Westwood, 1832 [36]. Basalys Westwood, 1832: 342–344 [36].

Ceratopria Ashmead, 1893: 407, 42 [34].

Acidopria Kieffer, 1913: 442 [37].

Loxotropa auct. nec Foerster, synonymized by Masner, 1964 [38].

Nesopria Muesebeck and Walkley, 1956: 319–419 [39].

Distribution. The genus is well represented in North and South America, rarely in Chile [5].

Biology. Several species were reared from various dipterous hosts, and some were collected in ant nests [5].

3.1.4. Basalys sp.

Material Studied. One female and 1 male (microscopic slide) collected with the "Argentine ant," *Linepithema humile* (Mayr), Argentina, Buenos Aires, J. C. Paz, 11-X-1934, Ogloblin coll.; 1 female (microscopic slide) collected with the Argentine ant *Linepithema humile*, Argentina, Buenos Aires, J. C. Paz, 8-IX-1945, Bezzi leg.

Biology. Associated with *Linepithema humile* (new record).

Remarks. Female and male studied were determined by Masner, who wrote a label: "*Basalys* sp. ♀♂(=*Loxotropa* auct.) aberrant sp. with !11-segm. ant. ♀, Det. L. Masner, '89"; and female specimen: "*Basalys* sp. ♀(=*Loxotropa* auct.) !11-segmented antenna, Det. L. Masner, '89." Specimens studied were determined by Ogloblin as a new species of *Doliopria*,

FIGURE 2: *Doliopria collegii* female in lateral view. Scale: 1 mm.

but he did not describe it. We also considered that material studied belong to genus *Basalys*, as it was established by Masner.

3.1.5. Doliopria Kieffer, 1910 [40]. Doliopria Kieffer, 1910: 48 [40].

Martinica Risbec, 1950: 533 [41].

Distribution. Doliopria is restricted to the New World, with only a few species in the Nearctic region and with a high number of undescribed species in tropical America [5].

Biology. Associated with ecitonini and attini ants [5].

Remarks. Three Neotropical species were described associated with ants [12, 35, 40]; hypothetically they parasitized synoeketic Diptera because they show no specialized morphology [5].

3.1.6. Doliopria collegii Ferrière, 1929 [35] (Figure 2). Doliopria collegii Ferrière, 1929 : 164 [35].

Distribution. Argentina (Buenos Aires and Misiones) [18, 35].

Biology. Associated with ecitonini ants, *Eciton burchellii* (Westwood) and *Eciton quadriglume* (Haliday) [35].

Material Studied. Two females alated, Argentina, Misiones, Loreto, 20-X-1919 and 18-IX-1923, Ogloblin coll. and det.

3.1.7. Doliopria myrmecobia Kieffer, 1921 [12] (Figure 3(a)). Doliopria myrmecobia Kieffer, 1921: 39 [12].

Distribution. Argentina (Buenos Aires; Misiones, new record) [12].

Biology. Associated with attini ants *Acromyrmex lundii* (Guérin-Méneville) [12] (Figure 3(b)).

Material Studied. One female, Argentina, Buenos Aires, La Plata, VIII, inside a nest of *Acromyrmex lundii*, Bruch coll.; 1 female, alated collected with *Acromyrmex* sp., Argentina, Misiones, Loreto, 3-XI-1928, Ogloblin coll. and det.

3.1.8. Notoxoides Ashmead, 1903 [42]. Notoxoides Ashmead, 1903: 30 [42].

Notoxopria Kieffer, 1910: 39 [40].

Philolestes Kieffer, 1922: 205 [13].

Psilogasteroides Brèthes, 1911: 209–210 [43].

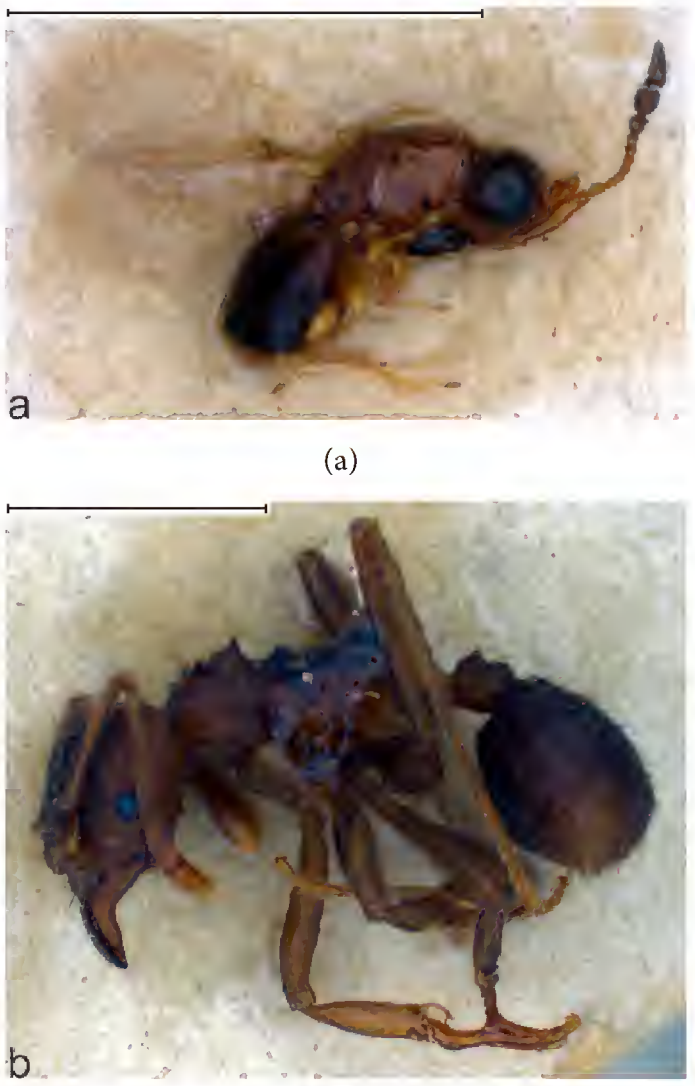


FIGURE 3: (a) *Doliopria myrmecobia* female in lateral view. (b) *Acromyrmex lundii*. Scale: 1 mm.

Distribution. Restricted to lowland rainforests of continental South America [5].

Biology. Members of *Notoxoides* display some of the most advanced associations with ants. So far, ants of genera *Neivamyrmex* and *Eciton* (Ecitonini) were recorded as hosts [19]. Adult wasps are frequently collected in light traps. Wings may be lost to typical alectomy as indicated by shriveled wing rudiments in some specimens [5].

3.1.9. Notoxoides pedissequus (Borgmeier, 1939) [44]. *Notoxopria pedissequa* Borgmeier, 1939: 538 [44].

Distribution. Argentina (Córdoba) [19].

Biology. Associated with *Neivamyrmex pseudops* (Forel) [44].

Remarks. Loiácono [20] studied a female alate collected by Bruch in Córdoba province.

3.1.10. Notoxoides pronotalis (Borgmeier, 1939) [44] (Figures 4(a), 4(b), and 5). *Philolestes rufus* Kieffer, 1922: 205 [13].
Philolestes pronotalis Borgmeier, 1939: 536 [44].
Notoxoides pronotalis: Masner, 1977: 34 [45].
Notoxoides kiefferi Loiácono, 1981: 305, 306 [19].

Distribution. Argentina (Córdoba, Salta, San Luis, and Santiago del Estero) [19, 44].

Biology. Associated with Eciton dulcium Forel and *Neivamyrmex sulcatus* (Mayr) [44].

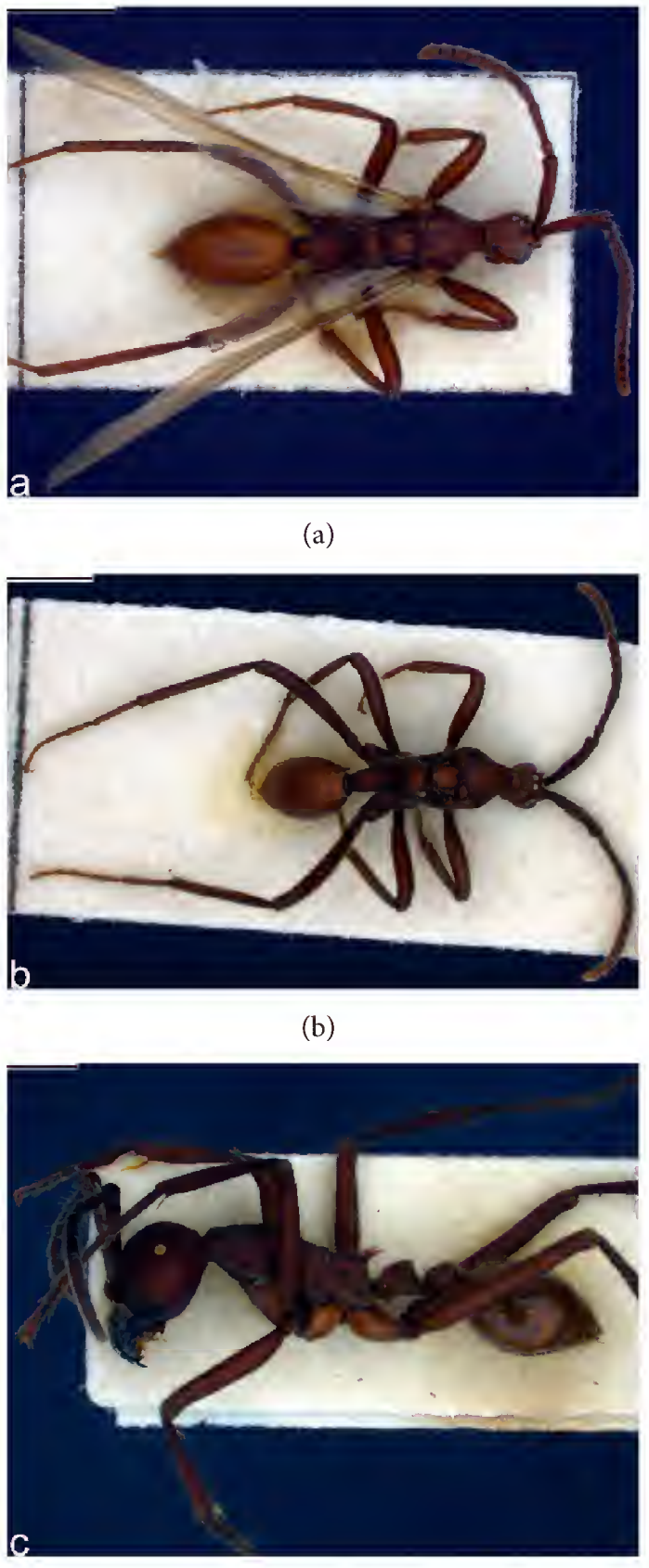


FIGURE 4: *Notoxoides pronotalis* female in dorsal view: (a) alate and (b) dealate specimens. Scale: 1 mm. (c) *Eciton dulcium* collected with *Notoxoides pronotalis*, in lateral view. Scale: 1 mm.

Material Studied. Syntype, female dealated, collected with *Eciton dulcium*, Argentina, Córdoba, Alta Gracia, 4-XII-I921, Bruch coll.; 2 syntype females alated, same data as syntype except II-1922, collected with *Neivamyrmex sulcatus*, Bruch coll. and det.; 21 females dealated, Argentina, Salta, Tartagal, I-1960, Martínez coll., with a *Eciton dulcium*, and 5 females alated, Argentina, Salta, Pocitos, III-1959, Martínez coll.; 3 females dealated and 1 alated, Córdoba, San Javier, La Paz, 15-31-XII-1928, Bruch coll., with *Eciton dulcium*; Córdoba, Alta Gracia: 1 female dealated, collected with *Eciton dulcium* (Figure 4(c)), 4-XII-1922, Bruch coll.; 1 female dealated, without date and collector; 1 female alated, La Granja, 21-VIII-1924, Bruch coll.; 2 females dealated, La Granja, 25-I-1925, Bruch coll.; 3 females alated, La Granja, 4-XI-1925, Bruch

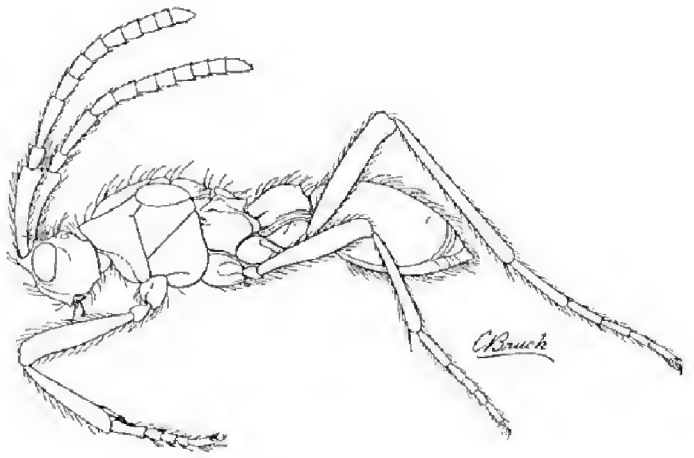
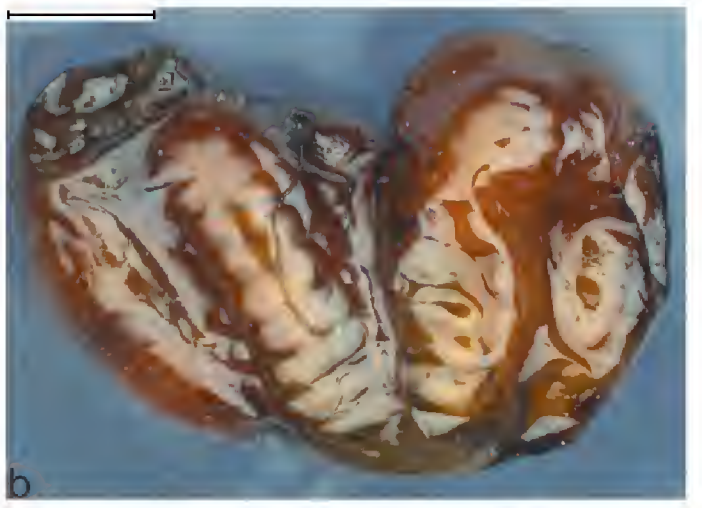


FIGURE 5: Original illustration of *Notoxoides pronotalis* female in lateral view, by Bruch.

FIGURE 6: *Neivamyrmex pseudops,* gravid queen in dorsal view, photographed by Bruch.

(a)



(b)

FIGURE 7: (a) *Szelenyiopria pampeana* female in lateral view. (b) *Acromyrmex lobicornis* larva showing immature instars of diapriines. Scale: 1 mm.

coll.; 2 females dealated, with *Eciton dulcium*, La Granja, 4-XI-1925, Bruch coll.; 2 females alated with *Eciton dulcium*, 13-III-1934, Bruch coll.; 3 females alated, without date, Bruch coll.; 1 female dealated; Córdoba, Unquillo, without date and collector; 9 females alated, Córdoba, Unquillo, without date and collector; 1 female with fore wings, Córdoba, Unquillo, without date and collector; 2 females alated, Santiago del Estero, Cerrillos, 2-V-1955, without collector, and 2 females alated, without date, Bruch coll.; 5 females alated, without locality, 21-II-1925, light collected, without collector; 2 females dealated and 3 alated, without locality, 22-II-1925, light collected, without collector; 5 females alated and 1 dealated, without locality, 23-II-1925, light collected, without collector; 1 female alated, without locality, 24-II-1925, light collected, without collector.

Remarks. Bruch always sent to Kieffer diapriid samples to be studied. As we mentioned, he was an excellent scientific illustrator (Figure 5) [46] and an important photographer as is shown in (Figure 6) *Neivamyrmex pseudops*, ant host of *Notoxoides pedisequus* [47].

We observed numerous both alate and dealate individuals found dependent on the phase of life. As is mentioned [2], during the alate phase, numerous adults were caught by light traps as we observed in the female material light collected by Bruch.

Lachaud [48] mentioned that ants search actively for some chemical substances produced by glands at the basis of the setae present on the diapriid cuticle; similarly we observed the presence of peculiar neck hairs in *N. pronotalis* [20].

3.1.11. Szelenyiopria Fabritius, 1974 [49]. *Szelenyiopria* Fabritius, 1974: 54 [49].

Gymnopria Loiácono, 1987: 130 [21].

Distribution. Wide distribution from Argentina to Guatemala [21, 49].

Biology. *Szelenyiopria lucens* (Loiácono) from Uruguay is the first member of the tribe Diapriini in the New World positively reared from ants. Loiácono [21] reports up to three wasps per mature larva of *Acromyrmex ambiguus* (Emery) (Attini). Members of *Szelenyiopria* show no specialized structures known among other myrmecophilic Diapriini; Masner and García [5] assumed that the specialized setae with truncate apices are outlet of chemical substances.

3.1.12. Szelenyiopria pampeana (Loiácono, 2000) [4] (Figure 7(a)). *Gymnopria pampeana* Loiácono, 2000: 10 in Loiácono et al., 2000 [4].

Szelenyiopria pampeana: Loiácono and Margaría, 2009: 63 [8].

Distribution. Argentina (La Pampa) [4, 8].

Biology. Koinobiont and gregarious endoparasitoids of late instar larvae of *Acromyrmex lobicornis* (Emery), it was also established simultaneous parasitoidism with *Trichopria* sp. [4] (Figure 7(b)).

Material Studied. Holotype female, Argentina, Santa Rosa, 8-XI-1995, Quirán and Corró Molas colls.; 25 paratypes females

and 3 males, Lihuel Calel, 4-XII-1997, Quirán and Corró Molas colls.

3.1.13. Szelenyiopria reichenspergeri (Ferrière, 1929) [35]. *Doliopria reinchespergeri* Ferrière, 1929: 165 [35].

Szelenyiopria reinchespergeri: Fabritius, 1974, 54 [49].

Distribution. Argentina (Salta and Tucumán) [35, 49].

Biology. Associated with *Eciton quadriglume* and *Neivamyrmex legionis* (Smith) [35, 49].

Material Studied. One female, Argentina, Salta, 2-6-II-1950, Golbach coll.

3.1.14. Szelenyiopria sp.

Distribution. Argentina (Córdoba) (new record).

Material Studied. Female and 3 males with an ecitonine ant, Argentina, Córdoba, San Javier, La Paz, 1-20-I-1929, Bruch coll.

Remarks. Most females of this genus have 11-segmented antennae, but material studied here presents antenna 12-segmented as mentioned by Masner and García [5] for undescribed species. We considered that these specimens belong to *Szelenyiopria* genus by the most important feature, the presence on entire body of specialized straight setae, truncate apically.

3.1.15. Trichopria Ashmead, 1893 [34]. *Trichopria* Ashmead, 1893: 407, 431 [34].

Ashmeadopria Kieffer, 1912: 8, 10, 59 [50].

Phaenopria Ashmead, 1893: 40, 436 [34].

Planopria Kieffer, 1906: 19 [51].

Orthopria Kieffer, 1911: 983, 984 [52]. *Distribution.* Worldwide [5].

Biology. Associated with the "fire ant," *Solenopsis richteri* Forel (Kieffer, 1921) and endoparasitoid of *Acromyrmex lobicornis* [4].

3.1.16. Trichopria formicans Loiácono, 2000 [4] (Figures 8(a) and 8(b)). *Trichopria formicans* Loiácono 2000 in Loiácono et al., 2000: 12 [4].

Distribution. Argentina (La Pampa) [4].

Biology. Reared from larvae of *Acromyrmex lobicornis* [4].

Material Studied. Holotype female, Argentina, La Pampa, Utracán, 22-XII-1997, Caramuti y Rodriguez colls.; paratypes 68 females and 43 males (MLP), same data as holotype.

3.1.17. Trichopria myrmecophila (Kieffer, 1921) [12]. *Phaenopria myrmecophila* Kieffer, 1921: 4 [12].

Trichopria myrmecophila: De Santis in De Santis and Esquivel, 1966: 50 [16].

Distribution. Argentina (Buenos Aires) [12].

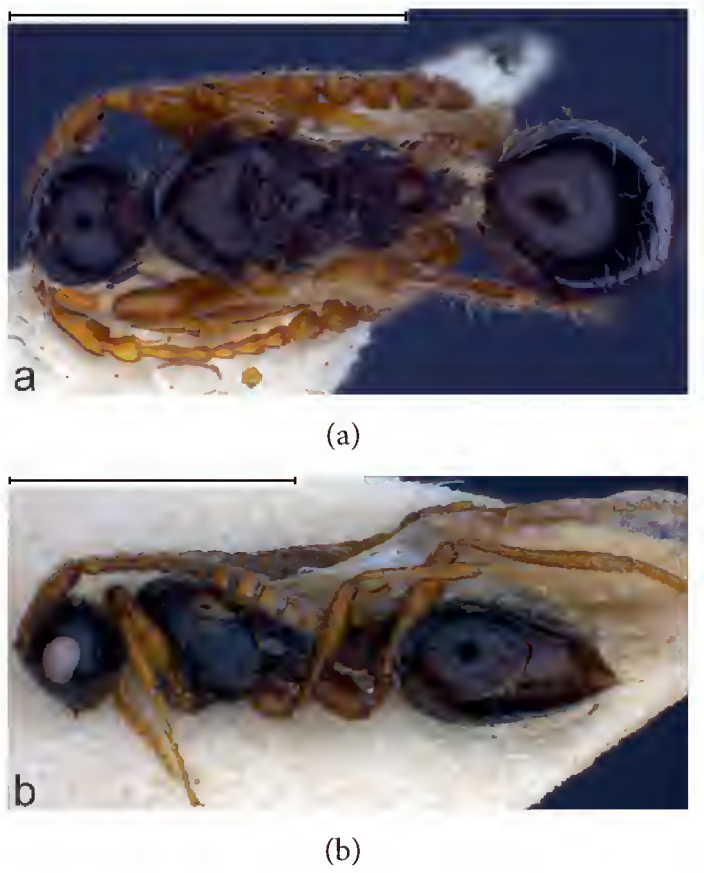


FIGURE 8: *Trichopria formicans* female (a) in dorsal view and (b) lateral view. Scale: 1 mm.

Biology. Associated with *Solenopsis richteri* [12].

3.1.18. Trichopria sp.

Distribution. Argentina Buenos Aires.

Biology. Collected with the "argentine ant," *Linepithema humile* (new record).

Material Studied. Female collected with *Linepithema humile*, Argentina, Buenos Aires, J. C. Paz, 8-II-1940, Ogloblin coll.

Remarks. Masner studied this material and determined specimens as *Trichopria* s. str. sp.

3.2. Tribe Spilomicrini Ashmead, 1893 [34]

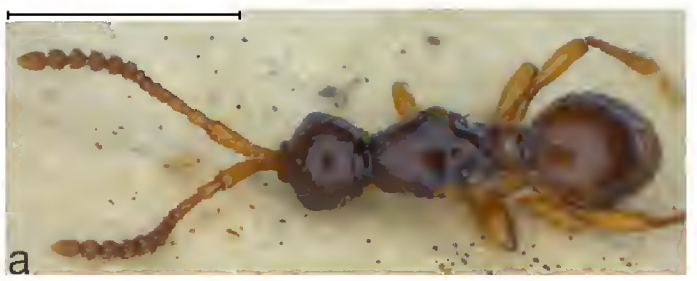
3.2.1. Bruchopria Kieffer, 1921 [12]. *Bruchopria* Kieffer, 1921: 38 [12].

Aulatopria Brèthes, 1927: 164 [53].

Distribution. Argentina (Buenos Aires, Córdoba, and Misiones) [12, 53].

Biology. Associated with ants of the genera *Solenopsis* Westwood (Solenopsidini) and *Acromyrmex* Mayr (Attini) [12].

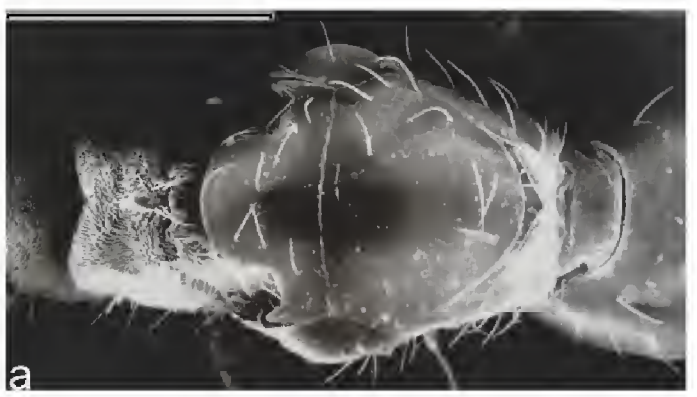
Remarks. Hölldobler and Wilson [54] mentioned specimens of genus *Bruchopria*, as *Solenopsis* guest. Masner and García [5] mentioned "wings often bitten off by ants." Loiácono et al. [26] studied alated and dealated individuals of *Bruchopria* species. The action of dealation has not been observed. The presence of tegulae with normal development and wing stumps demonstrates that the apterism has a secondary origin, caused by the autotomy or by bites of the host ants. The apices of the wing stumps of all individuals examined were



(a)

(b)

FIGURE 9: (a) *Bruchopria hexatoma* female dealate in dorsal view. (b) *Solenopsis richteri*. Scale: 1 mm.



(a)

(b)

FIGURE 10: *Bruchopria hexatoma* female. (a) mesosoma and petiole in dorsal view, scale: 0.5 mm; (b) wing stump, scale: 0.1 mm [26].

regular suggesting that the wings are bitten or torn off close to the tegulae. The fact that specimens are dealated allows them to move into the mound galleries and chambers.

3.2.2. Bruchopria hexatoma Kieffer, 1921 [12] (Figures 9(a), 10(a), and 10(b)). *Bruchopria hexatoma* Kieffer, 1921: 39 [12].

Bruchopria hexatoma: Borgmeier, 1939: 543 [44].

Distribution. Argentina (Misiones, Córdoba and Buenos Aires) [12, 44].

Biology. Associated with *Solenopsis richteri* (Figure 9(b)) and *Acromyrmex lundii* [12, 44].

Material Studied. One female dealated, Argentina, Misiones, Pastoreo Grande, 9-VII-1932, Ogloblin coll.; 1 female dealated, Argentina, Córdoba, XII-1920, Bruch coll., 1 female dealated, Córdoba, Sierras de Córdoba, La Granja, Bruch coll., without date; 1 male dealated, Argentina, Buenos Aires, without locality, 9-VII-1923, Bruch coll., with the ant; 4 females dealated, Argentina, Buenos Aires, Olivos, without date, Bruch coll., with the ant; 1 female dealated, Argentina, Buenos Aires, 10-IX-1925, Bruch coll.; 1 female dealated with *Acromyrmex lundii*, Argentina, Buenos Aires, without date, Bruch coll.

Remarks. Bruchopria hexatoma has been reported by Kieffer [12] in association with Solenopsis richteri and *Acromyrmex lundii* in Argentina; Borgmeier [44] also mentioned this species as a guest of S. *saevissima* (Smith), in Brazil.

The specimens from the provinces of Córdoba and Buenos Aires are dealated, with remains of wings (Figures 10(a) and 10(b)), and most of them are accompanied by the host ants (Figure 9(b)). Unfortunately, the types of the species described by Kieffer have become widely scattered or lost [55]. Bruch sent to Kieffer part of the same series of material to identify (De Santis, pers. comm.).

3.2.3. Bruchopria pentatoma Kieffer, 1921 [12]. Bruchopria pentatoma Kieffer, 1921: 38 [12].

Distribution. Argentina (Córdoba) [12].

Biology. Associated with *Solenopsis richteri* [12].

Material Studied. Syntype male dealated, Argentina, Córdoba, Alta Gracia; 1-8-IV-1920, Bruch coll.

Remarks. According to Kieffer's description, females of both species, *B. pentatoma* and *B. hexatoma*, are distinguished by the number of club antennomeres, five and six, respectively. Unfortunately, the unique female type is not available. *Bruchopria pentatoma* has also been reported by Kieffer [12] in association with S. richteri and *Acromyrmex lundii* (Guérin) in Argentina.

3.2.4. Pentapria Kieffer, 1905 [56]. Pentapria Kieffer, 1905: 34 [56].

Antipapria Fabritius, 1968: 844 [57].
Bakeria Kieffer, 1905: 34 [56].
Plutopria Kieffer, 1910: 48 [40].
Spilomicrinus Ogloblin, 1957: 425 [58].
Xenopria Fouts, 1939: 260 [59].

Distribution. The genus is distributed in the New World [5].

Biology. The principal host plausible to assume is Stratiomyidae (Diptera) [5]. Herein, we studied a female collected with *Solenopsis saevissima* (Hymenoptera: Formicidae).

Table 1

Diapriid tribe	Diapriids species	Argentine provinces	Ant subfamily	Ant tribe	Ant species
Diapriini	*Asolenopsia rufa*	Córdoba, Entre Ríos, Santa Fe	Ecitoninae	Ecitonini	*Neivamyrmex carettei*
	Basalys sp.	Buenos Aires	Dolichoderinae	Dolichoderini	*Linepithema humile*
	Doliopria collegii	Buenos Aires, Misiones	Ecitoninae	Ecitonini	*Eciton burchellii*, *Eciton quadriglume*
	Doliopria myrmecobia	Buenos Aires, Misiones	Myrmicinae	Attini	*Acromyrmex lundii*
	Notoxoides pedissequus	Córdoba	Ecitoninae	Ecitonini	*Neivamyrmex pseudops*
	Notoxoides pronotalis	Córdoba, Salta, San Luis, Santiago del Estero	Ecitoninae	Ecitonini	*Eciton dulcium*, *Neivamyrmex sulcatus*
	Szelenyiopria pampeana	La Pampa	Myrmicinae	Attini	*Acromyrmex lobicornis*
	Szelenyiopria reichenspergeri	Salta, Tucumán	Ecitoninae	Ecitonini	*Eciton quadriglume*, *Neivamyrmex legionis*
	Szelenyiopria sp.	Córdoba	Ecitoninae	Ecitonini	Ecitonini sp.
	Trichopria formicans	La Pampa	Myrmicinae	Attini	*Acromyrmex lobicornis*
	Trichopria myrmecophila	Buenos Aires	Myrmicinae	Solenopsidini	*Solenopsis richteri*
	Trichopria sp.	Buenos Aires	Dolichoderinae	Dolichoderini	*Linepithema humile*
Spilomicrini	*Bruchopria hexatoma*	Buenos Aires, Córdoba, Misiones	Myrmicinae Myrmicinae	Solenopsidini Attini	*Solenopsis richteri* *Acromyrmex lundii*
	Bruchopria pentatoma	Córdoba	Myrmicinae	Solenopsidini	*Solenopsis richteri*
	Pentapria cf. *nodicornis*	Córdoba	Myrmicinae	Solenopsidini	*Solenopsis saevissima*
	Spilomicrus sp.	Buenos Aires	Myrmicinae	Solenopsidini	Solenopsidini sp.

3.2.5. Pentapria cf. nodicornis

Distribution. Argentina (Córdoba).

Biology. Associated with *Solenopsis saevissima* (new record).

Material Studied. Female collected with *Solenopsis saevissima*, Argentina, Córdoba, Alta Gracia, La Granja, II-1927, Bruch. coll., with no more data.

3.2.6. Spilomicrus Westwood, 1832 [36]. *Spilomicrus* Westwood, 1832: 129 [36].

Loxotropa Foerster, 1856: 122, 123, 126 [60].
Hoplopria Ashmead, 1893: 385, 386, 388 [34].
Linkiola Kieffer, 1910: 39 [40].
Eriopria Kieffer, 1910: 693, 744 [40].
Tritopria Kieffer, 1910: 717, 748 [40].
Cologlyptus Crawford, 1910: 123 [61].
Scutellipria Szabó, 1961: 53–493 [62].

Distribution. America [5].

Biology. Primary parasitoidism solitary and gregarious of various Diptera; few species were reared from Coleoptera [5]. Herein, we studied samples associated with a Solenopsidini ant.

3.2.7. Spilomicrus sp.

Distribution. Argentina (Buenos Aires).

Biology. Associated with Solenopsidini ant.

Material Studied. Two females with a Solenopsidini ant, Argentina, Buenos Aires, 9-VIII-1923, Bruch coll.

Table 1 summarizes information about diapriids and their associates.

4. Discussion

The knowledge of the biology and behavior of these myrmecophilic diapriids and the nature of their interactions with ants has progressed in Argentina since 1980 [63] to present. There are nine genera recorded from Argentina, which represents about 50% of the genera mentioned by Masner and García [5] from the New World.

The study of Diapriidae Collection housed at División Entomología of Museo de La Plata, which includes Bruch and Ogloblin myrmecophilic diapriid specimens, allowed us to report 16 species of nine genera of Diapriinae associated with ants in Argentina. It is interesting to highlight that *Asolenopsia rufa, Notoxoides pronotalis*, *Bruchopria pentatoma,* and *B. hexatoma* are the species with a high degree of integration with the host ants, adaptation being both morphological and behavioral.

We mentioned for the first time the associations between the "argentine ant," *Linepithema humile,* and both *Basalys* sp. and *Trichopria* sp., *Pentapria* cf. *nodicornis* and *Solenopsis saevissima*, and *Spilomicrus* sp. and Solenopsidini ant.

Doliopria myrmecobia is a new record to Misiones. The only described species of *Szelenyiopria* occurs in La Pampa

province, *S. pampeana*; an undescribed species is known to us from Córdoba.

We considered that *Szelenyiopria pampeana* and *Trichopria formicans* parasitoids of *Acromyrmex* species in Argentina seem excellent potential models to explore how parasitoids impact ant colony demography, population biology, and ant community structure.

Acknowledgments

The authors would like to thank Dr. J.-P. Lachaud for inviting them to participate in the Special Issue of Psyche, "Ants and Their Parasites 2013." Special thanks go to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Secretaría de Ciencia y Técnica de la Universidad Nacional de La Plata (UNLP) for financial support, Dr. Carlos Margaría for his critical reading of the paper, and to reviewers for their suggestions.

References

[1] L. Huggert and L. Masner, "A review of myrmecophilic-symphilic diapriid wasps in the Holarctic realm, with descriptions of new taxa and a key to genera (Hymenoptera: Proctotrupoidea: Diapriidae)," *Contributions of the American Entomological Institute*, vol. 20, pp. 63–89, 1983.

[2] L. Masner, "A revision of Ecitophilous Diapriid-genus Mimopria Holmgren (Hym., Proctotrupoidea)," *Insectes Sociaux*, vol. 6, no. 4, pp. 361–367, 1959.

[3] J. P. Lachaud and G. Pérez-Lachaud, "Diversity of species and behavior of hymenopteran parasitoids of ants, a review," *Psyche*, vol. 2012, Article ID 134746, 24 pages, 2012.

[4] M. S. Loiácono, C. B. Margaría, E. M. Quirán, and B. Corró Molas, "Diápridos (Hymenoptera) parasitoides de larvas de la hormiga cortadora Acromyrmex lobicornis (Hymenoptera: Formicidae) en la Argentina," *Revista de la Sociedad Entomológica Argentina*, vol. 59, pp. 7–15, 2000.

[5] L. Masner and J. L. García, "The genera of Diapriinae (Hymenoptera: Diapriidae) in the new world," *Bulletin of the American Museum of Natural History*, no. 268, pp. 2–125, 2002.

[6] M. Yoder, "Mannomicrus (Hymenoptera: Diapriidae), a new genus of myrmecophilic diapriid, with a digital version of Masner and García's (2002) key to New World Diapriinae and an illustration of digital description and key markup using an ontology," *Zootaxa*, no. 1439, pp. 47–55, 2007.

[7] B. Pérez-Ortega, H. Fernández-Marín, M. S. Loiácono, P. Galgani, and W. T. Wcislo, "Biological notes on a fungus-growing ant, Trachymyrmex cf. zeteki (Hymenoptera, Formicidae, Attini) attacked by a diverse community of parasitoid wasps (Hymenoptera, Diapriidae)," *Insectes Sociaux*, vol. 57, no. 3, pp. 317–322, 2010.

[8] M. S. Loiácono and C. Margaría, "A note on *Szelenyiopria Pampeana* (Loiácono) n. comb., parasitoid wasps (Hymenoptera: Diapriidae) attacking the fungus growing ant, *Acromyrmex lobicornis* Emery (Hymenoptera: Formicidae: Attini) in La Pampa, Argentina," *Zootaxa*, vol. 2105, pp. 63–65, 2009.

[9] H. Fernández-Marín, J. K. Zimmerman, and W. T. Wcislo, "Acanthopria and Mimopriella parasitoid wasps (Diapriidae) attack Cyphomyrmex fungus-growing ants (Formicidae, Attini)," *Naturwissenschaften*, vol. 93, no. 1, pp. 17–21, 2006.

[10] A. Lanteri and J. Martínez, "Prohombres del Museo de la Plata: Carlos Bruch, organizador de la división entomología," *Revista de la Sociedad Entomológica Argentina*, vol. 71, no. 3-4, pp. 179–185, 2012.

[11] http://gap.entclub.org/taxonomists/Bruch/index.html, 2012.

[12] J. J. Kieffer, "Proctotrypides hôtes des fourmis en Argentine," *Anales de la Sociedad Científica Argentina*, vol. 91, pp. 36–41, 1921.

[13] J. J. Kieffer, "Trois nouveaux Hymenoptères d'Argentine recueillis par C. Bruch," *Anales de la Sociedad Científica Argentina*, vol. 94, pp. 205–208, 1922.

[14] S. V. Triapitsyn, J. T. Huber, G. A. Logarzo, V. V. Berezovskiy, and D. A. Aquino, "Review of Gonatocerus (Hymenoptera: Mymaridae) in the Neotropical region, with description of eleven new species," *Zootaxa*, no. 2456, pp. 1–243, 2010.

[15] M. S. Loiácono and C. B. Margaría, "Grado de curadoría de la colección de Diapriidae (Hymenoptera) del Museo de la Plata, Argentina," *Revista de la Sociedad Entomológica Argentina*, vol. 70, no. 3-4, pp. 293–300, 2011.

[16] L. De Santis and L. Esquivel, "Tercera lista de himenópteros parásitos y predatores de los insectos de la República Argentina," *Revista del Museo de la Plata*, vol. 9, pp. 47–215, 1966.

[17] L. De Santis, "Catálogo de los himenópteros argentinos de la Serie Parasítica, incluyendo Bethyloidea," *Publicación de la Comisión de Investigaciones Científicas de la Provincia de Buenos Aires*, pp. 1–337, 1967.

[18] L. De Santis, "Nuevas citas de himenópteros para la República Argentina," *Revista de la Facultad de Agronomía*, vol. 3, no. 47, pp. 201–207, 1971.

[19] M. S. Loiácono, "Diápridos ecitófilos sinfilos de la región Neotropical (Hymenoptera - Proctotrupoidea - Diapriidae)," *Revista de la Sociedad Entomológica Argentina*, vol. 40, pp. 297–310, 1981.

[20] M. S. Loiácono, "Notas sobre Diapriinae neotropicales (Hymenoptera, Diapriidae)," *Revista de la Sociedad Entomológica Argentina*, vol. 40, pp. 237–241, 1981.

[21] M. S. Loiácono, "Un nuevo diáprido (Hymenoptera) parasitoide de larvas de Acromyrmex ambiguus (Emery) (Hymenoptera, Formicidae) en el Uruguay," *Revista de la Sociedad Entomológica Argentina*, vol. 44, pp. 129–136, 1987.

[22] M. S. Loiácono and N. B. Díaz, "Los ejemplares tipo de Proctotrupoidea y Ceraphronoidea (Hymenoptera) depositados en la colección del Museo de la Plata," *Revista del Museo de la Plata, Serie Técnica y Didáctica*, vol. 23, pp. 1–13, 1996.

[23] M. S. Loiácono, "Diaprinos asociados a la hormiga Camponotus rufipes (Hymenoptera: Diapriidae)," *Revista de la Sociedad Entomológica Argentina*, vol. 59, pp. 198–200, 2000.

[24] M. S. Loiácono and C. B. Margaría, "Systematic study of the genus Gymnopria (Hymenoptera: Diapriidae)," *Insect Systematic Evolution*, vol. 31, no. 2, pp. 187–200, 2000.

[25] M. S. Loiácono and C. B. Margaría, "Ceraphronoidea, Platygastroidea, and Proctotrupoidea from Brazil (Hymenoptera)," *Neotropical Entomology*, vol. 31, no. 4, pp. 551–560, 2002.

[26] M. S. Loiácono, C. B. Margaría, E. Quirán, and B. Corró Molas, "Revision of the myrmecophilous diapriid genus Bruchopria Kieffer (Hymenoptera, Proctotrupoidea, Diapriidae)," *Revista Brasileira de Entomologia*, vol. 46, no. 3, pp. 231–235, 2002.

[27] M. S. Loiácono, C. B. Margaría, N. B. Díaz, and F. E. Gallardo, "Lista de himenópteros parasitoides y depredadores de los insectos de la República Argentina primer suplemento, por

De Santis, L. y C. Monetti," in *Contribuciones Taxonómicas en órdenes de Insectos Hiperdiversos*, J. L. Bousquets and A. Lanteri, Eds., pp. 91–139, Universidad Nacional Autónoma de México (UNAM) and RIBES-CYTED (Red Iberoamericano de Biogeografía y Entomología Sistemática-Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo), Mexico City, Mexico, 2008.

[28] M. Loiácono and C. Margaría, "Diapriidae," in *Biodiversidad de Artrópodos Argentinos*, L. Claps and S. Roig-Juñent, Eds., vol. 2, 2012.

[29] M. Loiácono, C. Margaría, D. O. Moreira, and D. Aquino, "A new species of Szelenyiopria Fabritius (Hymenoptera: Diapriidae), larval parasitoid of Acromyrmex subterraneus subterraneus (Forel) (Hymenoptera: Formicidae) from Brazil," *Zootaxa*. In press.

[30] A. Hadley, 2011, http://www.hadleyweb.pwp.blueyonder.co.uk/CZM/combinezm.htm.

[31] M. J. Sharkey, "Phylogeny and classification of Hymenoptera," in *Linnaeus Tercentenary: Progress in Invertebrate Taxonomy*, Z.-Q. Zhang and W. A. Shear, Eds., vol. 1668, pp. 521–548, *Zootaxa*, Auckland, New Zealand, 2007.

[32] B. Bolton, *Bolton's Catalogue of Ants of the World, 1758, 2005*, Harvard University Press, Cambridge, Mass, USA, 2006.

[33] M. J. Yoder, K. Dole, and A. R. Deans, "mx-A collaborative content management system for revisionary systematists'm," 2006, http://www.diapriid.org/.

[34] W. H. Ashmead, "Monograph of the North American Proctotrypidae," *United States National Museum Bulletin*, vol. 45, pp. 1–472, 1893.

[35] C. Ferrière, "Nouveaux diapriides du Brésil, hôtes des Eciton," *Zoologischer Anzeiger*, vol. 82, pp. 156–171, 1929.

[36] J. O. Westwood, "Descriptions of several new British forms amongst the parasitic hymenopterous insects," *London & Edinburgh Philosophical Magazine and Journal of Science*, vol. 1, pp. 127–129, 1832.

[37] J. J. Kieffer, "Serphides des Îles Philippines," *Insecta*, vol. 3, pp. 253–260, 1913.

[38] L. Masner, "A redescription of three species of Proctotrupoidea (Hymenoptera) from Jurine's collection," *Entomophaga*, vol. 9, no. 1, pp. 81–89, 1964.

[39] F. Muesebeck and L. M. Walkley, "Type species of the genera and subgenera of parasitic wasps comprising the superfamily Proctotrupoidea (order Hymenoptera)," *Proceedings of the United States Natural Museum*, vol. 105, no. 3359, pp. 319–419, 1956.

[40] J. J. Kieffer, "Beschreibung neuer südamerikanischer im Zoologischen Museum zu Berlin aufbewahrter Diapriiden," *Entomologische Rundschau*, vol. 27, pp. 39–56, 1910.

[41] J. Risbec, "La faune entomologique des cultures au Sénégal et au Soudan: II. Contribution à l'étude des Proctotrupidae (Serphiidae)," *Gouvernement général de l'Afrique occidentale française, [s.l.]*, pp. 509–638, 1950.

[42] W. H. Ashmead, "Classification of the pointed-tailed wasps, or the superfamily Proctotrypoidea- II," *Journal of the New York Entomological Society*, vol. 11, p. 30, 1903.

[43] J. Brèthes, "Himenópteros Argentinos," *Anales del Museo Nacional de Historia Natural de Buenos Aires*, vol. 20, pp. 205–316, 1911.

[44] T. Borgmeier, "Sobre algunos Diapriideos myrmecophilos, principalmente do Brasil (Hym. Diapriidae)," *Revista de Entomologia*, vol. 10, no. 3, pp. 530–545, 1939.

[45] L. Masner, "A new genus of ecitophilous diapriid wasps from Arizona (Hymenoptera: Proctotrupoidea: Diapriidae)," *Canadian Entomologist*, vol. 109, no. 1, pp. 33–36, 1977.

[46] C. Bruch, "Estudios mirmecológicos," *Anales del Museo Nacional de Historia Natural*, vol. 34, pp. 341–360, 1928.

[47] C. Bruch, "Estudios mirmecológicos con la descripción de nuevas especies de dípteros (Phoridae) por los Rr. Pp. H. Schmitz y Th. Borgmeier y de una araña (Gonyleptidae) por el Doctor Mello-Leitão," *Revista del Museo de la Plata*, vol. 27, pp. 172–220, 1923.

[48] J.-P. Lachaud, "*Etude des relations hôte-myrmécophile entre les Diapriidae Lepidopria pedestris Kieffer et Solenopsia imitatrix Wasmann et la fourmi Diplorhoptrum fugax Latreille [Ph.D. thesis]*," Paul Sabatier, Toulouse, France, 1981.

[49] K. Fabritius, "Die Gattung Doliopria Kieffer 1910 und nebenstehende Gattungen (Hymenoptera: Diapriidae)," *Folia Entomologica Hungarica*, vol. 27, pp. 53–57, 1974.

[50] J. J. Kieffer, "Hymenoptera fam. Diapriidae," in *Genera Insectorum*, P. Wytsman, Ed., vol. 124, pp. 1–75, Fascicle, Burlingame, Calif, USA, 1912.

[51] J. J. Kieffer, "Description de quelques nouveaux serphides," *Bulletin de la Societe D'Histoire Naturelle de Metz*, vol. 25, pp. 1–7, 1906.

[52] J. J. Kieffer, "Species des Hyménoptères d'Europe et d'Algérie," in *Librerie Scientifique A*, E. André, Ed., vol. 10, pp. 913–1015, Hermann & Fils, París, France, 1911.

[53] J. Brèthes, "Parásitos e hiperparásitos de "Diatraea saccharalis" en la caña de azúcar, en Tucumán," *Revista Industrial y Agrícola de Tucumán*, vol. 17, pp. 63–66, 1927.

[54] B. Hölldobler and E. O. Wilson, *The Ants*, Harvard University Press, Cambridge, Mass, USA, 1990.

[55] L. Masner, "The types of Proctotrupoidea (Hymenoptera) in the British Museum (Nat. Hist.) and Hope Department of Entomology (Oxford)," *Bulletin of the British Museum*, vol. 1, pp. 1–154, 1965.

[56] J. J. Kieffer, "Nouveaux Proctotrypides exotiquez conservés au Musée Civique de Gênes," *Annali del Museo Civico di Storia Naturale di Genova*, vol. 2, no. 2, pp. 9–39, 1905.

[57] K. Fabritius, "Antipapria n. gen., eine neue Diapriiden-Gattung aus Argentinien (Hymenoptera, Proctotrupoidea)," *Travaux du Muséum d'Histoire Naturelle "Grigore Antipa"*, vol. 8, pp. 841–844, 1968.

[58] A. Ogloblin, "Los Insectos de las Islas Juan Fernández. 35. Mymaridae, Ceraphronidae, Diapriidae y Scelionidae (Hymenoptera)," *Revista Chilena Entomología*, vol. 5, pp. 413–444, 1957.

[59] R. M. Fouts, "Descriptions of one new genus and three new species of Diapriidae (Hymenoptera)," *Proceedings of the Entomological Society of Washington*, vol. 41, pp. 260–263, 1939.

[60] A. Foerster, "Hymenopterologische studien," in *Chalcidiae und Proctotrupii*, vol. 2 of *II. Heft. Aachen*, pp. 1–152, Ernsteer Meer, Zoeterwoude, The Netherlands, 1856.

[61] J. C. Crawford, "New Hymenoptera from the Philippine Islands," *Proceedings of the United States National Museum*, vol. 38, no. 1733, pp. 119–133, 1910.

[62] J. B. Szabó, "Neue paläarktische Gattungen und Arten der Diapriiden in der Sammlung des Ungarischen Naturwissenschaftlichen Museums (Hymenoptera, Proctotrupoidea, Diapriidae)," *Annales Historico-Naturales Musei Nationalis Hungarici*, vol. 53, pp. 491–494, 1961.

[63] L. De Santis, "Entomología: evolución de las ciencias en la República Argentina 1923–1972," *Publicación de la Sociedad Científica Argentina*, vol. 12, pp. 1–323, 1992.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 601073, 5 pages
http://dx.doi.org/10.1155/2013/601073

Research Article

The Tergal Gland Secretion of the Two Rare Myrmecophilous Species *Zyras collaris* and *Z. haworthi* (Coleoptera: Staphylinidae) and the Effect on *Lasius fuliginosus*

Michael Stoeffler, Lea Boettinger, Till Tolasch, and Johannes L. M. Steidle

Universität Hohenheim, Institut für Zoologie, Fachgebiet Tierökologie 220c, Garbenstr. 30, 70593 Stuttgart, Germany

Correspondence should be addressed to Michael Stoeffler; m.stoeffler@t-online.de

Received 25 January 2013; Accepted 9 March 2013

Academic Editor: Jean-Paul Lachaud

The beetle species *Zyras collaris* and *Z. haworthi* belong to the rove beetle tribe Myrmedoniini (Staphylinidae: Aleocharinae), which comprises many myrmecophilous species. Due to their rareness, it is unknown how the two species interact with their host ants. GC-MS analyses revealed that both species release α-pinene, β-pinene, myrcene and limonene from their defensive tergal glands. This composition of tergal gland secretion is unique within the subfamily Aleocharinae. In biotests, *Lasius fuliginosus* ants showed increased antennation towards filter paper balls treated with mixtures of these substances in natural concentrations. Because these monoterpenes are also present in some aphid species which are attended by ants, we hypothesize that *Zyras* beetles mimic the presence of aphids and thereby achieve acceptance by their host ants.

1. Introduction

The rove beetles tribe Myrmedoniini (Staphylinidae: Aleocharinae) contains many myrmecophilous species. In Central Europe, it comprises the myrmecophilous genera *Lomechusa* and *Lomechusoides, Zyras, Myrmoecia,* and *Pella*, as well as the nonmyrmecophilous species *Drusilla canaliculata* Fabricius, 1787. *Myrmoecia* and *Pella* were formerly considered subgenera of *Zyras* but, meanwhile, have been elevated to genus rank [1–3], which is also supported by molecular data [4, 5].

Lomechusa and *Lomechusoides* are textbook examples for the integration of myrmecophiles in ant nests by the use of appeasement glands on their abdomen [6]. Different strategies are used by *Pella* species to escape from aggressions by their host ant *Lasius fuliginosus* (Latreille, 1798). While the Japanese species *P. comes* (Sharp, 1874) mimics the cuticular hydrocarbon (CHC) pattern of its host ant to be accepted [7], *P. laticollis* (Märkel, 1845) employs a specific appeasing behaviour [8]. *Pella cognata* (Märkel, 1842), *P. funesta* (Gravenhorst, 1806), and *P. humeralis* (Gravenhorst, 1802) repel ants by the use of their abdominal tergal gland. This tergal gland is only found within the Aleocharinae and is used by most species of the subfamily as defensive gland against aggressors [9]. In *P. funesta* and *P. humeralis,* the gland secretion specifically contains sulcatone, a panic alarm inducing pheromone of *L. fuliginosus.* By the release of this compound, beetles create an "ant free space" [8, 10]. In contrast to these species, only little is known on the biology of *Zyras* species, and it is unclear how they achieve acceptance by ants. For *Z. collaris* (Paykull, 1789) and *Z. haworthi* (Stephens, 1835), this is mainly due to their rarity. For South-West Germany, only 18 and 10 records exist from 1950 to 2000 for *Z. collaris* and *Z. haworthi*, respectively [11]. Our own collection efforts between 2001 and 2011 resulted in approximately 1200 specimens of different *Pella* species, but only one for each of the two *Zyras* species.

Here we report for the first time on the composition of the tergal gland secretion of *Z. collaris* and *Z. haworthi* and its potential role for the interaction with its putative host ant *L.*

Table 1: Substances found in the headspace of a flask containing rove beetles of the genus *Zyras*, which have been teased using a magnetic stir bar. Numbers in the table refer to numbers in Figure 1. Relative proportions of the substances between the beetles were calculated in accordance with [12]. The substance with the highest peak area for each row is the reference (= 1.00).

		Z. collaris		*Z. haworthi*	
	Substances	Rel. peak area	Rel. proportion	Rel. peak area	Rel. proportion
1[1]	α-pinene[2]	2.6	0.20	23.8	1.00
2	β-pinene[3]	41.3	1.00	57.0	0.76
3	Myrcene	51.9	1.00	13.6	0.14
4	Limonene	4.2	1.00	5.5	0.72

[1]Numbers refer to numbers in Figure 1.
[2,3]As proposed by the mass spectra database (see Section 2).

fuliginosus. Because the study is based on the analysis of only two *Zyras* specimen, more studies with these rare beetles are urgently needed to substantiate our findings.

2. Materials and Methods

2.1. Insects. One specimen of *Z. collaris* and one of *Z. haworthi* were collected in the state of Baden-Württemberg (Germany), the first in neglected grassland near Freiburg and the second in a rural area near Herrenberg, in the vicinity of a nest of *L. fuliginosus*. The nest was located in a stump between hedgerows along a brook.

In the lab, beetles were kept in plastic Petri dishes (diameter 90 mm) at room temperature under daylight conditions. The Petri dishes were filled with a 5 mm plaster layer, which was moistened daily to maintain humidity. A small piece of filter paper was provided as shelter. Beetles were fed with dead workers of *L. fuliginosus*. Ants used as food for the beetles and for behavioural observations were collected along ant trails near the nest entrances in the vicinity of Stuttgart (State of Baden-Württemberg, Germany). Insects were determined to species level using the identification keys by Lohse [13] for beetles and Seifert [14] for ants.

2.2. Chemical Analysis of the Tergal Gland Secretion. Volatiles released from the defensive tergal glands of the beetles were analysed as described in [10]. Beetles were placed in a flask and teased with a magnetic stir bar and a magnetic stick. The volatiles from the headspace of the flask were collected using a SPME-fiber coated with 65 μm Polydimethylsiloxane/Divinylbenzene [15]. The SPME-fiber was inserted into a gas chromatograph (Type 6890; Agilent Technologies, HP 5 column: 30 m long, 0.2 mm in diameter and 0.5 μm film thickness; splitless mode, programmed: 60°C for 3 min, 60°C to 300°C at 3°C/min and then constant over 30 min at 300°C, carrier gas: Helium 1.6 mL/min) coupled to a 5973 network mass selective detector (GC-MS) for identification of the collected substances. Chromatograms and mass spectra were analyzed with Agilent Technologies software (Enhanced Chemstation MSD Chem Station D 01.02.16, June 15, 2002) using Wiley- (Wiley275) and NIST-databases (NIST Mass Spectral Library 2002 Version). For identification, mass spectra and retention times of substances were compared with respective data from synthetic compounds.

2.3. Experiments on the Effect of the Tergal Gland Secretion. Ten *L. fuliginosus* ants were placed in a Petri dish with a filter paper ball in the center. The filter paper ball was treated with 10 μL terpene solution in hexane, containing a mixture of monoterpenes in a total concentration of either 1 μg/μL or 10 μg/μL. Control filter paper balls were treated with 10 μL hexane. Each test solution was tested 20 times with different ant specimen. Hexan as control was tested 40 times. The reaction of the ants to the filter paper balls was video-taped for 120 sec and analysed afterwards by counting the events of the different behaviours. Behaviour was considered as aggressive when ants touched the filter paper ball with both antennae and open mandibles or when they were biting into it. Antennation, that is, touching the filter paper ball with both antennae and closed mandibles, was considered as a nonaggressive behaviour.

The following test solutions containing mixtures of all four identified monoterpenes in hexane were prepared:

(1) mixture of α-pinene (3 mg), β-pinene (41 mg), myrcene (52 mg), and limonene (4 mg) in 100 mL hexane resembling the secretion of *Z. collaris*;

(2) mixture of α-pinene (24 mg), β-pinene (57 mg), myrcene (14 mg), and limonene (6 mg) in 100 mL hexane resembling the secretion of *Z. haworthi*.

Both mixtures contain terpenes in a total concentration of 1 μg/μL. For tests with 10 μg/μL, the mixtures were concentrated tenfold in a water bath. The relative concentrations of the single compounds matched the composition of the headspace analyses of the tergal gland secretion by GC/MS (Table 1). The concentration of either 1 μg/μL or 10 μg/μL is based on the assumption that the tergal gland reservoir of the two *Zyras* species is about 0.2 μL, equivalent to the volume of the similar sized *Aleochara curtula* Goeze [16] and that between 1/20 to 1/5 of the whole volume is released at one time.

2.4. Statistics. The results of the behavioural assays were analysed with the Mann-Whitney U-test using the software package STATISTICA 1999 Edition (StatSoft Inc., 1999).

3. Results

3.1. Chemical Analysis of the Tergal Gland Secretion. GC-MS analyses of volatiles released by *Z. collaris* and *Z. haworthi* revealed the presence of the monoterpenes α-pinene, β-pinene, myrcene, and limonene, which were identified by comparison of those of authentic reference samples (Figure 1, Table 1).

To compare the relative importance of each compound between the species, the relative proportions of the substances were calculated in accordance with [12]. This method reveals that *Z. haworthi* has a five times higher amount of α-pinene

than *Z. collaris* whereas the amount of myrcene in *Z. collaris* is approximately five times higher than in *Z. haworthi*. The amount of β-pinene and limonene is similar between the species.

3.2. Experiments on the Effect of the Tergal Gland Secretion. Filter paper balls treated with solutions mixed according to the results of the chemical analyses, representing the composition of the tergal secretion of *Z. collaris* and *Z. haworthi*, stimulated significantly more antennation by the ants than the control hexane. Furthermore, no significant aggression inducing effect was found (Figure 2).

4. Discussion

Using headspace SPME and GC-MS, the volatile compounds that were released by the two rove beetle species *Z. collaris* and *Z. haworthi* from their defensive tergal gland upon molestation were analysed. The analysis revealed the exclusive presence of the terpenes α-pinene, β-pinene, myrcene, and limonene. This is remarkable, because terpenes are absent from the tergal gland secretion of all the other 26 species from nine different tribes of this subfamily Aleocharinae which have been studied so far, including all the other species of the same tribe Myrmedoniini [8, 10, 16, 17]. Generally, the tergal gland secretion of the Aleocharine contains quinones as toxins, which are dissolved in alkanes, alkenes, aldehydes, ketones, acids, esters, and acetates [9]. Obviously, the composition of the secretion in the genus *Zyras* is unique within the subfamily.

This supports recent findings on the molecular phylogeny of Lomechusini [5], which show that the genus *Zyras* is much more distant to the genus *Pella* and that *Pella* should not be considered a subgenus of the former. This settles a long dispute on the phylogenetic relationship of these genera.

Due to the rarity of *Z. collaris* and *Z. haworthi*, the present study is based on the analysis of one specimen of each species only. So, it is not guaranteed that the mixtures found in the tergal glands of both specimens are representative of the entire species. Also possible methodological or sampling deviations cannot be excluded. However, in our earlier studies, we found that the qualitative composition of the defensive tergal gland secretion of the Aleocharinae is highly species specific and varies only quantitatively between individuals [9]. Thus, we consider that our results on the chemical composition of the tergal gland secretion are very likely to be valid. The uniqueness of the *Zyras* secretion within the Myrmedoniini is also supported by the fact that both *Zyras* specimens had qualitatively very similar secretions. Nevertheless, more studies on the chemical composition of the tergal gland secretion of *Zyras* species are required to substantiate our findings and to clarify the exact stereochemistry of the identified pinenes.

To study the role of the terpenes in the tergal gland secretion, the reaction of *L. fuliginosus* ants to mixtures of these compounds was studied in laboratory experiments. *L. fuliginosus* was chosen based on the literature where this species is described as host ant of *Z. haworthi* [13, 18] and because our *Z. haworthi* was collected in the vicinity of a nest of *L. fuliginosus*. This indicates that *L. fuliginosus* might be the host ant of *Z. haworthi,* whereas the host ants of *Z. collaris* remains unclear. Two different mixtures were tested, composed according to the ratio of single compounds in our chemical analysis of the secretion of both species. Mixtures were tested in two different concentrations covering the quantity of secretion released by the beetles under natural conditions. The experiments revealed no deterrent or aggression eliciting effect of these substances to the ants. Instead, increased antennation behaviour of ants towards filter balls treated with a mixture of these terpenes was observed. This reaction of the ants points to the fact that the terpenes might be used by the beetles to deal with their host ants in analogy to the ability of some myrmecophilous *Pella*-beetles, which repel aggressive host ants by the release of the ants' panic alarm pheromone sulcatone [8, 10]. However, none of the four identified monoterpenes have been described as pheromones in *L. fuliginosus* so far. Possibly, the antennation response of ants to the terpenes is based on their homobiosis with aphids. The aphids are protected by the ants, which receive the nutritious honeydew in return [6]. To obtain honeydew, ants antennate the aphid's abdominal tip. This behaviour strongly resembles the behaviour observed by us in interactions between myrmecophilous rove beetles and ants. In accordance with this idea, α-pinene, β-pinene, myrcene, and limonene have been reported to be present in some aphid species [19]. α- and β-Pinene as well as limonene occur in the aphid honeydew [20, 21]. Therefore, we hypothesise that these terpenes are used by ants to recognize aphids and that *Zyras* beetles mimic these compounds to calm down the aggressions of host ants during encounters. To address this hypothesis, it would be required (1) to unequivocally identify the host ants of both *Zyras* species, (2) to study in more details behavioural interactions between *Zyras* specimens and these host ants, (3) to identify aphid species that are relevant for the host ants, and (4) to examine the role of the identified terpenes on the interaction between these aphids and their host ants. This working plan is especially challenging because of the rarity of the beetles.

Taken together, the tergal gland secretion of *Z. collaris* and *Z. haworthi* is unique within the rove beetle subfamily Aleocharinae by its composition of the terpenes α-pinene, β-pinene, myrcene, and limonene. In biotests, *L. fuliginosus* ants were neither repelled nor did show aggressive behaviour towards these substances but were stimulated to antennation. Because terpenes are present in aphids, we hypothesize that *Zyras* beetles release these compounds to mimic aphids and achieve acceptance by their host ants.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

The authors would like to thank Tanja Maier and Alexander Szallies for their help in providing *Zyras* beetles. An unknown

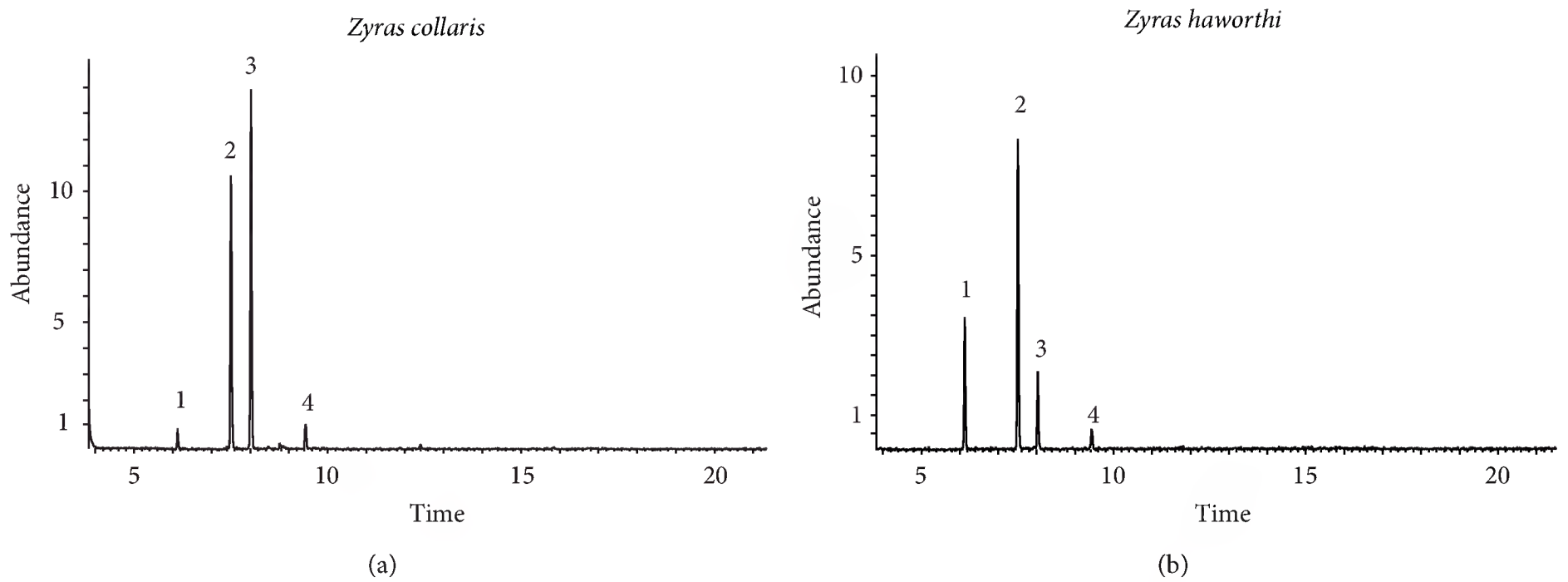


FIGURE 1: Gas chromatograms (TIC) of the tergal secretions obtained by stir bar irritation of *Zyras collaris* (a) and *Z. haworthi* (b). 1: α-pinene; 2: β-pinene; 3: myrcene; 4: limonene.

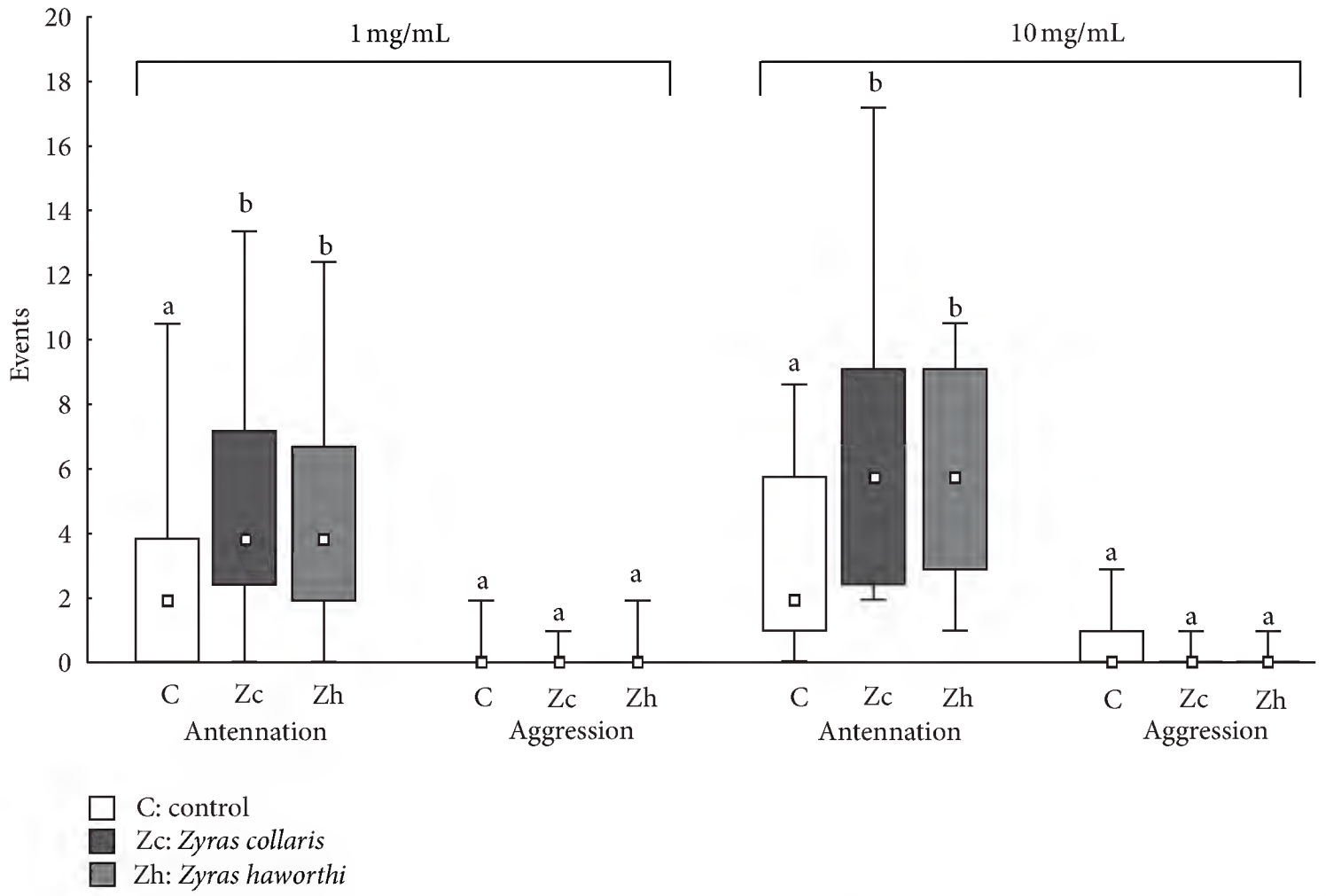


FIGURE 2: Antennation and aggressive behaviour (□: median, boxes: 25–75 percentiles, whiskers: min.–max.) by *Lasius fuliginosus* ants in a laboratory experiment towards a filter paper ball treated with mixtures of substances (1 mg/mL and 10 mg/mL), which are present in the tergal gland secretion of *Zyras collaris* and *Z. haworthi* rove beetles. Bars with different lower case letters are significantly different at $P \leq 0.05$ (Mann-Whitney U-test; control: $N = 40$; mixtures: $N = 20$).

reviewer carefully studied our paper and helped to improve it with his remarks.

References

[1] D. H. Kistner, "Studies of Japanese myrmecophiles, part I: the genera *Pella* and *Falagria* (Coleoptera, Staphylinidae)," in *Entomological Essays to Commemorate the Retirement of Professor K. Yasumatsu*, Z. Hidaka, Ed., pp. 141–165, Hokuryukan, Tokyo, Japan, 1972.

[2] M. Maruyama, "Revision of the Palearctic species of the myrmecophilous genus *Pella* (Coleoptera, Staphylinidae, Aleocharinae)," *National Science Museum Monographs*, vol. 32, pp. 1–207, 2006.

[3] P. Hlavác, A. F. Newton, and M. Maruyama, "World catalogue of the species of the tribe Lomechusini (Staphylinidae: Aleocharinae)," *Zootaxa*, no. 3075, pp. 1–151, 2011.

[4] H. Elven, L. Bachmann, and V. I. Gusarov, "Phylogeny of the tribe Athetini (Coleoptera: Staphylinidae) inferred from mitochondrial and nuclear sequence data," *Molecular Phylogenetics and Evolution*, vol. 57, no. 1, pp. 84–100, 2010.

[5] H. Elven, L. Bachmann, and V. I. Gusarov, "Molecular phylogeny of the Athetini-Lomechusini-Ecitocharini clade of aleocharine rove beetles (Insecta)," *Zoologica Scripta*, vol. 41, no. 6, pp. 617–636, 2012.

[6] B. Hölldobler and E. O. Wilson, *The Ants*, The Belknap Press, Cambridge, Mass, USA, 1990.

[7] T. Akino, "Chemical camouflage by myrmecophilous beetles *Zyras comes* (Coleoptera: Staphylinidae) and *Diaritiger fossulatus* (Coleoptera: Pselaphidae) to be integrated into the nest of *Lasius fuliginosus* (Hymenoptera: Formicidae)," *Chemoecology*, vol. 12, no. 2, pp. 83–89, 2002.

[8] M. Stöffler, T. Tolasch, and J. L. M. Steidle, "Three beetles-three concepts. Different defensive strategies of congeneric myrmecophilous beetles," *Behavioral Ecology and Sociobiology*, vol. 65, no. 8, pp. 1605–1613, 2011.

[9] J. L. M. Steidle and K. Dettner, "Chemistry and morphology of the tergal gland of free-living adult Aleocharinae (Coleoptera: Staphylinidae) and its phylogenetic significance," *Systematic Entomology*, vol. 18, no. 2, pp. 149–168, 1993.

[10] M. Stöffler, T. S. Maier, T. Tolasch, and J. L. M. Steidle, "Foreign-language skills in rove-beetles? Evidence for chemical mimicry of ant alarm pheromones in myrmecophilous *Pella* beetles (Coleoptera: Staphylinidae)," *Journal of Chemical Ecology*, vol. 33, no. 7, pp. 1382–1392, 2007.

[11] J. Frank and E. Konzelmann, *Die Käfer Baden-Württembergs 1950–2000*, Naturschutz-Praxis, Artenschutz 6, Landesanstalt für Umweltschutz Baden-Württemberg, Baden-Württemberg, Germany, 1st edition, 2002.

[12] J. T. Romeo, "New SPME guidelines, guidelines for quantitative analysis by SPME," *Journal of Chemical Ecology*, vol. 35, no. 12, p. 1383, 2009.

[13] G. A. Lohse, "Tribus zyrasini," in *Die Käfer Mitteleuropas*, H. Freude, K. W. Harde, and G. A. Lohse, Eds., Band 5, pp. 222–229, Goecke and Evers, Krefeld, Germany, 1974.

[14] B. Seifert, *Die Ameisen Mittel- und Nordeuropas*, Lutra Verlags- und Vertriebsgesellschaft, Tauer, Germany, 2007.

[15] C. L. Arthur and J. Pawliszyn, "Solid phase microextraction with thermal desorption using fused silica optical fibers," *Analytical Chemistry*, vol. 62, no. 19, pp. 2145–2148, 1990.

[16] K. Peschke and M. Metzler, "Defensive and pheromonal secretion of the tergal gland of Aleochara curtula - I. The chemical composition," *Journal of Chemical Ecology*, vol. 8, no. 4, pp. 773–783, 1982.

[17] C. Gnanasunderam, H. Young, C. F. Butcher, and R. F. N. Hutchins, "Ethyl decanoate as a major component in the defensive secretion of two new zealand aleocharine (staphylinidae) beetles—*Tramiathaea cornigera* (broun) and *Thamiaraea fuscicornis* (broun)," *Journal of Chemical Ecology*, vol. 7, no. 1, pp. 197–202, 1981.

[18] A. Horion, "Staphylinidae 3. Teil Habrocerinae bis Aleocharinae (ohne Subtribus *Athetae*)," in *Faunistik der Mitteleuropäischen Käfer*, W. Schmidt, Ed., vol. 11, Wechselnd, Überlingen, Germany, 1967.

[19] F. Francis, S. Vandermoten, F. Verheggen, G. Lognay, and E. Haubruge, "Is the (E)-β-farnesene only volatile terpenoid in aphids?" *Journal of Applied Entomology*, vol. 129, no. 1, pp. 6–11, 2005.

[20] J. A. Pickett and D. C. Griffiths, "Composition of aphid alarm pheromones," *Journal of Chemical Ecology*, vol. 6, no. 2, pp. 349–360, 1980.

[21] P. D. Leroy, A. Sabri, S. Heuskin et al., "Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies," *Nature Communications*, vol. 2, no. 1, article 348, 2011.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 538316, 9 pages
http://dx.doi.org/10.1155/2013/538316

Review Article

Review and Phylogenetic Evaluation of Associations between Microdontinae (Diptera: Syrphidae) and Ants (Hymenoptera: Formicidae)

Menno Reemer

Naturalis Biodiversity Center, European Invertebrate Survey, P.O. Box 9517, 2300 RA Leiden, The Netherlands

Correspondence should be addressed to Menno Reemer; menno.reemer@naturalis.nl

Received 11 February 2013; Accepted 21 March 2013

Academic Editor: Jean-Paul Lachaud

The immature stages of hoverflies of the subfamily Microdontinae (Diptera: Syrphidae) develop in ant nests, as predators of the ant brood. The present paper reviews published and unpublished records of associations of Microdontinae with ants, in order to discuss the following questions. (1) Are all Microdontinae associated with ants? (2) Are Microdontinae associated with all ants? (3) Are particular clades of Microdontinae associated with particular clades of ants? (4) Are Microdontinae associated with other insects? A total number of 109 associations between the groups are evaluated, relating to 43 species of Microdontinae belonging to 14 genera, and to at least 69 species of ants belonging to 24 genera and five subfamilies. The taxa of Microdontinae found in association with ants occur scattered throughout their phylogenetic tree. One of the supposedly most basal taxa (*Mixogaster*) is associated with ants, suggesting that associations with ants evolved early in the history of the subfamily and have remained a predominant feature of their lifestyle. Among ants, associations with Microdontinae are known from subfamilies Ponerinae, Dolichoderinae, Formicinae, Myrmicinae, and Pseudomyrmecinae. These subfamilies comprise more than 95% of all ant species. Interestingly, no associations are known with "dorylomorph" ants (army ants and relatives).

1. Introduction

Ants "run much of the terrestrial world," is the claim of Hölldobler and Wilson [1] in the opening lines of their landmark book *The ants*. This may be true, but the colonies of ants—on their turn—are to some extent affected by many species of myrmecophilous organisms which live in their nests, especially insects and other arthropods. Some of these are not detrimental to the ants or can even be considered beneficial, for example, because they clean up the nests or provide the ants with certain nutrients. Other species of myrmecophilous insects, however, are predators of the ant brood or the adult ants. The larvae of hoverflies of the subfamily Microdontinae (Diptera: Syrphidae) exemplify the latter category.

The nature of the feeding habits of the slug-like larvae of Microdontinae has long remained uncertain. Several authors have suggested that they live as scavengers or feed on pellets of food ejected by the worker ants [2–5]. More recently, however, accumulated evidence showed that larvae of at least a number of species of *Microdon* Meigen and *Omegasyrphus* Giglio-Tos are predators, feeding on eggs, larvae, and pupae of ants [6–10]. There are a few reports of Microdontinae larvae feeding on aphids and coccids attended by ants [11–13], but these could so far not be confirmed. Little is known about the degree of taxonomic specialization exhibited by Microdontinae with respect to their host ants, but available evidence suggests that *Microdon* species are highly specialized, although this may differ between species [14–17]. It seems probable that a certain degree of host specialization is required for predators living in ants nests, because the predators need to make sure that they are not recognized by the ants as hostile intruders. For some *Microdon* species it has been established that their larvae use "chemical mimicry" to prevent them from being attacked by the ants: the fly larvae possess cuticular hydrocarbons similar to those of the ants [14, 15].

The impact of larvae of Microdontinae on ant colonies is potentially large. Duffield [7] reported that third-instar *Microdon* larvae could consume 8–10 ant larvae in 30 minutes, and Barr [6] stated that a *Microdon* larva may consume

up to 125 ant larvae during its life. With an average number of five or six *Microdon* larvae per nest [6], over 700 ant larvae would be consumed per nest. A more indirect way in which *Microdon* larvae possibly affect the fitness of ant colonies was revealed by Gardner et al. [18]. They found that workers of a *Microdon*-infested polygynous ant colony are less closely related to each other than workers of uninfested colonies. They explained this by arguing that it is harder for a *Microdon* larva to intrude in a genetically homogeneous colony, because in such a colony the worker ants smell more alike and will therefore more easily recognize an intruder. So, a decreased genetic diversity will reduce the chance of becoming infested with *Microdon* larvae.

Worldwide, 454 valid species of Microdontinae are known [19], which may be only half or less of the actual species number (estimation by the author based on unpublished data). Approximately 12,500 species of ants are known [20]. Little is known about associations between species of Microdontinae and species of ants. Because of the potential impact of these flies on ant colonies, and hence on ecosystems, it is interesting to learn more about these associations. Besides, this information may be useful for research on subjects like the evolution of host association, chemical mimicry, and (triggers of) cryptic speciation. The present paper aims to summarize available knowledge of associations of Microdontinae with ants, in order to answer the following questions.

(1) Are all Microdontinae associated with ants?

(2) Are Microdontinae associated with all ants?

(3) Are particular clades of Microdontinae associated with particular clades of ants?

(4) Are Microdontinae also associated with other insects besides ants?

2. Material and Methods

2.1. Host Associations. The literature has been reviewed and records on associations of Microdontinae with ants and other insects were assembled. Omitted from the dataset were references to host associations for which considerable doubt exists as to whether the identifications are correct. This is especially the case with several older references to European species, since it became clear that certain taxa actually comprise cryptic species complexes, as in *Microdon analis* (Macquart)/*M. major* Andries and *M. mutabilis* (Linnaeus)/*M. myrmica* (Schönrogge et al.) [16, 21]. The following records were excluded because of this reason (names as in cited publication): *Microdon mutabilis* in nests of *Lasius niger* (Linneaus), *Myrmica ruginodis* Nylander, and *Formica fusca* Linnaeus [2]; *Microdon eggeri* Mik in nests of *Lasius niger* [2]; *Microdon eggeri* in nests of *Formica sanguinea* Latreille [22]; *Microdon devius* (Linnaeus) in nests of *Formica sanguinea* and *Lasius fuliginosus* (Latreille) [23–25]; *Microdon devius* in nests of *Formica fusca*, and *Formica rufa* Linnaeus [25]; *Microdon mutabilis* in nests of *Formica fusca*, *F. rufa*, *F. rufibarbis* Fabricius, *Lasius niger*, *L. brunneus* (Latreille), and *L. flavus* (Fabricius) [25]. These records were, however, included in a more generalized way, that is, as associations of species of *Microdon* s.s. with the ant genera *Formica* Linnaeus, *Lasius* Fabricius, and *Myrmica* Latreille. The records reported in the literature on European *Microdon* (the only genus of Microdontinae occurring in Europe) have not been fully surveyed, as this would not add information to the generic level at which this study was conducted.

Weber [26] reported larvae "of the *Microdon* type" from nests of the ant *Ectatomma ruidum* (Roger) (subfamily Ectatomminae). However, his figure does not show a *Microdon* larva but a larva belonging to another family of Diptera Cyclorrhapha (possibly Phoridae). Hence, this record was excluded from the dataset analyzed in this paper.

In addition to the survey of the literature, associations found in entomological collections were recorded. Such records were noted when an empty puparium was mounted together with an adult specimen, and the label mentioned a genus or species of host ant. Records were taken fro the following collections: Natural History Museum, London (BMNH); National Museums of Scotland, Edinburgh (RSME); United States National Museum, Washington D.C. (USNM); Zoölogisch Museum Amsterdam (ZMAN, recently included in the collection of Naturalis Biodiversity Center (RMNH), Leiden).

2.2. Taxonomy and Phylogeny. Classification of Microdontinae follows Reemer and Ståhls [19]. Classification of ants is updated to modern standards according to Bolton [27]. A recent phylogenetic hypothesis for intrageneric relationships of Microdontinae is obtained from Reemer and Ståhls [28], who presented a tree based on parsimony analysis of combined molecular and morphological characters. All specific taxa were pruned from this tree in order to obtain a tree of generic relationships only. For ants, several recent phylogenetic hypotheses are available (e.g., [29, 30]), which are incongruent at some points. Therefore, in the present study, the tree of extant subfamilies as compiled by Ward [31] is used, because this summarizes relationships which are well supported by all recent studies.

3. Results

Table 1 lists 109 recorded associations of Microdontinae with ants, 105 of which are based on the literature and four are based on collection surveys. These records concern 43 species of Microdontinae belonging to 14 genera, and at least 69 species of ants belonging to 24 genera and five subfamilies (Ponerinae, Dolichoderinae, Pseudomyrmecinae, Formicinae, and Myrmicinae). The distribution of recorded association over the major biogeographic regions is as follows: Nearctic 62, Palaearctic 18, Neotropical 18, Australia/Oceania 6, Afrotropical 4, and Oriental 1.

Figure 1 presents a phylogenetic hypothesis for 28 (out of 43) genera of Microdontinae, with indications of known associations with subfamilies of ants. Figure 2 presents a phylogenetic hypothesis for all extant subfamilies of ants, with indications of known associations with Microdontinae.

Table 1: List of all known records of immature stages of Microdontinae found in association with ants. The records are first sorted by ant subfamily, then alphabetically by ant genus and species. Observation: 1: larva(e) or pupa(e) found in nest; 2: freshly emerged specimens found near nest; 3: adult female(s) observed ovipositing near nest entrance; 4: adult specimens observed near nest.

Ant taxon	Microdontine taxon	Country/region	Source	Observation
Ponerinae				
Pachycondyla Smith	*Hypselosyrphus* spec.	Mexico	G. Pérez-Lachaud and J.-P. Lachaud, pers. comm.	1
Dolichoderinae				
Azteca trigona Emery	Microdontinae spec.	British Guiana	[32]	1
Azteca spec.	*Ceratophya* spec.	Costa Rica	Leg. M. Zumbado, G.E. Rotheray and G. Hancock, collection: RSME	1
Dolichoderus diversus Emery	Microdontinae spec.	Panama	[32]	1
Forelius pruinosus (Roger)	*Microdon* (*Dimeraspis*) *fuscipennis* (Macquart)	USA	[7]	1
Iridomyrmex chasei Forel	*Oligeriops dimorphon* (Ferguson)	Australia	[33]	1
Iridomyrmex rufoniger (Lowne)	*Oligeriops iridomyrmex* (Shannon)	Australia	[34]	1
Linepithema humile (Mayr)	*Mixogaster lanei* Carrera and Lenko	Argentina	[35]	1
Linepithema oblongum (Santschi)	Microdontinae spec.	Argentina	[36]	1
Tapinoma sessile (Say)	*Microdon* (*Dimeraspis*) *globosus* (Fabricius)	USA	[37, 38]	1
Technomyrmex albipes (Smith)	*Bardistopus papuanum* Mann	Solomon Islands	[39]	1
Technomyrmex fulvus (Wheeler)	Microdontinae spec.	Panama	[40]	1
Pseudomyrmecinae				
Pseudomyrmex ejectus (Smith)	*Rhopalosyrphus ramulorum* Weems and Deyrup	USA	[41]	1
Pseudomyrmex gracilis (Fabricius)	Microdontinae spec.	Mexico	[42]	1
Pseudomyrmex simplex (Smith)	*Rhopalosyrphus ramulorum* Weems and Deyrup	USA	[41]	1
Tetraponera penzigi (Mayr)	Microdontinae spec.	East Africa	[9]	1
Formicinae				
Brachymyrmex coactus Mayr	Microdontinae spec.	Brazil	[43]	1
Camponotus atriceps (Smith)	*Microdon* (*Chymophila*) *fulgens* Wiedemann	USA	[38]	
Camponotus herculeanus (Linnaeus)	*Microdon* (s.s.) *piperi* Knab	USA	[8, 38, 44]	1
Camponotus hildebrandti Forel	Microdontinae spec.	Madagascar	[25]	1
Camponotus laevigatus (Smith)	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Camponotus modoc Wheeler	*Microdon* (s.s.) *albicomatus* Novak	USA	[44]	1
Camponotus modoc Wheeler	*Microdon* (s.s.) *piperi* Knab	USA	[44, 45]	1
Camponotus mus Roger	*Masarygus planifrons* Brethes	Argentina	[46]	3
Camponotus nitidior (Santschi)	Microdontinae spec.	Costa Rica	[47]	
Camponotus novaeboracensis (Fitch)	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[38]	1
Camponotus novaeboracensis (Fitch)	*Microdon* (s.s.) *tristis* Loew	USA	[38]	1
Camponotus novogranadensis Mayr	Microdontinae spec.	Panama	[32]	
Camponotus obscuripes Mayr	*Microdon* (s.s.) *macrocerus* Hironaga and Maruyama	Japan	[48]	2
Camponotus pennsylvanicus (DeGeer)	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[38]	1
Camponotus pennsylvanicus (DeGeer)	*Microdon* (s.s.) *tristis* Loew	USA	[37]	1
Camponotus sp. cf. *textor* Forel	Microdontinae spec.	Mexico	[49]	
Camponotus vicinus Mayr	*Microdon* (s.s.) *piperi* Knab	USA	[44, 50]	1

Table 1: Continued.

Ant taxon	Microdontine taxon	Country/region	Source	Observation
Camponotus ?vicinus Mayr	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[50]	1
Camponotus spec.	*Microdon* (s.s.) *piperi* Knab	USA	[38]	1
Formica accreta Francoeur	*Microdon* (s.s.) *albicomatus* Novak	USA	[44]	1
Formica accreta Francoeur	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[44]	1
Formica accreta Francoeur	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Formica adamsi whymperi Wheeler	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[44]	1
Formica adamsi whymperi Wheeler	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Formica argentea Wheeler	*Microdon* (s.s.) *lanceolatus* Adams	USA	[51]	1
Formica aserva Forel	*Microdon* (s.s.) cf. *tristis* Loew	USA	[4]	1
Formica aserva Forel	*Microdon* (s.s.) *albicomatus* Novak	USA	[44]	1
Formica aserva Forel	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[8, 38, 44]	1
Formica aserva Forel	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Formica densiventris Viereck	*Microdon* (s.s.) *manitobensis* Curran	USA	[44]	1
Formica difficilis Emery	*Microdon* (s.s.) cf. *tristis* Loew	USA	[4]	1
Formica exsectoides Forel	*Microdon* (s.s.) *abstrusus* Thompson	USA	[38]	1
Formica fusca Linnaeus	*Microdon* (s.s.) *albicomatus* Novak	USA	[38]	1
Formica fusca Linnaeus	*Microdon* (s.s.) spec.	Europe	[25]	1
Formica japonica Motschoulsky	*Microdon* (s.s.) *kidai* Hironaga and Maruyama	Japan	[48]	2
Formica japonica Motschoulsky	*Microdon* (s.s.) *yokohamai* Hironaga and Maruyama	Japan	[48]	2
Formica lemani Bondroit	*Microdon* (s.s.) *murayami* Hironaga and Maruyama	Japan	[48]	4
Formica lemani Bondroit	*Microdon* (s.s.) *mutabilis* Linnaeus	United Kingdom	[16]	1
Formica neoclara Emery	*Microdon* (s.s.) *albicomatus* Novak	USA	[44]	1
Formica neoclara Emery	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[44]	1
Formica neoclara Emery	*Microdon* (s.s.) *manitobensis* Curran	USA	[44]	1
Formica neoclara Emery	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Formica neogagates Viereck	*Microdon* (s.s.) *lanceolatus* Adams	USA	[44]	1
Formica neorufibarbis Emery	*Microdon* (s.s.) *albicomatus* Novak	USA	[44]	1
Formica neorufibarbis Emery	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Formica obscuripes Forel	*Microdon* (s.s.) *albicomatus* Novak	USA	[38]	1
Formica obscuripes Forel	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[44, 51]	1
Formica obscuripes Forel	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Formica obscuripes Forel	*Microdon* (s.s.) cf. *tristis* Loew	USA	[4]	1
Formica obscuripes Forel	*Microdon* (s.s.) *xanthopilis* Townsend	USA	[44, 52]	1
Formica obscuriventris Mayr	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[44]	1
Formica obscuriventris Mayr	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Formica podzolica Francoeur	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[44]	1
Formica ravida Creighton	*Microdon* (s.s.) *cothurnatus* Bigot	USA	[44, 53]	1
Formica ravida Creighton	*Microdon* (s.s.) *piperi* Knab	USA	[44]	1
Formica rufa Linnaeus	*Microdon* (s.s.) spec.	Europe	[25]	1
Formica rufibarbis Fabricius	*Microdon* (s.s.) spec.	Europe	[25]	1
Formica sanguinea Latreille	*Microdon* (s.s.) spec.	Europe	[22–25]	1
Formica schaufussi Mayr	*Microdon* (s.s.) *ocellaris* Curran	USA	[38]	1
Formica schaufussi Mayr	*Microdon* (s.s.) cf. *tristis* Loew	USA	[4]	1
Formica subsericea Say	*Microdon* (s.s.) *megalogaster* Snow	USA	[38, 54]	1
Lasius alienus (Foerster)	*Microdon* (s.s.) *ruficrus* Williston	Canada	[38]	1

Table 1: Continued.

Ant taxon	Microdontine taxon	Country/region	Source	Observation
Lasius brunneus (Latreille)	*Microdon* (s.s.) spec.	Europe	[25]	1
Lasius fuliginosus (Latreille)	*Microdon* (s.s.) spec.	Europe	[23–25]	1
Lasius flavus (Fabricius)	*Microdon* (s.s.) spec.	Europe	[25]	1
Lasius niger (Linnaeus)	*Microdon (s.s.) ?mutabilis* (Linnaeus)	France	[55]	1
Lasius niger (Linnaeus)	*Microdon* (s.s.) spec.	Europe	[25]	1
Lasius pallitarsis (Provancher)	*Microdon* spec.	USA	[56]	
Lasius spec.	*Microdon* (s.s.) *ruficrus* Williston	USA	[38]	1
Lepisiota capensis (Mayr)	*Paramixogaster acantholepidis* (Speiser)	South Africa	[57]	1
Polyergus lucidus Mayr (slave: *Formica schaufusi* Mayr)	*Microdon* (*Chymophila*) *fulgens* Wiedemann	USA	[38]	1
Polyrhachis lamellidens Smith	*Microdon* (*Chymophila*) *katsurai* Maruyama and Hironaga	Japan	[58]	3
Polyrhachis spec.	*Microdon* (s.l.) *waterhousei* Ferguson	Australia	Collection: USNM; ant identified by J. Doyen	1
Myrmicinae				
Acromyrmex coronatus (Fabricius)	*Microdon* (*Chymophila*) *tigrinus* Curran	Brazil	[59, 60]	1
Aphaenogaster fulva Roger	*Omegasyrphus coarctatus* (Loew)	USA	[37]	1
Crematogaster brasiliensis Mayr	Microdontinae spec.	Costa Rica	[61]	1
Crematogaster crinosa Mayr	*Stipomorpha wheeleri* (Mann)	Panama	[62]	1
Crematogaster crinosa Mayr	Microdontinae spec.	Panama	[32]	1
Crematogaster cf. *crinosa* Mayr	Microdontinae spec.	British Guiana	[32]	1
Crematogaster limata Smith	*Pseudomicrodon biluminiferus* (Hull)	Brazil	[43]	1
Crematogaster spec.	*Paramixogaster crematogastri* (Speiser)	South Africa	[57]	1
Crematogaster spec.	*Stipomorpha* spec. Nov.	Brazil	Collection: BMNH; ant identified by O.W. Richards	1
Leptothorax spec.	*Microdon* (s.s.) *mutabilis* Linnaeus	United Kingdom	[16]	1
Monomorium minimum (Buckley)	*Omegasyrphus baliopterus* (Loew)	USA	[10, 63]	1
Monomorium minimum (Buckley)	*Omegasyrphus painteri* (Hull)	USA	[38]	1
Monomorium minimum (Buckley)*	*Omegasyrphus coarctatus* (Loew)	USA	[37, 64]	1
Myrmica incompleta Provancher	*Microdon* (s.s.) *albicomatus* Novak	USA	[15]	1
Myrmica scabrinodis Nylander	*Microdon* (s.s.) *myrmicae* Schonrogge et al.	United Kingdom	[16]	1
Pheidole dentata Mayr	*Serichlamys rufipes* (Macquart)	USA	[38]	1
Unidentified ants				
	Archimicrodon (s.l.) *brachycerus* (Knab and Malloch)	Australia	[65]	1
	Paramixogaster daveyi (Knab and Malloch)	Australia	[65]	1
	Paramixogaster vespiformis (Meijere)	Indonesia	Collection: ZMAN	1

*Reported as "*Monomorium minutum* (Buckley)" by Greene [37, 64]. The valid name fort that taxon is *Monomorium monomorium* Bolton, but that is an Old World species, whereas the records are from North America. Probably Greene erroneously mixed up the names *minimum* and *minutum*.

4. Discussion

4.1. Are All Microdontinae Associated with Ants? The larval habits remain unknown for the majority of microdontine taxa: 14 out of 43 genera are now known to be associated with ants. The present results, however, indicate that associations with ants are found well distributed over the tree representing the most recent phylogenetic hypothesis of Microdontinae (Figure 1). *Spheginobaccha* de Meijere (tribe Spheginobacchini) is the sister group to all other Microdontinae (tribe Microdontini), but the larvae of this taxon are presently unknown. Within the tribe Microdontini (the remaining part of the tree), *Mixogaster* Macquart is the first genus to branch off (a strongly supported clade; see Reemer and Ståhls [28]),

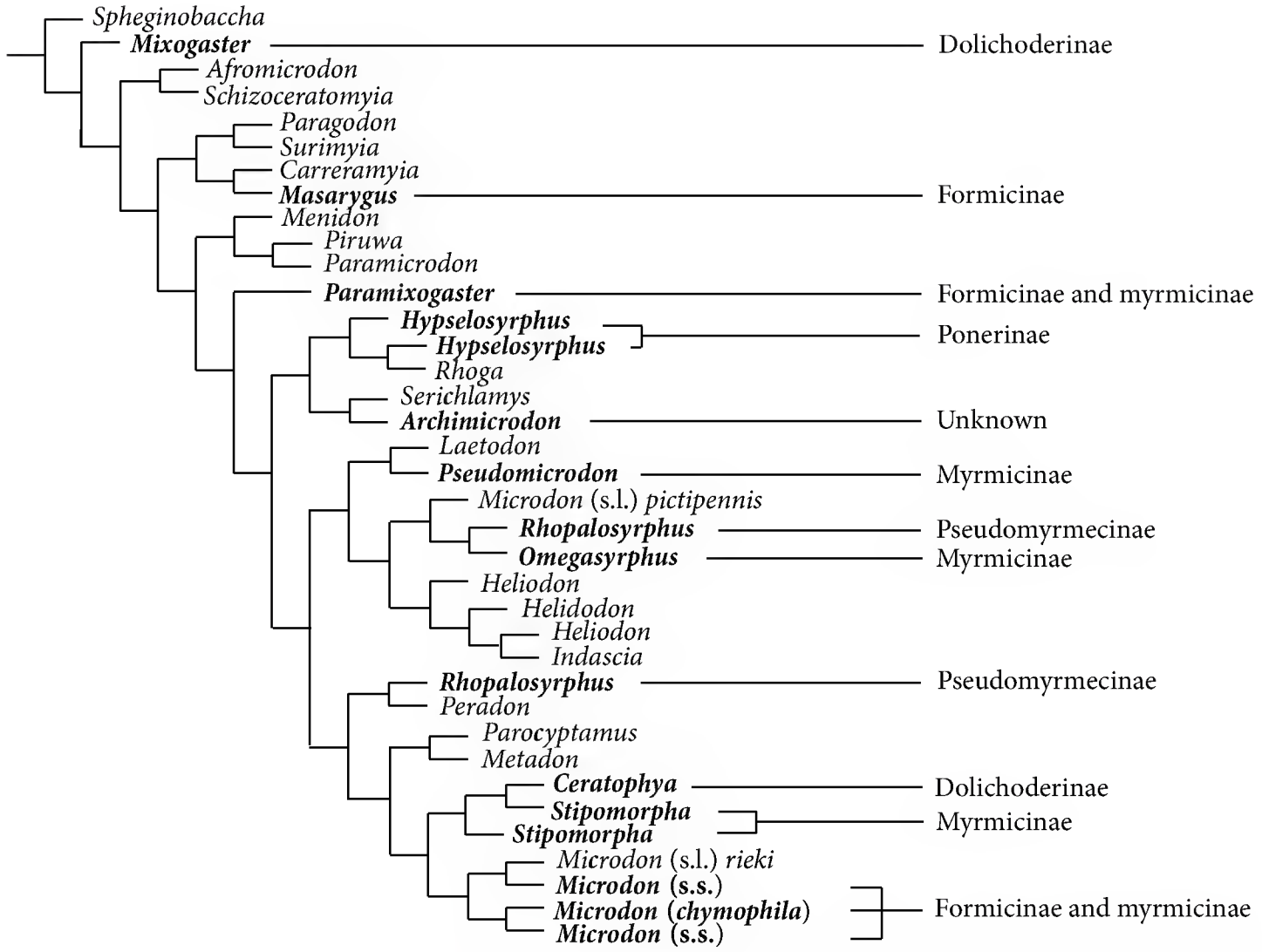


Figure 1: Phylogenetic hypothesis of 28 genera of Microdontinae (based on [28]), with indication of known associations with subfamilies of ants. Genera for which such associations are known are printed in bold. Note that several associations listed in Table 1 are lacking, because several taxa of Microdontinae were not included in the molecular dataset of [28].

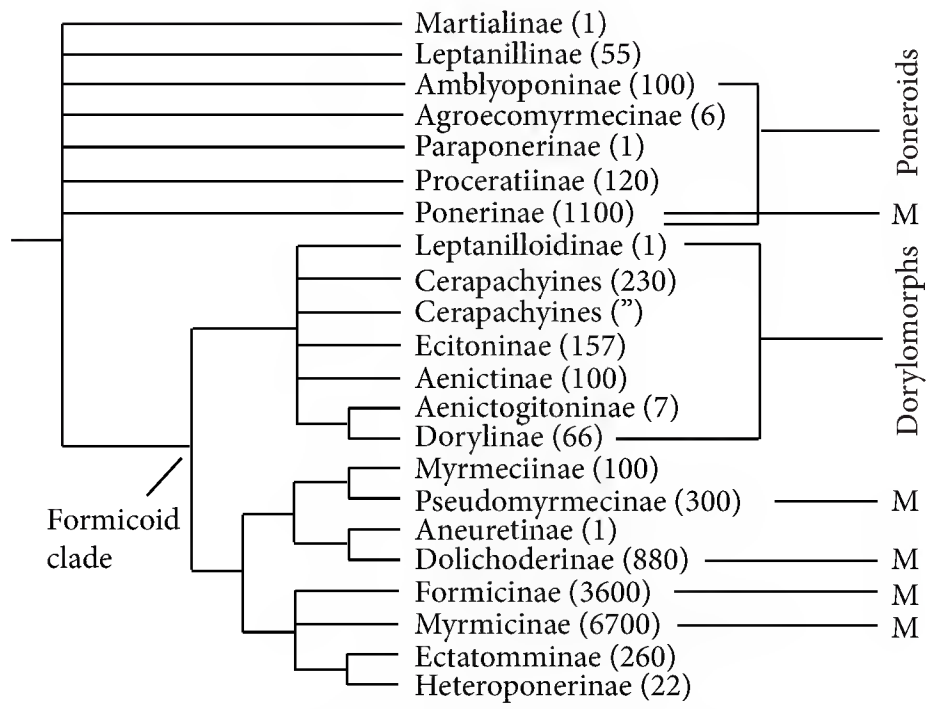


Figure 2: Phylogenetic tree summarizing well-supported relationships between extant subfamilies of ants (modified from [31]), with indication of known associations with Microdontinae ("M"). Numbers in parentheses are estimated numbers of described species per subfamily (based on [27, 31]).

and larvae of a species belonging to this genus have been found in an ant nest [35]. These results do not give a definite answer to the question, but they indicate that associations with ants are a dominant feature of larval biology for all Microdontinae, which has evolved early in the evolution of the group. Obviously, as already exclaimed by Cheng and Thompson [66], "one wants to know what the larvae of *Spheginobaccha* do!"

4.2. Are Microdontinae Associated with All Ants? The ant genera which have been recorded in association with Microdontinae belong to five subfamilies: Ponerinae, Dolichoderinae, Pseudomyrmecinae, Myrmicinae, and Formicinae. The four latter subfamilies all belong to the "formicoid clade" (Figure 2), as defined by Ward [31].

So far, no species of Microdontinae are known to be associated with the dorylomorph ant subfamilies (Figure 2), which also belong to the formicoid clade. This group includes the army ants: four subfamilies which are characterized by a nomadic lifestyle and mass foraging. The lack of records of associations of Microdontinae with army ants is remarkable, as these ants are relatively well studied and are known to host extremely rich communities of myrmecophiles [1]. It is tempting to hypothesize that the nomadic behaviour of these ants somehow prevents Microdontinae from getting adapted to them. However, when species numbers of the ant subfamilies are taken into account (Figure 2), it is clear that making such a statement would be jumping to conclusions. Together, the five subfamilies known to be associated with Microdontinae contain more than 12,000 species of ants, which is more than 95% of the world's ant diversity. With so few records available, chances that microdontine larvae are found in assocation with other groups of ants are small. These chances are even smaller when the geographical bias of the records is taken into consideration: a large majority

of the records originate from the Palaearctic and Nearctic regions, whereas the subfamilies outside of the formicoid clade are predominantly tropical.

4.3. Are Certain Clades of Microdontinae Associated with Certain Clades of Ants? So far, only one record of a poneroid ant associated with Microdontinae (*Hypselosyrphus* Hull) is known. Whether this is an exception or the tip of an iceberg remains uncertain until more data on associations of tropical taxa become available.

Figure 1 indicates that associations with the ant subfamilies Formicinae and Myrmicinae occur on several parts of the microdontine tree, without any obvious pattern. Associations with both subfamilies are even found within the same genus. For instance, *Microdon* (s.s.) *mutabilis* is associated with ants of the genus *Formica* (Formicinae), whereas the closely related *Microdon myrmicae*, which until recently was not separated from *M. mutabilis*, is associated with *Myrmica* ants [16]. Larvae of different species of *Paramixogaster* Brunetti were also recorded in association with ants of Formicinae and Myrmicinae (Table 1). These records suggest that shifts in host association between Formicinae and Myrmicinae occur relatively frequently. Whether this is also true for other ant subfamilies, or for other genera of Microdontinae, cannot be deduced from the presently available data. For most other genera of Microdontinae only one association is known (Table 1). An exception is *Stipomorpha* Hull, of which the larvae of two species were found in *Crematogaster* Lund nests. Another exception is *Oligeriops* Hull, of which two species were found in nests of *Iridomyrmex* Mayr. Whether these records indicate some degree of parallel evolution remains an open question, at least until a larger number of associations is be known.

4.4. Associations with Other Insects? Wasmann [23, 25] reported having found *Microdon* larvae in the nests of wasps and termites. This record was repeated by other authors [2, 4] but has never since been confirmed. Wheeler [32] reported a finding of *Microdon* larvae in the chambers of termite nests, but those were abandoned by the termites and occupied by ants of the genus *Camponotus* Mayr. He wrote "These ants regularly take possession of the chambers adjacent to the tree trunk supporting the termitarium and permit the termites to inhabit the remainder of the structure." A similar explanation may be true for Wasmann's reports of *Microdon* larvae in wasps and termites nests.

Another, apparently independent, record of an association of *Microdon* with termites was mentioned by Séguy [67], who stated that the larvae of a *Microdon* species were attracted to exuding saps on certain fruit trees that were attacked by termites. However, the source of this record is unclear and no figures of the larvae are provided, so whether this report really concerns *Microdon* larvae remains doubtful.

Pendlebury [68] described *Paramixogaster icariiformis* Pendlebury and hypothesized that its larva lives in the nest of the wasp species that it mimics, without presenting any other evidence than their similarity in appearance.

So, there are no convincing records of Microdontinae living in the nests of other insects than ants. All published records suggesting such associations can be considered doubtful.

5. Concluding Remarks

With so few associations known among the total of 12,500 described ant species and 454 described species of Microdontinae, any conclusion about evolutionary trends claiming general validity would be premature. Despite this, the present paper is the first to demonstrate in a phylogenetic context that it seems likely that all Microdontinae are associated with ants. Vice versa, associations with Microdontinae are found among a large diversity of ant subfamilies, suggesting that all ants may be prone to "infestation" by Microdontinae. Exceptions may occur, such as the army ants, with which no associations are known so far.

At least as interesting as the questions discussed in this paper is the question as to the exact nature of the associations between Microdontinae and ants. Available evidence for a few Palaearctic and Nearctic species shows that these species are predators of immature stages of ants (see Introduction). The species for which this feeding mode is known all belong to *Microdon* s.s. (in the sense of Reemer and Ståhls [19]) and *Omegasyrphus*. Whether the larvae of other genera of Microdontinae also feed this way remains to be discovered.

Acknowledgments

The author would like to thank the following persons for sharing information or helping with studying the collections they are curating: Ben Brugge (ZMAN), Jean-Paul Lachaud, Mirian Nunes Morales (DZUP), Gabriela Pérez-Lachaud, Graham Rotheray (RSME), and Manuel Zumbado (INBio). André van Loon and Gunilla Ståhls are thanked for commenting on an earlier version of the paper. Jean-Paul Lachaud was very helpful with updating the taxonomy of the ant hosts.

References

[1] B. Hölldobler and E. O. Wilson, *The Ants*, Harvard University Press, Cambridge, UK, 1990.

[2] J. K. Donisthorpe, *The Guests of British Ants, Their Habits and Life-Histories*, George Routledge & Sons, London, UK, 1927.

[3] J. C. Hartley, "A taxonomic account of the larvae of some British Syrphidae," *Proceedings of the Zoological Society of London*, vol. 136, no. 4, pp. 505–573, 1961.

[4] W. M. Wheeler, "Studies on myrmecophiles. III. *Microdon*," *Journal of the New York Entomological Society*, vol. 16, pp. 202–213, 1908.

[5] E. O. Wilson, *The Insect Societies*, Harvard University Press, Cambridge, UK, 1971.

[6] B. Barr, "Feeding behaviour and mouthpart structure of larvae of *Microdon eggeri* and *Microdon mutabilis* (Diptera, Syrphidae)," *Dipterists Digest*, vol. 2, pp. 31–36, 1995.

[7] R. M. Duffield, "Biology of *Microdon fuscipennis* (Diptera: Syrphidae) with interpretations of the reproductive strategies of *Microdon* species found north of Mexico," *Proceedings of the Entomological Society of Washington*, vol. 83, no. 4, pp. 716–724, 1981.

[8] W. B. Garnett, R. D. Akre, and G. Sehlke, "Cocoon mimicry and predation by myrmecophilous Diptera (Diptera: Syrphidae)," *Florida Entomologist*, vol. 68, no. 4, pp. 615–621, 1985.

[9] B. Hocking, "Insect associations with the swollen thorn acacias," *Transactions of the Royal Entomological Society of London*, vol. 122, no. 7, pp. 211–255, 1970.

[10] A. F. Van Pelt and S. A. Van Pelt, "Microdon (Diptera: Syrphidae) in nests of *Monomorium* (Hymenoptera: Formicidae) in Texas," *Annals of the Entomological Society of America*, vol. 65, no. 4, pp. 977–978, 1972.

[11] T. Borgmeier, "Beitrag zur Biologie der Feuerameise und ihrer Gäste (*Solenopsis geminata saevissima* Sm.)," *Deutscher Verein für Wissenschaft und Kunst in São Paulo*, vol. 3, no. 1, pp. 1–9, 1923.

[12] T. Borgmeier, "Syrphidenlarven in ameisennestern," *Die Naturwissenschaften*, vol. 40, no. 2, p. 36, 1953.

[13] M. H. Maneval, "Captures diverses au Mont Ventoux," *Bulletin de la Société Entomologique de France*, vol. 42, no. 1, pp. 66–67, 1937.

[14] R. W. Howard, R. D. Akre, and W. B. Garnett, "Chemical mimicry in an obligate predator of carpenter ants (Hymenoptera: Formicidae)," *Annals of the Entomological Society of America*, vol. 83, no. 3, pp. 607–616, 1990.

[15] R. W. Howard, D. W. Stanley-Samuelson, and R. D. Akre, "Biosynthesis and chemical mimicry of cuticular hydrocarbons from the obligate predator, *Microdon albicomatus* Novak (Diptera: Syrphidae) and its ant prey, *Myrmica incompleta* Provancher (Hymenoptera: Formicidae)," *Journal of the Kansas Entomological Society*, vol. 63, no. 3, pp. 437–443, 1990.

[16] K. Schönrogge, B. Barr, J. C. Wardlaw et al., "When rare species become endangered: cryptic speciation in myrmecophilous hoverflies," *Biological Journal of the Linnean Society*, vol. 75, no. 3, pp. 291–300, 2002.

[17] K. Schönrogge, M. G. Gardner, G. W. Elmes et al., "Host propagation permits extreme local adaptation in a social parasite of ants," *Ecology Letters*, vol. 9, no. 9, pp. 1032–1040, 2006.

[18] M. G. Gardner, K. Schönrogge, G. W. Elmes, and J. A. Thomas, "Increased genetic diversity as a defence against parasites is undermined by social parasites: *Microdon mutabilis* hoverflies infesting *Formica lemani* ant colonies," *Proceedings of the Royal Society B*, vol. 274, no. 1606, pp. 103–110, 2007.

[19] M. Reemer and G. Ståhls, "Generic revision and species classification of the Microdontinae (Diptera: Syrphidae)," *ZooKeys*, vol. 288, pp. 1–212, 2013.

[20] L. Lach, C. L. Parr, and K. L. Abbott, Eds., *Ant Ecology*, Oxford University Press, New York, NY, USA, 2010.

[21] U. Schmid, "*Microdon rhenanus* and *Microdon eggeri* var. major (Diptera, Syrphidae) revisited," *Volucella*, vol. 7, pp. 111–124, 2004.

[22] E. Wasmann, *Zur Kenntniss der Ameisen und Ameisengäste von Luxemburg. III. Teil*, Hofbuchdruckrei V. Bück, Luxemburg, 1909.

[23] E. Wasmann, "Vergleichende studien über ameisengäste und termitengäste," *Tijdschrift voor Entomologie*, vol. 33, no. 1, pp. 27–96, 1890.

[24] E. Wasmann, "Verzeichniss der ameisen und ameisengäste von holländisch limburg," *Tijdschrift voor Entomologie*, vol. 34, no. 1, pp. 39–64, 1890.

[25] E. Wasmann and E. Kritisches, *Verzeichniss der Myrmekophilen und Termitophilen Arthropoden*, Felix L. Dames, Berlin, Germany, 1894.

[26] N. A. Weber, "Two common ponerine ants of possible economic significance, *Ectatomma tuberculatum* (Olivier) and *E. ruidum* Roger," in *Proceedings of the Entomological Society of Washington*, vol. 48, no. 1, pp. 1–16, 1946.

[27] B. Bolton, "Synopsis and classification of formicidae," *Memoirs of the American Entomological Institute*, vol. 71, pp. 1–370, 2003.

[28] M. Reemer and G. Ståhls, "Phylogenetic relationships of Microdontinae (Diptera: Syrphidae) based on molecular and morphological characters," *Systematic Entomology*. In press.

[29] S. G. Brady, T. R. Schultz, B. L. Fisher, and P. S. Ward, "Evaluating alternative hypotheses for the early evolution and diversification of ants," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 48, pp. 18172–18177, 2006.

[30] C. S. Moreau, C. D. Bell, R. Vila, S. B. Archibald, and N. E. Pierce, "Phylogeny of the ants: diversification in the age of angiosperms," *Science*, vol. 312, no. 5770, pp. 101–104, 2006.

[31] P. S. Ward, "Taxonomy, phylogenetics, and evolution," in *Ant Ecology*, L. Lach, C. L. Parr, K. L. Abbott et al., Eds., pp. 3–17, Oxford University Press, New York, NY, USA, 2010.

[32] W. M. Wheeler, "Two extraordinary larval myrmecophiles from Panama," *Proceedings of the National Academy of Sciences*, vol. 10, no. 6, pp. 237–244, 1924.

[33] R. P. McMillan, "A contribution to the life history of a hover fly *Microdon dimorphon* Ferg., a dipterous inquiline," *Western Australian Naturalist*, vol. 5, no. 7, pp. 204–205, 1957.

[34] R. C. Shannon, "A new genus and five new species of Syrphidae from Australia (Diptera)," *Proceedings of the Entomological Society of Washington*, vol. 29, no. 4, pp. 82–87, 1927.

[35] M. Carrera and K. Lenko, "Descrição de duas espécies novas de *Mixogaster* (Diptera, Syrphidae) e observações sobre o inquilinismo de uma delas em ninhos de *Iridomyrmex humilis*, a 'formiga argentina'," *Studia Entomologica*, vol. 1, no. 3-4, pp. 465–486, 1958.

[36] A. Wild, Alex Wild Photography, 2012, http://www.alexanderwild.com/keyword/insects/1/1191657548_8rwSJ#!i=1292063955&k=bcGNtdn.

[37] C. T. Greene, "Larvae and pupae of the genera *Microdon* and *Mixogaster* (Diptera, Syrphidae)," *Transactions of the American Entomological Society*, vol. 81, no. 1, pp. 1–20, 1955.

[38] F. C. Thompson, "Revisionary notes on Nearctic *Microdon* flies (Diptera: Syrphidae)," *Proceedings of the Entomological Society of Washington*, vol. 83, no. 4, pp. 725–758, 1981.

[39] W. M. Mann, "Ant guests from Fiji and the British Solomon islands," *Annals of the Entomological Society of America*, vol. 13, no. 1, pp. 60–69, 1920.

[40] M. E. Schauff, "*Microdonophagus*, a new entedontine genus (Hymenoptera: Eulophidae) from Panama," *Proceedings of the Entomological Society of Washington*, vol. 88, no. 1, pp. 167–173, 1986.

[41] H. V. Weems, F. C. Thompson, G. E. Rotheray, and M. A. Deyrup, "The genus *Rhopalosyrphus* (Diptera: Syrphidae)," *Florida Entomologist*, vol. 86, no. 2, pp. 186–193, 2003.

[42] W. M. Wheeler, "Microdon larvae in *Pseudomyrma* nests," *Psyche*, vol. 9, no. 3, pp. 222–224, 1901.

[43] V. S. Schmid, M. N. Morales, L. Marinoni, R. Kamke, J. Steiner, and A. Zillikens, "Natural history and morphology of *Microdon biluminiferus* and its parasitic relationship with ants nesting in bromeliads," *Journal of Insect Science*. In press.

[44] R. D. Akre, W. B. Garnett, and R. S. Zack, "Ant hosts of *Microdon* (Diptera: Syrphidae) in the Pacific Northwest," *Journal of the Kansas Entomological Society*, vol. 63, no. 1, pp. 175–178, 1990.

[45] R. D. Akre, W. B. Garnett, and R. S. Zack, "Biology and behaviour of *Microdon piperi* in the Pacific northwest (Diptera: Syrphidae)," *Journal of the Kansas Entomological Society*, vol. 61, no. 4, pp. 441–452, 1988.

[46] J. Brèthes, "Masarygidae. Una nueva familia de dípteros," *Anales del Museo Nacional de Buenos Aires*, vol. 10, 3rd series, pp. 439–443, 1908.

[47] J. Longino, *Camponotus (Dendromyrmex) nitidior* (Santschi 1921), 2003, http://academic.evergreen.edu/projects/ants/genera/camponotus/species/nitidior/nitidior.html.

[48] T. Hironaga and M. Maruyama, "The myrmecophilous hoverfly genus *Microdon* (Diptera, Syrphidae, Microdontinae) in Hokkaidô, Japan, with descriptions of four new species," *Bulletin of the National Science Museum, Tokyo A*, vol. 30, no. 2, pp. 87–103, 2004.

[49] C. Hansson, J.-P. Lachaud, and G. Pérez-Lachaud, "Entedoninae wasps (Hymenoptera, Chalcidoidea, Eulophidae) associated with ants (Hymenoptera, Formicidae) in tropical America, with new species and notes on their biology," *ZooKeys*, vol. 134, pp. 65–82, 2011.

[50] F. R. Cole, "Notes on the early stages of the syrphid genus *Microdon* (Diptera)," *Pomona College Journal of Entomology and Zoology*, vol. 15, no. 1, pp. 19–20, 1923.

[51] T. D. A. Cockerell and H. Andrews, "Some Diptera (*Microdon*) from nests of ants," *Proceedings of the United States National Museum*, vol. 51, no. 2141, pp. 53–56, 1916.

[52] R. D. Akre, G. Alpert, and T. Alpert, "Life cycle and behaviour of *Microdon cothurnatus* in Washington (Diptera: Syrphidae)," *Journal of the Kansas Entomological Society*, vol. 46, no. 3, pp. 327–338, 1973.

[53] F. Knab, "On some North American species of *Microdon* (Diptera: Syrphidae)," *Proceedings of the Biological Society of Washington*, vol. 30, no. 1, p. 1, 1917.

[54] C. T. Greene, "The larva and pupa of *Microdon megalogaster* Snow (Diptera)," *Proceedings of the Entomological Society of Washington*, vol. 25, no. 4, pp. 140–141, 1923.

[55] A. Laboulbène, "Brief miscellaneous communications," *Annales de la Société Entomologique de France (6th Series)*, vol. 2, pp. 96–106, 1882.

[56] W. P. Mackay and E. Mackay, *The Ants of New Mexico (Hymenoptera: Formicidae)*, Edwin Mellen Press, Lewiston, NY, USA, 2002.

[57] P. Speiser, "Uber einige syrphiden und zwei für die deutsche fauna neue clythiiden," *Jahrbucher des Nassauischen Vereins für Naturkunde*, vol. 66, no. 1, pp. 117–146, 1913.

[58] M. Maruyama and T. Hironaga, "Microdon katsurai, a new species of myrmecophilous hoverfly (Diptera, Syrphidae) from Japan, associated with *Polyrhachis lamellidens* (Hymenoptera, Formicidae)," *Bulletin of the National Science Museum, Tokyo A*, vol. 30, no. 4, pp. 173–179, 2004.

[59] R. S. Camargo, L. C. Forti, R. T. Fujihara, A. A. Carlos, and C. A. O. Matos, "Nota sobre a biologia de *Microdon tigrinus* (Diptera: Syrphidae), parasito social de *Acromyrmex coronatus* (Hymenoptera: Formicidae)," *Boletin de Sanidad Vegetal Plagas*, vol. 34, no. 3, pp. 343–347, 2008.

[60] L. C. Forti, R. S. Camargo, S. S. Verza, A. P. P. Andrade, R. T. Fujihara, and J. F. S. Lopes, "*Microdon tigrinus* (Diptera, Syrphidae): populational fluctuations and specificity to the nest of *Acromyrmex coronatus* (Hymenoptera: Formicidae)," *Sociobiology*, vol. 50, no. 3, pp. 909–919, 2007.

[61] J. T. Longino, "The *Crematogaster* (Hymenoptera, Formicidae, Myrmicinae) of Costa Rica," *Zootaxa*, vol. 151, pp. 1–150, 2003.

[62] W. M. Mann, "A new *Microdon* from Panama," *Psyche*, vol. 35, no. 3, pp. 168–170, 1928.

[63] W. H. Clark and A. F. van Pelt, "Myrmecophiles in ant nests, Big Bend National Park, Texas," in *Proceedings of the 49th Annual Meeting of the Idaho Academy of Science*, Idaho Falls, Idaho, USA, April 2007.

[64] C. T. Greene, "A contribution to the biology of N.A. Diptera," *Proceedings of the Entomological Society of Washington*, vol. 25, no. 2, pp. 82–91, 1923.

[65] F. Knab and J. R. Malloch, "New Australian Diptera from ants' nests," *Transactions of the Royal Society of South Australia*, vol. 36, pp. 233–237, 1912.

[66] X.-Y. Cheng and F. C. Thompson, "A generic conspectus of the Microdontinae (Diptera: Syrphidae) with the description of two new genera from Africa and China," *Zootaxa*, vol. 1879, pp. 21–48, 2008.

[67] E. Séguy, "La biologie des diptères," *Encyclopédie Entomologique*, vol. 26, pp. 1–609, 1950.

[68] H. M. Pendlebury, "Notes on some mimetic insects from the Malay Peninsula," *Proceedings of the Entomological Society of London*, vol. 1, no. 1, pp. 37–39, 1927.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 696401, 8 pages
http://dx.doi.org/10.1155/2013/696401

Review Article

Declassifying Myrmecophily in the Coleoptera to Promote the Study of Ant-Beetle Symbioses

Glené Mynhardt

Department of Ecology, Evolution and Organismal Biology, Iowa State University, 253 Bessey Hall, Ames, IA 50011, USA

Correspondence should be addressed to Glené Mynhardt; mynhardt@iastate.edu

Received 7 February 2013; Accepted 5 April 2013

Academic Editor: Jean-Paul Lachaud

The symbiotic associations between beetles and ants have been observed in at least 35 beetle families. Among myrmecophiles, beetles exhibit the most diverse behavioral and morphological adaptations to a life with ants. These various associations have historically been grouped into discrete but overlapping behavioral categories, many of which are still used in the modern literature. While these behavioral classifications provide a rich foundation for the study of ant-beetle symbioses, the application of these systems in future studies may be less than effective. Since morphological characteristics often provide the only information of myrmecophilous beetles, they should be studied in a species-by-species fashion, as behavioral data are often limited or unavailable. Similarly, behavioral studies should focus on the target species at hand, avoiding discrete classification schemes. I formally propose the rejection of any classification scheme, in order to promote future studies of myrmecophily in both taxonomic and evolutionary studies.

1. Introduction

Myrmecophily is a charismatic biological phenomenon that defines the associations, whether casual or intimate, of various organisms with ants. Myrmecophilous life habits have been observed in at least 95 families of arthropods, including several genera of isopods, pseudoscorpions, many araneeid spiders, mites, millipedes, and close to 100 families of insects [1]. Among insects, the beetles are often the most easily recognized and morphologically distinct myrmecophiles, leading to a significant body of work. Currently, at least 35 beetle families are known to be associated with ants in some form or another [1, 2], but for at least fifteen of these families behavioral data are entirely absent. In many cases, presumed ant associates, both within the Coleoptera and other myrmecophilous groups, are cited as myrmecophiles based on unobserved interactions with ants, especially if specimens were collected in or near an ant nest. Specifically, beetles are considered to be myrmecophilous if they bear unique morphological characteristics presumed to be linked to myrmecophily. These morphological modifications frequently include combinations of enlarged or reduced antennae, reddish or "ant-red" integument, and, less often, modified mouthparts or appendages that are sometimes associated with a myrmecophilous habit [3]. Perhaps the most commonly documented and presumably convincing evidence for a life with ants is the presence of trichomes, or tufts of setae associated with exocrine glands, but similar clusters of putatively secretive hairs can be found in termitophilous beetles, as well [4–7], and are not necessarily unique to those beetles that share a life with ants.

Despite the great morphological diversity that exists among myrmecophilous Coleoptera, very little is known of the interactions that may be occurring between ant hosts and their respective associates. Detailed behavioral data are available for a few better-known species within the aleocharine and scydmaenine Staphylinidae [8, 9], the paussine Carabidae [10, 11], and various species within, for example, the Coccinellidae [12, 13], the Scarabaeidae [14–17], and the Ptinidae [18, 19]. The documented myrmecophilous habits of these few taxonomic groups capture the great diversity of ant-beetle interactions known for beetles, ranging from casual interactions, such as scavenging in and around middens and refuse deposits and preying on ants along migration trails, to more intimate associations involving being fed by ants or even being adopted as members of the colony.

The many interactions that have been observed in a few beetle groups have led to the creation of behavioral classification schemes, the first of which was proposed by Wasmann [4, 20]. Successive behavioral categories have since been suggested [3, 21–24], all of which have served as a shorthand in placing the many different kinds of myrmecophiles. While these systems have provided a basic framework from which to expand our current knowledge of myrmecophily, they have also posed some challenges. In order to bridge the gap between what is known and the many unanswered questions that remain, I pursue several objectives herein.

I provide a general overview of the existing classification systems of myrmecophily in the Coleoptera, discuss current applications and potential challenges of utilizing these systems, and propose the formal rejection of these classifications systems in order to reduce redundancy and better understand the complexities of myrmecophily, at least until more is known about the biology of ant-associated beetles and other myrmecophiles. Note that this review does not intend to discuss all the important biological facets involved in myrmecophilous associations, such as the innumerable types of morphological adaptations or the complexities of mimicry which are undoubtedly important in many ant-beetle associations.

2. Definitions and Classifications of Myrmecophily

In more than 140 papers the German myrmecologist, Erich Wasmann, laid the groundwork for studies of myrmecophily and termitophily, particularly within the Coleoptera. Before Wasmann's contributions, the first compilation of myrmecophilous arthropods estimated 284 species, including 274 beetle species that are associated with ants [25, 26]. Fifty years later, an approximation of 1246 species of arthropods was cited as ant associates, with 993 of those species belonging to the Coleoptera [20]. A few years later, at least 3000 beetles had been predicted to be myrmecophilous [3]. More than a century later, authors estimate that 80,000–100,000 species of insects [27] are presumed myrmecophiles and, undoubtedly, the majority of these belong within the Coleoptera.

Wasmann [20] provided descriptive comparisons between different myrmecophilous Coleoptera, and as a result of the various associations observed, he proposed several discrete behavioral categories, which successive authors, including Wheeler [3], Donisthorpe [22], Delamare-Deboutteville [28], Akre and Rettenmeyer [24], Paulian [29], Kistner [23], and Franc [30], have attempted to restructure or reconfigure. The categories proposed by Wasmann and his contemporaries are complex, although a great degree of overlap can be observed (see Table 1).

Wasmann [20] introduced the terms "synecthrans" (persecuted guests), "synoeketes" (tolerated guests), "symphiles" (true or symbiotic guests), "ecto- and endoparasites" (parasites on and within ant bodies), and "trophobiots" (those that feed ants with honeydew secretions and are provided protection in return). The only potential coleopterous ectoparasite belongs to the genus *Thorictus* in the family Dermestidae, which is found to latch onto the antennal scape of ants [3]. While authors originally cited that it "sucked blood" of ants [31, 32], no studies thus far have indicated that this is the case. The trophobiontic category applies largely to the two well-studied myrmecophilous groups that include heteropterans and the majority of genera within the Lycaenidae, both of which are associated with ants by secretions of either honeydew or nectar, respectively, in exchange for ants' protection. Since the latter two categories are not found in beetles, they will be excluded from further discussion but are reviewed in detail in other works [33–35].

I outline the different categories proposed by different authors but present them under the more specific, inclusive scheme of Wasmann, largely because this system serves as the basis for much of what is known of myrmecophilous beetles and not because it is more useful than other systems.

2.1. "Synecthrans". The synecthrans, as a whole, are classified as those associates that live in the vicinity of host nests, even within refuse deposits but only prey upon ants on raids and migrations [1, 4]. The synecthran classification is limited largely to staphylinids that often times bear defensive glands on the terminal abdominal segments and are able to either ward off ants in defense or may feed on ants during raids [24]. Taxa most often cited as being of the synecthran type include those staphylinids associated with army ants in the New World subfamily Ecitoninae. The singular species, *Eciton burchellii* (Westwood), hosts more than 300 species of ant associates, with 12 families and 59 species belonging to the beetles [36]. Most other authors have followed Wasmann's synecthran category, but the "extranidal" category of Donisthorpe [22] separated these associates from others, because they are found outside of the colony, unlike many other beetle species. Akre and Rettenmeyer [24] classified the typical synecthran types into what they named the "generalized species" (as opposed to specialized species), based on various behavioral characteristics as well as the absence of any morphological modifications found in these beetles. If following the categories of Delamare-Deboutteville [28], Wasmann's synecthrans would be considered as "accidental commensals;" similarly, if following Kistner's [23] groupings, the synecthrans would be considered as "nonintegrated" associates, as these beetles are not accepted as members of the colony.

2.2. "Synoeketes". Wasmann's second group, the "synoeketes," is a diverse group of myrmecophiles [3] and includes many species that are treated indifferently, being tolerated rather than attacked by ants. Synoeketes have been defined behaviorally as slow moving scavengers and occupy a range of morphological body types, including relatively small body size and being "neutral in odor," as well as the absence of morphological adaptations to the colony. In addition, mimetic beetles were grouped into this category. Because of the range of both morphological and behavioral types of presumed synoeketes, Wheeler [3] further subdivided the group into the "neutral synoeketes," which ignore hosts but live on nest materials and live in refuse piles; "mimetic synoeketes"

Table 1: Historical behavioral classification of myrmecophily by author. Categories marked with "—" indicate that the author did not consider the respective behavior in their classification scheme. Original terminology is used but translated if necessary.

Behavior	Wasmann [4, 20]	Silvestri [21]	Donisthorpe [22]	Paulian [29]	Delamare-Deboutteville [28]	Akre and Rettenmeyer [24]	Kistner [23]	Franc [30]
Scavengers or predators, ignored or tolerated by hosts	Synoeketes	Synoicoxeni "Synoeketes"	Passive/intranidal guests "Inside nest guests"	Les clients "Clients"	Accidental/preferred or obligate commensals	Specialized species	Nonintegrated species	Synoecious myrmecophiles
Scavengers or predators, treated with hostility; defensive	Synechthrans	Cleptocoxeni "Cleptoketes"	Passive/intranidal guests "Inside nest guests"	Les clients or les associes "Clients" or "associates"	Accidental/preferred commensals	Defensive/generalized species	Non-integrated species	Prosynecthricans, synecthricans, or hypersynecthricans
Accepted into colony by being groomed, fed, or reared	Symphiles	Euxeni "True guests"	Passive/intranidal guests "Inside nest guests"	Les associes "Associates"	Obligate commensals	—	Integrated species	Symphillous myrmecophiles
Live on body surface of host, feed on secretions or food particles	Ectoparasites	Parasitoxeni "Parasites"	Passive/intranidal guests "Inside nest guests"	Les associes "Associates"	Obligate commensals	—	Integrated species	—
Penetrate body to feed on blood; parasite	Endoparasites	Parasitoxeni "Parasites"	Passive/intranidal guests "Inside nest guests"	Les associes "Associates"	Obligate commensals	—	Integrated species	—
Exchange of honeydew or nectar for protection	Trophobionts	Euxeni "True guests"	Active/extranidal guests "Outside nest guests"	Les associes "Associates"	Obligate commensals	—	Integrated species	—
Follow hosts on raids	Synecthrans	—	Active/extranidal guests	Les suivants "Followers"	—	Generalized/specialized species	Non-integrated species	Synecthricans

that mimic ants; "loricate synoeketes" that are tear-drop shaped and therefore hard to capture or bite by an ant; and, "symphillid synoeketes," which resemble true guests but have not yet achieved perfection; where "perfection" describes those myrmecophiles that are integrated into the ant nest. In addition to the various supposed synoeketes, Wheeler also included "myrmecocleptics" to denote those which snatched food from ants. Paulian's [29] term "les clients" or ant clients includes all myrmecophiles that frequent debris piles and exploits ant bodies or excrement, as well as those that prey upon the insects that are attracted to these items, and is thus synonymous with synoeketes. Akre and Rettenmeyer [24] instead avoided the use of the term synoekete but proposed the term "specialized species," based on various behavioral characteristics and the fact that many of these species appear to be close mimics of their respective ant host species, matching hosts in both color and body shape [37]. If following any of the other authors' proposed categories, these species would be considered as "passive" or "intranidal" (within the nest) associates [22], "accidental" associates [28], or "nonintegrated associates" [23].

2.3. "Symphiles". The "symphiles," or true guests, is the most speciose group of myrmecophilous beetles, with likely more than 10,000 species being considered in this or synonymous categories [27]. The majority of authors including Wasmann and Wheeler cited "symphily" as the extreme form of myrmecophily or as the last step reached by myrmecophiles when compared to associates exhibiting more casual interactions with ants. This assumption of gradual, almost directional complexity has not been formally addressed, and no evidence supports the increasing complexity of any myrmecophilous group. This will be addressed in a separate paper.

One unique behavior, that is exhibited by the so-called symphiles, includes solicitation of liquid food from ant hosts, including larvae and adults, via trophallaxis [1, 35]. In nearly all known cases, beetles originally classified as symphiles also feed on brood, acting as obligate parasites.

The symphile category also typically includes beetles that are accepted into ant nests either by being carried in or entering without being detected and being successively integrated into the social life of the ant colony. The most likely cause for ants' accepting these associates into their colonies involves chemical mimicry exhibited by beetles [1]. Some elegant studies have indicated that beetles are able to adopt specific ant chemical signatures [14, 38], largely by means of physical contact with the ants themselves. Thus far, no studies have confirmed that ant associates are able to biosynthesize hydrocarbons or produce these chemicals *de novo*; however, it has been confirmed for the termitophilous staphylinid beetle, *Trichopsenius frosti* [39]. Instead, studies have indicated that certain aleocharine Staphylinidae produce nonhydrocarbon alarm pheromones similar to that of their hosts [38, 40, 41]. It is important to note that, thus far, no presumed "symphilous" beetles, which are accepted as part of the colony, are known to be able to biosynthesize compounds.

Perhaps the most interesting difference between the "symphiles" and other myrmecophilous beetles is that this group is almost always defined by the presence of trichomes, even without any behavioral information. These trichomes have been assumed to play a large role in the intimate associations between beetles that have them and their ant hosts. They are often discussed as being somehow attractive or "appeasing" to ants, with ants often licking, biting, or picking beetles up by these trichomes [3, 15]. It has also been demonstrated that exocrine glands associated with trichomes may play a role in ants' acceptance of beetles into the colony, as seen in the scarab genus *Cremastocheilus* [15]. Trichomes are even present in the ectoparasitic *Thorictus*, which further complicates the matter of accepting either "ectoparasite" or "symphile" as a classifier for this genus.

After Wasmann, symphiles have been reclassified into the "active" or "intranidal" (inside the nest) category of Donisthorpe [22], the "obligate commensals" group of Delamare-Deboutteville [28], or the "integrated" species of Kistner [23]. In all cases, except for Wasmann's and the subdivided system of Franc [30] are these highly integrated beetles grouped into broader categories that include many other ant associates. It is also evident that, while most of these beetles are highly "integrated," if using Kistner's terminology, the means by which these beetles become so is highly variable.

3. Problems with the Proposed Classifications of Myrmecophily

Several authors have mentioned the difficulty in accepting any one existing categorical scheme for myrmecophiles [1, 11, 23], and the most often cited problem associated with the use of any one scheme is the fact that many beetles fit into more than one category. Despite initial criticisms, Wasmann's system has been claimed as the most useful [1] and has been adopted by authors in modern studies or in reviews [30, 42]. In attempting to utilize any one of these schemes, it becomes apparent that a single type of association with an ant host may be classified differently depending on the author and even depending on the taxon. But perhaps most problematic is the fact that so little is known about the majority of myrmecophiles, which renders many of the existing classification systems obsolete or inadequate to capture the behavioral diversity likely to be discovered for these taxa. Attempts to place myrmecophiles into one of these ethological schemes can be cumbersome and inadvertently leads to the unintended rejection of complex species-specific behaviors in favor of placing a species in one or more of the categories. Various specific challenges limit what may otherwise lead to much more informative studies of myrmecophiles, although it should be noted that many studies do not use these classifications schemes.

3.1. Taxon-Specific Classifications. Several existing schemes are based on specific taxa and are less useful in identifying myrmecophilous associations at higher taxonomic levels. For example, the classification proposed by Paulian [29] can be applied only to staphylinid beetles that are closely associated with army ants in the subfamily Dorylinae. Akre and Rettenmeyer [24] also based their system on

staphylinids associated with the ecitoninae army ants. A separate subdivision of the various synecthran staphylinids was created by Franc [30] to recognize the varied behaviors observed for Slovakian staphylinids. The fact that several behavioral classifications have been created solely for myrmecophilous Staphylinidae illustrates the great diversity of myrmecophilous associations that exist within the family and suggests that it may be more appropriate to limit some of the previously proposed behavioral classes to the family.

3.2. Same Class, Different Behaviors. In many cases, the broadly defined classification schemes unintentionally capture vastly different associations in a single category [1]. For example, the very commonly used term "synoekete," which was used by nearly every author after Wasmann, is widely applied to many Coleoptera that vary greatly in their biology and in interactions with respective ant hosts. Wheeler's subdivision of the synoeketes into four different classes places potentially every kind of ant-associated beetle within the group, including the many beetles that are ignored by ant hosts, the numerous genera that feed on debris in refuse piles, several Staphylinidae that are mimics of ants, and those that resemble but are not really "true guests." In Wheeler's attempt to capture this diversity of behavior and morphology, it appears as if each type is mutually exclusive but is not. For instance, ant mimics, which Wheeler placed in their own category, actually are ignored by ants and may feed on debris in refuse piles [24], but this behavior is classified separately from the mimic category. It may be useful in these cases to separate morphology from behavior.

When comparing different groups of myrmecophiles at higher taxonomic levels, the terminology used for one group may not be applicable to those of another group [43], which supports the notion that creating overarching behavioral classes may be less effective than intended. For example, the term "symphile" may be interpreted differently in different groups of beetles. If one considers the symphilous spider beetles, for which we have data for only a few species, these beetles may be scavenging in refuse piles, while also involved in trophallaxis with ants. In contrast, the "symphilous" scarab genus *Cremastocheilus* is known to be carried into or walk into ant colonies undetected and subsequently feeding on ant larvae or pupae. While these two beetle groups are "integrated" into the ant nest, the mechanisms used to integrate themselves are vastly different. The term "symphile" falls apart when considering these different taxonomic groups. In addition, even if behaviors appear to be superficially identical in unrelated taxonomic groups, there may be niche-specific differences [43] or even host-specific adaptations that are not immediately visible. Factors such as colony size, the type of habitat, movement patterns and frequency, and other within-nest variables may all play roles in how associates are interacting with ants [11].

Most recently, Ellis and Hepburn [42] unsuccessfully attempted to classify the small hive beetle, a bee parasite, according to the schemes proposed by Wasmann [20] and Kistner [23]. They noted that beetles' associations with bee hosts differed depending on geographic range, the level of predation exhibited by the beetles and also varied among naturally occurring or introduced populations. Similar complex factors are likely to affect many myrmecophiles, especially if they are generalists, or are associates of multiple ant hosts where interactions may differ from one ant host to another. Most recently, Geiselhardt et al. [11] proposed the use of the terms "obligate" or "facultative" to capture myrmecophilous associations to avoid the use of Wasmann's system. Their scheme may be the most generalized, and probably the most practical, but still relies on authors knowing how closely species are associated with their ant hosts. For example, if one considers any of the staphylinid beetles that are associated with any of the various army ant genera, they could be considered obligate ant guests if associations are specific to the respective ant host; or, provided that many staphylinids are generalist predators and scavengers, they may all be considered facultative associates if the presence of ants or debris from ant nests are not required for survival. The usage of either of these terms is still problematic and may not be useful for many other myrmecophilous beetles, since few biological details are known for the majority of taxa.

3.3. Presumed Behaviors of Closely Related Taxa. In Hölldobler and Wilson's [1] list of myrmecophiles and their respective interactions with ants, much of the information needed to describe these interactions is cursory or entirely absent. Specifically, in the list of Coleoptera associated with ants, nearly half of the mentioned families are completely unknown in a behavioral sense. In addition, many are presumed to interact with ants in a certain way depending on what is known about a close relative. For example, the scarab genus *Stephanuca* was recently documented to be associated with ants, although the observations only indicated that beetles land close to or near plants that were covered with ants, and no beetles were ever collected in an ant mound [44]. It was compared to a closely related, presumably myrmecophilous species, *Euphoria inda*, which has been found to be carried into ant nests for the purpose of laying eggs in debris inside the ant colony [3]. *Euphoria hirtipes* has also been collected in *Formica* thatches [45], but interactions with ants have not been observed. These three beetles, while all similar in morphology, may use similar strategies to gain entrance into the ant colony, but behavioral data are incomplete.

In other cases where behavior is known, interactions of beetles with respective ant hosts can vary quite significantly among closely related taxa. The North American scarab genus *Cremastocheilus* is presumed to be exclusively myrmecophilous, and all known species bear conspicuous trichomes that would indicate a "symphilous" habit, if using the terminology of Wasmann. Most *Cremastocheilus* species have abundant ant-host records [15], but little is known about behavior, except for a few species. Two closely related species within the same subgenus *Trinodia* [15, 46], including *C. hirsutus* and *C. saucius*, use entirely different strategies to gain entrance into an ant mound. *Cremastocheilus hirsutus* enters *Pogonomyrmex* ant nests on its own, while *C. saucius*

feigns death and relies on the ants to carry it into the nest [15], suggesting that colony entrance behaviors are highly variable among closely related species within the genus. Similar studies of the rove beetle genus *Pella* [47] or the ladybird genus *Coccinella* [48] have also indicated vastly different behaviors among three congeners, which makes it nearly impossible to classify either genus as a specific type of myrmecophile and suggests that ant-beetle interactions are often species-specific, where each species may be classified differently according to Wasmann's or several other classification systems. The utility of behavioral categories becomes less reliable as one examines more taxa and may be little effective in truly understanding how complex phenomena like myrmecophily evolve.

3.4. Confounding Behavior and Morphology. The majority of categorical schemes include aspects of both behavior and morphology, no doubt because these two factors are inextricably connected. Therefore, the behavioral categories proposed by various researchers often hinge on morphological justifications to support purported behavioral interactions. Morphology often provides information, that is used to predict a certain behavior, but in many other cases such claims should be approached with caution, especially since various behavioral interactions with ant hosts may be occurring in taxa that bear similar morphological adaptations, such as the *Cremastocheilus* example cited earlier. The presence of trichomes is often immediately associated with a "symphilous" habit; while this appears to be true in many cases, behavioral information is absent for the majority of taxa that bear these trichomes. Even among taxa that bear trichomes, their interactions with ants still appear to be highly variable.

Wasmann's "symphile" category is almost always discussed in terms of trichomes [3], and the mere presence of trichomes has been cited as being immediately predictive of an intimate association with ants [3, 6, 49], even though trichomes are also found in many termite-associated beetles [4, 5, 7]. In other categories, particularly Wasmann's "synoeketes," the morphology among these beetles is highly varied, including various mimics "tear-drop shaped" beetles [3]. In addition, beetles often bear different combinations of morphological adaptations to a life with ants. These morphological modifications frequently include enlarged or reduced antennae, reddish or "ant-red" integument, and less frequently, modified mouthparts [18, 46] or "digging" appendages that are sometimes associated with myrmecophily [3, 15].

While it should not be assumed that each morphological modification is adaptive, that is, it serves a definite function in terms of behavior, it may be useful for future studies to investigate whether certain morphological characteristics are actually predictors of a certain behavior, instead of making *a priori* assumptions. In addition, both morphological and behavioral aspects of a presumed myrmecophile should be examined on a species-specific basis rather than on one that attempts to lump the target species into one of the existing categories for sake of simplicity.

4. Rejection of Previous Classification Systems

The descriptions used by authors often circumscribe significantly different behaviors and morphological character suites that may or may not be adaptations to myrmecophily. Many of these intended groupings of myrmecophilous interactions envelop the range of myrmecophilous interactions that have been observed, but none of the existing categories provide us with an effective method for describing these interactions. In part, creating categories for different ant associates may not be useful at any scale, particularly if applied to various unrelated taxa. Instead, examining each presumed myrmecophile as its own entity on its own evolutionary trajectory may be favorable.

Various factors that are discussed in the different categorical schemes should be considered when describing myrmecophiles. For example, the classification schemes of both Donisthorpe [22] and Kistner [23] focused on associates' relative occurrence inside or outside the ant colony. Those species that infrequently encounter ants are less likely to bear the behavioral or morphological adaptations than those which closely interact with ants on raids or inside the colony [24]. Therefore, behavioral descriptions should focus on the potential level of interaction between host and associate.

It is evident that myrmecophilous associations do not occur as discrete and easily identifiable interactions but rather on a behavioral gradient. The varying combinations of morphology found in different myrmecophiles may also be viewed as operating on a gradient, so that some body parts evolve in response to myrmecophilous interactions and others do not. While it is often easy to look at a myrmecophilous beetle and claim that it is an ant associate, based on the "typical myrmecophile" characteristics, these morphological traits may be relatively labile in an evolutionary context [35, 43] and are able to evolve rapidly in response to myrmecophilous interactions. Morphological convergence in response to myrmecophily may in itself be worth examining more closely.

5. Conclusion

I suggest that each target taxon, whether a single species or entire genera, should be studied in terms of its respective behavioral and morphological suite of characteristics. In the few cases where behavioral data are available, noting species-specific interactions with respective ant hosts is more likely to be informative than attempting to place taxa within a categorical scheme, at least until more is known of biology. A recent review of the Dermestidae suggests that examining taxa at lower levels, that is, below the family level [50], may provide insights into patterns of evolution that would not be possible if one attempted to group a diverse array of ecologically diverse taxa into a single behavioral category. Therefore, studies of myrmecophily, especially those attempting to elucidate patterns or processes underlying the evolution of myrmecophilous associations, may be pursued by viewing beetle-ant interactions from a declassified or deconstructed perspective.

Historically, the vast diversity of myrmecophilous interactions that occur within or around ant nests have both baffled and amazed biologists, and continued studies of ant associated beetles will undoubtedly fill in the gaps and answer some of the many questions that we have about this syndrome. It is this fascinating behavior and the bizarre morphological adaptations that evolve in response to it and that lure so many of us to the study of myrmecophily; however, relying on the need to classify or name myrmecophiles adds unnecessary confusion and redundancy to the field. Furthermore, the term "myrmecophily" should be approached with caution. I also suggest that studies should be pursued on a species-specific basis, both in terms of the associates and their respective ant hosts. Ants are rarely discussed in studies of myrmecophily, unless a specific ant host is mentioned. Instead, the focus is typically placed on those animals that are associated with ants, and it is likely that ant-specific behaviors may be just as interesting and complex as those of their respective associates. Finally, I urge amateurs, experts, and willing graduate students that are interested in rich, complex behavioral and morphological systems to begin to delve into the still largely unknown system of myrmecophily, especially in the Coleoptera. This phenomenon provides a rich area of research, both in terms of taxonomic and basic behavioral studies, as well as one that can be pursued to examine the evolution of complex morphology, behavior, and underlying molecular processes that may give greater insights into what we know as "myrmecophily."

References

[1] B. Hölldobler and E. O. Wilson, *The Ants*, Belknap Press, Cambridge, Mass, USA, 1990.

[2] E. O. Wilson, *The Insect Societies*, Belknap Press, Cambridge, Mass, USA, 1971.

[3] W. M. Wheeler, *Ants, Their Structure, Development and Behavior*, Columbia University Press, New York, NY, USA, 1910.

[4] E. Wasmann, "Vergleichende studien über ameisengäste und termitengäste," *Tijdschrift voor Entomologie*, vol. 33, pp. 27–96, 1890.

[5] J. Krikken and J. Huijbregts, "Southeast Asian *Termitodius*: a taxonomic review, with descriptions of four new species (Coleoptera: Aphodiidae)," *Zoologische Mededelingen Leiden*, vol. 61, no. 7, pp. 97–111, 1987.

[6] M. S. Caterino, "Descriptions of the first Chlamydopsinae (Coleoptera: Histeridae) from Wallacea," *Tijdschrift voor Entomologie*, vol. 143, no. 3, pp. 267–278, 2000.

[7] M. Maruyama, "A new genus and species of flightless, microphthalmic Corythoderini (Coleoptera: Scarabaeidae: Aphodiinae) from Cambodia, associated with Macrotermes termites," *Zootaxa*, vol. 3555, pp. 83–88, 2012.

[8] B. Hölldobler, "Communication between ants and their guests," *Scientific American*, vol. 224, pp. 86–93, 1971.

[9] S. T. O'Keefe, "Ant-like stone beetles, ants, and their associations (Coleoptera: Scydmaenidae; Hymenoptera: Formicidae; Isoptera)," *Journal of the New York Entomological Society*, vol. 108, no. 3-4, pp. 273–303, 2000.

[10] W. Moore, X. B. Song, and A. di Giulio, "The larva of *Eustra* (Coleoptera, Paussinae, Ozaenini): a facultative associate of ants," *ZooKeys*, vol. 90, pp. 63–82, 2011.

[11] S. F. Geiselhardt, K. Peschke, and P. Nagel, "A review of myrmecophily in ant nest beetles (Coleoptera: Carabidae: Paussinae): linking early observations with recent findings," *Naturwissenschaften*, vol. 94, no. 11, pp. 871–894, 2007.

[12] J. Orivel, P. Servigne, P. Cerdan, A. Dejean, and B. Corbara, "The ladybird *Thalassa saginata*, an obligatory myrmecophile of *Dolichodeurs bidens* ant colonies," *Naturwissenschaften*, vol. 91, no. 2, pp. 97–100, 2004.

[13] A. Vantaux, O. Roux, A. Magro, and J. Orivel, "Evolutionary perspectives on myrmecophily in ladybirds," *Psyche*, vol. 2012, Article ID 591570, 7 pages, 2012.

[14] R. K. Vander Meer and D. P. Wojcik, "Chemical mimicry in the myrmecophilous beetle *Myrmecaphodius excavaticollis*," *Science*, vol. 218, no. 4574, pp. 806–808, 1982.

[15] G. D. Alpert, "A comparative study of the symbiotic relationships between beetles of the genus *Cremastocheilus* (Coleoptera, Scarabaeidae) and their host ants (Hymenoptera, Formicidae)," *Sociobiology*, vol. 25, no. 1, pp. 1–266, 1994.

[16] F. Z. Vaz-De-Mello, J. N. C. Louzada, and J. H. Schoereder, "New data and comments on scarabaeidae (Coleoptera: Scarabaeoidea) associated with attini (Hymenoptera: Formicidae)," *Coleopterists Bulletin*, vol. 52, no. 3, pp. 209–216, 1998.

[17] F. T. Krell, "Dung beetles unharmed by army ants in tropical Africa (Coleoptera: Scarabaeidae; Hymenoptera: Formicidae, Dorylinae)," *Coleopterists Bulletin*, vol. 53, no. 4, pp. 325–328, 1999.

[18] J. F. Lawrence and H. Reichardt, "Revision of the genera *Gnostus* and *Fabrasia* (Coleoptera: Ptinidae)," *Psyche*, vol. 73, no. 1, pp. 30–45, 1966.

[19] T. K. Philips, "Phylogenetic analysis of the new world Ptininae (Coleoptera: Bostrichoidea)," *Systematic Entomology*, vol. 25, no. 2, pp. 235–262, 2000.

[20] E. Wasmann, *Kritisches Verzeichniss der Myrmecophilen und Termitophilen Arthropoden*, Berlin, Germany, 1894.

[21] F. Silvestri, "Contribuzioni alla conoscenza dei Mirmecofili, I: osservazioni su alcuni mirmecofili dei dintorni di Portici," *Annuario del Museo Zoologie della R. Universita di Napoli*, vol. 1, no. 13, pp. 1–5, 1903.

[22] H. Donisthorpe, "The ants (Formicidae), and some myrmecophiles, of Sicily," *The Entomologist's Record*, vol. 38, no. 12, pp. 6–9, 1927.

[23] D. H. Kistner, "Social and evolutionary significance of social insect symbionts," in *Social Insects*, H. R. Hermann, Ed., vol. 1, pp. 339–413, Academia Press, New York, NY, USA, 1979.

[24] R. D. Akre and C. W. Rettenmeyer, "Behavior of Staphylinidae associated with army ants (Formicidae: Ecitonini)," *Journal of the Kansas Entomological Society*, vol. 39, no. 4, pp. 745–782, 1966.

[25] F. Maerkel, "Beiträge zur Kenntniss der unter Ameisen lebenden, Insekten, Erstes Stück," *Germar's Zeitschrift für die Entomologie*, vol. 3, pp. 203–225, 1841.

[26] F. Maerkel, "Beiträge zur Kenntniss der unter Ameisen lebenden, Insekten, Zweites Stück," *Germar's Zeitschrift für die Entomologie*, vol. 5, pp. 193–271, 1844.

[27] K. Schönrogge, J. C. Wardlaw, J. A. Thomas, and G. W. Elmes, "Polymorphic growth rates in myrmecophilous insects," *Proceedings of the Royal Society B*, vol. 267, no. 1445, pp. 771–777, 2000.

[28] C. Delamare-Debouteville, "Recherches sur les Collemboles termitophiles et myrmecophiles (écologie, éthologie, systématique)," *Archives de Zoologie Experimentale et Generale*, vol. 85, no. 5, pp. 261–425, 1948.

[29] R. Paulian, "Observations sur les Coléoptères commensaux d'Anomma nigricans en Côte d'Ivoire," *Annales des Sciences Naturrelles*, vol. 10, no. 1, pp. 79–102, 1948.

[30] V. Franc, "Myrmecophilous beetles of Slovakia with special reference to their endangerment and perspectives for protection," *Acta Universitatis Carolinae Biologica*, vol. 36, pp. 299–324, 1992.

[31] A. Forel, "Les formicides de la Province D'Oran (Algerié)," *Bulletin de la Société Vaudoise des Sciences Naturelles*, vol. 30, no. 114, pp. 1–47, 1894.

[32] E. Wasmann, "Zur Kenntnis einiger schwieriger *Thorictus*-Arten," *Deutsche Entomologische Zeitschrift*, vol. 39, no. 1, pp. 41–44, 1895.

[33] K. Fiedler, "Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidopera: Papilionoidea)," *Bonner Zoologische Monographien*, vol. 31, pp. 1–210, 1991.

[34] N. E. Pierce, M. F. Braby, A. Heath et al., "The ecology and evolution of ant association in the Lycaenidae (Lepidoptera)," *Annual Review of Entomology*, vol. 47, pp. 733–771, 2002.

[35] A. W. Shingleton and D. L. Stern, "Molecular phylogenetic evidence for multiple gains or losses of ant mutualism within the aphid genus *Chaitophorus*," *Molecular Phylogenetics and Evolution*, vol. 26, no. 1, pp. 26–35, 2003.

[36] C. W. Rettenmeyer, M. E. Rettenmeyer, J. Joseph, and S. M. Berghoff, "The largest animal association centered on one species: the army ant eciton burchellii and its more than 300 associates," *Insectes Sociaux*, vol. 58, no. 3, pp. 281–292, 2011.

[37] D. H. Kistner and H. R. Jacobson, "Cladistic analysis and taxonomic revision of the ecitophilous tribe Ecitocharini with studies of their behavior and evolution (Coleoptera, Staphylinidae, Aleocharinae)," *Sociobiology*, vol. 17, pp. 333–480, 1990.

[38] T. Akino, "Chemical camouflage by myrmecophilous beetles *Zyras comes* (Coleoptera: Staphylinidae) and *Diaritiger fossulatus* (Coleoptera: Pselaphidae) to be integrated into the nest of *Lasius fuliginosus* (Hymenoptera: Formicidae)," *Chemoecology*, vol. 12, no. 2, pp. 83–89, 2002.

[39] R. W. Howard, C. A. McDaniel, and G. J. Blomquist, "Chemical mimicry as an integrating mechanism: cuticular hydrocarbons of a termitophile and its host," *Science*, vol. 210, no. 4468, pp. 431–433, 1980.

[40] R. W. Howard and G. J. Blomquist, "Ecological, behavioral, and biochemical aspects of insect hydrocarbons," *Annual Review of Entomology*, vol. 50, pp. 371–393, 2005.

[41] M. Stoeffler, T. S. Maier, T. Tolasch, and J. L. M. Steidle, "Foreign-language skills in rove-beetles? Evidence for chemical mimicry of ant alarm pheromones in myrmecophilous Pella beetles (Coleoptera: Staphylinidae)," *Journal of Chemical Ecology*, vol. 33, no. 7, pp. 1382–1392, 2007.

[42] J. D. Ellis and H. R. Hepburn, "An ecological digest of the small hive beetle (*Aethina tumida*), a symbiont in honey bee colonies (*Apis mellifera*)," *Insectes Sociaux*, vol. 53, no. 1, pp. 8–19, 2006.

[43] B. Stadler, P. Kindlmann, P. Šmilauer, and K. Fiedler, "A comparative analysis of morphological and ecological characters of European aphids and lycaenids in relation to ant attendance," *Oecologia*, vol. 135, no. 3, pp. 422–430, 2003.

[44] M. J. Paulsen, "Observations on possible myrmecophily in *Stephanucha pilipennis* Kraatz (Coleoptera: Scarabaeidae: Cetoniinae) in Western Nebraska," *The Coleopterists Bulletin*, vol. 56, no. 3, pp. 451–452, 2002.

[45] B. C. Ratcliffe, "Notes on the biology of *Euphoriaspis hirtipes* (Horn) and descriptions of the larva and pupa (Coleoptera: Scarabaeidae)," *The Coleopterists Bulletin*, vol. 30, no. 3, pp. 217–225, 1976.

[46] G. Mynhardt and J. W. Wenzel, "Phylogenetic analysis of the myrmecophilous *Cremastocheilus* Knoch (Coleoptera, Scarabaeidae, Cetoniinae), based on external adult morphology," *ZooKeys*, vol. 34, no. 2, pp. 129–140, 2010.

[47] M. Stoeffler, T. Tolasch, and J. L. M. Steidle, "Three beetles-three concepts. Different defensive strategies of congeneric myrmecophilous beetles," *Behavioral Ecology and Sociobiology*, vol. 65, no. 8, pp. 1605–1613, 2011.

[48] J. F. Godeau, J. L. Hemptinne, A. F. G. Dixon, and J. C. Verhaeghe, "Reaction of ants to, and feeding biology of, a congeneric myrmecophilous and non-myrmecophilous ladybird," *Journal of Insect Behavior*, vol. 22, no. 3, pp. 173–185, 2009.

[49] F. T. Krell and T. Keith Philips, "*Formicdubius* Philips & Scholtz from South Africa, a junior synonym of *Haroldius* Boucomont, and a survey of the trichomes in the African species (Coleoptera, Scarabaeidae, Onthophagini)," *ZooKeys*, vol. 34, no. 2, pp. 41–48, 2010.

[50] R. D. Zhantiev, "Ecology and classification of dermestid beetles (Coleoptera, Dermestidae) of the Palaearctic fauna," *Entomological Review*, vol. 89, no. 2, pp. 157–174, 2009.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 162964, 7 pages
http://dx.doi.org/10.1155/2013/162964

Research Article

Carcass Fungistasis of the Burying Beetle *Nicrophorus nepalensis* Hope (Coleoptera: Silphidae)

Wenbe Hwang and Hsiu-Mei Lin

Department of Ecoscience and Ecotechnology, National University of Tainan, 33, Section 2, Shu-Lin Street, Tainan 70005, Taiwan

Correspondence should be addressed to Wenbe Hwang; wenbehwang@mail.nutn.edu.tw

Received 14 January 2013; Accepted 8 April 2013

Academic Editor: Vladimir Kostal

Our study investigated the fungistatic effects of the anal secretions of *Nicrophorus nepalensis* Hope on mouse carcasses. The diversity of fungi on carcasses was investigated in five different experimental conditions that corresponded to stages of the burial process. The inhibition of fungal growth on carcasses that were treated by mature beetles before burial was lost when identically treated carcasses were washed with distilled water. Compared with control carcasses, carcasses that were prepared, buried, and subsequently guarded by mature breeding pairs of beetles exhibited the greatest inhibition of fungal growth. No significant difference in fungistasis was observed between the 3.5 g and the 18 to 22 g guarded carcasses. We used the growth of the predominant species of fungi on the control carcasses, *Trichoderma* sp., as a biological indicator to examine differences in the fungistatic efficiency of anal secretions between sexually mature and immature adults and between genders. The anal secretions of sexually mature beetles inhibited the growth of *Trichoderma* sp., whereas the secretions of immature beetles did not. The secretions of sexually mature females displayed significantly greater inhibition of the growth of *Trichoderma* sp. than those of sexually mature males, possibly reflecting a division of labor in burying beetle reproduction.

1. Introduction

Burying beetles (*Nicrophorus* spp.) use small vertebrate carcasses as food for their larval broods by depositing their eggs around a buried carcass [1, 2]. Carcasses are nutritious yet rare resources [3, 4]. During the lifetime of a beetle, it may find only one carcass that is suitable for reproduction [5]. Competition for carcasses is intense [6–8], and burying beetles of the same or different species may fight to maintain occupancy of the carcass [1, 9–11].

Bacterial and fungal decomposers destroy carcasses, and scavenging animals have evolved behavioral and physiological counterstrategies to maintain food sources [12]. Before burying a carcass, the burying beetles remove the fur or feathers from the carcass, compact the carcass by rolling it repeatedly, and smear its surface with their anal secretions [1]. Carcasses used by beetles typically vary in size from 1 to 75 g [9, 10, 13] and are encountered in variable states of decay. Burying beetles exhibit adaptive strategies that enable them to manage the carrion resources in such diverse conditions, such as adjusting the number of eggs laid [13, 14] and practicing infanticide [15, 16], with the number of surviving larvae positively correlated with carcass size [9, 10, 17].

Although the loss of biomass resulting from microbial growth on a carcass is not large, microorganisms often produce toxins that can affect beetle-larvae survival [18–20]. The oral and anal secretions of various burying beetle species have bacteriostatic effects [21]. The oral secretions contain phospholipase A_2 that may disrupt the cell membranes of bacteria [22]. Fungal growth may also be inhibited following the preburial treatments by burying beetles [23]. The temperature and the composition of food materials can influence the antimicrobial activity of the oral secretions [24], and the antibacterial activity of the anal secretions has been shown to be upregulated following the discovery of carrion [25].

Burying beetles' preference for appropriately sized carcasses for reproduction may be related to their capacity to secrete antimicrobial substances [26]. Although burying

beetles can feed more offspring on a larger carcass, the energy expenditure for the preburial preparation of larger carcasses is also higher. Scott [27] proposed that microorganisms are more serious competitors on larger carcasses because of the difficulties associated with preburial preparations. Scott [27] also reported that mold often renders substantial amounts of large carcasses unusable, and Hwang and Shiao [26] reported that large carcasses decay more rapidly than small carcasses, resulting in lower trophic efficiency for large carcasses. Therefore, communal breeding observed in some species of burying beetles may prevent the decay of a large carcass, contributing to better breeding efficiency [28–31].

Both uniparental and biparental breedings are commonly observed in burying beetles [8, 32]. Females reproducing without the assistance of a male do not display reduced reproductive success [33]. However, with the help of a male, the carcass can be better preserved [6, 34], and dipteran larvae and conspecific competitors can be more efficiently excluded [32, 35–38]. In addition, the male can also substitute for the female in brood care [39]. However, because the primary role of the male in brood care is providing protection against competitors, we propose that the female likely makes a greater antimicrobial contribution to the carcass.

In our current study, we assessed fungistasis in carcasses in the laboratory that were colonized by *Nicrophorus nepalensis*, a common burying beetle in southern Taiwan. We investigated whether the fungistatic efficiency of beetles correlated with the sexual maturity or the sex of the parent, and we examined whether fungistatic capacity of beetles was sex or age dependent.

2. Materials and Methods

2.1. Field Collection and Laboratory Rearing of Beetles. The *N. nepalensis* Hope beetles were collected using 15 hanging pitfall traps that were baited with 40 g of chicken meat each and placed at 100 m intervals along the Fengkang Forest Road (22°00′N, 120°41′E) in Kaohsiung City in southern Taiwan at altitudes of 1100 to 1600 m above sea level from January to July, 2009. All field-collected beetles were anesthetized with carbon dioxide, and any mites were removed under a microscope using forceps. To avoid the influence of parasites, only the laboratory-reared F1 and F2 offspring of field-collected beetles were used in our experiments. All beetle cages used in our study were 10.4 × 10.4 × 6 cm transparent plastic containers. All the beetles used in our study were reared at 20°C with 12 h light-dark cycling.

A breeding pair of field-collected beetles and a 20 g mouse carcass were added to a cage with 4 cm thick moist peat. Following oviposition, the eggs were removed and placed on wet toilet paper in an 8.5 cm Petri dish for hatching. The larvae and the parents were transferred to a new cage with 1 cm thick moist peat. When the larvae emerged from the burrow to pupate, up to 8 were placed in a new cage with 4 cm thick moist peat. Groups of up to 6 newly eclosed adults of the same sex were transferred to new cages with 3 cm thick moist peat. Prior to the fungistasis experiments, the laboratory-reared adult beetles were fed twice a week with freshly decapitated *Tenebrio molitor* or cut sections of *Zophobas morio*. No beetles were exposed to carcasses before being used in the fungistasis experiments.

2.2. Experimental Design. To investigate whether preburial preparations affect fungal growth on carcasses, fungal growth on mouse (ICR strain) carcasses was assessed in five different conditions. All fresh frozen mice were purchased in CMLAC in National Taiwan University and thawed before the experiments. Untreated (control) carcasses, treated carcass balls, washed carcass balls, protected carcass balls, and large protected carcass balls were examined for visible fungal growth over the course of 14 days under the standard rearing conditions. The control carcasses (approximately 3.5 g) were not exposed to burying beetles and were placed on the surface of the moist peat in an otherwise empty cage. The treated carcass balls were obtained at 3 days following presentation of a carcass (approximately 3.5 g) to a mating pair of sexually mature adults by removing the carcass immediately after burial. During this stage, the fur removal, the carcass compaction, and the deposition of anal excretions had occurred prior to the removal of the carcass, but larval hatch had not yet occurred. The treated carcasses were each transferred to a new cage with moist peat and no beetles. The washed carcass balls were obtained using the same procedure as the treated carcass balls, with an additional step in which the carcass balls were rinsed with distilled water before being transferred to a new cage. The protected and large protected carcass balls were obtained using the same procedure as the treated carcass balls, except that the same mating pair of beetles was added after the carcass was transferred to a new cage, and 18 to 22 g mouse carcasses were used for the large protected carcass balls. To remove newly oviposited eggs, the carcass ball and the adult beetles were transferred to a new cage daily, with the transfers performed under red light to avoid disrupting the light-dark cycle.

2.3. Cultivation and Identification of Carcass Fungi. Any fungus that grew on a carcass within 14 days in any of the 5 experimental conditions was cultivated for identification. Solid malt extract agar (MEA) medium was prepared from 26 g of malt extract agar (Fluka) in 500 mL distilled water and sterilized at 121°C for 15 min. To prepare the solid cornmeal agar (CMA) medium, 10 g of cornmeal was boiled in distilled water. Following filtration, the volume of the cornmeal filtrate was adjusted to 500 mL, and 10 g of agar (Sigma-Aldrich, St. Louis, MO, USA) was added before sterilization at 121°C for 15 min. After cooling the media to 50°C, 40 ppm of Streptomycin sulfate and 40 ppm of Penicillin G were added to both the MEA and CMA media, and the media were poured into Petri dishes before solidification. A hypodermic needle was used to remove a specimen of the carcass skin containing the fungi, and the specimen was used to inoculate MEA plates, and the fungi were cultured for 7 days at 25°C. The fungi cultured on MEA plates were used to inoculate CMA plates that were subsequently cultured at 25°C. The cultured fungi were dyed with cotton blue in lactoglycerol, and the various taxa were identified to genus or species.

2.4. Fungistasis Quantification Assays. To examine differences in the fungistatic capacity of the anal secretions from males versus females and sexually mature adults versus sexually immature adults, the anal secretions were collected from each and used in fungistasis quantification assays. We observed that *Trichoderma* sp. was the predominant species on control carcasses and that *Trichoderma* sp. growth was inhibited on carcasses treated by sexually mature beetles. Therefore, we used *Trichoderma* sp. growth as a biological indicator of the fungistatic efficiency of burying beetle secretions. Green colonies of *Trichoderma* sp. formed on CMA plates after culturing for 7 days at 25°C. One colony was suspended in 300 μL of ultrapure water. Inoculums were prepared by mixing 30 μL of the fungal suspension with 30 μL of ultrapure water (control group) or 30 μL of anal secretions from male or female beetles that were taken at 6 days (sexually immature) or 35 days (sexually mature) after eclosion, and 2 μL of each inoculum was separately used to inoculate CMA plates that were subsequently cultured at 25°C. The secretory volume of each individual was different; 30 μL of anal secretions were collected from different individuals. The number of *Trichoderma* sp. colonies present on the CMA plates at 7 days after inoculation was recorded. The number of days at which the fungal growth reached confluency on the CMA plates was also recorded.

2.5. Statistical Analysis. We used the Fisher exact test to compare the fungal growth in the various experimental conditions, and we used a χ^2 test to compare the differences in the fungal species that were isolated in each set of experimental conditions. An independent sample Student's t test was used to examine the differences in fungistatic efficiency between sexually mature males and females. All statistical analyses were conducted using the SPSS version 17.0 computer software, with an alpha value of 0.05 as the accepted level of statistical significance.

3. Results

3.1. Inhibition of Fungal Growth on Carcasses. Compared with the control carcasses ($n = 19$), fungal growth was significantly inhibited on the treated and protected carcass balls (Fisher exact test: treated carcass balls: $P < 0.01$, $n = 18$; protected carcass balls: $P < 0.001$, $n = 16$). However, the fungistatic effect was significantly greater on protected carcass balls than on treated carcass balls (Fisher exact test: $P = 0.01$). The fungistasis on the washed carcass balls ($n = 18$) was not significantly different than that of the control carcasses (Fisher exact test: $P = 0.08$), and the fungistasis on the protected carcass balls ($n = 16$) was not significantly different than that of the large protected carcass balls ($n = 17$; Fisher exact test: $P = 0.68$; Figure 1).

3.2. Fungus Diversity on Carcasses. The following 12 fungal species were isolated from the mouse carcasses in the various experimental conditions: *Alternaria* sp., *Aspergillus fumigates*, *Cladosporium herbarum*, *Cladosporium* sp. 1, *Cladosporium* sp. 2, *Conidiobolus* sp., *Dactylaria* sp., *Graphium* sp., *Mucor* sp., *Phoma* sp., *Trichoderma* sp., and *Verticillium* sp. On control carcasses, 23 fungal samples were acquired, which consisted of the following 7 species: *Aspergillus fumigates*, *Alternaria* sp., *Cladosporium* sp. 1, *Graphium* sp., *Mucor* sp., *Trichoderma* sp., and *Verticillium* sp. The *Trichoderma* sp. Had the highest frequency of occurrence on control carcasses, accounting for 72% of the acquired fungal samples. On the treated carcass balls, 10 fungal samples were acquired, which consisted of the following 4 species: *Aspergillus fumigates*, *Cladosporium herbarum*, *Mucor* sp., and *Phoma* sp. The *Mucor* sp. was the most predominant fungus on treated carcass balls, accounting for 70% of the acquired fungal samples. On the washed carcass balls, 14 fungal samples were acquired, which consisted of the following 8 species: *Aspergillus fumigates*, *Alternaria* sp., *Cladosporium* sp. 2, *Conidiobolus* sp., *Dactylaria* sp., *Graphium* sp., *Mucor* sp., and *Trichoderma* sp. The *Mucor* sp. was the predominant fungus on washed carcass balls, accounting for 62.5% of the acquired fungal samples (Figure 2). Only *Aspergillus fumigates* and *Cladosporium* sp. 1 were identified on the protected carcass balls, and only *Aspergillus fumigates* and *Mucor* sp. were identified on the large protected carcass balls.

The incidences of the various fungal species were not significantly different between the control carcasses and the washed carcass balls (χ^2 test: $P = 0.07$, control carcasses: $n = 23$, washed carcass balls: $n = 14$), or between the treated carcass balls and the washed carcass balls (χ^2 test: $P = 0.41$, treated carcass balls: $n = 10$). However, there were significant differences in the incidences of the various fungal species between the control carcasses and the treated carcass balls (χ^2 test: $P = 0.001$, treated carcass balls: $n = 10$).

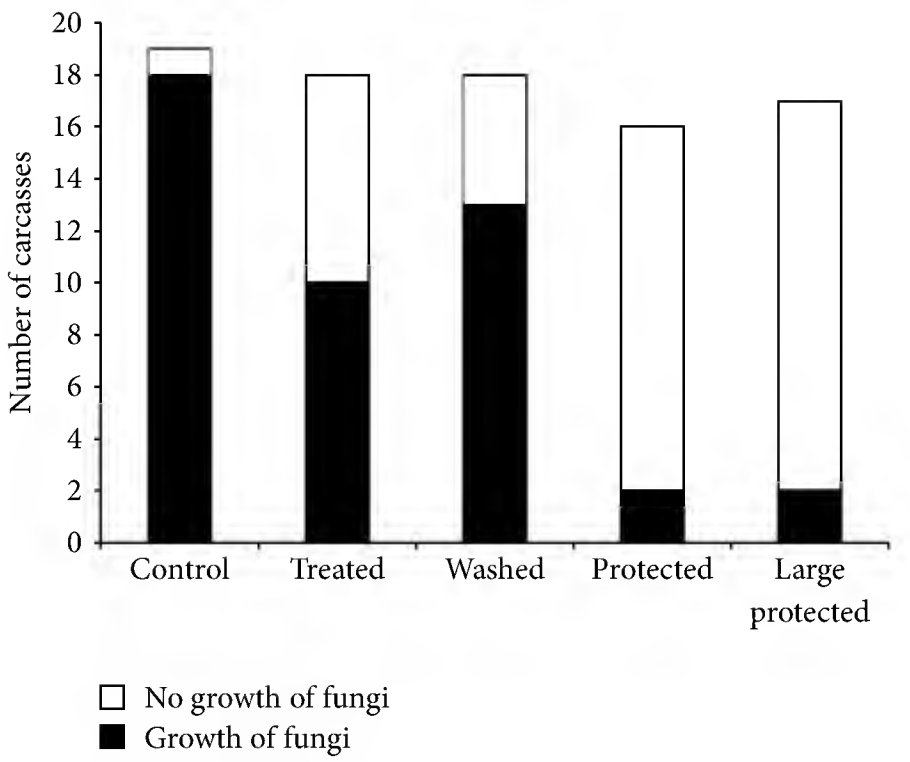


FIGURE 1: Number of control carcasses (untreated, $n = 19$), treated carcass balls ($n = 18$), washed carcass balls ($n = 18$), protected carcass balls (3.5 g, $n = 16$), and large protected carcass balls (18 to 22 g, $n = 17$) on which fungi grew within 14 d. The beetles had opportunity to come into contact with carcass on a protected carcass ball, while beetles were removed at 3 days after burial on a treated carcass ball.

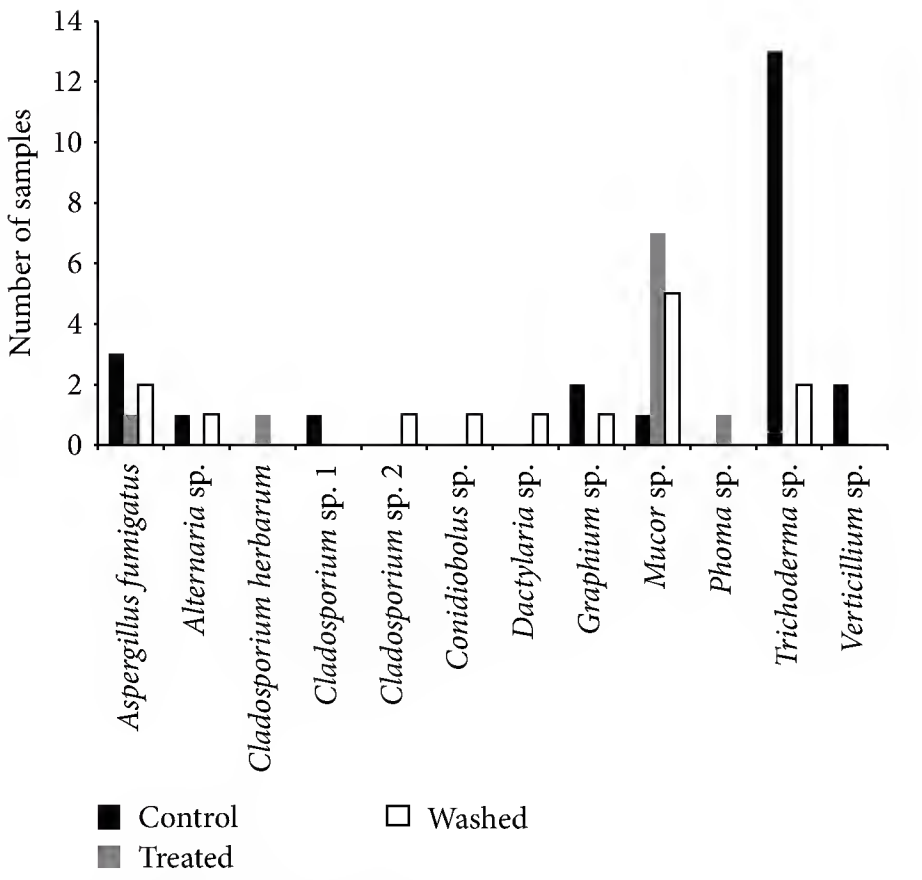


FIGURE 2: The sample number of the fungi species that were collected from control carcasses (n = 23), treated carcass balls (n = 10), and washed carcass balls (n = 14).

3.3. Effects of Beetle Sexual Maturity on Fungistasis. After cultivation for 7 days, 192.56 ± 70.41 colonies of *Trichoderma* sp. were present on the CMA plates that had been inoculated with the control inoculums (n = 16). The inoculums that contained the anal secretions of sexually mature beetles produced no colonies on the CMA plates. Compared to the control inoculums, the anal secretions from both sexually mature males and females could significantly inhibit fungal growth (independent sample Student's t test: $P < 0.001$; sexually mature male: n = 16, sexually mature female: n = 15). Inoculums containing the anal secretions of immature beetles produced fungal growth on the CMA plates, regardless of sex (immature male: 214.06 ± 48.86 colonies, n = 16; immature female: 264.38 ± 55.95 colonies, n = 16). The numbers of *Trichoderma* sp. colonies produced from the control inoculums were not significantly different than that produced from the immature male inoculums (independent sample Student's t test: P = 0.32). The inoculums that contained the anal secretions of sexually immature females produced a significantly greater number of colonies compared with the control inoculums (independent sample Student's t test: P = 0.003) and the inoculums that contained the anal secretions of sexually immature males (independent sample Student's t test: P = 0.011) (Figure 3).

The fungal growth reached confluency on the CMA plates in 3.0 ± 0.0 days using the control inoculums, 6.0 ± 0.0 days using the sexually mature male inoculums, and 9.0 ± 0.0 days using the sexually mature female inoculums. Thus, although the anal secretions from sexually mature beetles inhibited the growth of *Trichoderma* sp., the number of days required for fungal growth to reach confluency was significantly longer for inoculums containing mature female anal secretions than those produced from mature males (independent sample Student's t test: $P < 0.001$, sexually mature male: n = 16,

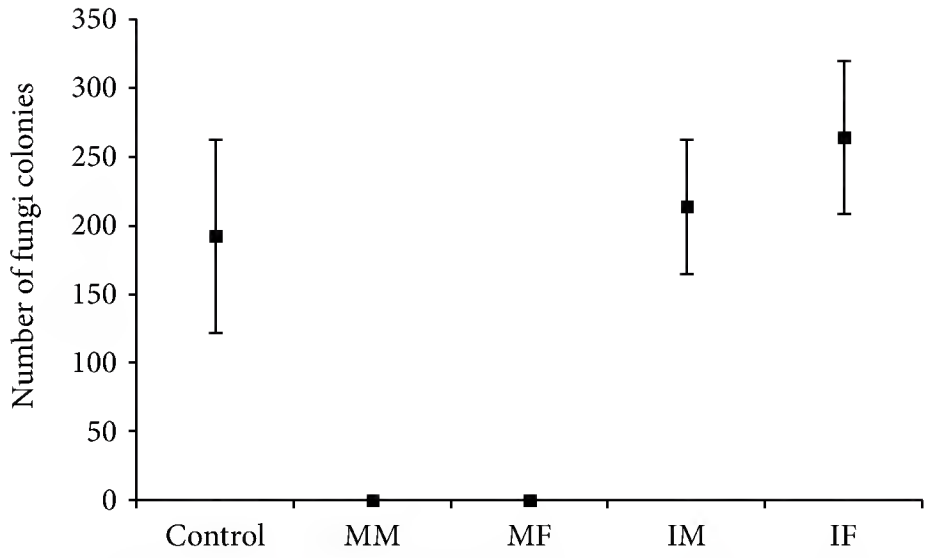


FIGURE 3: The number of *Trichoderma* sp. colonies (mean ± standard error) on CMA plates at 7 d after inoculation that were produced from the various inoculums. The inoculums were prepared from *Trichoderma* sp. suspended in ultrapure water (control group: n = 16) and the anal secretions of sexually mature males (MM: n = 16), sexually mature females (MF: n = 15), immature males (IM: n = 16), and immature females (IF: n = 16).

sexually mature female: n = 15), which is considered a better fungistasis from mature females.

4. Discussion

Vertebrate carcasses are a high-quality source of nutrition for many species, with insects, scavengers, and microbes competing for the food resources. Insects typically begin to consume carcasses before the arrival of the larger scavenging species, and microbes release toxins that may drive away competitors [40]. Burying beetles use a small vertebrate carcass as a source of nutrition of their larval broods [1, 14], putting them in direct competition with intraspecific or interspecific insects, bacteria, fungi, microorganisms, and so on [27].

The decomposition rate of a buried carcass is slower than that of an exposed carcass because subsequent access to the carcass may be hindered for many insects and other scavengers [40]. Thus, the burial behavior of burying beetles is an adaptation that reduces competition for food resources. Observed in our study, the adults of *N. nepalensis* often feed on the intestines of carcasses before removing the fur, which may reduce the rate of decay of the carcass by eliminating the bacteria that are normally present in intestines. However, soil is also rich in microorganisms, such as fungi, that may subsequently diminish the quality of a carcass after burial.

The efficiency of carcass preservation may thus directly affect the successful production of burying beetle offspring. The deposition of oral and anal secretions on a carcass is one burying beetle behavior that reduces the rate of decay [23, 27, 33, 35]. Before burying the whole carcass, *N. nepalensis* removes hair or feathers prior to coating the carcass with secretions first. Unlike other species in North America, burying beetles bury the whole carcass first [41]. In our study, fungistasis was most efficient when a breeding pair of beetles remained present with the carcass. Thus, it is likely that the anal secretions of beetles are continuously deposited on

the carcass. Our findings support the claim that the activity of the antimicrobial chemicals in the secretions is maintained over time [23, 24] because the fungistasis was also observed on the treated carcass balls without attending adult beetles. But when a prepared carcass was given a rinse in water, the protection of fungistatic effect was absent in the washed carcass balls. However, the diversity of fungal species isolated from washed carcass balls was nonetheless influenced by the preexisting anal secretions because the dominant fungal species on both treated and protected carcass balls was *Mucor* sp., whereas *Trichoderma* sp. was the dominant species on control carcasses.

The preparation of large carcasses for burial requires more time and energy and often leads to reduced quality of maintenance by beetles [27]. A previous study showed that *N. nepalensis* was unable to efficiently use the resources of a 130 g carcass, resulting in lower offspring weight to carcass weight ratios, compared with that of smaller carcasses, because the larger carcasses decayed rapidly [26]. Compared with the 3.5 g protected carcass balls, the 18 to 22 g protected carcass balls exhibited no significant difference in fungistasis. The 2 sizes of carcasses that were used in our study are within the size range of carcasses typically used by *N. nepalensis*. Nonetheless, the fungistatic capacity of burying beetle behavior in the field may be limited by the size of a carcass because the maintenance of carcasses in the field may involve greater competition with microorganisms and other competitors than was replicated in our laboratory experiments. Therefore, the effects of carcass size on reproduction success should be further investigated in the field.

In our study, the 12 fungal species that were collected from the carcasses are common in the natural environment of the burying beetle, especially in the soil and the decaying organic matter of leaf litter [42]. However, whether the source of the fungi in our experiments was the mouse carcasses, the beetles, or the moist peat used in the cages was not determined. *Conidiobolus* sp. and *Mucor* sp. belong to the Zygomycotina, and the other 10 species that were identified in our study are members of Ascomycotina. Fungi of the Zygomycotina are commonly found in leaf litter and soil, and some species may parasitize insects [43, 44]. Members of Ascomycotina may cause disease in certain plants, and other members, such as *Cordyceps sinensis*, may parasitize insects [45]. The dominant species on the control carcasses in our study was *Trichoderma* sp., which is widespread in soil [46, 47].

The oral and anal secretions of burying beetles contain antimicrobial chemicals [48]. The antimicrobial and lytic activities of the anal secretions in *N. vespilloides* are upregulated following the discovery of a carcass [25]. Cotter and Kilner [25] suggested that the antimicrobial activity may be influenced by juvenile hormone. Our findings support the role of juvenile hormone in the fungistatic properties of anal secretions because the secretions from sexually immature adults did not inhibit fungal growth. Thus, it is doubtful that the fungistatic properties of secretions from sexually immature beetles are upregulated following contact with a carcass, despite the burying of carcasses by sexually immature beetles [26]. We suggest that the burying behavior of sexually immature beetles may serve to protect the carcass for subsequent feeding or reproduction.

Burying beetles rear their offspring by biparentally caring for the brood [1]. The participation of the male in biparental care can significantly improve resistance to alien invaders, compared with uniparental care by a female [32, 37, 49]. However, no significant increase in larval weight or brood weight occurs with biparental care, compared with uniparental care by a female [33, 35, 36]. In biparental care, males spend more time protecting the larvae and carcass from invaders than females, whereas females spend more time feeding larvae than males [50–53]. The division of labor in reproduction among male and female burying beetles may extend to the deposition of oral and anal secretions on the carcass. Our results indicate that both sexually mature male and female burying beetles produce anal secretions that inhibit the growth of fungi. However, the degree of fungistasis conferred by the secretions was significantly different between the sexes.

Conflict of Interests

The authors have no conflict of interests with the SPSS used inside the paper.

Acknowledgments

The authors wish to thank Professor P.-J. Lee for her help with identifying the fungi. This research was supported by Grants from the National University of Tainan (99AB2-14 and AB100-319) and the National Science Council, Taiwan (NSC 97WFA0F00038).

References

[1] E. Pukowski, "Ökologische untersuchungen an *Necrophorus* F," *Zeitschrift für Morphologie und Ökologie der Tiere*, vol. 27, no. 3, pp. 518–586, 1933.

[2] S. B. Peck, "Nicrophorus (Silphidae) can use large carcasses for reproduction (Coleoptera)," *The Coleopterists Bulletin*, pp. 40–44, 1986.

[3] E. O. Wilson, *The Insect Societies*, Belknap Press, Cambridge, UK, 1971.

[4] I. Hanski and Y. Cambefort, *Dung Beetle Ecology*, Princeton University Press, Princeton, NJ, USA, 1991.

[5] M. P. Scott and D. S. Gladstein, "Calculating males? An empirical and theoretical examination of the duration of paternal care in burying beetles," *Evolutionary Ecology*, vol. 7, no. 4, pp. 362–378, 1993.

[6] D. S. Wilson and J. Fudge, "Burying beetles: intraspecific interactions and reproductive success in the field," *Ecological Entomology*, vol. 9, no. 2, pp. 195–203, 1984.

[7] D. S. Wilson, W. G. Knollenberg, and J. Fudge, "Species packing and temperature dependent competition among burying beetles (Silphidae, Nicrophorus)," *Ecological Entomology*, vol. 9, no. 2, pp. 205–216, 1984.

[8] S. T. Trumbo, "Interference competition among burying beetles (Silphidae, Nicrophorus)," *Ecological Entomology*, vol. 15, no. 3, pp. 347–355, 1990.

[9] J. Bartlett and C. M. Ashworth, "Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae)," *Behavioral Ecology and Sociobiology*, vol. 22, no. 6, pp. 429–434, 1988.

[10] M. Otronen, "The effect of body size on the outcome of fights in burying beetles (Nicrophorus)," *Annales Zoologici Fennici*, vol. 25, no. 2, pp. 191–201, 1988.

[11] J. K. Müller, A. K. Eggert, and E. Furlkröger, "Clutch size regulation in the burying beetle *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae)," *Journal of Insect Behavior*, vol. 3, no. 2, pp. 265–270, 1990.

[12] D. H. Janzen, "Why fruits rot, seeds mold, and meat spoils," *The American Naturalist*, vol. 111, no. 980, pp. 691–713, 1977.

[13] J. K. Müller, A. K. Eggert, and J. Dressel, "Intraspecific brood parasitism in the burying beetle, *Necrophorus vespilloides* (Coleoptera: Silphidae)," *Animal Behaviour*, vol. 40, no. 3, pp. 491–499, 1990.

[14] J. K. Müller, "Replacement of a lost clutch: a strategy for optimal resource utilization in *Necrophorus vespilloides* (Coleoptera: Silphidae)," *Ethology*, vol. 76, pp. 74–80, 1987.

[15] J. Bartlett, "Filial cannibalism in burying beetles," *Behavioral Ecology and Sociobiology*, vol. 21, no. 3, pp. 179–183, 1987.

[16] J. K. Müller and A. K. Eggert, "Time-dependent shifts between infanticidal and parental behavior in female burying beetles a mechanism of indirect mother-offspring recognition," *Behavioral Ecology and Sociobiology*, vol. 27, no. 1, pp. 11–16, 1990.

[17] C. W. Beninger and S. B. Peck, "Temporal and spatial patterns of resource use among *Nicrophorus* carrion beetles (Coleoptera: Silphidae) in a Sphagnum bog and adjacent forest near Ottawa, Canada," *Canadian Entomologist*, vol. 124, no. 1, pp. 79–86, 1992.

[18] T. N. Sherratt, D. M. Wilkinson, and R. S. Bain, "Why fruits rot, seeds mold and meat spoils: a reappraisal," *Ecological Modelling*, vol. 192, no. 3-4, pp. 618–626, 2006.

[19] D. E. Rozen, D. J. P. Engelmoer, and P. T. Smiseth, "Antimicrobial strategies in burying beetles breeding on carrion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 46, pp. 17890–17895, 2008.

[20] C. L. Hall, N. K. Wadsworth, D. R. Howard et al., "Inhibition of microorganisms on a carrion breeding resource: the antimicrobial peptide activity of burying beetle (Coleoptera: Silphidae) oral and anal secretions," *Environmental Entomology*, vol. 40, no. 3, pp. 669–678, 2011.

[21] W. W. Hoback, A. A. Bishop, J. Kroemer, J. Scalzitti, and J. J. Shaffer, "Differences among antimicrobial properties of carrion beetle secretions reflect phylogeny and ecology," *Journal of Chemical Ecology*, vol. 30, no. 4, pp. 719–729, 2004.

[22] R. L. Rana, W. Wyatt Hoback, N. A. A. Rahim, J. Bedick, and D. W. Stanley, "Pre-oral digestion: a phospholipase A_2 associated with oral secretions in adult burying beetles, *Nicrophorus marginatus*," *Comparative Biochemistry and Physiology B*, vol. 118, no. 2, pp. 375–380, 1997.

[23] S. Suzuki, "Suppression of fungal development on carcasses by the burying beetle *Nicrophorus quadripunctatus* (Coleoptera: Silphidae)," *Entomological Science*, vol. 4, no. 4, pp. 403–405, 2001.

[24] B. J. Jacques, S. Akahane, M. Abe, W. Middleton, W. W. Hoback, and J. J. Shaffer, "Temperature and food availability differentially affect the production of antimicrobial compounds in oral secretions produced by two species of burying beetle," *Journal of chemical ecology*, vol. 35, no. 8, pp. 871–877, 2009.

[25] S. C. Cotter and R. M. Kilner, "Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*," *Journal of Animal Ecology*, vol. 79, no. 1, pp. 35–43, 2010.

[26] W. Hwang and S. F. Shiao, "Use of carcass by burying beetles *Nicrophorus nepalensis* Hope (Coleoptera: Silphidae)," *Formosan Entomologist*, vol. 28, no. 2, pp. 87–100, 2008.

[27] M. P. Scott, "The ecology and behavior of burying beetles," *Annual Review of Entomology*, vol. 43, pp. 595–618, 1998.

[28] A.-K. Eggert and J. K. Müller, "Joint breeding in female burying beetles," *Behavioral Ecology and Sociobiology*, vol. 31, no. 4, pp. 237–242, 1992.

[29] S. T. Trumbo, "Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (Nicrophorus)," *Ecological Entomology*, vol. 17, no. 3, pp. 289–298, 1992.

[30] M. P. Scott, "Competition with flies promotes communal breeding in the burying beetle, *Nicrophorus tomentosus*," *Behavioral Ecology and Sociobiology*, vol. 34, no. 5, pp. 367–373, 1994.

[31] M. P. Scott, W. J. Lee, and E. D. Van Der Reijden, "The frequency and fitness consequences of communal breeding in a natural population of burying beetles: a test of reproductive skew," *Ecological Entomology*, vol. 32, no. 6, pp. 651–661, 2007.

[32] M. P. Scott, "The benefit of paternal assistance in intra- and interspecific competition for the burying beetle, *Nicrophorus defodiens*," *Ethology Ecology and Evolution*, vol. 6, no. 4, pp. 537–543, 1994.

[33] J. K. Müller, A. K. Eggert, and S. K. Sakaluk, "Carcass maintenance and biparental brood care in burying beetles: are males redundant?" *Ecological Entomology*, vol. 23, no. 2, pp. 195–200, 1998.

[34] G. S. Halffter, C. Huerta, and A. Huerta, "Nidification des *Nicrophorus* (Col. Silphidae)," *Bulletin de la Société Entomologique de France*, vol. 88, pp. 648–666, 1983.

[35] J. Bartlett, "Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae)," *Behavioral Ecology and Sociobiology*, vol. 23, no. 5, pp. 297–303, 1988.

[36] S. T. Trumbo, "Reproductive benefits and the duration of paternal care in a biparental burying beetle, *Necrophorus orbicollis*," *Behaviour*, vol. 117, no. 1-2, pp. 82–105, 1991.

[37] I. C. Robertson, "Nest intrusions, infanticide, and parental care in the burying beetle, *Nicrophorus orbicollis* (Coleoptera: Silphidae)," *Journal of Zoology*, vol. 231, no. 4, pp. 583–593, 1993.

[38] S. T. Trumbo, "Interspecific competition, brood parasitism, and the evolution of biparental cooperation in burying beetles," *Oikos*, vol. 69, no. 2, pp. 241–249, 1994.

[39] I. A. Fetherston, M. P. Scott, and J. F. A. Traniello, "Behavioural compensation for mate loss in the burying beetle *Nicrophorus orbicollis*," *Animal Behaviour*, vol. 47, no. 4, pp. 777–785, 1994.

[40] D. O. Carter, D. Yellowlees, and M. Tibbett, "Cadaver decomposition in terrestrial ecosystems," *Naturwissenschaften*, vol. 94, no. 1, pp. 12–24, 2007.

[41] L. J. Milne and M. J. Milne, "The social behavior of the burying beetles," *Scientific American*, vol. 235, pp. 84–89, 1976.

[42] D. H. Larone, *Medically Important Fungi: A Guide to Identification*, ASM Press, Washington, DC, USA, 4th edition, 2002.

[43] C. M. S. Kumar, K. Jeyaram, and H. B. Singh, "First record of the entomopathogenic fungus Entomophaga aulicae on the Bihar hairy caterpillar Spilarctia obliqua in Manipur, India," *Phytoparasitica*, vol. 39, no. 1, pp. 67–71, 2011.

[44] C. Nielsen and A. E. Hajek, "Diurnal pattern of death and sporulation in Entomophaga maimaiga-infected Lymantria dispar," *Entomologia Experimentalis et Applicata*, vol. 118, no. 3, pp. 237–243, 2006.

[45] X. L. Wang and Y. J. Yao, "Host insect species of ophiocordyceps sinensis: a review," *ZooKeys*, vol. 127, pp. 43–59, 2011.

[46] A. Hagn, K. Pritsch, M. Schloter, and J. C. Munch, "Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of Trichoderma spp.," *Biology and Fertility of Soils*, vol. 38, no. 4, pp. 236–244, 2003.

[47] D. J. Roiger, S. N. Jeffers, and R. W. Caldwell, "Occurrence of Trichoderma species in apple orchard and woodland soils," *Soil Biology and Biochemistry*, vol. 23, no. 4, pp. 353–359, 1991.

[48] T. Degenkolb, R. A. Düring, and A. Vilcinskas, "Secondary metabolites released by the burying beetle *Nicrophorus vespilloides*: chemical analyses and possible ecological functions," *Journal of Chemical Ecology*, vol. 37, no. 7, pp. 724–735, 2011.

[49] S. T. Trumbo, "Defending young biparentally: female risk-taking with and without a male in the burying beetle, *Nicrophorus pustulatus*," *Behavioral Ecology and Sociobiology*, vol. 61, no. 11, pp. 1717–1723, 2007.

[50] I. A. Fetherston, M. P. Scott, and J. F. A. Traniello, "Parental care in burying beetles: the organization of male and female brood-care behavior," *Ethology*, vol. 95, no. 3, pp. 179–190, 1990.

[51] E. V. Jenkins, C. Morris, and S. Blackman, "Delayed benefits of paternal care in the burying beetle *Nicrophorus vespilloides*," *Animal Behaviour*, vol. 60, no. 4, pp. 443–451, 2000.

[52] P. T. Smiseth and A. J. Moore, "Behavioral dynamics between caring males and females in a beetle with facultative biparental care," *Behavioral Ecology*, vol. 15, no. 4, pp. 621–628, 2004.

[53] P. T. Smiseth, C. Dawson, E. Varley, and A. J. Moore, "How do caring parents respond to mate loss? Differential response by males and females," *Animal Behaviour*, vol. 69, no. 3, pp. 551–559, 2005.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 465108, 11 pages
http://dx.doi.org/10.1155/2013/465108

Research Article

Ecological Observations of Native *Geocoris pallens* and *G. punctipes* Populations in the Great Basin Desert of Southwestern Utah

Meredith C. Schuman, Danny Kessler, and Ian T. Baldwin

Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, 07745 Jena, Germany

Correspondence should be addressed to Ian T. Baldwin; baldwin@ice.mpg.de

Received 5 November 2012; Accepted 16 April 2013

Academic Editor: David G. James

Big-eyed bugs (*Geocoris* spp. Fallén, Hemiptera: Lygaeidae) are ubiquitous, omnivorous insect predators whose plant feeding behavior raises the question of whether they benefit or harm plants. However, several studies have investigated both the potential of *Geocoris* spp. to serve as biological control agents in agriculture and their importance as agents of plant indirect defense in nature. These studies have demonstrated that *Geocoris* spp. effectively reduce herbivore populations and increase plant yield. Previous work has also indicated that *Geocoris* spp. respond to visual and olfactory cues when foraging and choosing their prey and that associative learning of prey and plant cues informs their foraging strategies. For these reasons, *Geocoris* spp. have become models for the study of tritrophic plant-herbivore-predator interactions. Here, we present detailed images and ecological observations of *G. pallens* Stål and *G. punctipes* (Say) native to the Great Basin Desert of southwestern Utah, including observations of their life histories and color morphs, dynamics of their predatory feeding behavior and prey choice over space and time, and novel aspects of *Geocoris* spp.'s relationships to their host plants. These observations open up new areas to be explored regarding the behavior of *Geocoris* spp. and their interactions with plant and herbivore populations.

1. Introduction

Geocoris spp. Fallén (Hemiptera: Lygaeidae), commonly known as big-eyed bugs, are generalist insect omnivores which occur naturally worldwide [1]. *Geocoris* spp. are well known to prey on a variety of insects, including several economically important agricultural pests [1, 2] but have also been reported to feed on plant material [1–6], particularly seeds [1, 5, 7]. Several studies in laboratories [1, 4, 6, 8–14], agricultural fields [1, 8, 15–21], and natural habitats [22–31] have investigated the potential of multiple *Geocoris* spp.—including *G. bullatus* (Say) [1], *G. ochropterus* (Fieber) [10], *G. pallens* Stål [1, 20–22, 24, 26–31], *G. proteus* Distant [32], *G. punctipes* (Say) [4, 6, 9, 11–18, 20, 21, 33], *G. uliginosus* (Say) [16, 19, 33], and *G. varius* (Uhler) [32]—to serve as biological control agents to protect plants against herbivores. These studies have found that individual *Geocoris* spp. accept a variety of insect prey, and the field studies have also shown that *Geocoris* spp. reduce herbivore populations [1, 15, 17, 18, 20–24, 26–29] (but see [25]) and increase plant yield [23, 31]. Thus, despite plant feeding, the net effect of *Geocoris* spp.-plant interactions is usually beneficial to plants [34], and *Geocoris* spp. can be effective biological control agents in many agricultural systems. The most important consequence of plant feeding by *Geocoris* spp. may be that it renders them more directly susceptible to agricultural pesticides [6].

Geocoris spp. adults lay their eggs on plants in nature, or on moist cotton or paper cellulose in the laboratory. Life history traits have been characterized in laboratory colonies of *G. atricolor* Montandon [35], *G. bullatus* [1], *G. lubra* Kirkaldy [36], *G. pallens* [1, 35], and *G. punctipes* [35, 37]. The speed of development from egg to adult correlates positively with temperature between 21°C and 37°C; outside this range, eggs are not viable [1, 35, 36]. The photoperiod associated with the most rapid development differs among species; the photoperiod for which development is slowest may correspond to the diapause-inducing photoperiod for a species [36]. Eggs hatch after ca. 1–3 weeks depending on temperature (higher

temperature = faster development) [1, 36, 37], and nymphs develop through five stages over ca. 1 month before reaching adulthood [36, 37]; nymph viability was found to be higher at 27°C than at 24°C for *G. lubra*, but higher at 24°C than at 27°C for *G. punctipes* [35, 36]. Adults can survive from 1 week to nearly 4 months in captivity [37].

Geocoris spp. feed on a combination of insect prey and plant material [1, 2, 4, 5, 7]. They can survive if given a water source and either insect prey or plant seeds, but diets combining insects with seeds or seed pods decrease development time and increase survival rates and fecundity; *Geocoris* spp. may even require seeds or seed pods in order to complete development [1, 5, 38]. This may be due in part to the fact that *Geocoris* spp. prey on many different insects of varying nutritional value. Interestingly, although *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) eggs are higher quality food for *G. punctipes* than are *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), *G. punctipes* more often preyed on *A. pisum* in choice tests [5, 38]. Seed pods and seeds are thus important nutritional resources for *Geocoris* spp. [1, 2, 5, 7, 38]. However, because leaf feeding has not been shown to increase survival in comparison to a water-only diet, it is thought that leaves serve only as a water source [3, 4].

We study the ecological interactions of the wild tobacco *Nicotiana attenuata* Torr. ex S. Watson (Solanales: Solanaceae) in its native habitat, the Great Basin Desert of the southwestern USA. The postfire germination behavior of *N. attenuata* creates large monocultures of plants that host a diverse insect herbivore community [39]. This herbivore community includes several specialists on Solanaceae: *Corimelaena extensa* Uhler (Hemiptera: Thyreocoridae) [39], *Epitrix hirtipennis* (Melsheimer) and *E. subcrinita* LeConte (Coleoptera: Chrysomelidae) [28], *Manduca quinquemaculata* (Haworth) and *M. sexta* (Linnaeus) (Lepidoptera: Sphingidae), and *Tupiocoris notatus* (Distant) (Hemiptera: Miridae) [23]; the generalist herbivores *Spodoptera* spp. Guenée (Lepidoptera: Noctuidae) and *Trimerotropis* spp. (Orthoptera: Acrididae) [40]; and opportunistic herbivores which attack only poorly-defended plants, such as *Empoasca* spp. Walsh (Hemiptera: Cicadellidae) [41, 42] and *Heliothis* spp. Ochsenheimer (Lepidoptera: Noctuidae) [43]. *G. pallens* is a common predator of herbivores on *N. attenuata*, and both *G. pallens* and *G. punctipes* can be found on *N. attenuata* or neighboring plant species during *N. attenuata*'s growing season [22, 31]. *Geocoris* spp. respond to volatiles emitted from *N. attenuata* after herbivory by removing more herbivores from emitting plants [22, 26, 27, 29–31], resulting in a fitness benefit for plants [31].

Here, we present quantitative and qualitative observations and high-resolution images of morphology and behavior for *G. pallens* and *G. punctipes* co-occurring with *N. attenuata*. We have observed aspects of the life history, host plant, and insect prey preferences of both species. For *G. pallens*, we have also made detailed recordings of feeding behavior with a high-resolution macro lens (courtesy of A. Shillabeer with Merit Motion Pictures); quantified variation in predation activity of subpopulations with respect to the lepidopteran herbivore *M. sexta*; assayed the inclination of different generations of nymphs and adults from a single wild population to feed on *M. sexta* over a season; recorded increased occurrence of *Geocoris pallens* on wilting *N. attenuata* plants; and demonstrated that *G. pallens* can, in fact, survive when provided only with water and vegetative plant tissue.

2. Methods

2.1. Study Sites and Insect Collections. *Geocoris pallens* and *G. punctipes* were assayed in and collected from Lytle Preserve in the Great Basin Desert of southwestern Utah, USA (latitude 37.146, longitude −114.020), where we have annual field plantations of *N. attenuata*, and from a nearby location where a native *N. attenuata* population could be found from 2007 to 2009 after a 2006 burn (latitude 37.077, longitude −113.833). In May and June 2009, we collected adults and nymphs of *G. pallens* from the native *N. attenuata* population (four collections of 73 insects (19% adults), 31 insects (71%), 99 insects (58%), and 107 insects (95%)), allowed adults to mate and lay eggs, and observed the eggs through development to adults. These collections, together with ca. 100 insects collected from Lytle and a nearby wash, were used to start a colony of *G. pallens* at our institute in Jena, Germany. This colony has since received annual inputs from field collections. *G. punctipes* adults were also collected in June 2009 and used to start a colony in Jena. The colonies are fed a diet of Nutrimac (sterilized *Ephestia kuehniella* eggs, Biobest N. V.), *Manduca sexta* and *Spodoptera littoralis* (Boisduval) eggs and larvae, and *N. attenuata* green tissue and seeds, with additional water provided using moist dental rolls in microcentrifuge tubes containing tap water. They are kept in 9 L food-quality plastic boxes (Lock & Lock) with two holes in each lid of ca. 8 cm diameter each covered with a fine mesh, and containing paper towels to provide structure for oviposition and hiding places, inside a growth chamber (Snijders Scientific, http://www.snijders-scientific.nl/cooling-and-freezing-systems/) with 16 h D/8 h N (06:00–22:00 D/22:00–06:00 N), 26/22°C, daylight provided by Osram L 36 W/77 fluorescent lamps (http://www.osram.com/) at 50% power, 65% RH, and ventilation by PAPST type 4656 N fans (http://www.ebmpapst.com/en/).

2.2. Images of Geocoris pallens and G. punctipes. Pictures were taken of insects collected from the native *N. attenuata* population in 2009 (Figures 1, 2, and 3) or from Lytle in 2011 (Figure 4). Images in Figures 1–3 are from an Axiocam HRc connected to a stereomicroscope SV 11 and captured with AxioVision 4.0 software (Zeiss, http://corporate.zeiss.com/gateway/en_de/home.html; Figure 1, Figure 2 instar 3 and adult), or from a Powershot SD1000 camera (Canon, Inc., http://www.usa.canon.com/cusa/home; Figure 2 instars 1, 2, 4, and 5; Figure 3). Images in Figure 4 were taken with a probe lens (Innovision Optics, http://www.innovision-optics.com/) by A. Shillabeer and kindly provided by Merit Motion Pictures (Winnipeg, MB, Canada). The probe lens permits the capture of HD macro images with an unusually large depth of field.

2.3. Egg Predation Assays. Although *M. quinquemaculata* and *M. sexta* moths oviposit in native *N. attenuata* populations, the number of eggs is usually not sufficient for

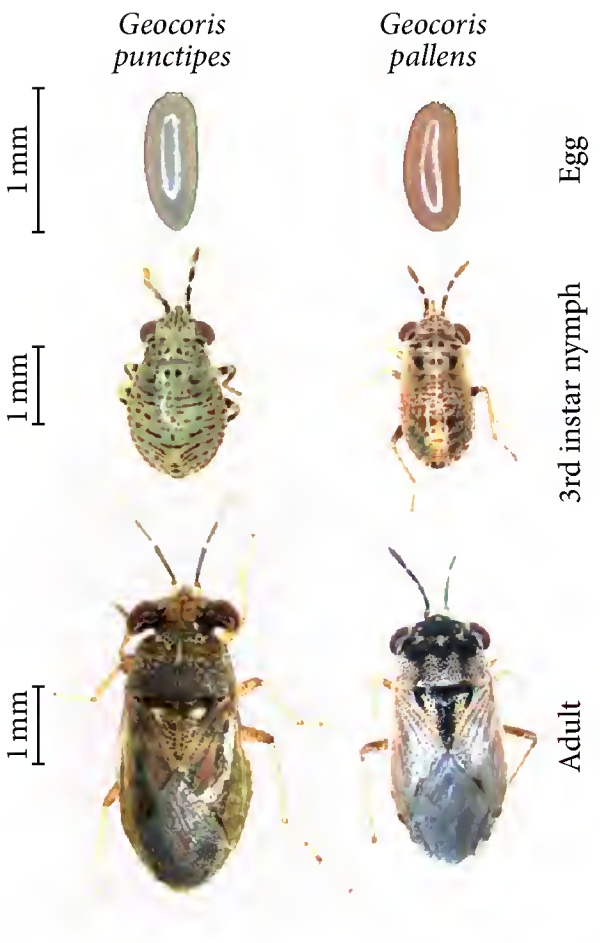


FIGURE 1: Comparison between *Geocoris punctipes* and *G. pallens* collected from the Great Basin Desert in southwestern Utah.

experiments except in outbreak years. Thus, we use *M. sexta* eggs and larvae from lab colonies for many field assays. In June 2007, *M. sexta* eggs purchased from North Carolina State University were frozen to kill developing larvae and thus prevent hatching, then thawed and used to assay native *Geocoris* spp. predation activity in a wild population of *Nicotiana attenuata* growing on a recent burn (see Section 2.1). Five eggs were glued with an α-cellulose glue (KVS, Leuna, Germany)—which does not damage plants, induce volatile emission, or prevent egg predation—to the underside of a similarly sized, intact lower stem leaf in a standardized position (as in [22]) on 35 plants per location, in three locations within the native *N. attenuata* population (Figure 5). After 36 h, empty eggs with intact shells containing visible puncture holes typical of *Geocoris* feeding (Figure 4(c)) were counted as predated, and intact eggs were counted as non-predated. Missing eggs were not included in counts.

2.4. "Feeder/Non-Feeder" Assays. We observed in many years that *G. pallens* prey on small bugs from invasive stork's bill ground cover plants (*Erodium cicutarium* (L.) L'Hér. ex Aiton, Geraniales: Geraniaceae) in April and May and move to *N. attenuata* plants later in the spring, as *E. cicutarium* plants are drying up. On *N. attenuata*, *G. pallens* prey on flea beetles (*Epitrix* spp.), which are usually the first herbivores on *N. attenuata*, and mirids (*T. notatus*) which arrive on *N. attenuata* as plants begin to elongate. If *Manduca* spp. moths oviposit on *N. attenuata* (which they often do when pollinating flowers), *G. pallens* will begin to eat *Manduca* spp. eggs and young larvae [23, 30]. We conducted feeding assays to quantify the tendency of two generations of *G. pallens* nymphs and adults to feed on *M. sexta* eggs (Figure 6). On four separate days in May and June 2009, *G. pallens* were collected from a native *N. attenuata* population (see Section 2.1); Table 1 shows the distribution of adults and nymphs in each collection. May collections were tested at the field station immediately after collection, and June collections were tested after transportation to the laboratory in Germany, within 48 h after collection (during which *G. pallens* individuals had access to a variety of field-collected plant and insect food and could adapt to the new conditions). Each individual was put with a piece of damp cotton and a single *M. sexta* egg into a 30 mL Dixie plastic cup (http://www.dixie.com/) with a lid containing small air holes and left for 72 h; cups were kept by a window in a shaded travel trailer at the field station (May assays) or in a laboratory (June assays), and water from an underground spring (May assays) or from a tap (June assays) was added to the cotton daily. *G. pallens* individuals which had eaten the egg within 72 h were counted as feeders, and those which had not were counted as nonfeeders. From the June 15th collection, two of the *M. sexta* eggs hatched and the larvae were eaten; these *G. pallens* were also counted as feeders. Native *Manduca* spp. oviposition in the field at this time was not sufficient for feeding assays, and the *M. sexta* for the May assays in 2009 were kindly provided by C. Miles of the State University of New York at Binghamton; the *M. sexta* for June assays came from an in-house colony of the same original stock as the Binghamton colony.

2.5. G. pallens Populations around Wilting versus Healthy Plants. In several years we observed *Geocoris* spp. individuals associated with diseased or damaged *N. attenuata* plants which began to wilt. In 2012, when a massive disease outbreak occurred which killed a huge number of plants, we investigated this phenomenon by counting the presence of *Geocoris* spp. on dying plants versus the two nearest healthy plants (Figure 7). On two days in May 2012 we searched for wilting plants and directly checked for the presence of *Geocoris* spp. and then checked the nearest neighboring healthy plant (approximately 1-2 m away) and the second nearest neighboring healthy plant (approximately 2-3 m away) for *Geocoris* spp. presence. Except for one plant, only single *G. pallens* individuals were found on plants.

2.6. G. pallens Survival on Leaf Tissue and Water versus Water Alone. Geocoris spp. have been reported to feed on seeds and insects. In 2006, to test the potential of *G. pallens* to survive on leaf tissue, we conducted a feeding assay in which *G. pallens* adults collected in Lytle were offered either water from an underground spring, or spring water and an *N. attenuata* leaf (Figure 8). Each individual ($n = 12$ collected immediately prior to the start of the assay) was caged in a 50 mL food-quality plastic container (Huhtamaki; http://www.huhtamaki.com/) secured with miniature claw-style hair clips and padded on the rim with foam to avoid damaging plant leaves. These "clip cages" contained a cotton ball moistened with spring water or the moist cotton ball and part of an *N. attenuata* leaf. The leaf was still attached to a living plant and thus did not have to be replaced for the duration of the experiment. The plant did not harbor any insect herbivores. The cotton ball with water was exchanged every second day. Mortality was monitored once a day at noon.

FIGURE 2: Larval and adult stages of *G. pallens* from the Great Basin Desert in southwestern Utah. *Geocoris* spp. have five nymphal instars.

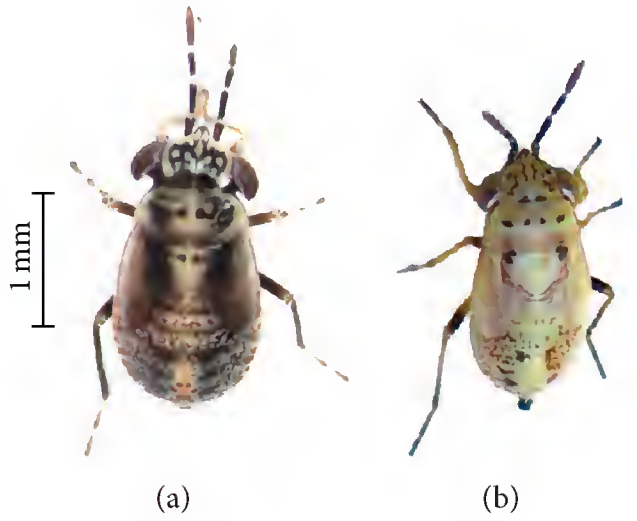


FIGURE 3: Color morphs of *G. pallens* nymphs. The dark (a) and the more common light (b) color morph are shown in the fourth instar. Size differences are not characteristic of the morphs but are rather due to individual differences.

2.7. Statistics. Fisher's exact tests conducted using a spreadsheet (J. H. Macdonald, http://udel.edu/~mcdonald/statfishers.html) for Excel (Microsoft) [44] were used to compare counts of predated eggs, *M. sexta*-feeding *G. pallens*, plants harboring *G. pallens* individuals, and *G. pallens* individuals surviving on a water-only versus water and live leaf diet. When necessary, Bonferroni post hoc corrections were calculated using Excel to correct for multiple testing.

3. Results

3.1. Geocoris pallens and G. punctipes Populations at the Study Site. *G. pallens* and *G. punctipes* can be easily distinguished by differences in the coloration of their eggs and by the size and coloration of their nymph and adult stages (Figures 1 and 2). In 10 years of field research at Lytle Preserve and in the surrounding areas, we have almost exclusively found *G. pallens* associated with invasive *Erodium cicutarium* (L.) L'Hér. ex Aiton (Geraniales: Geraniaceae) plants and alfalfa (*Medicago sativa* Linnaeus, Fabales: Fabaceae) plantations in the early spring (mid-April to mid-May) and with *N. attenuata* plants in the late spring to summer (from the end of May). In contrast, we have observed *G. punctipes* primarily on *Cucurbita foetidissima* Kunth in Humb. (Cucurbitales: Cucurbitaceae) and *Datura wrightii* Regel (Solanales: Solanaceae) plants. We have not observed *Geocoris* spp. in areas where ants are abundant.

Our observations over several years indicate that the main food for *G. punctipes* on *C. foetidissima* is *Empoasca* spp., and on *D. wrightii*, *Lema trilineata* (Olivier) (Coleoptera: Chrysomelidae) eggs and *Manduca* spp. eggs and larvae. The main foods for *G. pallens* on *N. attenuata* appear to be *Epitrix* spp., *T. notatus*, *Manduca* spp. eggs and young larvae depending on their abundance, and, when plants are setting seed, *C. extensa*, the seed-feeding negro bug. On *N. attenuata*, *G. pallens* begin by eating primarily flea beetles (*Epitrix* spp.), which are usually the first herbivores on *N. attenuata*, and mirids (*T. notatus*) which arrive on *N. attenuata* as plants begin to elongate but will switch to eating *M. sexta* and *M. quinquemaculata* eggs and young larvae when *Manduca* spp. are abundant, usually after *N. attenuata* begins to flower and attract *Manduca* spp. as pollinators [23, 30]. We have also found *G. pallens* sheltering in open *N. attenuata* seed capsules overnight and eating ripe seed (M. C. Schuman and M. Stanton, observation). In 2008–2010 we observed that the number of *Manduca* spp. eggs preyed on *Geocoris* spp. increased in locations which received oviposition from native *Manduca* spp. moths (2008, M. C. Schuman and S. Allmann, observation; 2009, [30]; 2010, I. T. Baldwin and C. Diezel, observation). In 2011, we found that *Geocoris* spp. began to prey on *M. sexta* larvae within 24 h after plants were experimentally infested with larvae, in the absence of wild *Manduca* spp. oviposition [31].

We have observed that *Geocoris* spp. adults emerge from overwintering sites in March and April and lay eggs which hatch in May, giving rise to a second generation; the adults of this second generation overwinter to the following year. We have generated laboratory colonies of both *Geocoris* species from field collections. *G. punctipes* can be easily reared in captivity, and there are multiple other colonies of this species in captivity, primarily for use in biological control [4, 6, 9, 11–18, 20, 21, 33]. The *Geocoris* spp. in our colonies have similar developmental and survival times as reported in the literature (ca. 1 month for nymph development and 1–3 months survival as adults, see Section 1), and adults reproduce year-round.

3.2. Developmental Stages and Color Morphs of G. pallens. In the 2009 field collections of *G. pallens* we observed five

FIGURE 4: *G. pallens* feeding on a *Manduca sexta* egg (a)–(c) or larva (d)–(f). Note the flexible stylet clearly visible inside the egg in (c). Copyright: Merit Motion Pictures, Winnipeg, MB, Canada.

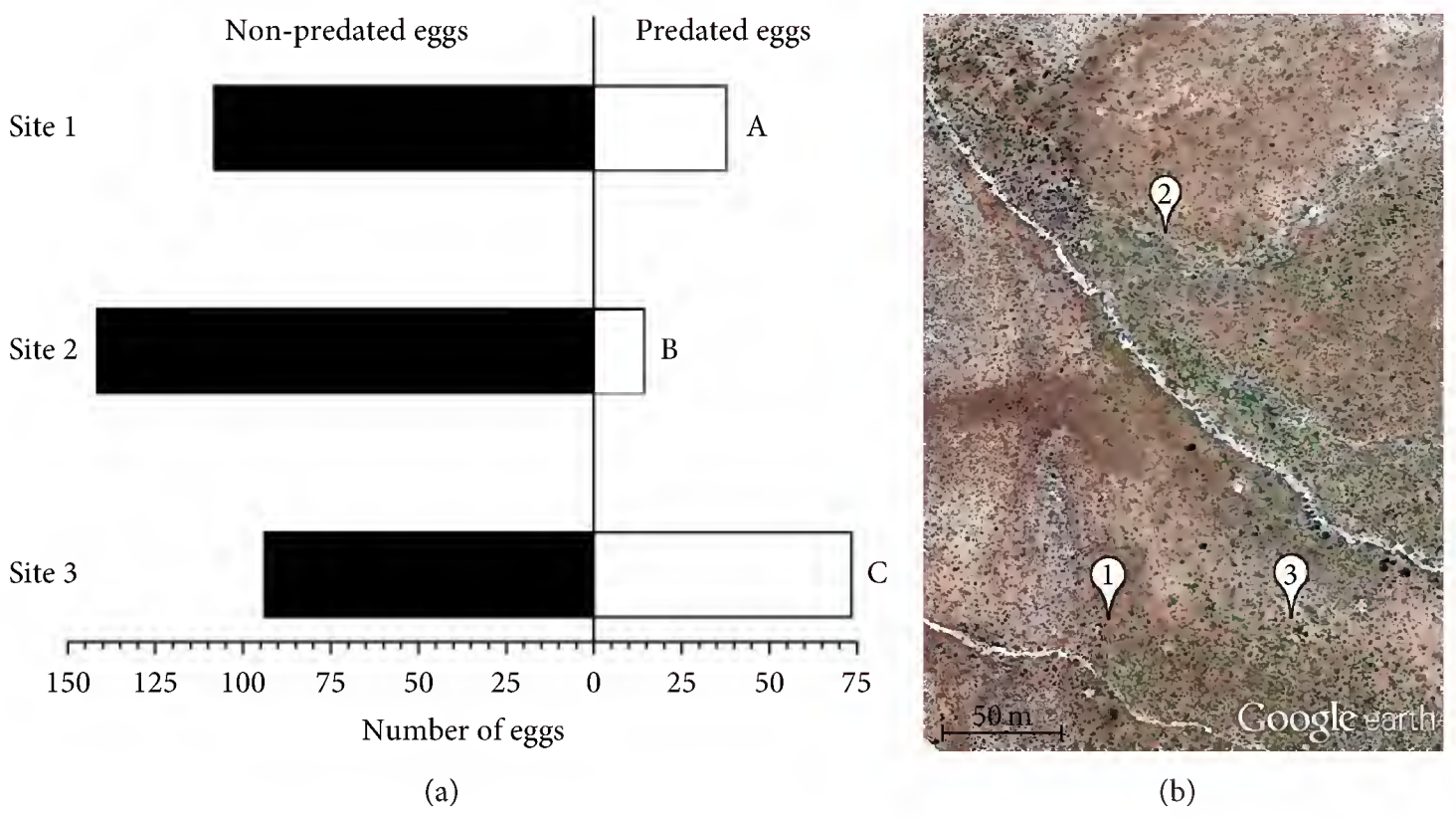


FIGURE 5: *Geocoris* spp. predation activity differs among sites within a native *N. attenuata* population. (a) The graph shows numbers of *M. sexta* eggs predated over 36 h by *Geocoris* spp. Letters indicate significant differences between sites in Bonferroni-corrected pairwise Fisher's exact tests, $P < 0.003$. (b) Sites were clusters of plants ca. 50–100 m apart.

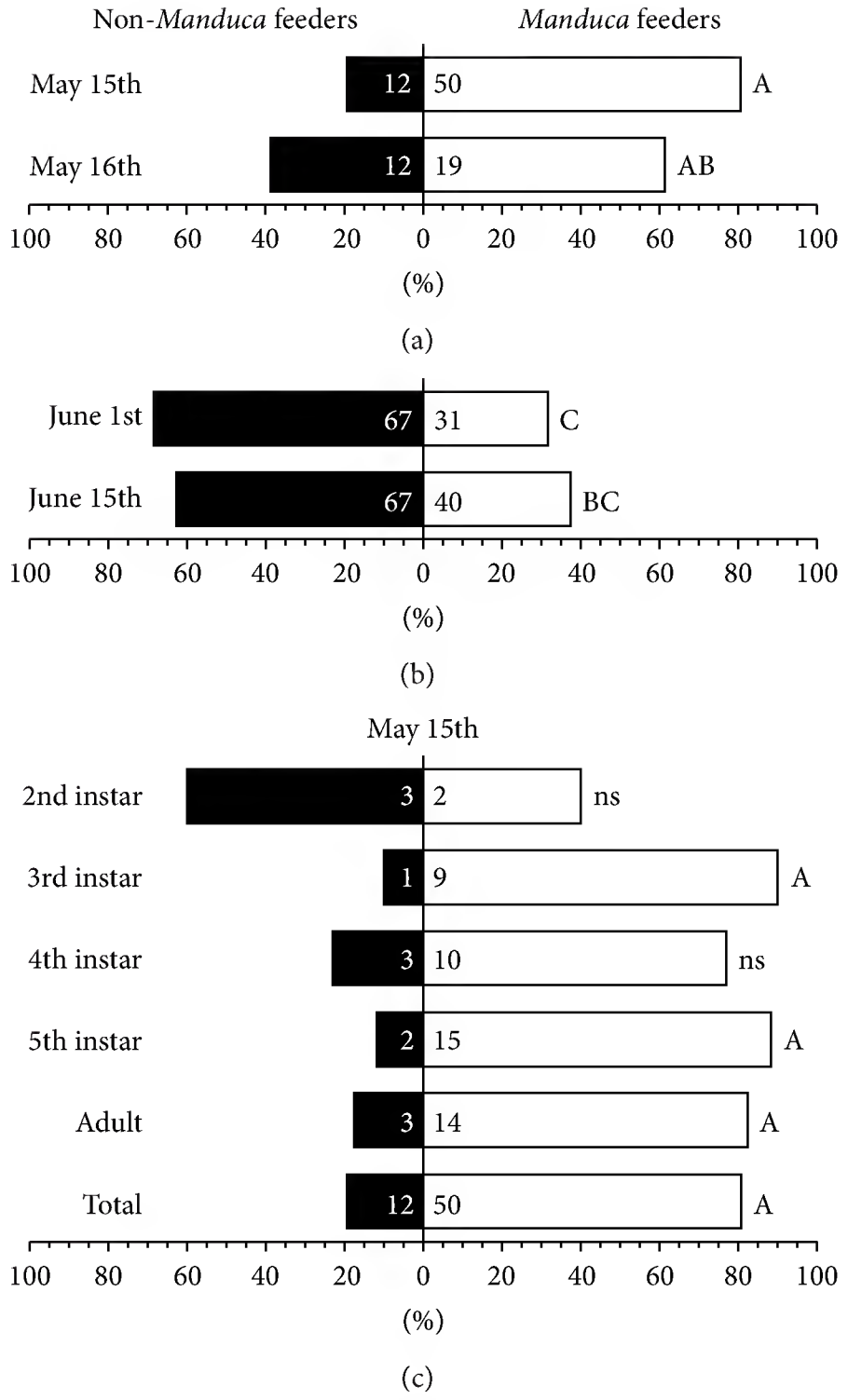


Figure 6: *G. pallens* collected from a single wild *N. attenuata* population vary in their tendency to eat *M. sexta* eggs, but different stages in a single collection do not. Graphs show percentages of *G. pallens* in collections which ate *M. sexta* eggs within 72 h in no-choice assays; counts are in bars. Letters indicate significant differences in Bonferroni-corrected pairwise Fisher's exact tests across all groups, $P < 0.05$; ns: no significant difference to any other group. (a) Individuals collected in May and tested immediately after collection show a similar tendency to eat *M. sexta* eggs (61–81%). (b) Individuals collected at two dates in June and tested 24–48 h later, after transportation to a laboratory and a short adjustment period, also show a similar tendency to eat *M. sexta* eggs (32–37%), although the tendency is lower than for the May collections. This could be due either to a shift in the population's tendency to eat *M. sexta* eggs or to transportation and changed environmental conditions. (c) The May 15th population had a fairly even distribution of different nymphal stages and adults, which did not differ significantly in their tendency to eat *M. sexta* eggs.

nymphal stages (instars) (Figure 2) occasionally present as dark morphs (Figure 3(a)) but dominated by a light morph (Figure 3(b)). Adults from dark and light morphs were able to interbreed, and both morphs have since reoccurred in our colony in Jena, which is propagated from annual field collections in Lytle Preserve and the surrounding areas.

Table 1: Distribution of nymphal and adult stages in *G. pallens* collections tested for their tendency to eat *M. sexta* eggs (Figure 6).

Stage	May 15th	May 16th	June 1st	June 15th
Nymphs				
1	—	—	—	—
2	8.1%	—	1.0%	—
3	16.1%	—	—	—
4	21.0%	—	9.2%	1.9%
5	27.4%	29.0%	21.4%	2.8%
Adults	27.4%	71.0%	68.4%	95.3%
n	**62**	**31**	**98**	**107**

3.3. Feeding Behavior of G. pallens. A. Shillabeer with Merit Motion Pictures filmed one of our field-collected *G. pallens* feeding on *M. sexta* eggs and larvae in high-resolution macro focus (Figure 4). In these pictures, one can clearly see how the proboscis sheath is used to penetrate prey and then bends at three joints, permitting the flexible stylets to emerge and suck out the prey's contents.

3.4. Geocoris spp. Predation Activity Varied Significantly within a Single N. attenuata Population. We found that *Geocoris* spp. predation of *M. sexta* eggs varied significantly for patches in a single wild *N. attenuata* population in 2007 (Figure 5, $n = 146$–167 eggs per site, pairwise Fisher's exact tests followed by a Bonferroni correction for multiple testing: site 1 versus site 2, $P = 0.0002$; site 2 versus site 3, $P < 0.0001$; site 1 versus site 3, $P = 0.0027$). This difference was driven by total *Geocoris* spp. predation activity and not necessarily by the attractiveness of plants for *Geocoris* spp. in each site: between 91% and 100% of plants at each site had at least one egg predated. There were no significant differences among sites in the numbers of plants from which eggs were predated ($P > 0.4$).

3.5. G. pallens Generations Varied in Their Tendency to Eat M. sexta Eggs and Larvae, but Nymphs and Adults Did Not. We tested field collections of *G. pallens* from a native *N. attenuata* population in 2009 for their tendency to eat *M. sexta* eggs or larvae (Figure 6, Table 1). Between 61 and 81% of *G. pallens* collected and tested in the field in mid-May (15th or 16th) ate *M. sexta*. Collections from the same *N. attenuata* population were tested again in June, within 48 h after collection and transport to the lab in Jena. In a collection from June 1st, 32% of individuals ate *M. sexta*, and this increased slightly (but not significantly) to 37% in a collection from June 15th ($n = 31$–107 individuals per collection, pairwise Fisher's exact tests followed by a Bonferroni correction for multiple testing: $P < 0.0001$ for the May 15th versus the June 1st collection, $P = 0.0163$ for the May 16th versus the June 1st collection, $P < 0.0001$ for the May 15th versus the June 15th collection, but $P = 0.0699$ [not significant] for the May 16th versus the June 15th collection). *G. pallens* individuals from collections made within the same month did not significantly differ in their tendency to eat *M. sexta* ($P > 0.2$). Mortality over the course of the 72 h assay was less than 15% and did not

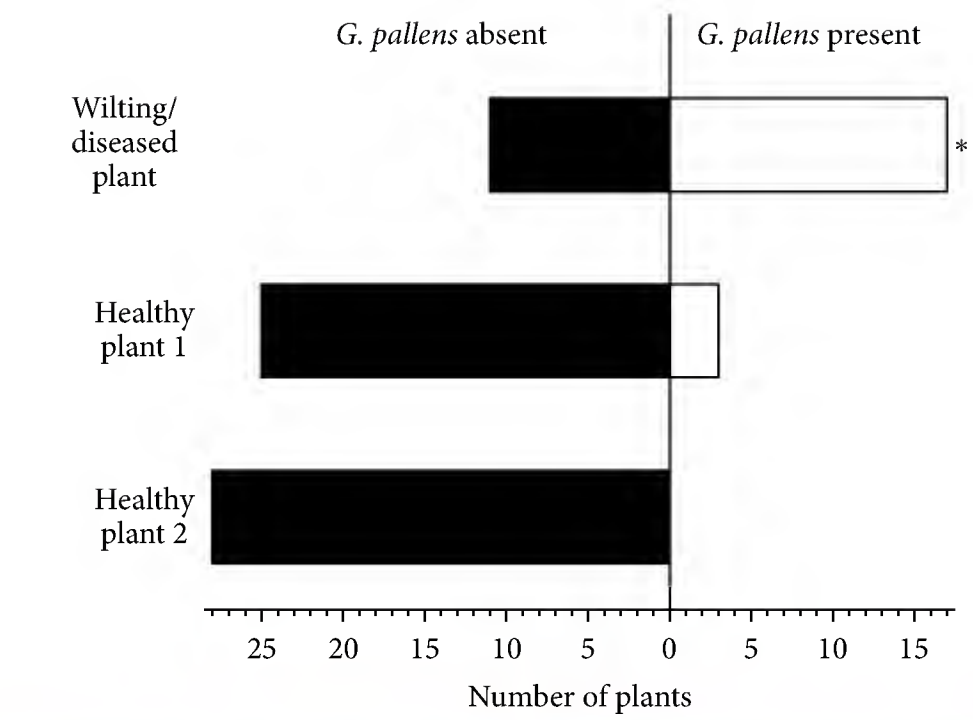


FIGURE 7: *G. pallens* is more likely to be found on wilting plants. The graph shows the number of wilting, diseased, and neighboring healthy plants found to harbor *G. pallens* individuals (a). Healthy plant 1 (b) was on average ca. 1.5 m, and healthy plant 2 was ca. 2-3 m away from the wilting plant (c). The asterisk indicates significant differences between the presence of *G. pallens* in both sets of healthy plants and wilting plants in a Fisher's exact test, $P < 0.0001$.

differ significantly among collections (pairwise Fisher's exact tests followed by a Bonferroni correction for multiple testing, $P > 0.06$).

All collections comprised both adults and nymphs, and the May 15th collection had a particularly good representation of most nymphal stages and adults (Table 1). There was no significant difference among different developmental stages in their tendency to eat *M. sexta* eggs (Figure 6(c), pairwise Fisher's exact tests followed by a Bonferroni correction for multiple testing, $P = 1$), although in the case of second-instar nymphs, which tended to eat fewer eggs, this was likely due to low replicate numbers.

3.6. G. pallens Individuals Associated with Wilting and Diseased Plants. When *N. attenuata* plants wilted in the field due to various stresses, for example, uprooting by wind, cattle damage, or disease, we often observed *Geocoris* spp. around the dying plants. When a fungal disease outbreak killed a large number of *N. attenuata* plants in 2012, we found *G. pallens* more frequently on wilting plants (Figure 7). *G. pallens* was present on 61% of the wilting plants, but only on 11% of the nearest healthy neighboring plants (1.5 m away), and no *Geocoris* spp. could be found on the second-nearest healthy plants (approximately 2-3 m away from wilting plants) in any of 28 replicates (Fisher's exact test of numbers of healthy versus wilting plants harboring *G. pallens*, $P < 0.0001$).

3.7. G. pallens Can Use Leaf Tissue as a Food Source. *G. pallens* adults survived significantly longer if reared on *N. attenuata* leaves and a water-soaked cotton ball than only on the wet cotton ball. After six days without any insect prey, twice as many *G. pallens* individuals died if given only water than if given leaf material and water ($n = 12$ individuals per treatment, Fisher's exact test, $P = 0.0498$; Figure 8). This effect lasted until the end of the experiment after eight days, when all *G. pallens* individuals living only on water had died. After seven days, only four individuals had died if they were allowed to feed on plant diet, while 11 individuals had died in the water-only group ($P = 0.0047$), and after eight days all

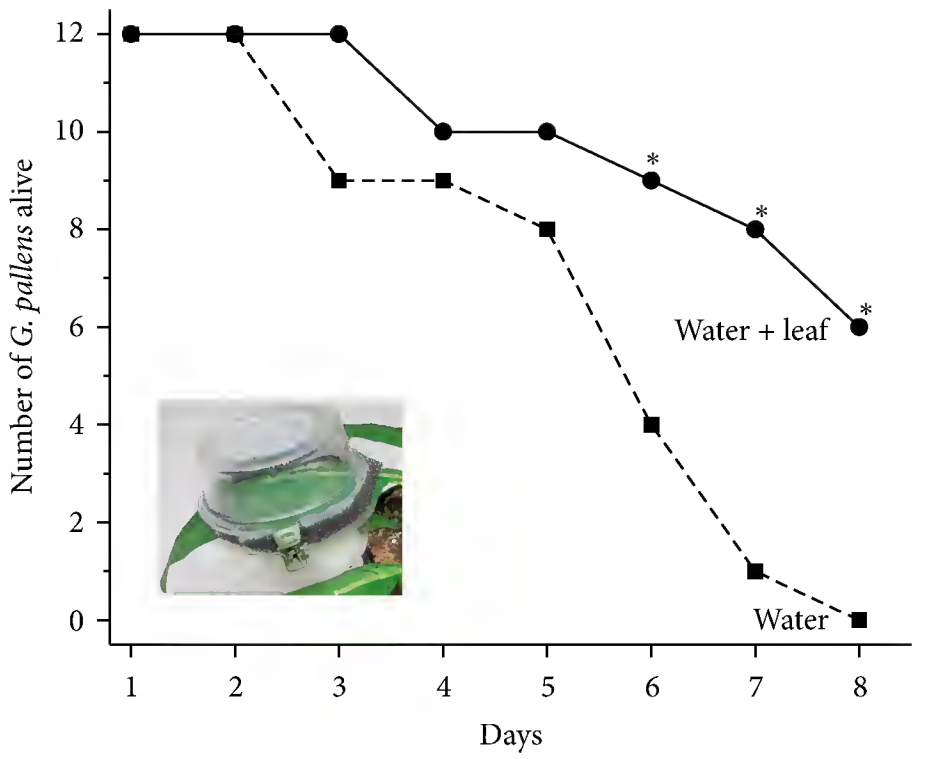


FIGURE 8: Use of *N. attenuata* leaf tissue as a food source by *G. pallens*. The graph shows the number of *G. pallens* individuals surviving in clip cages with either a cotton ball soaked with water (water) or water plus an *N. attenuata* leaf still attached to a living plant (water + leaf). Inset: clip cage on a leaf with a moist cotton ball in the lower right (water + leaf treatment); each half of the cage has a hole covered with netting to permit transpiration (only visible for top half). Asterisks indicate significant differences between treatments obtained by Fisher's exact tests on indicated days, $P < 0.05$.

animals reared only on water had died, while six individuals given *N. attenuata* leaves remained alive ($P = 0.0069$).

4. Discussion

We have observed aspects of the life history, host plant, and insect prey preferences of *G. pallens* and *G. punctipes* that co-occur with the native tobacco *N. attenuata* in the Great Basin Desert of southwestern Utah. For *G. pallens*, we have also captured images of feeding behavior with a high-resolution macro lens (courtesy of A. Shillabeer and Merit Motion Pictures), quantified variation in the predation of one insect prey species, *M. sexta*, in space and time, recorded increased occurrence around wilting or sick *N. attenuata* plants, and demonstrated the ability to survive on only water and vegetative plant tissue, which has not otherwise been demonstrated for any species of *Geocoris*. Furthermore, we describe how we have maintained laboratory colonies of both *Geocoris* species from field collections.

4.1. Geocoris pallens and G. punctipes May Have Overlapping Ranges but Separate Niches in Southwestern Utah. *G. pallens* and *G. punctipes* can be easily distinguished based on size and morphology at all life stages (Figures 1 and 2). We have found these species feeding on a partially overlapping diet of insect prey but almost always on different plant species: *G. pallens* is associated with *E. cicutarium* plants and *M. sativa* plantations in the early spring and with *N. attenuata* plants in the late spring and summer; this apparent host shift is likely due to the fact that *E. cicutarium*, a shallow-rooted ground cover, dries up by the end of May or beginning of June. We have observed *G. punctipes* primarily on *C. foetidissima* and *D. wrightii* plants. Both *Geocoris* spp. will feed on *Manduca sexta* and *M. quinquemaculata* eggs and young larvae, and both eat the same food in our colonies, but in nature their diets may overlap very little except for *Manduca* spp. and the mirid *T. notatus*.

Within the native populations of *G. pallens*, we have observed two color morphs (Figure 3). The light morph seems to be the more prevalent. It would be interesting to know whether the difference in pigmentation is genetically or environmentally based, because dark and light morphs co-occur in the same populations without any obvious differences in microclimate, a genetic basis seems likely. *G. pallens* nymphs and adults spend most of their time foraging on plants and moving between plants over the sandy ground or sheltering in the shade of plants. The dark morph may be better camouflaged in the shade, while the light morph would be better camouflaged on the sunlit sand. Potential behavioral differences associated with the color morphs, however, remain to be investigated. To our knowledge, such a strong color contrast has not been reported as a morphotype in any other species of *Geocoris*.

4.2. G. pallens Predation Activity Varied Significantly within an N. attenuata Population. We found that the number of *M. sexta* eggs predated by *G. pallens* (Figure 4) varied significantly for *N. attenuata* plants in different locations within a single population (Figure 5). This might have been due to local variation in *G. pallens* population density or differences in feeding behavior within a host plant population, perhaps dependent on local *Manduca* spp. oviposition events or differences in the abundance of other prey. We do not know how far *G. pallens* individuals travel in search of prey, but the assay sites we chose were ca. 50–100 m apart, and it is possible that *G. pallens* at the different sites represented local subpopulations with little exchange of individuals between them.

It is also possible that differences in *G. pallens* predation activity were due to differences in *N. attenuata* plant phenotypes. *N. attenuata* plants within a population vary greatly both in neutral genetic markers [45] and in their response to herbivore attack, particularly the volatiles they emit and their degree of induced defense upon herbivore feeding [46]; the variation within populations is as great as the variation between populations in these plant traits [28, 47]. *G. pallens* and *G. punctipes* respond to specific herbivore-induced volatiles of *N. attenuata* [22, 26], but it is not known how quickly or how well they learn to respond to the differing volatile profiles of plants in native populations. The phenomena of associative susceptibility and associative resistance, in which plant traits increase or decrease the herbivore loads of neighboring plants, are widespread in ecological communities [48], and associative susceptibility or resistance due to neighbor volatile emission may contribute to the site-by-site variation in *Geocoris* predation activity.

4.3. G. pallens Prey Choices May Be Learned Anew with Each Generation but Did Not Differ between Nymphs and Adults Tested Simultaneously. We tested field-collected *G. pallens*

adults and nymphs from the same wild population over a season (Table 1) for their inclination to eat *M. sexta* eggs in no-choice assays (Figure 6). Based on known emergence and generation times for these insects, the May collections must have been from the first generation of eggs laid in 2009; we found that 61–81% of the individuals consumed *M. sexta* eggs in no-choice assays. From these collections, we can conclude that there is no significant difference between nymphs and adults in their propensity to eat *M. sexta* eggs, because the May 15th collection comprised 27% adults, whereas the May 16th collection comprised 71% adults (Table 1), and the two collections did not significantly differ in their tendency to eat *M. sexta* eggs (Figure 6(a)). Furthermore, different developmental stages within the May 15th collection also did not differ significantly in their tendency to eat *M. sexta* eggs (Figure 6(c)), although in the case of 2nd instar nymphs this was likely due to low replicate numbers. It should be noted that the composition of nymphs in collections may not accurately reflect the composition of the sampled *G. pallens* population: later nymphal stages and adults are probably overrepresented, because they are easier to see and catch.

The June 1st collection made two weeks later comprised 68% adults; in this collection, the nymphs were certainly the offspring of the May collections, and the adults may have been a mix of May-nymphs and May-offspring. This collection was transported to the lab in Jena for testing, and although they were allowed to adapt for 24–48 h, transport and laboratory conditions may have negatively affected feeding rates. Only 32% of these *G. pallens* fed on *M. sexta* eggs in the same no-choice assay. A final collection made two weeks later (June 15th) and also tested after transport to the laboratory comprised 95% adults, all of which were likely offspring of the May collections. In the June 15th collection, the number of egg feeders had increased slightly to 37%.

The May and June generations may have experienced separate *Manduca* spp. oviposition events which influenced their propensity to eat *M. sexta* eggs. There are typically two *Manduca* spp. oviposition peaks in the Lytle area and surroundings: one at the end of April to the first week of May (mainly on *D. wrightii*) and one in the middle of June (*D. wrightii* and *N. attenuata*). (*Manduca* spp. oviposition, however, occurs to a minor degree also between those two peaks.) Given that lepidopteran eggs are more nutritious for *Geocoris* spp. than aphids [5, 38] and likely other hemipteran prey such as *T. notatus*, it is interesting that *G. pallens* does not always readily eat *M. sexta* eggs but might need to learn to prey on them. *G. punctipes* seem to be strongly influenced by prey mobility rather than nutritional quality [38]; a preference for mobile prey might explain why *G. pallens* does not always seem to recognize *M. sexta* eggs as prey [23, 30]. Perhaps *G. pallens* must first learn to associate *Manduca* spp. eggs with feeding larvae and the associated herbivore-induced plant volatiles.

4.4. G. pallens May Scavenge from Dying Plants. We found *G. pallens* individuals to be significantly (5-fold) more abundant on dying, wilting *N. attenuata* plants than on nearby healthy plants. In fact, in a 2012 plant disease outbreak, *G. pallens* were not found on healthy plants unless they were next to wilting plants. This could be due to greater foraging success for *G. pallens* when hunting insects fleeing from dying plants, or it might be that dying plants are a better nutritional supplement to *G. pallens*'s insect diet, which may also be more nutritious when herbivores feed on dying plants. It has long been known that nutrients, including amino acids, are mobilized from water-stressed and senescing plant tissue, although some reports indicate that the lack of turgor pressure in wilting plants reduces sap flow so that phloem feeders may not be able to access these increased resources [49]. If *G. pallens* are feeding directly from cells or the apoplastic space, they may be able to harvest the products of cellular senescence and degradation from dying plants. A more speculative hypothesis would be that *G. pallens* itself transfers disease when feeding on plant tissue, as is known for herbivores (e.g., [42]). If *T. notatus* is more fecund on diseased plants, as a consequence of impaired host plant resistance, *Geocoris* could benefit from spreading disease and thus increasing the current population of its prey. However, herbivores likely spread plant disease more efficiently than omnivores such as *Geocoris* spp.

4.5. G. pallens Feeds on Seeds and Leaves. Although it has been reported that *Geocoris* spp. feed from vegetative plant tissue, all prior reports indicated that *Geocoris* individuals could not survive any better on vegetative tissue than on water alone [4]. Here, we show that mortality of *G. pallens* individuals offered water and living leaf tissue *on planta* is 50% over 8 d, but for individuals given only water is 100%. The discrepancy between our results and previous results could be due to the appropriateness of the plant tissue for the particular *Geocoris* spp.; differences in nutritional quality of cut leaves [4] versus leaves left on a plant; or even the increased importance of relative humidity provided by leaf cover in a desert environment, which is unlikely to be a factor in a laboratory. We have seen *G. pallens* individuals drinking from *N. attenuata* leaves in wild populations without leaving visible leaf damage (S. Allmann and M. C. Schuman, observation).

5. Conclusions

Wild populations of *G. pallens* change plant hosts and adapt to changes in host quality and herbivore prey abundance over their lifetimes. *G. punctipes*, though co-occurring with *G. pallens*, uses different host plant and herbivore resources than does *G. pallens* in southwestern Utah. *Geocoris* spp. are phenotypically plastic generalists which, though omnivorous, benefit plants by reducing their herbivore loads. These insects have become a model system to study the development of plant-herbivore-predator tritrophic interactions, and how predators learn plant cues, and have great promise as effective biological control agents for agriculture.

Authors' Contributions

Meredith C. Schuman and Danny Kessler contributed equally. Meredith C. Schuman and Danny Kessler conceived and designed studies, performed research, analyzed data, and wrote the paper. Ian T. Baldwin conceived and designed studies, performed research, and wrote the paper.

Acknowledgments

The authors would like to thank A. Shillabeer and Merit Motion Pictures for the film stills of *G. pallens* (Figure 4), S. Allmann for help with *M. sexta* feeding screens (Figure 6), the members of the 2009 Utah field research crew for help with *Geocoris* spp. collections, and Brigham Young University for the use of Lytle Preserve. This work was funded by the International Max Planck Research Schools (MCS), the Max Planck Society (all), and ERC advanced to ITB, number 293926 (all).

References

[1] G. Tamaki and R. E. Weeks, *Biology and Ecology of Two Predators,* Geocoris pallens *Stål and* G. bullatus *(Say)*, vol. 1446 of *Technical Bulletin of the US Deparmtent of Agriculture*, 1972.

[2] A. Stone, "Plant feeding by a predacious insect, *Geocoris punctipes*," *Journal of Economic Entomology*, vol. 63, no. 6, pp. 1911–1915, 1970.

[3] S. Lockwood, "The relation of weeds to insect pests," *California Department of Agriculture Monthly Bulletin*, vol. 22, no. 6, pp. 279–282, 1933.

[4] G. T. York, "Food studies of *Geocoris* spp., predators of the beet leafhopper," *Journal of Economic Entomology*, vol. 37, no. 1, pp. 25–29, 1944.

[5] M. D. Eubanks and R. F. Denno, "The ecological consequences of variation in plants and prey for an omnivorous insect," *Ecology*, vol. 80, no. 4, pp. 1253–1266, 1999.

[6] P. G. Tillman and B. G. Mullinix, "Effect of prey species on plant feeding behavior by the big-eyed bug, *Geocoris punctipes* (Say) (Heteroptera: Geocoridae), on cotton," *Environmental Entomology*, vol. 32, no. 6, pp. 1399–1403, 2003.

[7] M. H. Sweet, "The seed bugs: a contribution to the feeding habits of the Lygaeidae (Hemiptera: Heteroptera)," *Annals of the Entomological Society of America*, vol. 53, no. 3, pp. 317–321, 1960.

[8] G. M. Orphianides, D. Gonzalez, and B. R. Bartlett, "Identification and evaluation of pink bollworm predators in southern California," *Journal of Economic Entomology*, vol. 64, no. 2, pp. 421–425, 1971.

[9] S. Chiravathanapong and H. N. Pitre, "Effects of *Heliothis virescens* larval size on predation by *Geocoris punctipes*," *The Florida Entomologist*, vol. 63, no. 1, pp. 146–151, 1980.

[10] M. N. Kapadia and S. N. Puri, "Biology and comparative predation efficacy of three heteropteran species recorded as predators of *Bemisia tabaci* in Maharashtra," *Entomophaga*, vol. 36, no. 4, pp. 555–559, 1991.

[11] J. C. Medal, A. J. Mueller, T. J. Kring, and E. E. Gbur, "Predation of *Spissistilus festinus* (Homoptera: Membracidae) nymphs by hemipteran predators in the presence of alternative prey," *Florida Entomologist*, vol. 80, no. 4, pp. 451–456, 1997.

[12] G. S. McCutcheon, "Consumption of tobacco budworm (Lepidoptera: Noctuidae) by Hooded Beetle (Coleoptera: Anthicidae) and bigeyed bug (Hemiptera: Lygaeidae)," *Journal of Agricultural and Urban Entomology*, vol. 19, no. 1, pp. 55–61, 2002.

[13] S. I. Rondon, D. J. Cantliffe, and J. F. Price, "The feeding behavior of the bigeyed bug, minute pirate bug, and pink spotted lady beetle relative to main strawberry pests," *Environmental Entomology*, vol. 33, no. 4, pp. 1014–1019, 2004.

[14] J. Hagler, "Comparative studies of predation among feral, commercially-purchased, and laboratory-reared predators," *BioControl*, vol. 54, no. 3, pp. 351–361, 2009.

[15] K. O. Bell and W. H. Whitcomb, "Field studies on egg predators of the bollworm, *Heliothis zea* (Boddie)," *The Florida Entomologist*, vol. 47, no. 3, pp. 171–180, 1964.

[16] W. H. Whitcomb and K. Bell, "Predacious insects, spiders, and mites of Arkansas cotton fields," *Arkansas Experiment Station Bulletin*, vol. 690, pp. 1–84, 1964.

[17] P. D. Lindgren, R. L. Ridgway, and S. L. Jones, "Consumption by several common arthropod predators of eggs and larvae of two *Heliothis* species that attack cotton," *Annals of the Entomological Society of America*, vol. 61, no. 3, pp. 613–618, 1968.

[18] M. D. Eubanks and R. F. Denno, "Host plants mediate omnivore-herbivore interactions and influence prey suppression," *Ecology*, vol. 81, no. 4, pp. 936–947, 2000.

[19] S. K. Braman, R. R. Duncan, W. W. Hanna, and M. C. Engelke, "Arthropod predator occurrence and performance of *Geocoris uliginosus* (Say) on pest-resistant and susceptible turfgrasses," *Environmental Entomology*, vol. 32, no. 4, pp. 907–914, 2003.

[20] S. E. Naranjo and P. C. Ellsworth, "Mortality dynamics and population regulation in *Bemisia tabaci*," *Entomologia Experimentalis et Applicata*, vol. 116, no. 2, pp. 93–108, 2005.

[21] S. E. Naranjo and P. C. Ellsworth, "The contribution of conservation biological control to integrated control of *Bemisia tabaci* in cotton," *Biological Control*, vol. 51, no. 3, pp. 458–470, 2009.

[22] A. Kessler and I. T. Baldwin, "Defensive function of herbivore-induced plant volatile emissions in nature," *Science*, vol. 291, no. 5511, pp. 2141–2144, 2001.

[23] A. Kessler and I. T. Baldwin, "Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*," *Plant Journal*, vol. 38, no. 4, pp. 639–649, 2004.

[24] A. J. Gassmann and J. D. Hare, "Indirect cost of a defensive trait: variation in trichome type affects the natural enemies of herbivorous insects on *Datura wrightii*," *Oecologia*, vol. 144, no. 1, pp. 62–71, 2005.

[25] R. Karban, "Damage to sagebrush attracts predators but this does not reduce herbivory," *Entomologia Experimentalis et Applicata*, vol. 125, no. 1, pp. 71–80, 2007.

[26] R. Halitschke, J. A. Stenberg, D. Kessler, A. Kessler, and I. T. Baldwin, "Shared signals: 'alarm calls' from plants increase apparency to herbivores and their enemies in nature," *Ecology Letters*, vol. 11, no. 1, pp. 24–34, 2008.

[27] M. Skibbe, N. Qu, I. Galis, and I. T. Baldwin, "Induced plant defenses in the natural environment: *Nicotiana attenuata* WRKY3 and WRKY6 coordinate responses to herbivory," *Plant Cell*, vol. 20, no. 7, pp. 1984–2000, 2008.

[28] A. Steppuhn, M. C. Schuman, and I. T. Baldwin, "Silencing jasmonate signalling and jasmonate-mediated defences reveals different survival strategies between two *Nicotiana attenuata* accessions," *Molecular Ecology*, vol. 17, no. 16, pp. 3717–3732, 2008.

[29] S. Meldau, J. Wu, and I. T. Baldwin, "Silencing two herbivory-activated MAP kinases, SIPK and WIPK, does not increase *Nicotiana attenuata*'s susceptibility to herbivores in the glasshouse and in nature," *New Phytologist*, vol. 181, no. 1, pp. 161–173, 2009.

[30] S. Allmann and I. T. Baldwin, "Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles," *Science*, vol. 329, no. 5995, pp. 1075–1078, 2010.

[31] M. C. Schuman, K. Barthel, and I. T. Baldwin, "Herbivory-induced volatiles function as defenses increasing fitness of the native plant *Nicotiana attenuata* in nature," *eLife*, vol. 1, no. 1, Article ID e00007, 2012.

[32] H. Oida and F. Kadono, "Prey consumption by *Geocoris varius* and *G. proteus* (Heteroptera: Geocoridae) provided with horticultural major pests in greenhouses," *Japanese Journal of Applied Entomology and Zoology*, vol. 55, no. 4, pp. 217–225, 2011.

[33] S. V. Joseph and S. K. Braman, "Influence of plant parameters on occurrence and abundance of arthropods in residential turfgrass," *Journal of Economic Entomology*, vol. 102, no. 3, pp. 1116–1122, 2009.

[34] M. D. Hunter, "Trophic promiscuity, intraguild predation and the problem of omnivores," *Agricultural and Forest Entomology*, vol. 11, no. 2, pp. 125–131, 2009.

[35] D. M. Dunbar and O. G. Bacon, "Influence of temperature on development and reproduction of *Geocoris atricolor*, *G. pallens*, and *G. punctipes* (Heteroptera: Lygaeidae) from California," *Environmental Entomology*, vol. 1, no. 5, pp. 596–599, 1972.

[36] S. Mansfield, B. Scholz, S. Armitage, and M. L. Johnson, "Effects of diet, temperature and photoperiod on development and survival of the bigeyed bug, *Geocoris lubra*," *BioControl*, vol. 52, no. 1, pp. 63–74, 2007.

[37] R. A. Champlain and L. L. Sholdt, "Life history of *Geocoris punctipes* (Hemiptera: Lygeidae) in the laboratory," *Annals of the Entomological Society of America*, vol. 60, no. 5, pp. 881–885, 1967.

[38] M. D. Eubanks and R. F. Denno, "Health food versus fast food: the effects of prey quality and mobility on prey selection by a generalist predator and indirect interactions among prey species," *Ecological Entomology*, vol. 25, no. 2, pp. 140–146, 2000.

[39] I. T. Baldwin, "Jasmonate-induced responses are costly but benefit plants under attack in native populations," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 14, pp. 8113–8118, 1998.

[40] A. Steppuhn, K. Gase, B. Krock, R. Halitschke, and I. T. Baldwin, "Nicotine's defensive function in nature," *PLoS Biology*, vol. 2, no. 8, 2004.

[41] A. Kessler, R. Halitschke, and I. T. Baldwin, "Silencing the jasmonate cascade: induced plant defenses and insect populations," *Science*, vol. 305, no. 5684, pp. 665–668, 2004.

[42] M. Kallenbach, G. Bonaventure, P. A. Gilardoni, A. Wissgott, and I. T. Baldwin, "PNAS Plus: *Empoasca* leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 24, pp. E1548–E1557, 2012.

[43] D. Kessler, K. Gase, and I. T. Baldwin, "Field experiments with transformed plants reveal the sense of floral scents," *Science*, vol. 321, no. 5893, pp. 1200–1202, 2008.

[44] J. H. McDonald, *Handbook of Biological Statistics*, Sparky House Publishing, Baltimore, Md, USA, 2nd edition, 2009.

[45] R. A. Bahulikar, D. Stanculescu, C. A. Preston, and I. T. Baldwin, "ISSR and AFLP analysis of the temporal and spatial population structure of the post-fire annual, *Nicotiana attenuata*, in SW Utah," *BMC Ecology*, vol. 4, article 12, 2004.

[46] M. C. Schuman, N. Heinzel, E. Gaquerel, A. Svatos, and I. T. Baldwin, "Polymorphism in jasmonate signaling partially accounts for the variety of volatiles produced by *Nicotiana attenuata* plants in a native population," *New Phytologist*, vol. 183, no. 4, pp. 1134–1148, 2009.

[47] J. Wu, C. Hettenhausen, M. C. Schuman, and I. T. Baldwin, "A comparison of two *Nicotiana attenuata* accessions reveals large differences in signaling induced by oral secretions of the specialist herbivore *Manduca sexta*," *Plant Physiology*, vol. 146, no. 3, pp. 927–939, 2008.

[48] P. Barbosa, J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei, "Associational resistance and associational susceptibility: having right or wrong neighbors," *Annual Review of Ecology, Evolution, and Systematics*, vol. 40, pp. 1–20, 2009.

[49] A. F. Huberty and R. F. Denno, "Plant water stress and its consequences for herbivorous insects: a new synthesis," *Ecology*, vol. 85, no. 5, pp. 1383–1398, 2004.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 242037, 14 pages
http://dx.doi.org/10.1155/2013/242037

Review Article

Ant-Associated Beetle Fauna in Bulgaria: A Review and New Data

Albena Lapeva-Gjonova

Department of Zoology and Anthropology, Faculty of Biology, Sofia University, 8 Dragan Tzankov Boulevard, 1164 Sofia, Bulgaria

Correspondence should be addressed to Albena Lapeva-Gjonova; gjonova@abv.bg

Received 8 February 2013; Revised 22 April 2013; Accepted 23 April 2013

Academic Editor: Alain Lenoir

The rich myrmecofauna in Bulgaria, comprising about 170 species, constitutes favorable settings for a diverse associated fauna. An attempt to summarize the fragmented faunal data on this ecological group in Bulgaria, together with inclusion of new data, has resulted in a comprehensive list of 121 beetle species from 14 families, obligate or facultative ant related. The extent of current knowledge on the various beetle families, host specificity, the nature of relations between guests and their ant hosts, and the regional characteristics of the myrmecophilous fauna are discussed.

1. Introduction

The social organization of ants and the conditions found within their nests are favorable to a number of organisms that coexist with them. These guests are mainly arthropods, and they form a variety of relationships with their hosts. Some guests enter the nests, where they feed as predators, scavengers, temporary commensals, or as ecto- and endoparasites. Others, commonly known as myrmecophiles, are dependent on ant communities for the whole or part of their life cycle [1]. Beetles are one of the ant-associated fauna groups that are the richest in number of species [2, 3]. Studies on these specific multispecies interactions are of particular faunistic, ecological, and evolutionary interest.

The number of documented ant-associated species has been steadily increasing since the beginning of intensive research on the myrmecophilous fauna in the 19th century. Even in 1841 and 1844 Märkel [4, 5] published detailed lists of about 280 beetle species associated to ant nests in Europe. The first significant review was made by Wasmann [6], who reported a total of 1,177 myrmecophilous species in the world. Soon after this, the number grew to a total of 1,500, of which 1,000 species are beetles [1]. Nearly a century later, Wilson [7], and after that Hölldobler and Wilson [2], listed 35 beetle families all over the world documented to have links with ants. According to the latest taxonomic changes in Coleoptera, there are actually only 28 such families [8], but the families with myrmecophilous members expand their range. Here, we should add the first recently established myrmecophilous member of Buprestidae family [9]. Currently, it is estimated that the number of the ant-associated insects is not less than 10,000 species [10].

The diversity of ant-related fauna is closely connected with nest size [2, 11]. As a rule, larger colonies exist for longer and offer a wider variety of ecological niches that are useful to more guests. For these reasons, in the Palaearctic, the highest species richness of guests is found in the mound-building ants of the *Formica* genus and also in the *Lasius* species, which nest in tree trunks [2, 11–13].

There is a great variety of associated beetle species and a multitude of combinations of features from different behavioral categories that they might display. Different classifications have been suggested to describe the relationships between ants and their guests (e.g., [1, 6, 14–16]). Additionally, the natures of their relationships with ants are often not understood. For these reasons, I am using the broadly accepted definition of myrmecophiles, that is, that they are closely associated with ants and their nests and usually not found outside the ants' nests.

2. Ant-Associated Beetles in Bulgaria: A List with Comments

Bulgarian species of myrmecophilous beetles have not been thoroughly investigated, with the exception of a few faunistic contributions. Most data comes from single publications on specific beetle families, with information on their hosts frequently missing. Information about beetles associated with ants was found in 58 scientific publications, with 10 being devoted entirely to the Bulgarian myrmecophilous species.

The geographic location of Bulgaria in Southern Europe, the combination of typical temperate continental and transitional-Mediterranean climates, its diverse topography with inclination from sea level to 2925 m above sea level, and the presence of a diverse ant fauna of nearly 170 species [17, 18] suggest the presence of a rich myrmecophilous fauna.

A review of the current data on ant-associated beetles in Bulgaria will extend our knowledge on the degree about to which this specific ecological group has been studied.

The prepared list (Table 1) contains beetle species found in ants' nests in Bulgaria based on literature sources and new data. Some species are well-known myrmecophiles from other countries, even though ant hosts and nest collection are not always recorded from Bulgaria. Other parts of the beetle species collected from ants' nests in Bulgaria occur also in habitats outside them but regularly or accidentally enter into the ant' nests. Ant host species are also listed, with corresponding references, where information is available. Species that are widely accepted as typical inhabitants of ants' nests without using of subdivisions according to different classifications are highlighted as myrmecophiles. The beetle list is arranged using the classification proposed by Bouchard et al. [8], and the arrangement of species within the families is given by subfamilies.

2.1. Family Carabidae. Ground beetles from subfamily Paussinae are commonly known as "ant nest beetles" and "flanged bombardier beetles." There are around 800 species, distributed mainly throughout the tropical and subtropical regions [76]. All 329 species in the genus *Paussus* (tribe Paussini) are myrmecophiles [77]. They prey on ant eggs, larvae and adults, piercing ants' bodies with mandibles and sucking out the fluid inside [10]. Extremely modified antennas with glandular hairs, secreting substances which ants lick, and the use of stridulatory organs are examples of adaptations that favor close integration with ant society.

Two species of the genus *Paussus* occur in Europe—*Paussus favieri* Fairmaire, 1851 and *P. turcicus* Frivaldszky von Frivald, 1835 [19]. The first of them occurs mainly in the Western Mediterranean. *P. turcicus* was described from the territory of Bulgaria, then still part of the Ottoman empire, and thus it is the first-known myrmecophilous species to be recorded in Bulgaria that is also distributed in Central Asia, the Middle East, Asia Minor, and the Balkans [78]. In Bulgaria, it is a rare species, located in the southern regions, and always found in the subterranean nests of its ant host *Pheidole pallidula* (Nylander, 1849) [21, 22], although it has also been collected from *Tetramorium semilaeve* Andre, 1883 and *Messor barbarus* (Linnaeus, 1767) nests [79].

2.2. Family Histeridae. Histeridae is worldwide in distribution with just under 4,300 known species, grouped into about 350 genera [80, 81], and reaches its highest diversity in the tropics. Both subfamilies Chlamydopsinae, mainly distributed in southern Asia, Pacific, and Australia, and Haeteriinae contain myrmeco- or termitophilous species. It is accepted that myrmecophiles feed on the larvae of ants or other insects or even regurgitated food from the host ants [2].

Haeteriinae is very rich in species, especially in the neotropics. In the Palaearctic it is represented by four genera *Eretmotus*, *Sternocoelis*, *Hetaerius*, and *Satrapes,* which include species living exclusively in ants' nests. *Eretmotus* and *Sternocoelis* are widespread in the Mediterranean region. Two species—*Sternocoelis merklii* (Schmidt, 1885) [26] with the ant *Messor structor* (Latreille, 1798) and *Haeterius ferrugineus* (Olivier, 1789), found in the nests of various *Formica* spp.—have been reported in Bulgaria so far [23, 25]. Unlike the wider distribution of *H. ferrugineus* in many European countries, *Sternocoelis merklii* also has been reported from several localities in Greece and Turkey [26].

In addition, it is the first time the presence of a member of the genus *Satrapes* is established in Bulgaria with the following collecting data.

Satrapes sartorii (L. Redtenbacher, 1857). Western Bulgaria, near Dolni Koriten vill., N422839 E223503, 889 m a.s.l., 10.04.2010: 1 specimen.

This rare species, more common in Central Europe [82], was found in a *Tetramorium* cf. *caespitum* (Linnaeus, 1758) nest under a stone in early spring. The sample locality is in a low-mountainous region with features determined by a typical temperate climate; hence, this finding was expected.

The fourth myrmecophilous member is a Dendrophilinae species—*Dendrophilus pygmaeus* (Linnaeus, 1758)—that typically occurs in the mound nests of *Formica*, which are built using plant materials [23, 24].

Two other species—*Acritus nigricornis* (Hoffmann, 1803) and *Onthophilus affinis* L. Redtenbacher, 1849—were also found with ants without being obligate inhabitants. The presence of *Acritus nigricornis* in ants' nests also was reported by Roubal [83] as well as in a termite nest of *Reticulitermes lucifugus* (Rossi, 1792) [84], but the presence of *Onthophilus affinis* may seem rather accidental.

2.3. Family Ptiliidae. Feather-winged beetles are among the smallest beetles, and, together with Staphylinidae, they can reach high numbers in ants' nests. Family Ptiliidae includes about 600 described species across some 80 genera [85]. In Europe, approximately 140 species of Ptiliidae are known [86]. Most species dwell in leaf litter and rotting organic matter in shady woodland areas, feeding on the spores and hyphae of fungi, as well as other organic food sources [86, 87].

Associations with ants range from an accidentally entering nests through to regular entry and strict myrmecophily. This has led to significant morphological changes in the subfamily Cephaloplectinae, known to inhabit America and Australia. There are a few ptiliid species in Europe which often inhabit ant nests, typically of species from the genera

Table 1: List of ant-associated beetles and their hosts (where data is available) according to the studied literature and new records. Facultative or undefined ant relations are not indicated.

Beetle families, genera, and species	Recorded ant hosts in Bulgaria	References	Ant-relation	Endemic beetles
Carabidae				
Paussus turcicus I. Frivaldszky von Frivald, 1835		[19–21]	Myrmecophile	
	Pheidole pallidula (Nylander, 1849)	[22]		
Histeridae				
Acritus nigricornis (Hoffmann, 1803)	*Formica rufa* Linnaeus, 1761	[23]		
Dendrophilus pygmaeus (Linnaeus, 1758)	*Formica exsecta* Nylander, 1846	[23, 24]	Myrmecophile	
	Formica lugubris Zetterstedt, 1838	[23]		
	Formica rufa Linnaeus, 1761	[23]		
Haeterius ferrugineus (Olivier, 1789)	*Formica fusca* Linnaeus, 1758	[23]	Myrmecophile	
	Formica cinerea Mayr, 1853	[25]		
	Lasius niger (Linnaeus, 1758)	New ant host record for Bulgaria		
Sternocoelis merklii (Schmidt, 1885)	*Messor structor* (Latreille, 1798)	[26]	Myrmecophile	Balkans
Satrapes sartorii (L. Redtenbacher, 1857)	*Tetramorium* cf. *caespitum* (Linnaeus, 1758)	New species for Bulgaria	Myrmecophile	
Margarinotus ruficornis (Grimm, 1852)		[27, 28]		
Onthophilus affinis L. Redtenbacher, 1849	*Formica fusca* Linnaeus, 1758	[23]		
	Myrmica sp.	[23]		
Ptiliidae				
Ptenidium pusillum (Gyllenhal, 1808)		[29]		
Ptiliolum oedipus (Flach, 1886)	*Formica pratensis* Retzius, 1783	New ant host record for Bulgaria		
Ptilium myrmecophilum (Allibert, 1844)	*Formica pratensis* Retzius, 1783	New species for Bulgaria	Myrmecophile	
	Formica rufa Linnaeus, 1761			
Pteryx suturalis (Heer, 1841)	*Formica rufa* Linnaeus, 1761	New ant host record for Bulgaria		
Acrotrichis atomaria (De Geer, 1774)	*Formica pratensis* Retzius, 1783	New species for Bulgaria		
Leiodidae				
Cholevinae				
Anemadus strigosus strigosus Kraatz, 1852		[30, 31]		
Eocatops pelopis (Reitter, 1884)		[30, 32]	Myrmecophile	
	Messor sp.	[33]		
Eocatops skopjensis Karaman, 1957		[34]	Myrmecophile	Balkans
Nemadus colonoides (Kraatz, 1851)		[30, 31]	Myrmecophile	
Dreposcia umbrina Erichson, 1837		[31, 34]		
Attaephilus arenarius (Hampe, 1852)		[30, 33]	Myrmecophile	
	Messor structor (Latreille, 1798)	[25], New record		
Attaephilus cf. *funebris* (Reitter, 1888)	*Messor* sp.	[25]		Balkans
Attaephilus rambouseki Jeannel, 1936		[32, 33]		Bulgaria
Catopsimorphus marani Roubal, 1936	*Messor* sp.	[33]	Myrmecophile	Bulgaria

Table 1: Continued.

Beetle families, genera, and species	Recorded ant hosts in Bulgaria	References	Ant-relation	Endemic beetles
Staphylinidae				
Pselaphinae				
Batrisodes buqueti (Aubé, 1833)		[25]	Myrmecophile	
	Lasius brunneus (Latreille, 1798)	[35]		
Batrisodes delaporti (Aubé, 1833)		[36]	Myrmecophile	
	Lasius brunneus (Latreille, 1798)	[37]		
	Lasius alienus (Förster, 1850)	[25]		
Batrisodes hubenthali Reitter, 1913		[25, 38]	Myrmecophile	
Batrisodes oculatus (Aubé, 1833)		[39]	Myrmecophile	
Batrisodes sulcaticeps Besuchet, 1981		[25, 40]	Myrmecophile	
Batrisodes venustus (Reichenbach, 1816)		[25, 36, 38]	Myrmecophile	
Batrisus formicarius Aubé, 1833	*Lasius brunneus* (Latreille, 1798)	[35, 37, 41]	Myrmecophile	
	Lasius alienus (Förster, 1850)	[25]		
Claviger elysius Reitter, 1884		[25]	Myrmecophile	Balkans
Claviger emgei Reitter, 1885	*Lasius alienus* (Förster, 1850)	[25]	Myrmecophile	Balkans
Claviger handmanni Wasmann, 1898	*Lasius flavus* (Fabricius, 1782)	[37]	Myrmecophile	Balkans
	Lasius alienus (Förster, 1850)	[25]		
Claviger longicornis P.W.J. Müller, 1818		[42]	Myrmecophile	
	Lasius alienus (Förster, 1850)	[37]		
	Lasius niger (Linnaeus, 1758)	[37]		
	Lasius brunneus (Latreille, 1798)	[37]		
Claviger merkli Reitter, 1885		[35, 43]	Myrmecophile	Balkans
	Lasius niger (Linnaeus, 1758)	[25]		
	Lasius alienus (Förster, 1850)	[25]		
Claviger testaceus Preyssler, 1790		[25, 40]	Myrmecophile	
	Lasius flavus (Fabricius, 1782)	[35, 44]		
Euplectus frater Besuchet, 1964	*Formica rufa* Linnaeus, 1761	[37]		
Euplectus nanus (Reichenbach, 1816)		[39]		
	Formica rufa Linnaeus, 1761	[37]		
Euplectus signatus (Reichenbach, 1816)		[39]		
	Formica pratensis Retzius, 1783	[37]		
	Formica sp.	[45]		
Trichonyx sulcicollis (Reichenbach, 1816)		[36]		
	Lasius alienus (Förster, 1850)	[25]		
Trimium carpathicum Saulcy, 1875	*Formica rufa* Linnaeus, 1761	[35]		
Trimium puncticeps Reitter, 1880	*Formica pratensis* Retzius, 1783	[37]		
Bryaxis romaniae Raffray, 1904	*Formica rufa* Linnaeus, 1761	[37]		Balkans
Centrotoma brucki Saulcy, 1874		[25]	Myrmecophile	Balkans
Centrotoma lucifuga C. Heyden, 1849	*Tetramorium caespitum* (Linnaeus, 1758)	[37]	Myrmecophile	
Chennium bituberculatum Latreille, 1807	*Tetramorium caespitum* (Linnaeus, 1758)	[37]	Myrmecophile	
Chennium steigerwaldi Reitter, 1882	*Tetramorium* cf. *caespitum* (Linnaeus, 1758)	[25]	Myrmecophile	
	Tetramorium ferox Ruzsky, 1903	[25]		

Table 1: Continued.

Beetle families, genera, and species	Recorded ant hosts in Bulgaria	References	Ant-relation	Endemic beetles
Tachyporinae				
Ischnosoma splendidum (Gravenhorst, 1806)	*Lasius brunneus* (Latreille, 1798)	[46]		
Tachinus rufipes (Linnaeus, 1758)	*Formica rufa* Linnaeus, 1761	[46]		
Tachyporus hypnorum (Fabricius, 1775)	*Formica pratensis* Retzius, 1783	[46]		
Tachyporus nitidulus (Fabricius, 1781)	*Lasius fuliginosus* (Latreille, 1798)	[46]		
Aleocharinae				
Piochardia reitteri (Wasmann, 1894)	*Cataglyphis nodus* (Brullé, 1833)	[46]	Myrmecophile	
Lyprocorrhe anceps (Erichson, 1837)	*Formica pratensis* Retzius, 1783	[46, 47]	Myrmecophile	
Notothecta flavipes (Gravenhorst, 1806)	*Formica pratensis* Retzius, 1783	[46, 47]	Myrmecophile	
	Formica rufa Linnaeus, 1761	[46]		
Euryusa sinuata Erichson, 1837	*Lasius brunneus* (Latreille, 1798)	[46]	Myrmecophile	
Anaulacaspis nigra (Gravenhorst, 1802)	*Formica pratensis* Retzius, 1783	[46]		
Oligota pumilio Kiesenwetter, 1858	*Formica pratensis* Retzius, 1783	[46]		
Oligota inflata (Mannerheim, 1830)	*Formica pratensis* Retzius, 1783	[46]		
Lomechusa emarginata (Paykull, 1789)		[48]	Myrmecophile	
	Formica sp.	[25]		
	Myrmica rugulosa Nylander, 1849	[46]		
	Formica cinerea Mayr, 1853	[46]		
Drusilla canaliculata (Fabricius, 1787)	*Tetramorium caespitum* (Linnaeus, 1758)	[46]		
Myrmoecia plicata (Erichson, 1837)		[49]	Myrmecophile	
Pella funesta (Gravenhorst, 1806)		[39]	Myrmecophile	
Pella hampei (Kraatz, 1862)		[50]	Myrmecophile	
Pella humeralis (Gravenhorst, 1802)		[51, 52]	Myrmecophile	
	Formica pratensis Retzius, 1783	[47]		
Pella laticollis (Märkel, 1844)		[39]	Myrmecophile	
Pella limbata (Paykull, 1789)		[48]	Myrmecophile	
Pella lugens (Gravenhorst, 1802)		[53]	Myrmecophile	
Pella ruficollis (Grim, 1845)		[50]	Myrmecophile	
Zyras collaris (Paykull, 1789)		[45]		
Zyras fulgidus (Gravenhorst, 1806)		[54]		
Zyras haworthi (Stephens, 1832)		[48]	Myrmecophile	
Dinarda hagensii Wasmann, 1889	*Formica exsecta* Nylander, 1846	[24, 46]	Myrmecophile	
Dinarda maerkelii Kiesenwetter, 1843	*Formica pratensis* Retzius, 1783	[46, 47]	Myrmecophile	
Dinarda sp.	*Formica sanguinea* Latreille, 1798	[25]	Myrmecophile	
Haploglossa gentilis (Märkel, 1845)	*Lasius fuliginosus* (Latreille, 1798)	[55]		
Haploglossa pulla (Gyllenhal, 1827)		[51]		
Oxypoda formiceticola Märkel, 1841	*Formica rufa* Linnaeus, 1761	[46]	Myrmecophile	
	Formica lugubris Zetterstedt, 1838	[46]		
Oxypoda haemorrhoa (Mannerheim, 1830)	*Formica exsecta* Nylander, 1846	[24]	Myrmecophile	
	Formica pratensis Retzius, 1783	[46, 47]		
Oxypoda pratensicola Lohse, 1970	*Formica pratensis* Retzius, 1783	[46, 47]	Myrmecophile	
Oxypoda rugicollis Kraatz, 1856	*Formica exsecta* Nylander, 1846	[24, 46]	Myrmecophile	
	Formica pressilabris Nylander, 1846	[46]		
Oxypoda vittata Märkel, 1842		[54, 56]	Myrmecophile	

Table 1: Continued.

Beetle families, genera, and species	Recorded ant hosts in Bulgaria	References	Ant-relation	Endemic beetles
Thiasophila angulata (Erichson, 1837)	*Formica rufa* Linnaeus, 1761	[46]	Myrmecophile	
Thiasophila canaliculata Mulsant and Rey, 1875	*Formica exsecta* Nylander, 1846	[24, 46]	Myrmecophile	
Thiasophila lohsei Zerche, 1987	*Formica pratensis* Retzius, 1783	[46, 47]	Myrmecophile	
Scydmaeninae				
Euconnus chrysocomus (Saulcy, 1864)	*Tetramorium* cf. *ferox* Ruzsky, 1903	[25]	Myrmecophile	
Euthiconus conicicollis (Fairmaire and Laboulbene, 1854)		[57]		
Microscydmus nanus (Schaum, 1844)		[39, 58]		
Neuraphes parvulus Rambousek, 1909	*Lasius fuliginosus* (Latreille, 1798)	[39]		
Scydmoraphes minutus (Chaudoir, 1845)		[58]		
Scydmaenus perrisi Reitter, 1881		[39, 59]		
Steninae				
Stenus aterrimus Erichson, 1839	*Formica pratensis* Retzius, 1783	[46, 47]	Myrmecophile	
Stenus heydeni L. Benick, 1915	*Formica rufa* Linnaeus, 1761	[46]		Balkans
Paederinae				
Lithocharis nigriceps Kraatz, 1859	*Formica pratensis* Retzius, 1783	[47]		
Astenus gracilis (Paykull, 1789)	*Formica pratensis* Retzius, 1783	[46]		
Sunius melanocephalus (Fabricius, 1793)	*Formica rufa* Linnaeus, 1761	[46]		
Scopaeus pusillus Kiesenwetter, 1843	*Formica pratensis* Retzius, 1783	[46]		
	Formica rufa Linnaeus, 1761	[46]		
Scopaeus sulcicollis (Stephens, 1833)	*Formica pratensis* Retzius, 1783	[46]		
Staphylininae				
Leptacinus formicetorum Märkel, 1841	*Formica pratensis* Retzius, 1783	[47]	Myrmecophile	
	Formica rufa Linnaeus, 1761	[46]		
	Formica exsecta Nylander, 1846	[46]		
Gyrohypnus angustatus Stephens, 1833	*Formica pratensis* Retzius, 1783	[46]		
	Formica rufa Linnaeus, 1761	[46]		
Xantholinus linearis (Olivier, 1795)	*Formica pratensis* Retzius, 1783	[46]		
Gabrius splendidulus (Gravenhorst, 1802)	*Lasius brunneus* (Latreille, 1798)	[46]		
Quedius brevis Erichson, 1840	*Formica rufa* Linnaeus, 1761	[46]	Myrmecophile	
Monotomidae				
Rhizophagus bipustulatus (Fabricius, 1792)	*Lasius niger* (Linnaeus, 1758)	New ant host record for Bulgaria		
Monotoma conicollis Aubé, 1837		[29]	Myrmecophile	
	Formica pratensis Retzius, 1783	New ant host record for Bulgaria		
Cryptophagidae				
Hypocoprus latridioides Motschulsky, 1839		[54]	Myrmecophile	
	Formica exsecta Nylander, 1846	[24]		
	Formica rufa Linnaeus, 1761	New ant host record for Bulgaria		
	Formica lugubris Zetterstedt, 1838	New ant host record for Bulgaria		
Nitidulidae				
Amphotis marginata (Fabricius, 1781)	*Lasius fuliginosus* (Latreille, 1798)	[29, 60, 61]	Myrmecophile	
Amphotis orientalis Reiche, 1861		[34, 62]	Myrmecophile	
Cerylonidae				
Cerylon histeroides (Fabricius, 1792)	*Lasius brunneus* (Latreille, 1798)	New ant host record for Bulgaria		

Table 1: Continued.

Beetle families, genera, and species	Recorded ant hosts in Bulgaria	References	Ant-relation	Endemic beetles
Endomychidae				
Pleganophorus bispinosus Hampe, 1855	*Lasius brunneus* (Latreille, 1798)	[63–66]	Myrmecophile	
Cholovocera major Reitter, 1887	*Messor structor* (Latreille, 1798)	[67]	Myrmecophile	Balkan-Anatolian
Merophysia oblonga Kiesenwetter, 1872		[68]	Myrmecophile	
	Tetramorium cf. *caespitum* (Linnaeus, 1758)	[67]		
Mycetaea subterranea Fabricius, 1801	*Formica rufa* Linnaeus, 1761	[67]		
Latridiidae				
Dienerella costulata (Reitter, 1877)	*Formica pratensis* Retzius, 1783	[67]		
	Formica rufa Linnaeus, 1761	[67]		
Dienerella vincenti Johnson, 2007	*Formica pratensis* Retzius, 1783	[67]		
	Formica exsecta Nylander, 1846	[67]		
Corticaria longicollis (Zetterstedt, 1838)	*Formica pratensis* Retzius, 1783	[67]	Myrmecophile	
	Formica rufa Linnaeus, 1761	[67]		
	Formica exsecta Nylander, 1846	[67]		
	Tetramorium cf. *caespitum* (Linnaeus, 1758)	[67]		
Tenebrionidae				
Dichillus carinatus (Küster, 1848)		[34, 69]	Myrmecophile	Balkan-Anatolian
	Pheidole pallidula (Nylander, 1849)	New ant host record for Bulgaria		
Eutagenia smyrnensis (Solier, 1838)		[69]	Myrmecophile	Balkan-Anatolian
Palorus depressus (Fabricius, 1790)	*Formica rufa* Linnaeus, 1761	New ant host record for Bulgaria		
Myrmechixenus subterraneus Chevrolat, 1835	*Formica pratensis* Retzius, 1783	New species for Bulgaria	Myrmecophile	
	Formica rufa Linnaeus, 1761			
	Formica pressilabris Nylander, 1846			
	Formica cinerea Mayr, 1853			
Chrysomelidae				
Clytra laeviuscula Ratzeburg, 1837		[70, 71]	Myrmecophile	
	Formica sanguinea Latreille, 1798	New ant host record for Bulgaria		
Clytra quadripunctata (Linnaeus, 1758)		[70, 71]	Myrmecophile	
	Formica rufa Linnaeus, 1761	New ant host record for Bulgaria		
	Formica pratensis Retzius, 1783	New ant host record for Bulgaria		
Brentidae				
Amorphocephala coronata (Germar, 1817)		[29, 34, 72–75]	Myrmecophile	
	Camponotus aethiops Latreille, 1798	New ant host record for Bulgaria		

Formica and *Lasius*, where there is a significant amount of decaying organic material without these beetles being limited to these habitats.

Feather-winged beetles are exceptionally under-researched in Bulgaria, with only scarce data being available. Ioakimov [29] reported the finding of *Ptenidium pusillum* (Gyllenhal, 1808) in ants' nests without this species being related to living with ants. During my investigation on the myrmecophilous fauna in some *Formica* species, I collected 4 more ptiliid species, which were kindly identified by Mikael Sörensson. *Ptilium myrmecophilum* (Allibert, 1844) and *Acrotrichis atomaria* (De Geer, 1774) were not previously known for the Bulgarian fauna. Collection and habitat data for these two species are presented below.

Ptilium myrmecophilum (Allibert, 1844). Southwestern Bulgaria, Vitosha Mt., near Bistritsa and Jeleznitsa vill., from January to October in 1994-1995: about 150 specimens in nests of *F. pratensis*; Vitosha Mt., near Simeonovo vill., 06.10.1998, 25.10.1998: 4 specimens in nests of *Formica rufa*.

Pt. myrmecophilum commonly lives in nests of *Formica rufa* and *F. pratensis*, recorded in Central and North Europe. The new data from Bulgaria affirms the preferred ant host species. Out of the 5 feather-winged beetle species collected in ants' nests, only *Ptilium myrmecophilum* is a tolerated guest, occurring in the explored nests in large numbers.

Acrotrichis atomaria (De Geer, 1774). Southwestern Bulgaria, Vitosha Mt., near Bistritsa vill., 1000 m a.s.l., 15.08.1995: 11 specimens; 15.10.1995: 110 specimens; 20.06.1997: 10 specimens; 14.11.1997: 31 specimens; 27.06.1998: specimens. It was found in and around *Formica pratensis* nests.

A. atomaria is a western Palaearctic species, which typically inhabits wet mosses, leaf-litter of *Castanea*, *Fagus* and *Quercus*, at the bases of *Ulmus* and *Salix* trees [86, 88].

2.4. Family Leiodidae. Family Leiodidae is represented by 111 species in Bulgaria [34, 89, 90], most of which inhabit forest habitats. They are saprophagous and mycophagous feeders, living on various decaying organic materials, and also in specific habitats such as ants' nests, caves or nests, and burrows of vertebrates [90].

Reports exist for 9 leiodids associated with ants in Bulgaria. Four of them—*Eocatops pelopis* (Reitter, 1884), *E. skopjensis* Karaman, 1957, *Nemadus colonoides* (Kraatz, 1851), and *Attaephilus arenarius* (Hampe, 1852)—are treated in widest sense as myrmecophiles. The rest of the documented species are common both in nests and in other habitats. Arboricolous leiodids usually cohabit with *Lasius* ant species, while soil species are more likely to be found with *Messor* and *Aphaenogaster*. Most members of *Attaephilus* are known as ant associated or cavernicolous.

Four of the 9 leiodid beetles show local distribution: 2 are endemic to the Balkans (*Eocatops skopjensis* Karaman, 1957, and *Attaephilus* cf. *funebris* (Reitter, 1888)), and the other 2 have been established in Bulgaria without being reported from anywhere else (*Attaephilus rambouseki* Jeannel, 1936, and *Catopsimorphus marani* Roubal, 1936). Until recently, *Eocatops skopjensis* Karaman, 1957, has been known only from Macedonia [34, 91].

2.5. Family Staphylinidae. Rove beetles are the most diverse beetles found in ants' nests and display varying degrees of the ant-association. There are more than 200 staphylinid species in different relationships with ants in the Palaearctic [92]. The degree of relatedness ranges from occasional visits to indifferent relationships or full dependency on ants. In the latter case, different morphological modifications (modified antennae, glandular trichomes on the body, reduction of the mouthparts, specific body shape and coloration) and behavioral adaptations (depending on the ants to be fed, care for the offspring, moving under unfavorable conditions) have been involved. Close integration with the ant colony is mediated by morphological mimicry (Wasmannian mimicry) [93]. Chemical mimicry is also used. The entry of alien species into a highly discriminatory environment of ants is accomplished using cuticular hydrocarbons similar to those of ants, as well as "soothing substances" from special glands [94, 95]. The most integrated guests, categorized by Wasmann as "symphiles" [6], show the most diverse integrative mechanisms. This group of species is limited in number when compared with the facultative and obligate predators and commensals.

Although data on the ant-associated staphylinids in Bulgaria is reported in certain faunistic publications, there is still great scope for their exploration. Strictly myrmecophilous genera (such as *Thoracophorus*, *Lamprinus*, *Lamprinodes*, *Lomechusoides*) are widely distributed in Europe but have not been recorded from Bulgaria so far. From all of the 121 ant-associated beetle species listed in this paper, 79 species belong to family Staphylinidae where Pselaphinae (24 species) and Aleocharinae (33 species) are the richest subfamilies.

2.5.1. Pselaphinae. Members of the tribes Clavigerini, Ctenistini, and Batrisini are recognized as true myrmecophiles amongst the European pselaphines. The most specialized myrmecophiles are Clavigerini species, represented in Bulgaria by 6 species of *Claviger*. They are clearly distinguished by their reduced eyes and their modified mouthparts, which are adapted for regurgitated feeding by ant hosts, and for preying on ant eggs, larvae, and pupae [2]. The presence of trichome glands is another adaptation found in these species. The *Claviger* species form relationships with different *Lasius* ant species. Probably, all previous records for *Claviger longicornis* in Bulgaria should refer to *C. handmanni*, which is an endemic to the Balkans.

All 4 members of the genera *Centrotoma* and *Chennium* (tribe Ctenistini), which are known to occur in Bulgaria, are obligate myrmecophiles with ant species of the genus *Tetramorium*. One of them, *Centrotoma brucki* Saulcy, 1874, has been only recorded from Greece, but was recently added to the Bulgarian myrmecophilous fauna [25]. Ants care for these species and feed them with regurgitated food. In the *Centrotoma* species, the mouthparts are well developed, whereas in the case of *Chennium* the maxillary palps are reduced [14]. The trichomes are less developed, in contrast to the *Clavigerini* species of both genera.

Species from the tribe Batrisini (*Batrisus* and *Batrisodes*) are often found in the nests of different *Lasius* ants. They have no trichome glands, but despite this, ant workers seem to tolerate them. These species mainly eat mites found in the nests [14].

Some pselaphines, such as species from the genera *Euplectus*, *Trichonyx*, and *Trimium*, appear to be well adapted to both decaying plant material and ants' nests.

2.5.2. Aleocharinae. Aleocharines are the most successful group of beetles found in ants' nests. Thirty three ant-associated species have been recorded in Bulgaria. Despite the increase in their known number, the available records from Bulgaria are singular and often lack data on ant hosts.

A western Palaearctic member of the myrmecophilous genus *Piochardia* belonging to the tribe Aleocharini has recently been identified in a few localities in Southern Bulgaria [46]. *Piochardia reitteri* (Wasmann, 1894) is the only known myrmecophile in the nests of *Cataglyphis nodus* (Brullé, 1833) in Bulgaria, which is found in locations from the Southern Balkans to Anatolia, Caucasus, Iraq, Syria, and Iran [96].

Lomechusini are well known to be associated with ants, either being totally dependent on ant societies (like *Lomechusa*, *Lomechusoides, Myrmoecia*) or as predators of ants (*Zyras*, *Pella*, *Drusilla*). Altogether, 435 Lomechusini species or subspecies have been recorded living with ants all over the world [50]. Only 13 species have been established in Bulgaria. The high integrated *Lomechusa* species change ant hosts according to the seasons, wintering in *Myrmica* nests and spending the summer with *Formica* spp.

Different species of the genera *Oxypoda* and *Thiasophila* live in mound-built *Formica* ants' nests. Because they are tolerated by the ants, they often reach a significant number of specimens [21, 43, 47].

2.5.3. Scydmaeninae. Scydmaeninae, commonly known as ant-like stone beetles, have long been treated as a separate beetle family. They are known to live mostly in moist leaf litter and rotting logs in forests, feeding on oribatid mites and even collembolans [97, 98]. According to O'Keefe [97], 117 ant-associated species all over the world are known, but there are few really integrated Scydmaeninae guests. Only 1 European ant-like stone beetle—*Euconnus chrysocomus* (Saulcy, 1864)—is recognized as a true myrmecophile (symphile), while the relationships between neutral and facultative Scydmaeninae guests and their hosts remain to be studied [97].

2.6. Family Monotomidae. Mound-building *Formica* ants provide suitable conditions for 2 Euro-Siberian monotomids—*Monotoma conicollis* Aubé, 1837, and *M. angusticollis* (Gyllenhal, 1827). Only *M. conicollis* has been listed in Bulgaria so far. It is the first time that the association with *F. pratensis* has been reported. It is considered that *Monotoma* species are mycophagous as a whole [99].

2.7. Family Cryptophagidae. The species of family Cryptophagidae are typically small (0,8–5,2 mm), most diverse in cool temperate environments. Most members are free living and mycophagous; inquilines in the nests of social insects have also been known [100].

Hypocoprus latridioides Motschulsky, 1839, lives both inside and outside the nests of *Formica* species and cohabits particularly frequently with *Formica exsecta.* It has been reported in few localities in Bulgaria from sea level to 2000 m above sea level [24, 54]. The new data confirms its presence with *F. exsecta* but also adds 2 new ant host species for the country—*F. rufa* and *F. lugubris.*

2.8. Family Nitidulidae. Two European sap beetle species have close relationships with ants: *Amphotis marginata* (Fabricius, 1781), known to occur in the Palaearctic, and *A. orientalis* Reiche, 1861, restricted to the Mediterranean region of Europe and the Near East. *A. marginata* has long been known to cohabit with *Lasius fuliginosus* in Bulgaria, whereas *Amphotis orientalis* was recently found for the first time in soil traps in Southwestern Bulgaria in a region with increased mild Mediterranean climate [62]. It is believed that *A. orientalis* is more xerothermic than *A. marginata,* and that it lives in the nests of *Crematogaster scutellaris* [101]. The characteristic body shape of *Amphotis* species provides secure protection of the appendages in case of ant attacks. Ant workers have been observed feeding the adult beetles through regurgitation. Their larvae are mycophagous and phytosaprophagous [102].

2.9. Family Cerylonidae. Only few cerylonid species from Ceryloninae and Euxestinae show myrmecophilous life habits. *Cerylon histeroides* (Fabricius, 1792) found in a nest of *Lasius brunneus* in Bulgaria usually lives under the bark of rotting deciduous trees [103]. Sieber [104] established it in a *Formica rufa* L. nest in Germany and treated this species as a winter guest.

2.10. Family Endomychidae. The majority of genera in the subfamily Merophysiinae (*Cholovocera*, *Merophysia*, *Reitteria*) as well as in Pleganophorinae (*Pleganophorus*, *Trochoideus*) are closely related to ants and their nests [67]. Three species from Endomychidae family—*Cholovocera major* Reitter, 1887, *Merophysia oblonga* Kiesenwetter, 1872, and *Mycetaea subterranea* Fabricius, 1801—have been reported in ants' nests in Bulgaria. In Europe, *Cholovocera major* has only been collected in Bulgaria and Macedonia, after its description in Anatolia [67, 105]. It is thus the only representative of the genus *Cholovocera* in Bulgaria. *Mycetaea subterranea* can be found both inside and outside of ants' nests, for example, in birds' nests, and it has also been found in caves in Bulgaria [106, 107].

2.11. Family Latridiidae. Family Latridiidae, commonly known as minute brown scavenger beetles, has scarcely been investigated in Bulgaria. These beetles are frequently found in decaying vegetation, where they feed in a predominantly mycophagous manner. Only *Corticaria longicollis* (Zetterstedt, 1838) is a myrmecophile in nests of different *Formica* species [67], recorded in Bulgaria.

2.12. Family Tenebrionidae. Darkling beetles are one of the most diverse family within Coleoptera [108] with more than 15,000 species all over the world. They inhabit a wide range of localities and show a particular affinity to dry, warm habitats.

Myrmechixenus subterraneus Chevrolat, 1835, from family Tenebrionidae has not been reported for the Bulgarian fauna until now. It is a well-known Euro-Siberian species, common in the nests of *Formica* ant species and, more rarely, of *Lasius* [16]. The collecting data from Bulgaria are as follows.

In nests of Formica pratensis. Southwestern Bulgaria, Vitosha Mt., near Bistritsa vill., 1000 m a.s.l., from February to November 1994–2002: 130 specimens; near Zheleznitsa vill., 1250 m a.s.l., 17.08.1998: 21 specimens, 02.03.2002: 1 specimen; Lozen Mt., 900 m a.s.l., 23.02.2002: 1 specimen; Zemen gorge, 580 m a.s.l., 28.02.1998: 14 specimens, 18.10.1998: 3 specimens, 05.11.1998: 8 specimens; 27.03.2001: 3 specimens.

In nests of Formica rufa. Southwestern Bulgaria, Vitosha Mt., above Bistritsa vill., 1050 m a.s.l., 14.11.1997: 1 specimen; Rila Mt., 1400 m a.s.l., 24.07.1998: 2 specimens.

In a nest of Formica cinerea. Vitosha Mt., above Zheleznitsa vill., 1200 m a.s.l., 02.03.2002: 1 specimen.

In a nest of Formica pressilabris. Zemen gorge, the ridge above the town of Zemen, 900 m a.s.l., 26.09.1998: 33 specimens.

Picka [69] was the first to document 2 Balkan-Anatolian Stenosini species: *Eutagenia smyrnensis* (Solier, 1838) and *Dichillus carinatus* (Küster, 1848) as myrmecophilous in Bulgaria. Here, I include an ant host *Pheidole pallidula* for *D. carinatus,* observed under a stone in Southwestern Bulgaria (Zemen gorge).

2.13. Family Chrysomelidae. The larvae of *Clytra laeviuscula* Ratzeburg, 1837, and *C. quadripunctata* (Linnaeus, 1758), enclosed in cases, live in nests of *Formica* where they feed partly on vegetable refuse, but also on ant droppings and pellets [109]. The former mostly inhabits the ground nests of *Formica sanguinea,* in comparison with *C. quadripunctata,* which occurs in mound-built *Formica* nests.

2.14. Family Brentidae. Family Brentidae is distributed mainly within the tropics. The tribe Eremoxenini is represented in the Palaearctic by 2 myrmecophilous species—*Eremoxenus chan* Semenow, 1892 (living with *Camponotus turkestanicus* Emery, 1887 in Middle Asia) and *Amorphocephala coronata* (Germar, 1817).

Amorphocephala coronata occurs in the Mediterranean region, almost always in *Camponotus* ants' nests but also, more rarely, in *Lasius*, *Pheidole*, and *Crematogaster* nests where 2-way regurgitation feeding with the aim of close integration of beetles and ant workers has been observed [2, 110, 111].

The species has been recorded in a few localities in Bulgaria, but it is the first time that the association of *A. coronata* with *Camponotus aethiops* has been established. The new collecting locality was in Southeastern Bulgaria, near the Turkish border (Strandzha Mt., Kalovo vill.).

3. Conclusions

Based on investigation of the available literature as well as new data on ant-associated beetles in Bulgaria, a total of 121 species from 14 Coleoptera families have been listed, and 71 of these species are referred to as myrmecophilous. Not surprisingly, the family Staphylinidae, with 79 species, are the most diverse and species-rich beetles found in ants' nests.

Of about 170 ant species in Bulgaria, only 22 ant host species have been documented in singular reports on the myrmecophilous beetle fauna. The largest proportion of the known ant-related beetles in Bulgaria inhabit the nests of the Formicinae ant species of *Formica* (76 species) and *Lasius* (25 species) genera, similar with findings from other countries in the Palaearctic [12, 13, 16]. Most beetle species have been reported in nests of the meadow ant *Formica pratensis* and the red wood ant *Formica rufa* (30 and 25 species, resp.). Together with species from other mound-building *Formica* (*F. exsecta*—9, *F. lugubris*—3, and *F. pressilabris*—2), the number of species totals 69. This is because, on the one hand, there have been more intensive studies on the nests of the above-mentioned species, and on the other hand, mound nests provide more of a variety of microhabitats which are suitable for a greater number of cohabitants. There is a lack of available information on myrmecophiles found with ant species from subfamilies Ponerinae and Dolichoderinae, although the Dolichoderinae such as *Tapinoma erraticum* (Latreille, 1798) and *Liometopum microcephalum* (Panzer, 1798) are common ant species in Bulgaria, and many myrmecophiles are known to inhabit their nests. Ant hosts for 31 ant-associated beetles listed for Bulgaria in previous studies have not been noted at all.

Bulgaria's location favors the existence of a diverse ant-associated fauna mainly composited by species with a wide range in the western Palaearctic, especially in Europe, but some species, are known to occur in limited regions only: 10 are endemic to the Balkans, 3 are Balkan-Anatolian species and 2 are currently known from Bulgaria (Table 1).

Ants' nests are unique habitats with a high local biodiversity, and the associated beetle species contributes to species richness in Bulgaria. The presence of only singular records for most of the listed species and the lack of data from the nests of most ant species in Bulgaria are valid reasons for more intensive investigation on this group of beetles in the future.

Acknowledgments

The author wishes to express her sincere gratitude to Mikael Sörensson for the identification of the species from family Ptiliidae and to Borislav Guéorguiev and Peter Hlaváč for providing additional literature sources. She is also indebted to the anonymous referees for their useful comments on an earlier version of the paper.

References

[1] W. M. Wheeler, *Ants: Their Structure, Development and Behavior*, Columbia University Press, New York, NY, USA, 1910.

[2] B. Hölldobler and E. O. Wilson, *The Ants*, Harvard University Press, Cambridge, Mass, USA, 1990.

[3] D. J. C. Kronauer and N. E. Pierce, "Myrmecophiles," *Current Biology*, vol. 21, no. 6, pp. R208–R209, 2011.

[4] F. Märkel, "Beiträge zur Kenntniss der unter Ameisen lebenden, Insekten, Erstes Stück," *Germar's Zeitschrift für die Entomologie*, vol. 3, pp. 203–225, 1841.

[5] F. Märkel, "Beiträge zur Kenntniss der unter Ameisen lebenden, Insekten, Zweites Stück," *Germar's Zeitschrift für die Entomologie*, vol. 5, pp. 193–271, 1844.

[6] E. S. J. Wasmann, *Kritisches Verzeichniss der myrmekophilen und termitophilen Arthropoden mit Angabe der Lebensweise und mit Beschreibung neuer Arten*, Berlin, Germany, 1894.

[7] E. O. Wilson, *The Insect Societies*, The Belknap Press of Harvard University Press, Cambridge, Mass, USA, 1971.

[8] P. Bouchard, Y. Bousquet, A. E. Davies et al., "Family-group names in Coleoptera (Insecta)," *ZooKeys*, vol. 88, pp. 1–972, 2011.

[9] S. Bílý, M. Fikáček, and P. Šípek, "First record of myrmecophily in buprestid beetles: immature stages of *Habroloma myrmecophila* sp. nov. (Coleoptera: Buprestidae) associated with *Oecophylla* ants (Hymenoptera: Formicidae)," *Insect Systematics and Evolution*, vol. 39, no. 2, pp. 121–131, 2008.

[10] E. Maurizi, S. Fattorini, W. Moore, and A. Di Giulio, "Behavior of *Paussus favieri* (Coleoptera, Carabidae, Paussini): a myrmecophilous beetle associated with *Pheidole pallidula* (Hymenoptera, Formicidae)," *Psyche*, vol. 2012, Article ID 940315, 9 pages, 2012.

[11] J. Päivinen, P. Ahlroth, V. Kaitala, J. S. Kotiaho, J. Suhonen, and T. Virola, "Species richness and regional distribution of myrmecophilous beetles," *Oecologia*, vol. 134, no. 4, pp. 587–595, 2003.

[12] G. M. Dlussky, *The Ants from the Genus Formica (Hymenoptera, Formicidae, G. Formica)*, Nauka, Moscow, Russia, 1967.

[13] J. Päivinen, P. Ahlroth, and V. Kaitala, "Ant-associated beetles of Fennoscandia and Denmark," *Entomologica Fennica*, vol. 13, no. 1, pp. 20–40, 2002.

[14] R. Jeannel, *Coléoptères Psélaphides*, vol. 53 of *Faune de France*, 1950.

[15] D. H. Kistner, "Social and evolutionary significance of social insect symbionts," in *Social Insects, Vol. I*, H. R. Hermann, Ed., pp. 339–413, Academia Press, New York, NY, USA, 1979.

[16] V. Franc, "Myrmecophilous beetles of Slovakia with special reference to their endangerment and perspectives for protection," *Acta Universitatis Carolinae Biologica*, vol. 36, pp. 299–324, 1992.

[17] A. Lapeva-Gjonova, V. Antonova, A. G. Radchenko, and M. Atanasova, "Catalogue of the ants (Hymenoptera, Formicidae) of Bulgaria," *ZooKeys*, vol. 62, pp. 1–124, 2010.

[18] A. Lapeva-Gjonova and K. Kiran, "Ants from Strandzha Mountain (Bulgaria, Turkey) and adjacent Black Sea coast," *North-Western Journal of Zoology*, vol. 8, no. 1, pp. 72–84, 2012.

[19] I. Frivaldszky von Frivald, *Közlések, a'Balkány vidékén tett természettudományi utazásról*, Budán: Magyar Királyi Egyetem Nyomtatása, 1835.

[20] F. Hieke and D. Wrase, "Faunistik der Laufkafer Bulgariens (Coleoptera, Carabidae)," *Deutsche Entomologische Zeitschrift*, vol. 35, no. 1–3, p. 12, 1988.

[21] V. Guéorguiev and B. V. Guéorguiev, *Catalogue of the Ground-Beetles of Bulgaria (Coleoptera: Carabidae)*, vol. 2 of *Series Faunistica*, Pensoft Publishers, Sofia-Moscow, Russia, 1995.

[22] A. Lapeva-Gjonova, R. Kostova, and B. V. Guéorguiev, "New records of the myrmecophilous beetle *Paussus turcicus* (Coleoptera, Carabidae) in Southeastern Balkans," *ZooNotes*, vol. 21, pp. 1–3, 2011.

[23] A. Lapeva-Gjonova and E. Chehlarov, "Contribution to the knowledge of myrmecophilous Histeridae (Insecta: Coleoptera) in Bulgaria," *Acta Zoologica Bulgarica*, vol. 55, no. 1, pp. 11–14, 2003.

[24] L. Zerche, "Myrmecobionte Aleocharinae bei *Formica exsecta* (Nylander) aus Bulgarien (Coleoptera, Staphylinidae)," *Entomologische Nachrichten und Berichte*, vol. 32, pp. 271–272, 1988.

[25] P. Hlaváč, R. Bekchiev, J. Růžička, and T. Lackner, "Contribution to the knowledge of myrmecophilous beetles (Coleoptera) of Bulgaria," *Acta Societatis Zoologicae Bohemicae*, vol. 71, pp. 127–132, 2007.

[26] T. Yélamos, "Revision of the genus *Sternocoelis* Lewis, 1888 (Coleoptera: Histeridae), with a proposed phylogeny," *Revue Suisse de Zoologie*, vol. 102, no. 1, pp. 113–174, 1995.

[27] P. Angelov, "Die Arten der Familie Histeridae (Coleoptera) aus Bulgarien," *Travaux Scientifiques des Universite d'Plovdiv* , vol. 26, no. 6, pp. 163–175, 1988.

[28] E. Chehlarov, "Histeridae (Insecta: Coleoptera) from the Eastern Rhodopes (Bulgaria)," in *Biodiversity of Bulgaria. 2. Biodiversity of Eastern Rhodopes (Bulgaria and Greece)*, P. Beron and A. Popov, Eds., pp. 413–418, Pensoft & National Museum of Natural History, Sofia, Bulgaria, 2004.

[29] D. Ioakimov, "A contribution to the Bulgarian insect fauna," *Sbornik Narodni Umotvorenija, Nauka i Knižnina*, vol. 20, pp. 1–43, 1904.

[30] J. Růžička, "A new species of *Catops* and notes on Cholevinae (Coleoptera: Leiodidae) from Bulgaria," *Klapalekiana*, vol. 31, pp. 121–129, 1995.

[31] P. Hlaváč and T. Lackner, "Contribution to the knowledge of myrmecophilous beetles of Slovakia," *Entomofauna Carpathica*, vol. 10, pp. 1–9, 1998.

[32] R. Jeannel, *Monographie des Catopidae (Insectes Coléoptères)*, Mémoires du Museum National d'Histoire Naturelle (N.S.), 1936.

[33] W. Szymczakowski, "Ergebnisse der Albanien-Expedition 1961 des Deutschen Entomologischen Institutes: Coleoptera Catopidae (exclusive Bathysciinae)," *Beiträge zur Entomologie*, pp. 689–699, 1965.

[34] B. Guéorguiev and T. Ljubomirov, "Coleoptera and Hymenoptera (Insecta) from Bulgarian section of the Maleshevska planina Mountain: study of an until recently unknown biodiversity," *Acta Zoologica Bulgarica*, vol. 61, no. 3, pp. 235–276, 2009.

[35] R. Bekchiev, "A study of the Pselaphinae (Coleoptera, Staphylinidae) in the Rhodopes Mountain (Bulgaria)," in *Biodiversity of Bulgaria. 4. Biodiversity of Western Rhodopes (Bulgaria and Greece). II*, P. Beron, Ed., pp. 267–278, Pensoft & National Museum of Natural History, Sofia, Bulgaria, 2011.

[36] Z. Karaman, "Über einige neue und interessante Pselaphiden vertreter der Balkanhalbinsel," *Fragmenta Balcanica*, vol. 7, no. 3, pp. 9–20, 1969.

[37] A. Lapeva-Gjonova, "Pselaphinae (Coleoptera, Staphylinidae) from ant nests (Hymenoptera: Formicidae) in Southwestern Bulgaria," *Acta Zoologica Bulgarica*, vol. 56, no. 1, pp. 67–73, 2004.

[38] C. Besuchet, "Contribution à l'étude des *Batrisodes* paléarctiques (Coleoptera: Pselaphidae)," *Revue Suisse de Zoologie*, vol. 88, pp. 275–296, 1981.

[39] J. Rambousek, "Příspěvek k poznaní bulharských Pselaphidů a Scydmaenidů," *Časopis České Společnosti Entomologické*, vol. 6, pp. 16–24, 1909.

[40] I. Löbl and C. Besuchet, "Pselaphinae," in *Catalogue of the Palaearctic Coleoptera*, I. Löbl and A. Smetana, Eds., vol. 2 of *Hydrophiloidea-Histeroidea-Staphylinoidea*, pp. 272–329, Stenstrup: Apollo Books, 2004.

[41] R. Bekchiev, "The subfamily Pselaphinae (Coleoptera: Staphylinidae) of Southwestern Bulgaria II: Belasitsa Mts," *Biologia*, vol. 66, no. 3, pp. 506–508, 2011.

[42] Z. Karaman, "Vier balkanische und eine italienische neue Pselaphiden Arten," *Fragmenta Balcanica Musei Macedonici Scientiarum Naturalium*, vol. 9, no. 4, pp. 37–51, 1972.

[43] J. Mařan, "Nový druh rodu *Claviger* Preyss. z Řecka a poznámky o geografickém rozšíření některých Clavigeridů," *Časopis Československé Společnosti Entomologické*, vol. 33, no. 1-2, pp. 26–27, 1936.

[44] R. Bekchiev and M. Shishiniova, "A contribution to the Pselaphinae fauna (Coleoptera: Staphylinidae) of the Vitosha Mountain," *Acta Zoologica Bulgarica*, vol. 59, no. 1, pp. 33–39, 2007.

[45] R. Bekchiev, "The subfamily Pselaphinae (Coleoptera: Staphylinidae) of Southwestern Bulgaria I," *Historia Naturalis Bulgarica*, vol. 19, pp. 51–71, 2008.

[46] A. Lapeva-Gjonova and O. Ilieff, "Ant-associated rove beetles (Coleoptera, Staphylinidae) in Bulgaria," *Acta Entomologica Slovenica*, vol. 20, no. 1, pp. 73–84, 2012.

[47] O. llieff and A. Lapeva, "Rove beetles (Coleoptera, Staphylinidae) from nests of the meadow ant *Formica pratensis* (Hymenoptera, Formicidae)," *Acta Zoologica Bulgarica*, vol. 49, pp. 102–104, 1997.

[48] I. Vasilev and O. Ilieff, "New rove beetle species (Coleoptera, Staphylinidae) for the fauna of Bulgaria," *Acta Zoologica Bulgarica*, vol. 35, pp. 90–92, 1987.

[49] A. Smetana, "Aleocharinae," in *Catalogue of the Palaearctic Coleoptera*, I. Löbl and A. Smetana, Eds., vol. 2 of *Hydrophiloidea-Histeroidea-Staphylinoidea*, pp. 353–494, Stenstrup: Apollo Books, 2004.

[50] P. Hlaváč, A. F. Newton, and M. Maruyama, "World catalogue of the species of the tribe Lomechusini (Staphylinidae: Aleocharinae)," *Zootaxa*, vol. 3075, pp. 1–151, 2011.

[51] O. Scheerpeltz, "Wissenschaftliche Ergebnisse einer von Herrn Hofrat F. Schubert seinem Sohne Herrn cand. Phil. F. Schubert und Herrn Prof. Ing. K. Mandl im Sommer 1935 (1936) nach Bulgarien unternommenen Studienreise. Coleoptera: I. Staphylinidae. (24. Beitrag zur Kenntnis der palaearctischen Staphylinidae, gleichzeitig 1. Beitrag zur Kenntnis der Staphylinidae Bulgariens)," *Mitteilungen aus den Königlichen Naturwissenschaftlichen Instituten in Sofia-Bulgarien*, vol. 10, pp. 185–246, 1937.

[52] V. B. Guéorguiev, "Coleopters (Coleoptera) in the orophyte zone of the Pirin Mountain," in *Fauna of Southwestern Bulgaria, Part 2*, B. Botev, V. Golemanski, V. Beshovski, and V. Guéorguiev, Eds., pp. 74–87, Bulgarian Academy of Sciences, Sofia, Bulgaria, 1988.

[53] O. Ilieff and I. Vasilev, "A contribution to the study of the rove beetles (Staphylinidae, Coleoptera)," in *Annuaire de l'Universitete de Sofia "St. Kliment Ohridski", Faculte de Biologie, Livre 1—Zoologie*, vol. 79, pp. 45–48, 1985.

[54] J. Roubal, "Fragmente zur Koleopterenfaunistik des balkanischen Festlandes," *Entomologischer Anzeiger*, vol. 11, no. 23, 1931.

[55] N. Nedelkov, "Vierter Beitrag zur Insectenfauna Bulgariens," *Sbornik Narodni Umotvorenia Nauka Knizhnina*, vol. 25, pp. 1–32, 1909.

[56] O. Ilieff, "New and rare species staphylinid beetles from subfamily Aleocharinae (Coleoptera, Staphylinidae) from Southwestern Bulgaria," *Annuaire de l'Universitete de Sofia "St. Kliment Ohridski", Faculte de Biologie, Livre 1—Zoologie*, vol. 84, pp. 127–131, 1994.

[57] M. A. Alonso-Zarazaga and A. Sanchez-Terron, "Fauna Europaea: Scydmaenidae," Fauna Europaea version 1. 1, 2004, http://www.faunaeur.org.

[58] A. Davies, "Scydmaenidae, tribe Cyrtoscydmini," in *Catalogue of the Palaearctic Coleoptera*, I. Löbl and A. Smetana, Eds., vol. 2 of *Hydrophiloidea-Histeroidea-Staphylinoidea*, pp. 206–223, Stenstrup: Apollo Books, 2004.

[59] S. Vít, "Scydmaenidae, Scydmaeninae, Scydmaenini," in *Catalogue of the Palaearctic Coleoptera*, I. Löbl and A. Smetana, Eds., vol. 2 of *Hydrophiloidea-Histeroidea-Staphylinoidea*, pp. 224–227, Stenstrup: Apollo Books, 2004.

[60] *Collections du Musée d'Histoire Naturelle de son Altesse Royale Ferdinand I Prince de Bulgarie*, Sofia, Bulgaria, 1907.

[61] J. Jelínek, "Ergebnisse der Albanien-Expedition 1961 des Deutschen Entomologischen Institutes. 37 Beitrag. Coleoptera: Nitidulidae," *Beiträge zur Entomologie*, vol. 15, no. 5-6, pp. 673–688, 1965.

[62] B. Guéorguiev, "*Amphotis orientalis* Reiche, 1861 in Bulgaria (Coleoptera: Nitidulidae)," *Historia Naturalis Bulgarica*, vol. 16, pp. 109–112, 2004.

[63] J. Roubal, "*Pleganophorus bispinosus* Hampe aus der Čechoslovakei," *Entomologische Nachrichtsblatt*, vol. 2, pp. 24–25, 1928.

[64] K. Dorn, "*Pleganophorus bispinosus* Hampe (Coleopt.)," *Entomologische Blätter*, vol. 32, pp. 137–139, 1936.

[65] A. Horion, *Faunistik der Mitteleuropäischen Käfer*, Band 8: Clavicornia 2. Teil (Thorictidae bis Cisidae), Teredilia, Coccinellidae, Überlingen, Germany, 1961.

[66] J. Sár, Gy. Dudás, and O. Merkl, "*Pleganophorus bispinosus* Hampe, 1855 in Hungary (Coleoptera: Endomychidae)," *Somogyi Múzeumok Közleményei*, vol. 16, pp. 329–332, 2004.

[67] A. Lapeva-Gjonova and W. H. Rücker, "Latridiidae and Endomychidae beetles (Coleoptera) from ant nests in Bulgaria," *Latridiidae—Mitteilungsblatt für Systematik und Taxonomie der Latridiidae*, vol. 8, pp. 5–8, 2011.

[68] J. Roubal, "Fragmente zur Koleopterenfaunistik des balkanischen Festlandes," *Entomologischer Anzeiger Wien*, vol. 12, pp. 18–19, 1932.

[69] J. Picka, "Zweiter Beitrag zur Kenntnis der Familie Tenebrionidae Bulgariens (Coleoptera)," *Acta Entomologica Musei Nationalis Pragae*, vol. 42, pp. 307–309, 1987.

[70] B. Gruev and V. Tomov, "Coleoptera, Chrysomelidae. I," in *Fauna Bulgarica*, vol. 13, Academie Scientiarum Bulgarica, 1984.

[71] B. Gruev and V. Tomov, "Coleoptera: Chrysomelidae," in *Catalogus Faunae Bulgaricae*, vol. 3, Bulgarian Academy of Sciences, Pensoft Publishers, Sofia, Bulgaria, 1998.

[72] E. Csiki, "Coleopteren von Alibotusch-Gebirge in Süd-Bulgarien," *Mitteilungen aus den Königlichen Naturwissenschaftlischen Instituten, Sofia*, vol. 16, pp. 214–218, 1943.

[73] A. Popov, "An insect of tropical origin found in Bulgaria," *Priroda*, vol. 9, no. 4, pp. 76–77, 1960.

[74] P. Angelov, "Coleoptera: Rhynchophora: Urodonidae, Anthribidae, Brenthidae, Rhinomaceridae, Attelabidae," in *Fauna Bulgariae*, M. Josifov, Ed., vol. 11, Bulgarian Academy of Sciences, Sofia, Bulgaria, 1981.

[75] J. Simandl, "New and interesting records of beetles (Coleoptera) from Bulgaria," *Acta Zoologica Bulgarica*, vol. 54, no. 2, pp. 59–66, 2002.

[76] P. Nagel, "Flanged bombardier beetles from Laos (Carabidae, Paussinae)," *Entomologica Basiliensia et Collectionis Frey*, vol. 31, pp. 101–113, 2009.

[77] S. F. Geiselhardt, K. Peschke, and P. Nagel, "A review of myrmecophily in ant nest beetles (Coleoptera: Carabidae: Paussinae): linking early observations with recent findings," *Naturwissenschaften*, vol. 94, no. 11, pp. 871–894, 2007.

[78] P. Nagel, "Carabidae: Paussinae," in *Catalogue of Palaearctic Coleoptera*, I. Löbl and A. Smetana, Eds., vol. 1, pp. 208–211, Apollo Books, Stenstrup, 2003.

[79] P. Nagel, "Insects of Saudi Arabia. Coleoptera: Fam. Carabidae, Subfam. Paussinae," in *Fauna of Saudi Arabia*, W. Wittimer and W. Buttiker, Eds., vol. 4, pp. 99–107, Basel, Switzerland, 1982.

[80] S. Mazur, *A World Catalogue of Histeridae (Coleoptera, Histeroidea)*, Biologica Silesiae, Wroclaw, Poland, 1997.

[81] F. Penati, "An updated catalogue of the Histeridae (Coleoptera) of Sardinia, with faunistic, zoogeographical, ecological and conservation remarks," in *Research on the Terrestrial Arthropods of Sardinia (Italy)*, P. Cerretti, F. Mason, A. Minelli, G. Nardi, and D. Whitmore, Eds., vol. 2318 of *Zootaxa*, pp. 1–602, 2009.

[82] T. Yélamos, "Description of a new species of *Satrapes* Schmidt, 1885 with proposed phylogeny of the Palearctic genera of Hetaeriinae (Coleoptera: Histeridae)," *Sessions Entomològiques ICHN-SCL, Barcelona*, vol. 9, pp. 63–74, 1997.

[83] J. Roubal, "Catalogue of myrmecophilous Coleoptera in northeast Bohemia, with bibliography and notes of Bohemians myrmecophilous Coleoptera," *Časopis České Společnosti Entomologické*, vol. 46, no. 3-4, pp. 157–165, 1949.

[84] O. L. Kryzhanovskij and A. N. Reihardt, *Fauna USSR. 5. Superfamily Histeroidea (Sphaeritidae, Histeridae, Sinteliidae)*, Nauka, Leningrad, Russia, 1976.

[85] C. G. Majka and M. Sörensson, "Featherwing beetles (Coleoptera: Ptiliidae) of the Atlantic Maritime Ecozone," in *Assessment of the Species Diversity in the Atlantic Maritime Ecozone*, D. F. McAlpine and I. M. Smith, Eds., pp. 433–438, NRC Research Press, Ottawa, Canada, 2010.

[86] M. Sörensson, "The Ptiliidae of Sardinia—new records, distribution and zoogeography, with a checklist of Italian species (Coleoptera)," in *Biodiversity of Marganai and Montimannu (Sardinia), Research in the Framework of the ICP Forests Network, Conservazione Habitat Invertebrati*, G. Nardi, D. Whitmore, M. Bardiani et al., Eds., vol. 5, pp. 313–329, 2011.

[87] W. E. Hall, "Ptiliidae Erichson, 1845," in *Handbook of Zoology. Vol. IV. Arthropoda: Insecta. Part 38. Coleoptera, B. Volume 1: Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim)*, R. G. Beutel and R. A. B. Leschen, Eds., pp. 251–261, Walter de Gruyter, New York, NY, USA, 2005.

[88] F. Angelini and M. Sörensson, "Materiali per una coleotterofauna dell'Italia meridionale e della Sicilia. Ptiliidae. (Coleoptera)," *Annali del Museo civico di Storia naturale, "G. Doria"*, vol. 91, pp. 555–587, 1997.

[89] B. Guéorguiev, "First records of beetles (Insecta: Coleoptera) for the fauna of Bulgaria from the Belasitsa Mountain," *Historia Naturalis Bulgarica*, vol. 20, pp. 75–78, 2012.

[90] P. Kočárek, "Small carrion beetles (Coleoptera: Leiodidae: Cholevinae) in Central European lowland ecosystem: seasonality and habitat preference," *Acta Societatis Zoologicae Bohemicae*, vol. 66, pp. 37–45, 2002.

[91] Z. Karaman, "Mirmekofilni Koleopteri makedonije I," *Godishen zbornik Zemjodelsko-šumarski fakultet Skopje*, vol. 10, pp. 5–15, 1957.

[92] V. A. Kastcheev, "Classification of eco-morphological types of adult staphylinids," *Tethys Entomological Research*, vol. 1, pp. 157–170, 1999.

[93] J. D. McIver and G. Stonedahl, "Myrmecomorphy: morphological and behavioral mimicry of ants," *Annual Review of Entomology*, vol. 38, pp. 351–379, 1993.

[94] R. K. Vander Meer and D. P. Wojcik, "Chemical mimicry in the myrmecophilous beetle *Myrmecaphodius excavaticollis*," *Science*, vol. 218, no. 4574, pp. 806–808, 1982.

[95] B. Hölldobler, M. Möglich, and U. Maschwitz, "Myrmecophilic relationship of *Pella* (Coleoptera: Staphylinidae) to *Lasius fuliginosus* (Hymenoptera, Formicidae)," *Psyche*, vol. 88, pp. 347–374, 1981.

[96] V. Assing, "A revision of the genus *Piochardia* Heyden, 1870 (Insecta: Coleoptera: Staphylinidae: Aleocharinae)," *Annalen des Naturhistorischen Museums in Wien*, vol. 101, pp. 277–301, 1999.

[97] S. T. O'Keefe, "Ant-like stone beetles, ants, and their associations (Coleoptera: Scydmaenidae; Hymenoptera: Formicidae; Isoptera)," *Journal of the New York Entomological Society*, vol. 108, no. 3-4, pp. 273–303, 2000.

[98] P. Jałoszyński, "Adults of European ant-like stone beetles (Coleoptera: Staphylinidae: Scydmaeninae) *Scydmaenus tarsatus* Müller & Kunze and *S. hellwigii* (Herbst) prey on soft-bodied arthropods," *Entomological Science*, vol. 15, pp. 35–41, 2012.

[99] Y. Bousquet, "Monotomidae Laporte, 1840," in *Handbook of Zoology—Coleoptera, Vol. II*, R. A. B. Leschen and R. G. Beutel, Eds., pp. 319–324, De Gruyter, New York, NY, USA, 2010.

[100] R. A. B. Leschen, "Origins of symbiosis: phylogenetic patterns of social insect inquilinism in Cryptophagidae (Coleoptera: Cucujoidea)," in *Entomological Contributions in Memory of Byron A. Alexander*, G. W. Byers, R. H. Hagen, and R. W. Brooks, Eds., vol. 24, pp. 85–101, University of Kansas Natural History Museum Special Publication, 1999.

[101] P. Audisio, "Sulla presenza in Italia di *Amphotis orientalis* Reiche, con alcune considerazioni sul genere *Amphotis* Erichson (Coleoptera, Nitidulidae)," *La vori Societa Veneziana di Scienze Naturali*, vol. 10, pp. 17–19, 1985.

[102] P. Audisio, *Coleoptera Nitidulidae—Kateretidae. Fauna d'Italia*, vol. 32, Calderini, Bologna, Italy, 1993.

[103] S. A. Ślipiński, "A monograph of the world Cerylonidae (Coleoptera: Cucujoidea) Part I—Introduction and higher classification," *Annali del Museo Civico di Storia Naturale "Giacomo Doria"*, vol. 88, 1990.

[104] M. Sieber, "Ergebnisse einer Siebeprobe zum quantitativen Verhältnis von Coleopteren in *Formica rufa*-Nestern," *Entomologische Nachrichten und Berichte*, vol. 26, pp. 137–138, 1982.

[105] W. H. Rücker, "Ergebnisse von Typenstudien der Gattungen *Metophthalmus* Motschulsky, 1850 (Coleoptera: Latridiidae), *Cholovocera* Motschulsky, 1838, *Reitteria* Leder, 1872, *Merophysia* Lucas, 1852 (Endomychidae: Merophysiinae) und *Holoparamecus* Curtis, 1833 (Endomychidae: Holoparamecinae)," *Latridiidae—Mitteilungsblatt für Systematik und Taxonomie der Latridiidae*, vol. 8, pp. 11–20, 2011.

[106] V. Guéorguiev and P. Beron, "Essai sur la faune cavernicole de Bulgarie," *Annales de Speleologie*, vol. 17, no. 2-3, pp. 285–441, 1962.

[107] P. Beron, "Essai sur la faune cavernicole de Bulgarie. III. Resultats des recherches biospeologique de 1966 à 1970," *International Journal of Speleology*, vol. 4, p. 317, 1972.

[108] P. Bouchard, J. F. Lawrence, A. E. Davies, and A. F. Newton, "Synoptic classification of the world Tenebrionidae (Insecta: Coleoptera) with a review of family-group names," *Annales Zoologici*, vol. 55, no. 4, pp. 499–530, 2005.

[109] H. Donisthorpe, *The Guests of British Ants, Their Habits and Life-Histories*, G. Routledge and Sons, London, UK, 1927.

[110] C. Bach-Piella and A. M. Cárdenas-Talaverón, "Cita para Andalucía de *Amorphocephalus coronatus* (Germar 1817) (Col. Brenthidae)," *Miscellania. Zoologíca*, vol. 8, pp. 287–288, 1984.

[111] G. Le Masne and C. Torossian, "Observations sur le comportement du coléoptère myrmécophile *Amorphocephalus coronatus* Germar (Brenthidæ) hôte des *Camponotus*," *Insectes Sociaux*, vol. 12, no. 2, pp. 185–194, 1965.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 736939, 10 pages
http://dx.doi.org/10.1155/2013/736939

Review Article

Evaluating Alpha and Beta Taxonomy in Ant-Nest Beetles (Coleoptera, Carabidae, Paussini)

Simone Fattorini,[1,2] Emanuela Maurizi,[3] and Andrea Di Giulio[3]

[1] *Water Ecology Team, Department of Biotechnology and Biosciences, University of Milano Bicocca, Piazza della Scienza 2, 20126 Milan, Italy*
[2] *Azorean Biodiversity Group (ABG, CITA-A) and Portuguese Platform for Enhancing Ecological Research & Sustainability (PEERS), University of the Azores, Rua Capitão João de Àvila, 9700-042 Angra do Heroísmo, Portugal*
[3] *Department of Sciences, University of Roma Tre, Viale G. Marconi 446, 00146 Rome, Italy*

Correspondence should be addressed to Andrea Di Giulio; digiulio@uniroma3.it

Received 7 March 2013; Accepted 24 April 2013

Academic Editor: Alain Lenoir

We evaluated completeness, accuracy, and historical trend of the taxonomic knowledge on the myrmecophilous ground beetle tribe Paussini (Coleoptera, Carabidae, Paussinae). Accumulation curves for valid names and synonyms of species, subgenera, and genera were modelled using logistic functions. Analyses of trends in synonymies suggest that few currently accepted taxa will be recognized to be synonymous in the future. This may indicate that Paussini are a taxonomically relatively stable tribe of carabid beetles. However, this result might also be due to the lack of recent taxonomic work in some biogeographical regions.

1. Introduction

Arthropods are the most diversified animal group [1, 2]. Although it is widely acknowledged that only a small fraction of the extant arthropod species has been described, the magnitude of the so-called Linnean shortfall (i.e., the discrepancy between the number of described species and the number of living species) is a matter of discussion [2]. Also for relatively well-investigated arthropod groups, there is few information about the quality of the taxonomic knowledge [3, 4]. The most basic question is to establish how complete and accurate the taxonomic status of a given group is. With the word completeness we refer here to the problem whether the species list of a given group can be considered fairly complete or if there are still many species to describe. A completely known group is one for which there is no longer a need of an alpha taxonomic work (the discovering and naming of new species [5]). With accuracy we refer to taxonomic stability. An accurately known group is one for which there is no more need of a beta taxonomic work (the study of the relationships between the already described taxa, through systematic revisional work of higher taxa [5]). Because it is not rare that species are redundantly described under different names (i.e., synonyms), a group is known with accuracy when no relevant taxonomic change is expected.

Although the two aspects tend to be interrelated, they are not necessarily redundant, because revisional works are much rarer than descriptions of new taxa.

In this paper, we evaluated the completeness and accuracy of the taxonomic knowledge about a group of myrmecophilous beetles, the tribe Paussini ("ant-nest beetles") of the family Carabidae (Coleoptera, Adephaga, Paussinae), at a global level.

All Paussini are highly specialized social parasites, depending on ants (mainly associated with Myrmicinae and Formicinae) during any stage of their development [6–9]. Adults prey on ants and their broods without any obvious benefit for the ant colonies [10–15]. Because of their specialised behavioural and morphological adaptations, Paussini have long attracted the interest of entomologists working on myrmecophilous insects [13], and they have been recently into focus because of strong incertitude about their relationships with other Paussinae lineages [6, 7]. These studies have prompted our knowledge of Paussini biology,

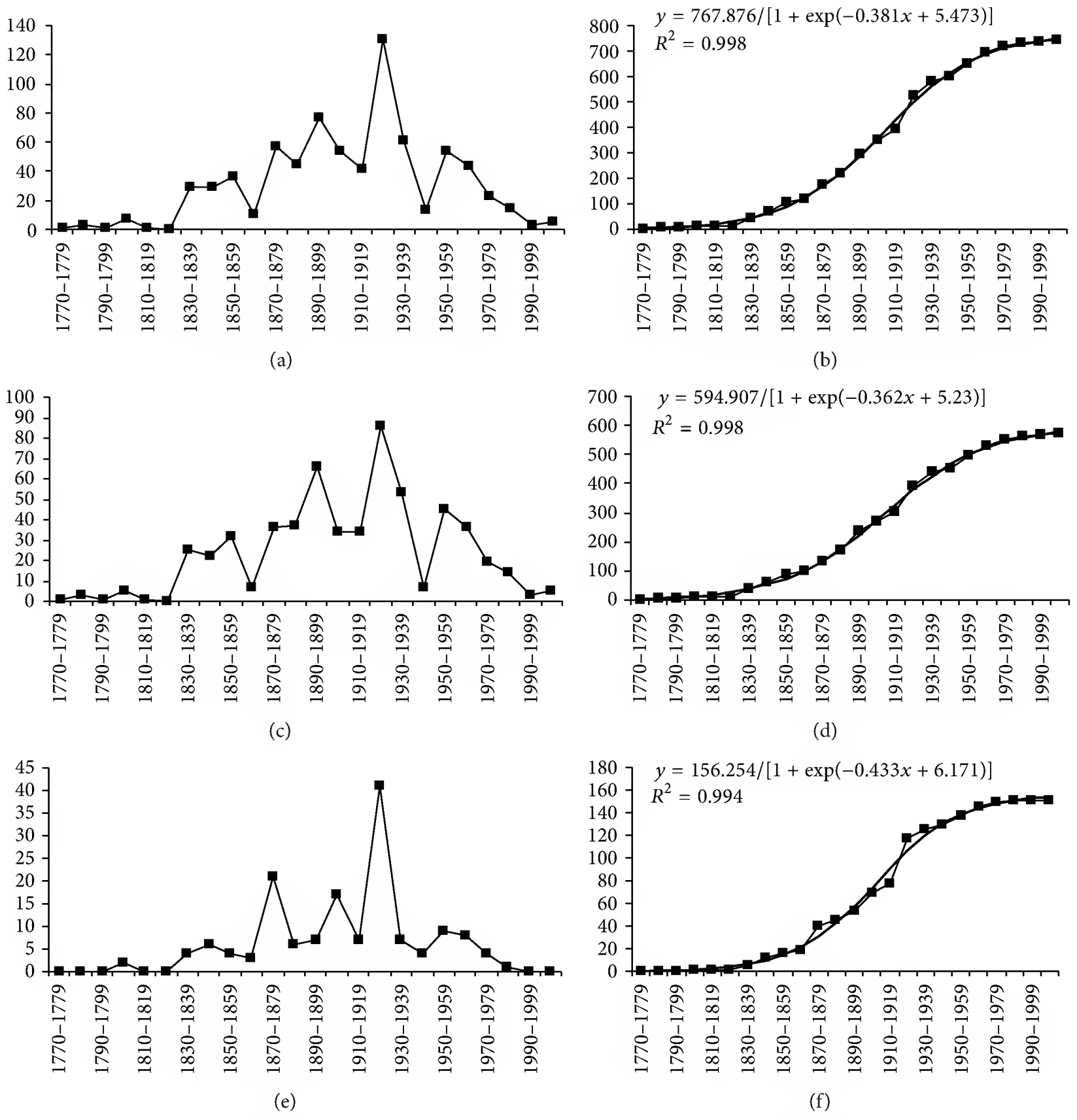


Figure 1: Numbers of total described taxa (a, b), valid species (c, d), and synonyms (e, f) of Paussini by decade. Figures (a), (c), and (e) report the absolute numbers, and Figures (b), (d), and (f) the cumulative numbers along with the equations of the fitted curves.

with emphasis on their immature stages and microscopic morphology, but taxonomical work seems to receive little attention.

In general, for assessing the status of the taxonomical process in a given group, the study should be addressed to describe (1) the growth through time of the cumulative number of valid names to estimate the number of species that remain to be discovered in a given taxonomic group, globally or regionally [4, 16–19], (2) the progression of the cumulative number of invalid names (synonyms), and (3) the temporal trends in the proportion of synonyms [20, 21]. Presence of a plateau is considered evidence that no, or few, species remain to be described, but it can be also due to a stop in taxonomic research [22]. In this paper we present an extension and continuation of a recently published study [22] where we have presented a comprehensive treatment of point 1. In the present paper we will treat the additional aspects of points 2 and 3 taking advantage of the statistical methodologies developed in the former paper.

2. Material and Methods

2.1. Data Collection. We used a computerized database including 572 species and 17 subspecies of the tribe Paussini.

The following information was recorded for each species and subspecies: generic assignment, subgeneric assignment, author, year of description, synonyms, and the biogeographical region of species distribution. We also recorded authorship and year of description of genera (see [22] for details).

2.2. Historical Accumulation Curves of Valid Names and Synonyms. We extracted the year of description of all valid

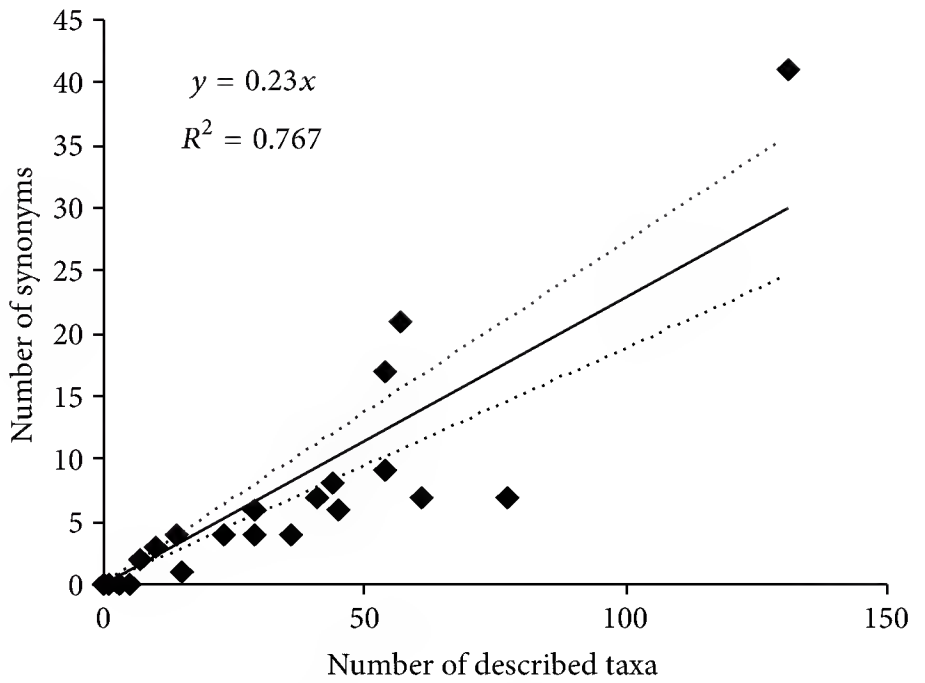


FIGURE 2: Relationship between number of synonyms and total number of described taxa per decade. Ordinary least square (OLS) regression forced to pass through the origin.

species and subspecies names, as well as the year of description of the names that are currently considered synonyms and grouped years into decades. We plotted the raw number of described taxa, and the raw number of valid taxa, the raw number of synonyms, as well as their cumulative number, against the decade of description.

To model species accumulation curves we used the logistic function $y = b_0/(1 + \exp(b_1 x + b_2))$, where b_0, b_1, and b_2 are estimated parameters, because it gave excellent fits and the first parameter (b_0) is the upper asymptote, thus providing an immediate estimate of the expected number of taxa. Similar analyses were conducted for genera and subgenera. The use of subgenera in the tribe Paussini is very controversial. For this reason, as in our companion study [22], we used subgenera as currently accepted by most authors [23].

2.3. Trends in Synonymies. Both the historical accumulation of species names and the relationship between valid species and synonyms may provide information about the status of the taxonomical knowledge in a given group [24].

Thus, we modelled synonym accumulation curves and measured the temporal variation in the taxonomical efficiency through time in three ways: (1) as the relationship between the number of synonyms versus the number of total described taxa in each decade, (2) as the proportion of names that are now regarded as synonyms over the total number of taxa described in that decade, (3) as the cumulative proportion of synonyms through decades.

Relationship between the number of synonyms versus the number of total described taxa was substantially linear, and we used an ordinary least squares (OLS) regression to model it. We forced the regression to pass through the origin, because when no taxon is described, the number of synonyms must be zero. We used the coefficient of the regression line as a measure of the number of synonyms introduced—on average—for each species in each decade. We used the 95% confidence limits to identify decades with exceptional number of synonyms.

Proportion of synonyms was used as a measure of the relationship between descriptive (alpha) and revisional (beta) taxonomy. We calculated the proportion of synonymous taxa described in each decade to identify a possible temporal trend in synonym proliferation.

The cumulative proportion of synonyms through decades was used as a rough measure of the quality of currently valid names. Following Baselga et al. [24] we assumed that the more taxonomical revisions are carried out, the higher is the probability for a given species name to be synonymized. Given that the synonyms are assigned to the date when the name was introduced, rather than the date when it was recognized as a synonym, the percentage of synonyms will show a diminishing trend with time, as newly described species will have had less time to be reviewed and eventually synonymized [24]. Irrespective of that, the steepness of the decay of this percentage through time can help us to measure the quality of currently valid names.

3. Results

The rate of species description per decade, when the absolute numbers are considered, is very irregular (Figure 1(a)). Between 1775 (when the first species of Paussini was described by Linnaeus) and 1840 only 31 species were described, and no species was described in the decade 1820–1829. In the latter half of the 19th century species were described at an increasing rate, with two peaks, respectively, in the decades 1870–1879 and 1890–1899, in which a total of 38 Paussini taxa were described. However, the description of species peaked between 1920 and 1929, during which period 131 taxa were described, covering almost 17% of the available names. The low level of species descriptions in the decades 1910–1919 and 1940–1949 may be explained by the effects of the First and Second World Wars. Since the 1950s descriptions decreased progressively. When the cumulative numbers are considered, the increase per decade was low until the 1840s. The cumulative numbers of species/subspecies descriptions have reached a plateau, the estimated asymptotic value for the fitted curve being 768 taxa (Figure 1(b)).

Analyses omitting synonyms and subspecies revealed similar patterns (Figure 1(c)), with an estimated asymptotic value of species number at 595 (Figure 1(d)). Because the number of currently recognized valid species is 572, the model predicts the existence of 23 undescribed species, with about 96 per cent of the world fauna described.

Patterns in synonyms were also similar to the general trend (Figures 1(e) and 1(f)). The asymptotic value for the number of synonyms is 156 names, very close to the current number of recognized synonyms.

Number of synonyms per decade was directly proportional to the number of described taxa, with a mean rate of one synonym per four taxa each decade (Figure 2). However, the decades 1870–1879, 1900–1909, and 1920–1929 were characterized by an exceptional high number of synonyms. An in-depth analysis of the percentage of synonyms per

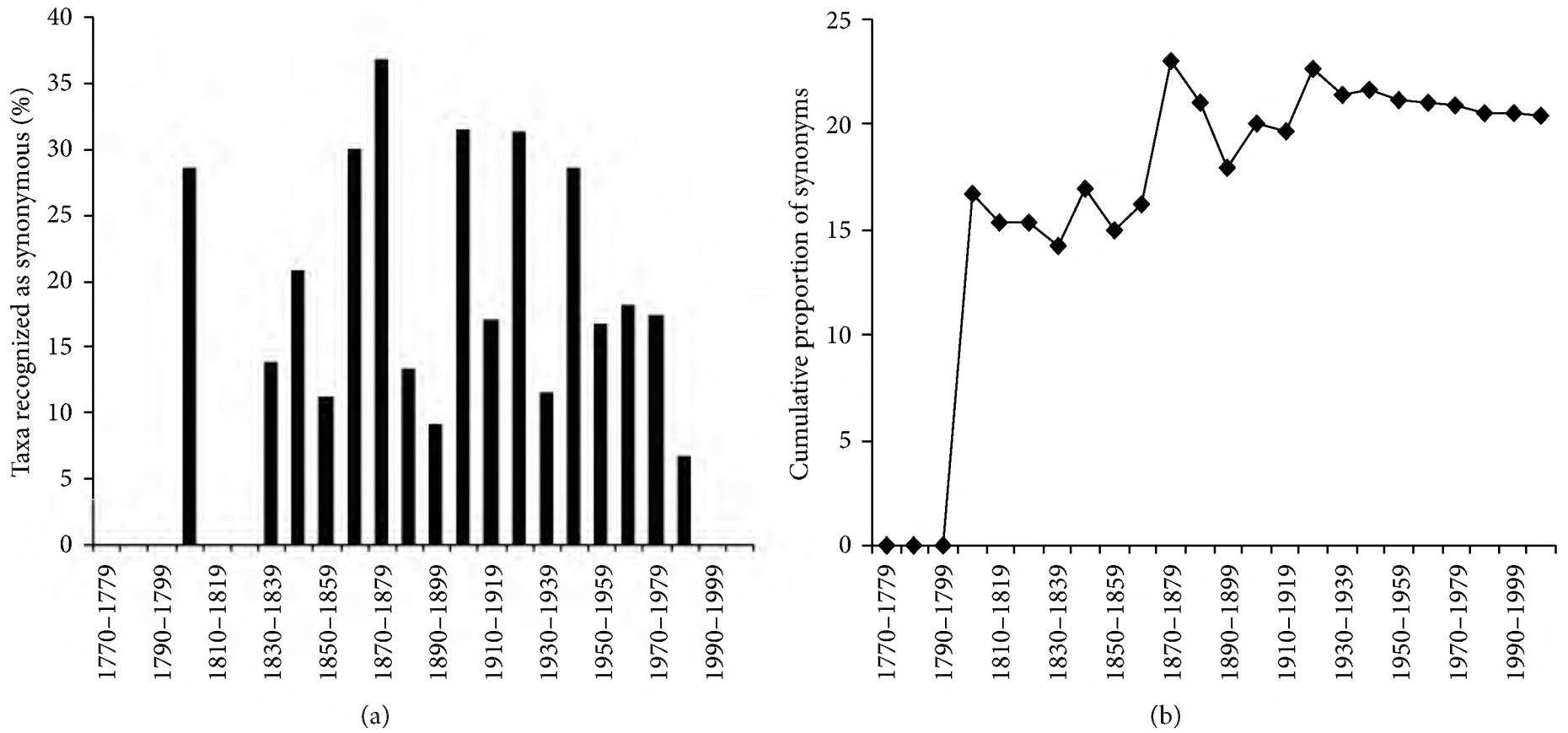


FIGURE 3: Percentages of synonymous taxa described in each decade (a) and their historical process of accumulation (b).

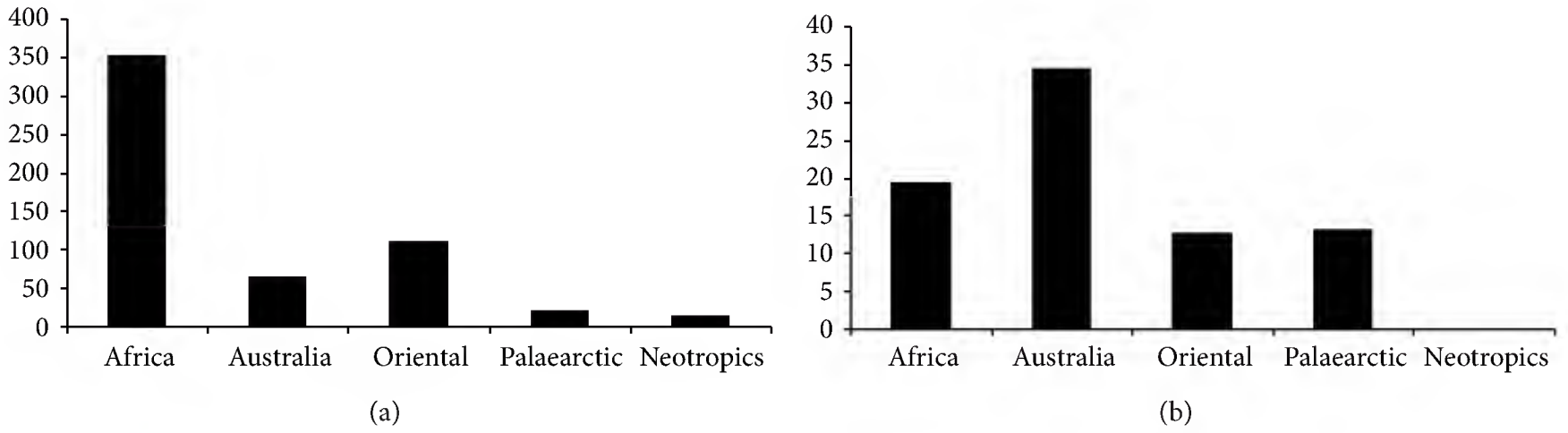


FIGURE 4: Number of valid species of Paussini per biogeographical region (a) and percentage of synonymous taxa of Paussini per biogeographical region (b).

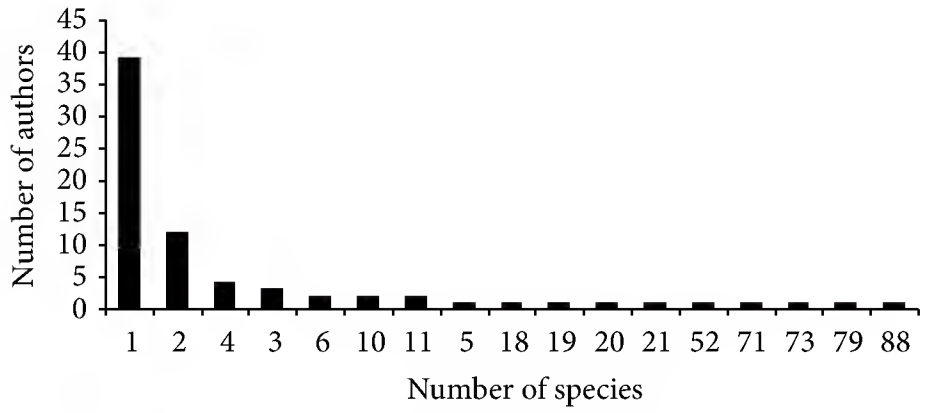


FIGURE 5: Number of authors in relation to the numbers of species of Paussini that they described.

decade shows a roughly humped trend, with proportion of synonymous taxa increasing from 1830–1849 to 1870–1879, and then decreasing to very low values (Figure 3(a)), which determines a plateau in the accumulation curve of synonymies (Figure 3(b)).

The historical process of variation in proportion of synonymized names defines the following time spans that correspond to periods of roughly homogenous taxonomical work (Figure 3(a)): (1) the very early stage was obviously characterized by few descriptions (cf. Figure 1) which are still valid species; (2) the relative rate of redescriptions was nearly constant between 1800 and 1870; (3) between 1870 and 1930 we found that at increasing description of species there was also an increasing number of species subsequently found to be synonymous; and (4) finally, from 1930 to present time, the relative rate of descriptions subsequently synonymized diminishes drastically, as less than 20% of the species described during this period have been synonymized (Figure 3(b)).

The largest numbers of described species occur in Africa, followed by the Oriental and Australian regions (Figure 4(a)). This pattern is not paralleled by proportion of synonymies, with the Australian fauna being that with the highest percentage of synonymized taxa (Figure 4(b)).

The distribution of the numbers of authors that have described Paussini taxa is strongly right-skewed (Figure 5). Over 52% of authors have described only one species. The most productive author, Reichensperger, described 88

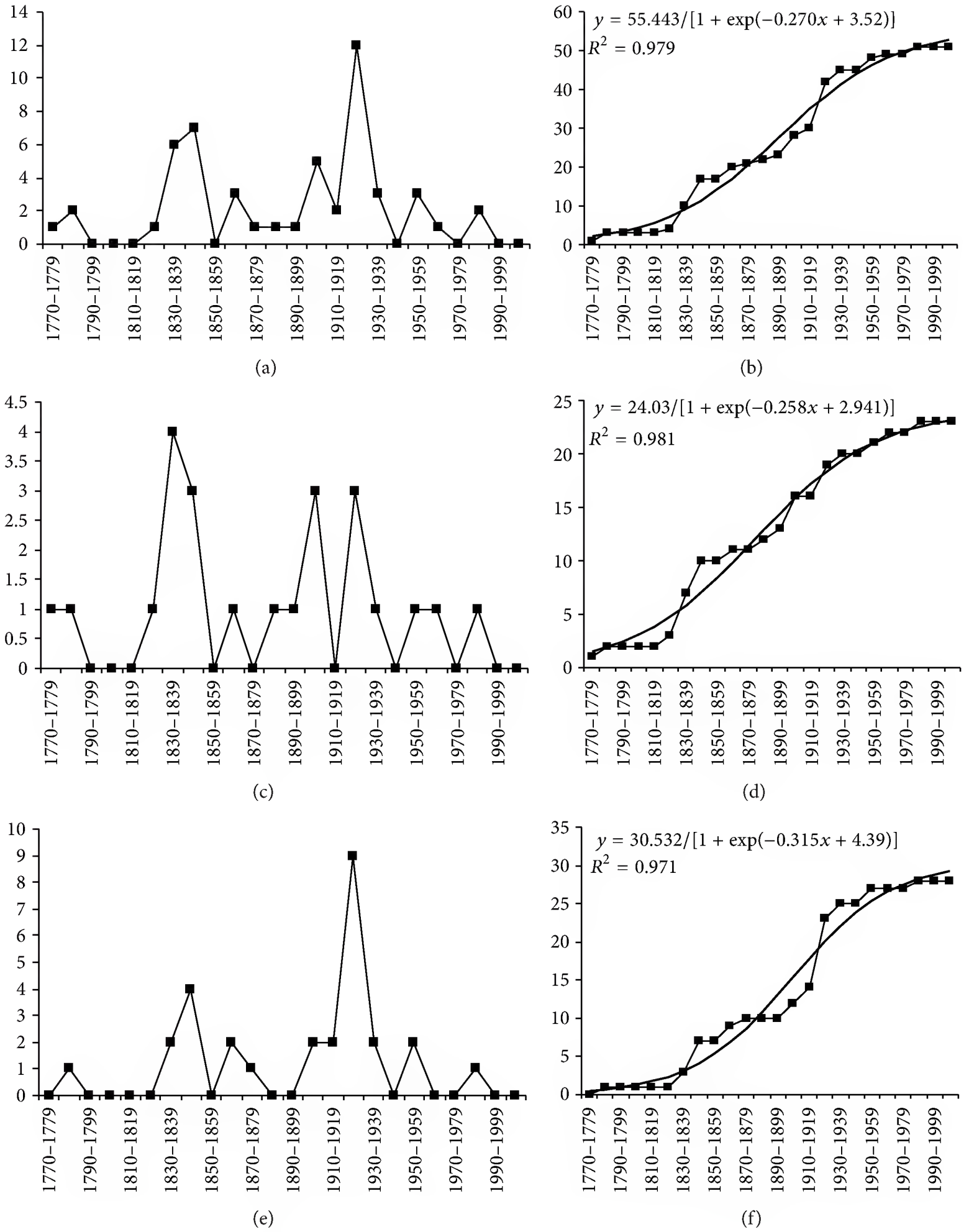


FIGURE 6: Numbers of total described genera (a, b), valid genera (c, d), and synonyms (e, f) of Paussini by decade. Figures (a), (c), and (e) report the absolute numbers, and Figures (b), (d), and (f) the cumulative numbers along with the equations of the fitted curves.

species, followed by Westwood (79), Wasmann (73), and Luna de Carvalho (71). Altogether, these four authors described more than 40% of known species.

Reichensperger published his descriptions between 1913 and 1958 (with an average of ca. 2 species per year), covering all biogeographical regions except the Australian. Most of his species (ca. 94%) were described from Africa. Westwood made his descriptions between 1833 and 1874 (with an average of more than 6 species per year) covering all biogeographical regions with a high proportion (ca. 41%) of Oriental taxa. Wasmann also covered all biogeographical regions between 1892 and 1930, with similar proportion of African (49%) and Oriental (42%) taxa and a mean rate of ca. 2 species per year. Finally, Luna de Carvalho described most of his species from Africa (ca. 85%), with a few species from the Oriental and the Palaearctic regions.

Paussini species are currently allocated in 23 genera. The total number of described genera is 51, with 28 synonyms

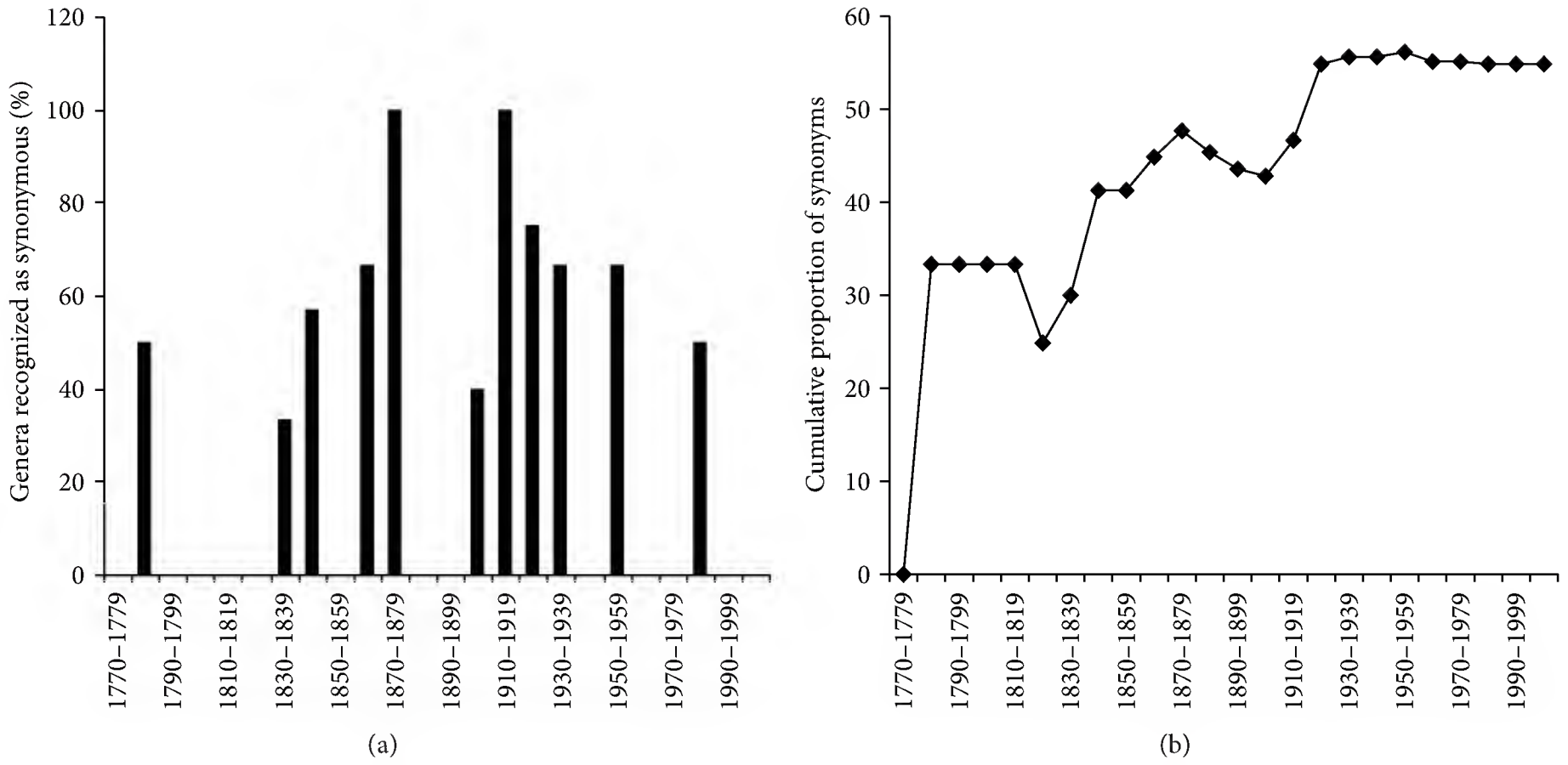


FIGURE 7: Percentage of synonymous genera described in each decade (a) and their historical process of accumulation (b).

(55%). Most of the genera were described in the decade 1920–1929 (Figure 6(a)). Although some decades were characterized by a high number of descriptions of genera, many were recognized as synonyms (especially among those described in the decade 1920–1929) (Figure 6(e)), so the decade with the highest number of valid genera (4 genera) was 1830–1839 (Figure 6(c)): 75% of the genera described in the decade 1920–1929, and 50% of those described in 1980–1989, were subsequently recognized as synonyms.

Patterns of genera accumulation through time indicate a good sigmoid shape for the total number of species (Figure 6(b)), valid genera (Figure 6(d)), and synonyms (Figure 6(f)). In all cases, a plateau has been reached, so virtually no new genus is expected for the future. The historical process of variation in proportion of synonymized genera indicates that after the 1930s there is a substantial stabilization (Figure 7).

The study of subgenera indicates a proliferation of names in the periods 1920–1929 and 1980–1989 as for the genera (Figure 8). Although these were the two decades which mostly contributed to the current accepted subgenera, these were also the decades in which a large number of synonymous subgenera were described, with proportions of synonyms of more than 54% and 64%, respectively. Accumulation curves showed a stair shape pattern, with apparent plateaus, and were therefore not modelled with fitting curves (Figure 8). Moreover, the historical process of variation in proportion of synonymized genera indicates that there is no substantial stabilization (Figure 9). This was mostly due to the large number of subgenera proposed in a recent time (1980–1989) and subsequently synonymized (Figure 9). These patterns suggest that subdivision into subgenera is not reaching a definitive solution.

Species allocation among genera is strongly dominated by the richest genus (*Paussus*), with 342 ascribed species (Figure 10) and 25 subgenera. Species distribution among subgenera is also very uneven: the subgenus with the highest number of species is *Cochliopaussus* (Figure 11).

4. Discussion

Species accumulation curves of the world Paussini fauna indicate that this tribe of carabid beetle is taxonomically stable but do not prove that knowledge is exhaustive. According to the trends analyzed in the present paper, relatively few species are expected to be described in the future on morphological basis and few currently accepted taxa will be recognized to be synonymous. However, if this situation may reflect a true state of affairs in the best explored regions, it may be an artefact when stabilization is merely due to prolonged taxonomic inactivity.

In general, temporal trends in species descriptions mirror dramatic events in human history. The first peak in African species description occurred in the decade 1880–1899, which can be considered an indirect reflection of the first phase of African explorations that occurred between 1840 and 1870 and especially a direct effect of the German expansion in Africa in the 1880s. The second peak occurred in the decade 1920–1939, which coincides with the third phase of the African colonialism, during which the most influential European states organized and stabilised their territories. The overall trend in species descriptions shows two falls in correspondence with the First and Second World Wars. If taxonomic research was frozen at those dates, we would have a completely false signal of stability. For example, taxonomic

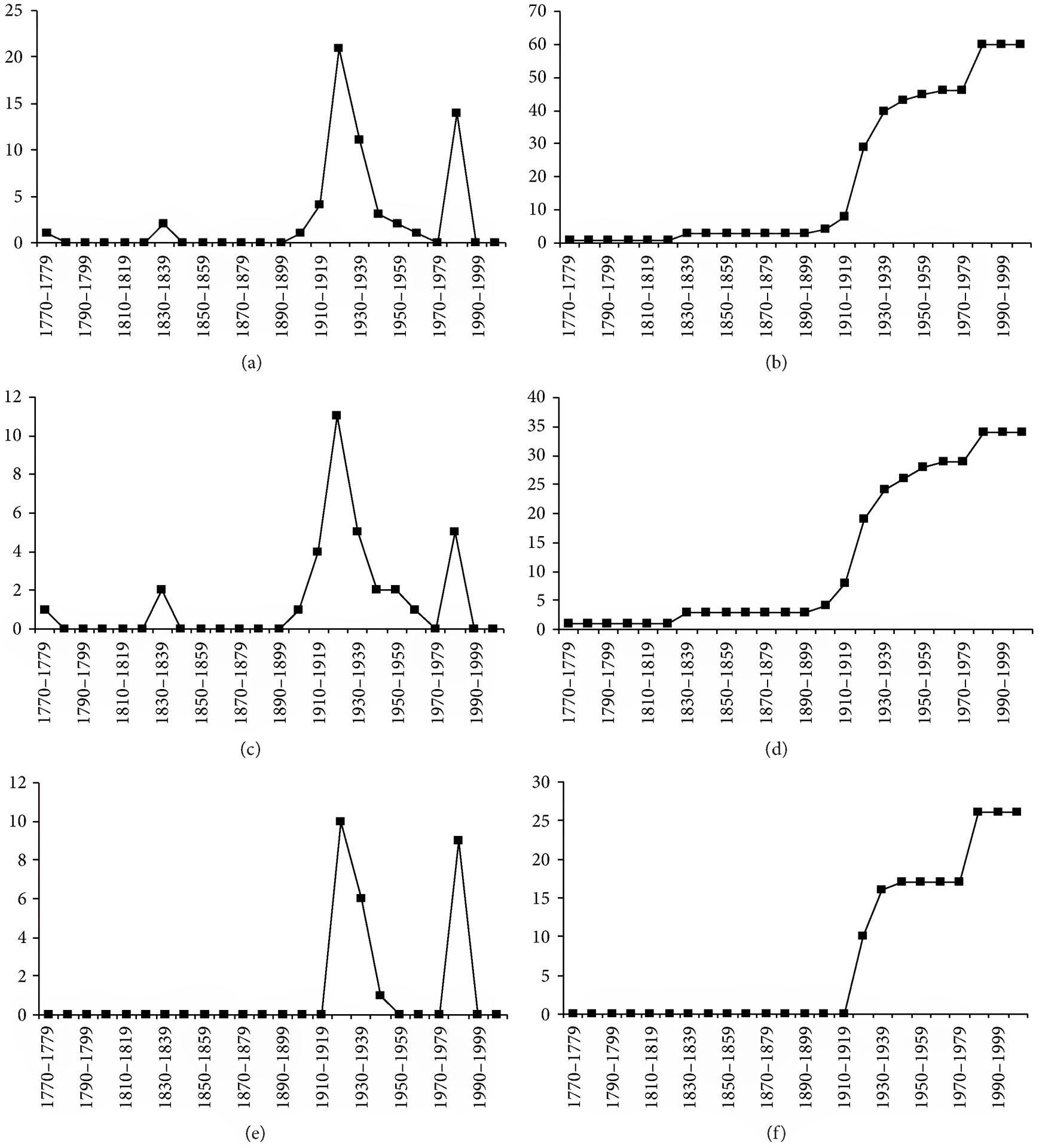


FIGURE 8: Numbers of total described subgenera (a, b), valid subgenera (c, d), and synonyms (e, f) of Paussini by decades. Figures (a), (c), and (e) report the absolute numbers, and Figures (b), (d), and (f) the cumulative numbers.

knowledge in Australia rested at the 1930s [22]. The lack of recent taxonomic activity, coupled with the low number of described species and the high percentage of synonyms, indicates that the fauna of this region is still poorly known.

Most of taxonomic work on Paussini has been produced by few but very prolific authors. Moreover, the authors that described most species during the 20th century were the same that realised the most comprehensive revisions. This has created a self-referenced system, with an almost complete lack of plurality of views. Therefore, taxonomic stability is largely an effect of the "monopolistic" position of certain taxonomists (e.g., Reichensperger, Westwood, Wasmann, and Luna de Carvalho) for long times. Moreover, each of the most active taxonomists was mostly interested in a different biogeographical region, thus with limited taxonomic overlap.

At global level, the asymptotic value calculated for the synonym curve is very close to the current number of synonyms (151); thus we expect that virtually no taxa will be recognized as synonymous in the next future. This indicates that new species are still being described (alpha taxonomy), albeit at decreasing rate in the best explored regions, whereas virtually no synonyms are currently being

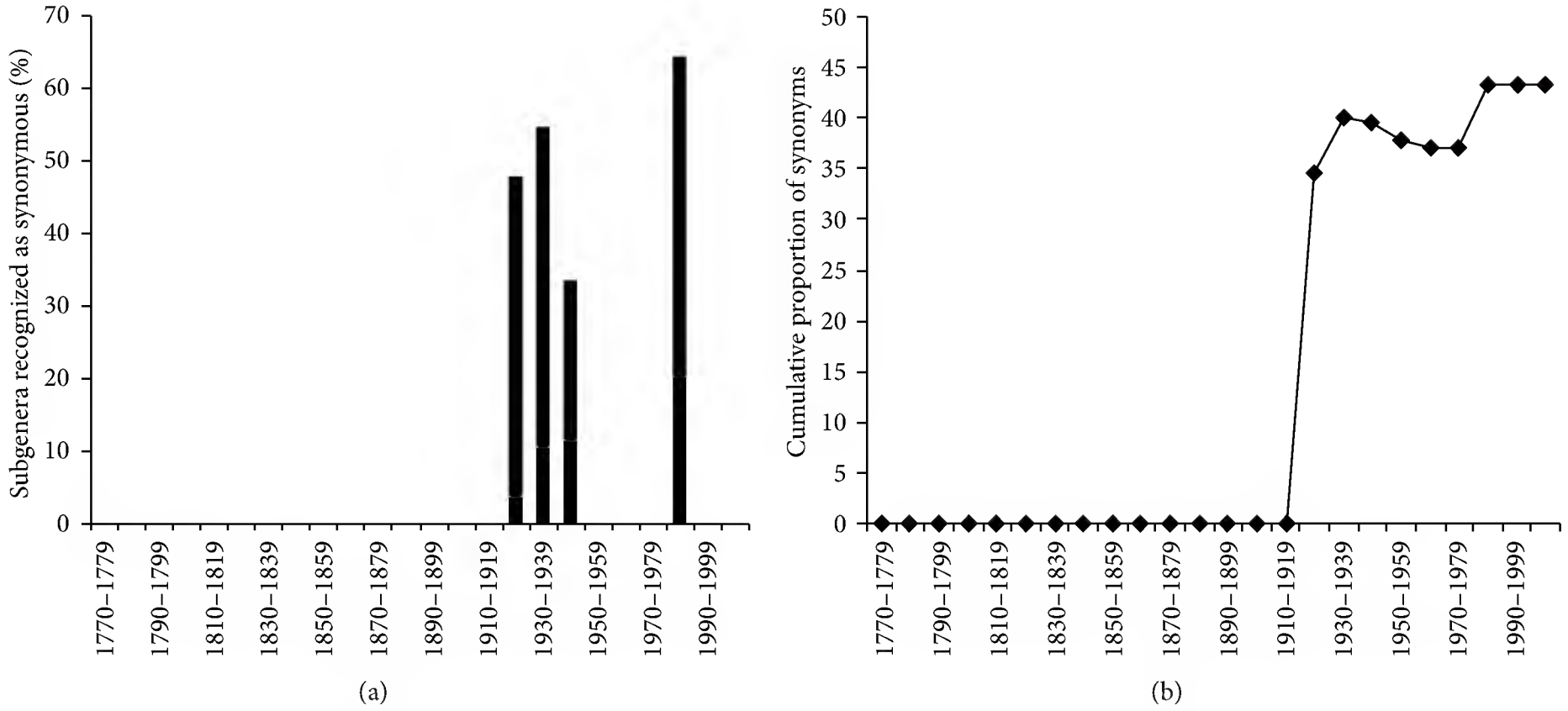


FIGURE 9: Percentage of synonymous subgenera described in each decade (a) and historical process of accumulation of percentage of synonyms over the total number of names in the Paussini, according to the date of their description (b).

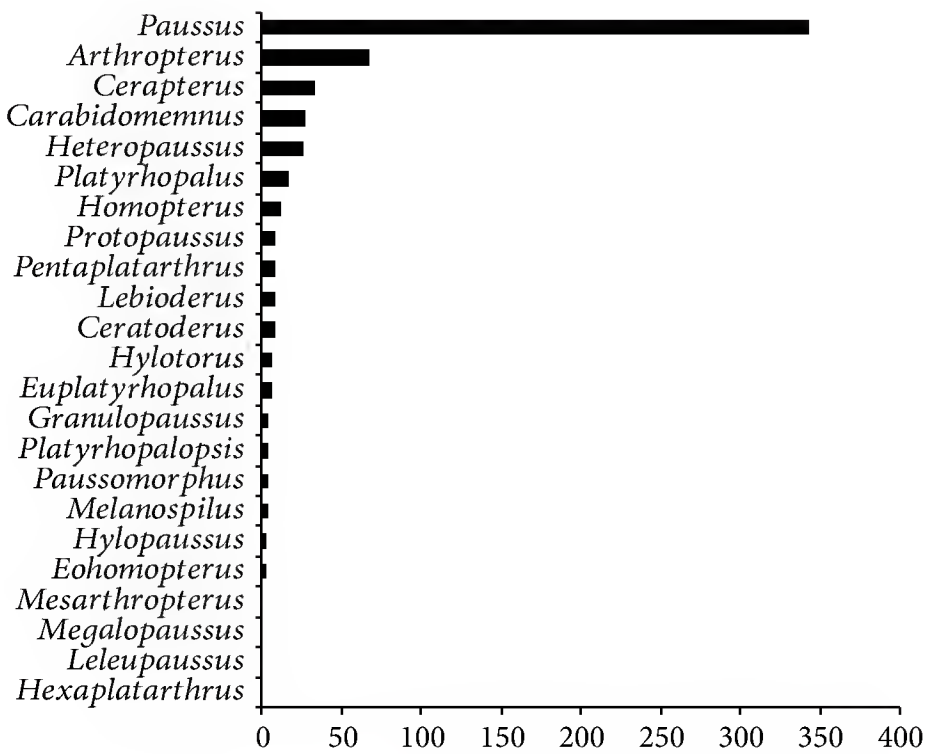


FIGURE 10: Number of species per genus in the tribe Paussini.

described, implying either a lack of beta taxonomy (i.e., redundant descriptions are still considered valid because of the reduced revisional work) or an excellent efficiency of alpha taxonomy (i.e., all new species are valid and none is redundantly described) [24]. We think that failure to recognize synonymies is likely high in the less studied faunas, for which most species have been described from sparse individuals, but this is balanced by the presence of still undescribed species. This may be the case of the Oriental region, which seems to have few species and a moderate percentage of synonymies, but from which so many species are being discovered and no further synonymies established.

Stability in species beta taxonomy indicates that Paussini species are recognized as discrete entities by most researchers. Paussini species were described and are currently recognized on the basis of morphological traits, that is, as groups of phenetically similar individuals that can be separated from other analogous groups by means of phenetic gaps, thus corresponding to a morphological concept of species [25]. Stabilization in synonymies suggests that most taxonomists agree in considering the diagnostic characters presented in species descriptions as gaps sufficiently strong to mark discontinuities among populations. Morphologically defined species do not necessarily correspond to "biological" species (defined as reproductively isolated populations [5]). However, the application of a morphological approach for discriminating species was the practical methodology most frequently used by taxonomists in the past, and the same approach still dominates (and likely will dominate) daily work of the majority of taxonomists. Stability in beta taxonomy of

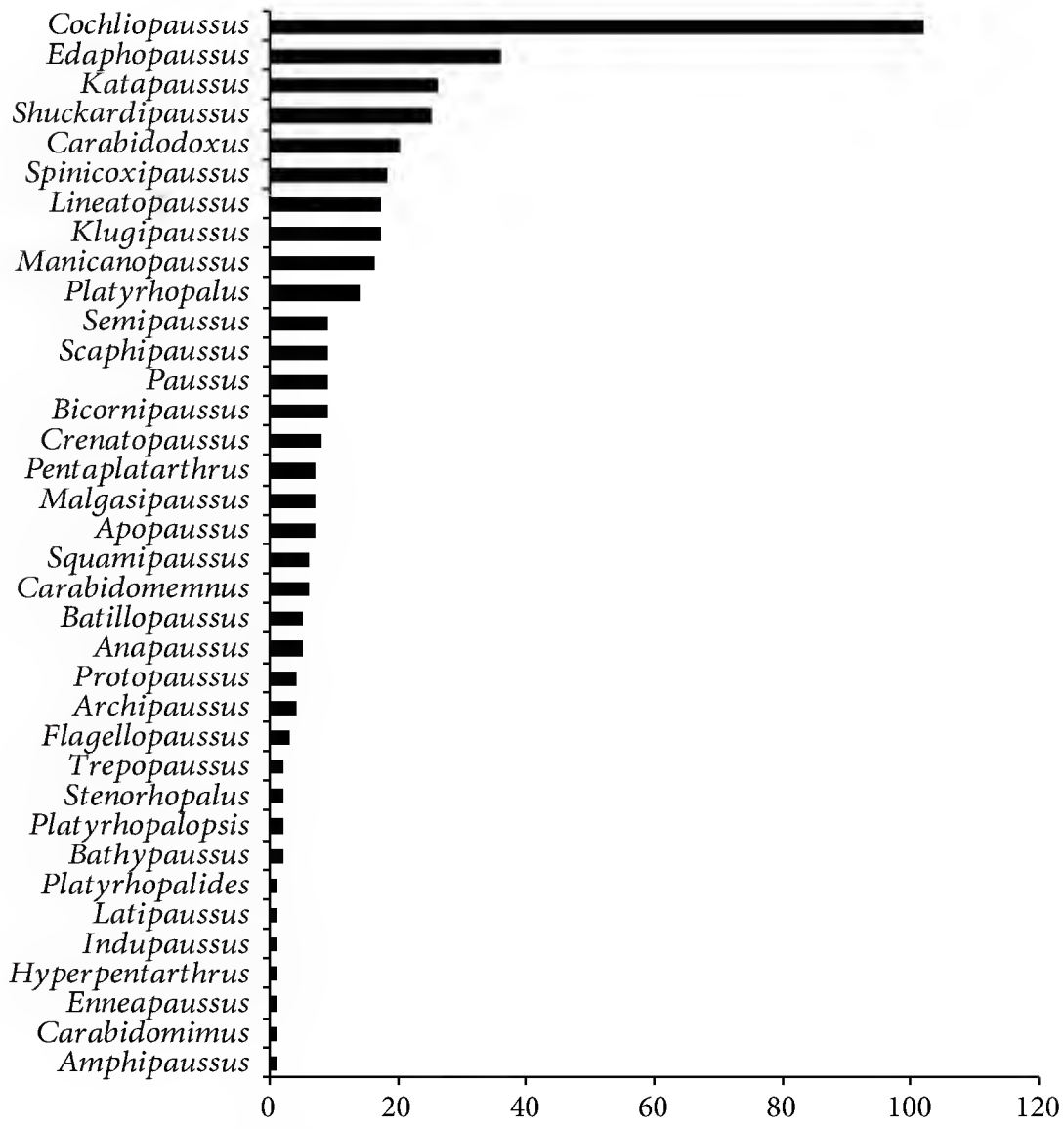


Figure 11: Number of species per subgenus in the tribe Paussini.

morphological species makes Paussini an ideal candidate for future works using molecular approach to investigate how morphological discontinuities are paralleled by molecular divergences. This would be particularly important to clarify relationships among species. Current taxonomic patterns suggest that most species were allocated into the genus *Paussus* probably reflecting a real phylogenetic proximity. However, subgeneric divisions appear instable and based on subtle and controversial morphological characters. This suggests that morphological characters are not fully adequate to resolve infrageneric relationships.

Acknowledgments

The authors are grateful to Wendy Moore (AZ, USA), Augusto Vigna Taglianti (Roma, Italy), and Peter Nagel (Basel, Switzerland) for the continued stimulating discussions about the taxonomy of Paussini and two anonymous referees for their useful comments. Wendy Moore also provided them with an electronic species level database of Paussini and revised the language of the paper. The authors thank Valerio Viglioglia for his help in the data entry and Peter Hlaváč for his suggestions.

References

[1] K. J. Gaston, "The magnitude of global insect species richness," *Conservation Biology*, vol. 5, no. 3, pp. 283–296, 1991.

[2] R. J. Ladle and R. J. Whittaker, *Conservation Biogeography*, Springer, West Sussex, UK, 2011.

[3] A. Baselga, J. Hortal, A. Jiménez-Valverde, J. F. Gómez, and J. M. Lobo, "Which leaf beetles have not yet been described? Determinants of the description of Western Palaearctic *Aphthona* species (Coleoptera: Chrysomelidae)," *Biodiversity and Conservation*, vol. 16, no. 5, pp. 1409–1421, 2007.

[4] J. Hortal, A. Jiménez-Valverde, J. F. Gómez, J. M. Lobo, and A. Baselga, "Historical bias in biodiversity inventories affects the observed environmental niche of the species," *Oikos*, vol. 117, no. 6, pp. 847–858, 2008.

[5] E. Mayr, *Principles of Systematic Zoology*, McGraw-Hill, New York, NY, USA, 1969.

[6] A. Di Giulio, S. Fattorini, A. Kaupp, A. V. Taglianti, and P. Nagel, "Review of competing hypotheses of phylogenetic relationships of Paussinae (Coleoptera: Carabidae) based on larval characters," *Systematic Entomology*, vol. 28, no. 4, pp. 509–537, 2003.

[7] A. Di Giulio and W. Moore, "The first-instar larva of the genus *Arthropterus* (Coleoptera:Carabidae: Paussinae): implications for evolution of myrmecophily and phylogenetic relationships within the subfamily," *Invertebrate Systematics*, vol. 18, no. 2, pp. 101–115, 2004.

[8] A. Di Giulio, "Fine morphology of the myrmecophilous larva of *Paussus kannegieteri* (Coleoptera: Carabidae: Paussinae: Paussini)," *Zootaxa*, no. 1741, pp. 37–50, 2008.

[9] W. Moore, X. B. Song, and A. di Giulio, "The larva of *Eustra* (Coleoptera, Paussinae, Ozaenini): a facultative associate of ants," *ZooKeys*, vol. 90, pp. 63–82, 2011.

[10] C. Escherich, "Zur naturgeschichte von *Paussus favieri* Fairm," *Verhandlungen der Zoologisch-Botanischen Gesellschaft*, vol. 49, pp. 278–283, 1899.

[11] G. Le Masne, "Recherches sur la biologie des animaux myrmécophiles IV: observations sur le comportement de *Paussus favieri* Fairm, hôte de la fourmi Pheidole pallidula Nyl.," *Annales de la Faculté des Sciences de Marseille*, vol. 31, pp. 111–130, 1961.

[12] G. Le Masne, "Recherches sur la biologie des animaux myrmécophiles I: L'adoption des *Paussus favieri* Fairm, par une nouvelle societe de *Pheidole pallidula* Nyl.," *Comptes Rendus Hebdomadaires des Scéances de L'Academie des Scéances*, vol. 253, pp. 1621–1623, 1961.

[13] S. F. Geiselhardt, K. Peschke, and P. Nagel, "A review of myrmecophily in ant nest beetles (Coleoptera: Carabidae: Paussinae): linking early observations with recent findings," *Naturwissenschaften*, vol. 94, no. 11, pp. 871–894, 2007.

[14] A. di Giulio, E. Maurizi, P. Hlaváč, and W. Moore, "The long-awaited first instar larva of *Paussus favieri* (Coleoptera: Carabidae: Paussini)," *European Journal of Entomology*, vol. 108, no. 1, pp. 127–138, 2011.

[15] E. Maurizi, S. Fattorini, and A. Di Giulio, "Behavior of *Paussus favieri* (Coleoptera, Carabidae, Paussini), a myrmecophilous beetle associated with *Pheidole pallidula* (Hymenoptera, Formicidae)," *Psyche*, vol. 2012, Article ID 940315, 9 pages, 2012.

[16] G. C. Steyskal, "Trend curves of the rate of species description in zoology," *Science*, vol. 149, no. 3686, pp. 880–882, 1965.

[17] R. A. Medellín and J. Soberón, "Predictions of mammal diversity on four land masses," *Conservation Biology*, vol. 13, no. 1, pp. 143–149, 1999.

[18] F. J. Cabrero-Sañudo and J. M. Lobo, "Estimating the number of species not yet described and their characteristics: the case of Western Palaearctic dung beetle species (Coleoptera, Scarabaeoidea)," *Biodiversity and Conservation*, vol. 12, no. 1, pp. 147–166, 2003.

[19] A. Baselga and F. Novoa, "Diversity of Chrysomelidae (Coleoptera) in Galicia, Northwest Spain: estimating the completeness of the regional inventory," *Biodiversity and Conservation*, vol. 15, no. 1, pp. 205–230, 2006.

[20] A. R. Solow and W. K. Smith, "On estimating the number of species from the discovery record," *Proceedings of the Royal Society B*, vol. 272, no. 1560, pp. 285–287, 2005.

[21] J. Alroy, "How many named species are valid?" *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 6, pp. 3706–3711, 2002.

[22] S. Fattorini, E. Maurizi, and A. Di Giulio, "Tackling the taxonomic impediment: a global assessment for ant-nest beetle diversity (Coleoptera: Carabidae: Paussini)," *Biological Journal of the Linnean Society*, vol. 105, pp. 330–339, 2012.

[23] W. Lorenz, *A Systematic List of Extant Ground Beetles of the World (Coleoptera "Geadephaga": Trachypachidae and Carabidae Incl. Paussinae, Cicindelinae, Rhysodinae)*, W. Lorenz, Tutzing, Germany, 2nd edition, 2005.

[24] A. Baselga, J. M. Lobo, J. Hortal, A. Jiménez-Valverde, and J. F. Gómez, "Assessing alpha and beta taxonomy in eupelmid wasps: determinants of the probability of describing good species and synonyms," *Journal of Zoological Systematics and Evolutionary Research*, vol. 48, no. 1, pp. 40–49, 2010.

[25] R. R. Sokal and J. Crovello, "The biological species concept: a critical evaluation," *The American Naturalist*, vol. 104, pp. 127–153, 1970.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 230601, 6 pages
http://dx.doi.org/10.1155/2013/230601

Research Article

New Host Record for *Camponotophilus delvarei* (Hymenoptera: Eurytomidae), a Parasitoid of Microdontine Larvae (Diptera: Syrphidae), Associated with the Ant *Camponotus* sp. aff. *textor*

Gabriela Pérez-Lachaud,[1] Michael W. Gates,[2] and Jean-Paul Lachaud[1,3]

[1] *El Colegio de la Frontera Sur, Avenida Centenario Km 5.5, 77014 Chetumal, Quintana Roo, Mexico*
[2] *Systematic Entomology Laboratory, USDA, ARS, PSI, c/o National Museum of Natural History, Washington, DC 20013-7012, USA*
[3] *Centre de Recherches sur la Cognition Animale, CNRS-UMR 5169, Université de Toulouse UPS, 118 route de Narbonne, 31062 Toulouse Cedex 09, France*

Correspondence should be addressed to Gabriela Pérez-Lachaud; igperez@ecosur.mx

Received 8 March 2013; Accepted 22 April 2013

Academic Editor: Alain Lenoir

Microdontine syrphid flies are obligate social parasites of ants. Larvae prey on ant brood whereas adults live outside the nests. Knowledge of their interaction with their host is often scarce, as it is information about their natural enemies. Here we report the first case of parasitism of a species of microdontine fly by a myrmecophilous eurytomid wasp. This is also the first host record for *Camponotophilus delvarei* Gates, a recently described parasitic wasp discovered in Chiapas, Mexico, within the nests of the weaver ant, *Camponotus* sp. aff. *textor* Forel. Eleven pupal cases of a microdontine fly were found within a single nest of this ant, five of them being parasitized. Five adult *C. delvarei* females were reared from a puparium and 29 female and 2 male pupae were obtained from another one. The eurytomid is a gregarious, primary ectoparasitoid of larvae and pupae of Microdontinae, its immature stages developing within the protective puparium of the fly. The species is synovigenic. Adult females likely locate and parasitize their hosts within the ant nest. As some species of Microdontinae are considered endangered, their parasitoids are likewise threatened and in need of accurate and urgent surveys in the future.

1. Introduction

Although hoverflies or flower flies (Diptera: Syrphidae) are best known for their role as important plant pollinators [1, 2] or as potential agents in aphid biological control [3–5], many species have long been reported as associated with ants [6–10]. Current classification of Syrphidae recognizes three subfamilies: Microdontinae, Eristalinae, and Syrphinae [11, 12], with Microdontinae being the least known group [10] and yet the most intriguing, considering their apparent obligatory relationships with ants (see [13]). In fact, all of the microdontine species for which the natural history is known have been found within ant nests or in their immediate vicinity (for a review see [10, 13, 14]). According to the most recent generic revision [10, 15], 43 valid genera are currently assigned to this subfamily. Larval taxonomy for the group is virtually undeveloped; therefore, there are no ways of distinguishing these genera at the larval stage. Historically, the genus *Microdon* Meigen was used as a collective genus for more than 300 specific taxa of uncertain taxonomic affinities, and records of microdontines associated with ants include taxa known only from the immature stages. Presently, only 126 of 454 valid species of Microdontinae remain in the genus *Microdon* [15]. For such reasons, all mentions of "*Microdon* sp." larvae or puparia from previous literature will be referred here as "unknown microdontine species."

Members of the Microdontinae are non-typical syrphids. Their larvae live in ant nests as predators on ant brood [16, 17] and resemble slugs to such an extent that they have been described as mollusks on at least four independent occasions

(see [7, 10]). Larvae of Microdontinae are tolerated by their ant hosts, and chemical mimicry of the host has been reported [18]. Early larval instars can be transported when nests are disturbed, but mature larvae are not [7, 16]. By contrast, adults are fiercely attacked by the ants after their wings were distended, at least under laboratory conditions [19, 20].

There are 454 valid species of Microdontinae found in all zoogeographical regions [10, 15], with the greatest diversity in the Tropics [8, 15]. Because larvae of Microdontinae develop within the protective ant nest and because adults are rarely collected, they are poorly known. Particularly, their life cycle, feeding habits, inquilinism, as well as the interactions between the larvae and their specific ant hosts have not been thoroughly studied [21, 22], even though some species are considered endangered [17, 23, 24]. Consequently, there is even less information concerning their natural enemies, including those of the European and Nearctic Microdontinae species which have received more attention than their Neotropical relatives.

Camponotophilus delvarei Gates is a recently described species of Eurytomidae (Hymenoptera: Chalcidoidea) discovered in Chiapas, Mexico, within the arboreal nests of *Camponotus* (*Myrmobrachys*) sp. aff. *textor* Forel (Hymenoptera: Formicidae), a weaver ant that builds oval to round nests by sewing leaves together with larval silk [25]. Females of the wasp were found within colonies collected during the dry season along with brood and adult ants, albeit in very low numbers—only one or two females per nest, among 16 700 workers per colony on average (G. Pérez-Lachaud and J.-P. Lachaud, unpub. data). No immature stages of the wasp could be found at that time and its biology, as well as the exact nature of the interaction with the ants, remained unknown. Adult wasps resemble worker ants in color, shape, and size and may be confused with them on cursory examination, suggesting that *C. delvarei* may be a visual mimic of *C.* sp. aff. *textor* [25]. Because the ant nests harbored very few arthropods that could be considered as potential host candidates for the eurytomid, it was hypothesized that *C. delvarei* females parasitized the ant brood. Here we report complementary biological data on *C. delvarei* that confirm its myrmecophilic status but provide new evidence that the actual hosts are the larvae and pupae of an unknown species of syrphid fly of the subfamily Microdontinae associated with *C.* sp. aff. *textor*. This is the first report of true primary parasitoidism of a syrphid fly by a eurytomid wasp.

2. Material and Methods

Two complete nests of *Camponotus* sp. aff. *textor* were collected during the rainy season, one in September 2011 and another one on October 3rd, 2012. Both nests were located in a private orchard situated about 10 km to the southwest of the type locality of *C. delvarei*, adjacent to Izapa archaeological site, Tuxtla Chico Municipality, Chiapas, Mexico (14°55′18″ N, 92°10′56″ W). No nests could be located at the type locality where the experimental shaded coffee plantation has since been transformed into a *Jatropha* spp. (Euphorbiaceae) biofuel plantation with no shade trees. The nest collected in 2011 measured 12 × 17 cm and was located on a rose apple tree *Syzygium jambos* (Linnaeus) Alston (Myrtaceae) at a height of about 2.5 m. The nest collected in 2012 measured 12 × 15 cm and was situated at a height of about 6 m on a cocoplum tree, *Chrysobalanus icaco* Linnaeus (Chrysobalanaceae).

Evaluation of the nest collected on rose apple yielded no evidence of immature stages of *C. delvarei*, but the nest collected in 2012 contained several puparia of an unknown microdontine species. One puparium found in the superficial layers of the nest was detected upon collection and was isolated in a vial glass plugged with cotton. The rest of the nest was preserved in alcohol for later examination. The isolated puparium was checked once a week, and by October 23th several developing larvae could be observed through the puparial case. It contained 16 wasp larvae at different developmental stages, some of them already in a decaying state, and 6 pupae. Wasp pupae were placed in a separate vial along with some filter paper as support and to absorb excess humidity.

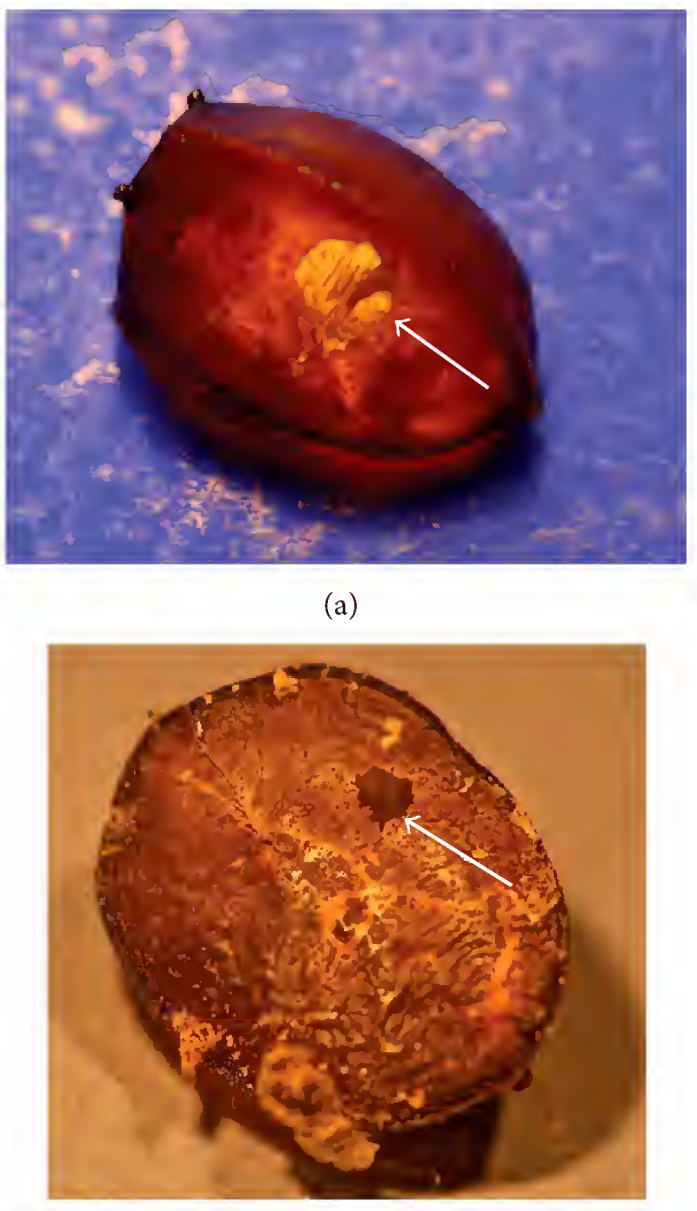
Several *Camponotophilus delvarei* female wasps emerged from the puparium. Two females were dissected under a stereomicroscope (Wild M3) upon emergence and two other females were placed in a glass vial provided with honey and water *ad libitum* and dissected when 5 days old in order to determine their egg load. A fifth female from the same nest and another from a previous collection [25], both of unknown age, were also dissected and their eggs were counted. Upon examination of the nest, several other puparia were discovered. They were dissected and their contents were inspected. Voucher specimens of the wasp (adult females and pupae of both sexes) and pupal cases of the fly were deposited at the Arthropod Collection of El Colegio de la Frontera Sur-Chetumal, Quintana Roo, Mexico (ECO-CH-AR). Images were captured using a digital camera (Olympus μ1020) affixed to the ocular of the microscope. Lighting was provided by a fiber optic light source.

3. Results

Overall, the *Camponotus* sp. aff. *textor* nest collected in 2012 contained 11 pupal cases of a microdontine fly, and one *C. delvarei* adult female was also found among workers. Five out of the 11 puparia were parasitized (45%). The other six were empty and showed evidence of previous emergence of the adult fly (Figure 1). Consequently, no adults of the microdontine syrphid fly were obtained and its identity remains unknown. It is worth noting that the puparia were found enclosed within the structural walls of the nest, entirely covered with silk, at different depths from its outer surface. This suggests that ants covered them with silk as they enlarged the nest, in the same manner that they covered with silk any debris, refuse, or plant part (Figure 1).

Of the parasitized puparia, two presented an exit hole on their dorsal surface (Figure 2(a)), from which wasp parasitoids had already emerged. Another puparium contained 31 *C. delvarei* pupae (29 females: 2 males). These pupae filled the entire space inside the host puparium (Figure 3). Another parasitized puparium contained many small larvae, probably

FIGURE 1: Empty puparium from which an adult microdontine fly has emerged, as found included with silk in the nest walls of its host *Camponotus* sp. aff. *textor*. Photo: J.-P. Lachaud and G. Pérez-Lachaud.



(a)

(b)

FIGURE 2: Parasitized puparia: (a) puparium (dorsal view) showing the emergence hole chewed by the eurytomids (arrow); (b) puparium (ventral view) showing the emergence hole chewed by eulophids (arrow). Photos: J.-P. Lachaud and G. Pérez-Lachaud.

Horismenus microdonophagus Hansson et al. (as suggested by their number and size), a species of Eulophidae also known to parasitize this unidentified species of Microdontinae ([26], Figure 2(b)). Finally, from the puparium isolated on October 3rd, five *C. delvarei* females successfully emerged on October 30th, one individual died during the pupal stage, and the 16 larvae did not proceed development. Since the nest was

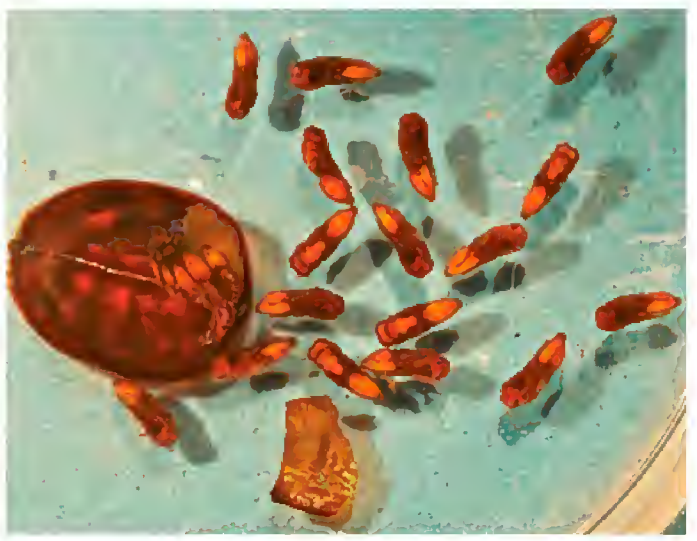


FIGURE 3: Microdontine syrphid fly pupa parasitized by *Camponotophilus delvarei*. The host puparium has been cut open to show the wasp pupae filling up the whole inner space. Photo: G. Pérez-Lachaud and J.-P. Lachaud.

collected on October 3rd, development from egg to adult takes at least 27 days, considering that the host was recently parasitized.

Inspection of the host remains showed that larvae of the eurytomid fed externally upon the larva/prepupa (2 cases) or upon the transforming(-ed) pupa (wing primordia were detected in the remains of one host). The eurytomid thus develops as a gregarious, idiobiont, ectoparasitoid. Dissection of newly emerged *C. delvarei* females and also of those aged of 5 days and fed on honey, revealed that they had no mature eggs and that their ovaries were undeveloped. Dissection of a female from a previous collection (February 2010) and of the female found within the ant nest showed that older females may have up to 20 mature eggs ($n = 2$). The species is thus synovigenic; that is, no mature egg is present at emergence.

4. Discussion

Exceedingly few studies on myrmecophagous microdontine syrphid flies and their parasitoids have been conducted in the Neotropics, in contrast to the numerous reports documenting natural enemies of aphidophagous syrphids. The latter are attacked by a wide range of parasitoids in the families Ichneumonidae, Braconidae, Chalcididae, Encyrtidae, Pteromalidae, Megaspilidae, and Figitidae [27–29]. The commonest syrphid parasitoids belong to the Ichneumonoidea subfamily Diplazontinae [29]. This is not surprising since aphidophagous syrphids pupate in open spaces and may be easy to locate by both natural enemies and researchers. By contrast, larvae of Microdontinae live and pupate within the protective walls of the ant nests and may be more difficult for parasitoids to locate/parasitize given that they must cope with ant aggressiveness.

To our knowledge, only two species of Eulophidae and one of Encyrtidae are recorded as parasitizing members of the Microdontinae: *Microdonophagus woodleyi* Schauff (Eulophidae: Entedoninae), which parasitizes larvae of an unidentified species of microdontine (reported as *Microdon* sp.) living in nests of *Technomyrmex fulvus* (Wheeler)

(referred to as *Tapinoma fulvum*) (Formicidae: Dolichoderinae) in Panama [30], *Horismenus microdonophagus* (Eulophidae: Entedoninae), which parasitizes the unidentified microdontine species found in nests of *Camponotus* sp. aff. *textor* (Formicidae: Formicinae) in Chiapas, Mexico [26], and *Exoristobia ugandensis* Subba Rao (Encyrtidae: Encyrtinae), reported to parasitize larvae of another unidentified species of Microdontinae in Uganda [31]. The associated ant for *E. ugandensis* is unknown, but both eulophids are gregarious endoparasitoids of larvae of Microdontinae living in nests of arboreal ants. *Technomyrmex fulvus* builds conspicuous carton nests in the low arboreal zone [32], while *Camponotus* sp. aff. *textor* builds silk nests (Figure 4, G. Pérez-Lachaud and J.-P. Lachaud, unpub. data). Up to 70 pupae of *M. woodleyi* were obtained from a single host [30], while 85 adults of *H. microdonophagus* (79 females, 6 males) were obtained from a microdontine larva [26]. There are two other *Microdonophagus* species described to date, which are presumed to be associated with ants, but their biology is unknown [26].

FIGURE 4: The silk nest of the weaver ant host *Camponotus* sp. aff. *textor*. Photo: G. Pérez-Lachaud.

Our record is thus the fourth reliable report of a parasitoid attacking Microdontinae. From our observations, it could be concluded that *Camponotophilus delvarei* is a gregarious, primary ectoparasitoid of larvae and pupae of microdontine flies, whose immature stages develop within the protective puparium of the fly. The initial stage of the host used for oviposition is not known, but the presence of adult females, with plenty of mature eggs, inside ant nests in the absence of suitable hosts (see [25]) strongly suggests that adult females locate and parasitize their hosts within the nests of the ants and that they wait for their hosts within the protective walls of the ant nest. Being a visual mimic of *Camponotus* sp. aff. *textor* ants may be a strategy to cope with the ant recognition system. Our data also showed that the species is synovigenic; that is, females emerge without mature eggs. Furthermore, females fed on honey for 5 days did not have mature eggs. It is unknown if females host feed in order to produce eggs or whether they need some other sources of energy to initiate ovigenesis. It is interesting to note that *C. delvarei* individuals were found attacking both the larvae and pupae of the syrphid as shown by the host remains found in the puparia. Similarly, some other species attacking Diptera may emerge from either the larvae or the host puparia as it is the case for the species of the genus *Bothriothorax* Ratzeburg (Encyrtidae) that attack aphidophagous syrphids [28].

Only very limited information is available on the habitat preferences and host ant specificity of microdontines [13, 33]. As already stated, larvae are tolerated by ants, and several studies on their interaction with ants have been performed (e.g., [16]), but interactions of adults and ants have rarely been reported. Microdontine larvae migrate to the superficial part of the ant nest (near the exit) when about to pupate [16], and adults are thought to emerge early in the morning and to exit the nest unnoticed by ants. In the case of *Microdon major* (Andries), larvae were found inside the ant brood chambers of *Formica lemani* Bondroit and *F. fusca* Linnaeus, while pupal cases were found closer to the outer nest surface. *Microdon* larvae showed a clear preference for remaining among the part of the nest containing wooden debris and were ignored by the ant workers [33]. In *M. tigrinus* Curran, larvae and pupae were well accepted in the nests and the adults were not attacked by the workers immediately after eclosion, suggesting that they produce semiochemicals for a short time period until they arrive outside the *Acromyrmex coronatus* (Fabricius) nest [20]. In our case, empty microdontine puparia were found at different depths in the nest, completely covered with silk, suggesting that ants covered them with silk as they enlarged the nest.

Eurytomidae is a diverse group within Chalcidoidea [34], with some clades showing a quick evolution of diet habits and feeding behavior (e.g., [35]). Most eurytomids are primary parasitoids typically attacking eggs, larvae, or pupae of holometabolous insects (Coleoptera, Orthoptera, Diptera, and Hymenoptera [36, 37]), but this group also includes hyperparasitoids, and phytophagous eurytomines are known from at least 12 plant families (plant miners, gall inducers, and seed predators [38]; MW Gates, unpub. data). Certain eurytomines are also known to switch to phytophagy before and/or after consuming an insect host [39, 40]. Several dipteran families include species that are the hosts of eurytomids, especially larvae and pupae of Tephritidae (e.g., [41]). However, this is the first time a eurytomid is recorded as parasitoid of Syrphidae. Association with ants is also very uncommon in Eurytomidae, and so far only *Aximopsis aztecicida* (Brues) and *A. affinis* (Brues) have been documented as parasitoids of ants [42, 43]. These species are known ectoparasitoids of foundress queens of several species of *Azteca* Forel (Formicidae: Dolichoderinae), commonly found within hollow stems of *Cecropia* Loefl. [44]. However, these eurytomids are not associated with an active ant colony; that is, they are not myrmecophilous, as they attack only foundresses. *Camponotophilus delvarei* is thus the first myrmecophilic eurytomid reported to date [25].

It is worth noting that microdontine larvae were more abundant during the rainy season (up to 11 puparia in a single nest) than during the dry season, when only one puparium was found out of three ant nests collected (G. Pérez-Lachaud and J.-P. Lachaud, unpub. data). Likewise, in *M. tigrinus*, a Neotropical microdontine exclusively associated with the

fungus-growing ant *A. coronatus* in Brazil, a greater population was found during September-October, with a mean of more than 60 larvae per nest [20].

Microdontine flies are obligate social parasites of ants, the larvae prey on ant brood, but knowledge of their interaction with their hosts is often scarce. Many species of ants' social parasites are rare and are considered endangered, since their strong relationship with their hosts makes them more vulnerable to habitat change [45, 46]. However, due to their rarity, this vulnerability to habitat loss is even more blatant in the case of the parasitoids of these endangered myrmecophiles. Even for the best studied species, *M. mutabilis* (Linnaeus) and *M. myrmicae* Schönrogge et al. [23, 24, 47], no parasitoids have been recorded to date. As for many other poorly studied parasites and parasitoids associated with ants, which represent a significant unknown "hidden biodiversity" [26, 43, 48–50], there is an urgent need to improve our understanding of the biology of both microdontine flies and their natural enemies before their natural habitat is lost.

Acknowledgments

The authors thank José Antonio López Méndez and Annie Damon (ECOSUR) for their help with tree identification and Don Emilio Hernández Colomo, who owns the orchard where the ant nests were collected, for not destroying the host ant nests. They also thank John Heraty, Menno Reemer, and John Noyes for their helpful comments on a previous version of the paper. USDA is an equal opportunity provider and employer.

References

[1] J. F. Tooker, M. Hauser, and L. M. Hanks, "Floral host plants of Syrphidae and Tachinidae (Diptera) of central Illinois," *Annals of the Entomological Society of America*, vol. 99, no. 1, pp. 96–112, 2006.

[2] A. Ssymank, C. A. Kearns, T. Pape, and F. C. Thompson, "Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production," *Biodiversity*, vol. 9, no. 1-2, pp. 86–89, 2008.

[3] F. Schneider, "Bionomics and physiology of aphidophagous Syrphidae," *Annual Review of Entomology*, vol. 14, pp. 103–124, 1969.

[4] S. Haenke, B. Scheid, M. Schaefer, T. Tscharntke, and C. Thies, "Increasing syrphid fly diversity and density in sown flower strips within simple vs. complex landscapes," *Journal of Applied Ecology*, vol. 46, no. 5, pp. 1106–1114, 2009.

[5] E. A. Laubertie, S. D. Wratten, and J. L. Hemptinne, "The contribution of potential beneficial insectary plant species to adult hoverfly (Diptera: Syrphidae) fitness," *Biological Control*, vol. 61, no. 1, pp. 1–6, 2012.

[6] W. M. Wheeler, "An extraordinary ant-guest," *The American Naturalist*, vol. 35, no. 420, pp. 1007–1016, 1901.

[7] W. M. Wheeler, "Studies of myrmecophiles. III. *Microdon*," *Journal of the New York Entomological Society*, vol. 16, no. 4, pp. 202–213, 1908.

[8] R. M. Duffield, "Biology of *Microdon fuscipennis* (Diptera: Syrphidae) with interpretations of the reproductive strategies of *Microdon* species found North of Mexico," *Proceedings of the Entomological Society of Washington*, vol. 83, no. 4, pp. 716–724, 1981.

[9] G. E. Rotheray, B. Barr, and S. M. Hewitt, "The myrmecophilous larvae of *Chrysotoxum arcuatum*, *Pipizella varipes* and *Xanthogramma pedissequum* from Europe and *Platycheirus milleri* from New Zealand (Dip.: Syrphidae)," *The Entomologist's Record and Journal of Variation*, vol. 108, no. 9-10, pp. 257–265, 1996.

[10] M. Reemer, *Unravelling a hotchpotch: phylogeny and classification of the Microdontinae (Diptera: Syrphidae) [Ph.D. thesis]*, Leiden University, Leiden, The Netherlands, 2012.

[11] F. C. Thompson and G. E. Rotheray, "Family Syrphidae," in *Contributions to a Manual of Palaearctic Diptera (with Special Reference to Flies of Economic Importance)*, L. Papp and B. Darvas, Eds., vol. 3, pp. 81–139, Science Herald, Budapest, Hungary, 1998.

[12] G. Ståhls, H. Hippa, G. Rotheray, J. Muona, and F. Gilbert, "Phylogeny of Syrphidae (Diptera) inferred from combined analysis of molecular and morphological characters," *Systematic Entomology*, vol. 28, no. 4, pp. 433–450, 2003.

[13] M. Reemer, "Review and phylogenetic evaluation of associations between Microdontinae (Diptera: Syrphidae) and ants (Hymenoptera: Formicidae)," *Psyche*, vol. 2013, Article ID 538316, 9 pages, 2013.

[14] X.-Y. Cheng and F. C. Thompson, "A generic conspectus of the Microdontinae (Diptera: Syrphidae) with the description of two new genera from Africa and China," *Zootaxa*, no. 1879, pp. 21–48, 2008.

[15] M. Reemer and G. Ståhls, "Generic revision and species classification of the Microdontinae (Diptera: Syrphidae)," *ZooKeys*, vol. 288, pp. 1–213, 2013.

[16] W. B. Garnett, R. D. Akre, and G. Sehlke, "Cocoon mimicry and predation by myrmecophilous diptera (Diptera: Syrphidae)," *The Florida Entomologist*, vol. 68, no. 4, pp. 615–621, 1985.

[17] M. Witek, S. Canterino, E. Balletto, and S. Bonelli, "Life cycle and growth pattern of the endangered myrmecophilous *Microdon myrmicae* (Diptera: Syrphidae)," *European Journal of Entomology*, vol. 109, no. 3, pp. 457–461, 2012.

[18] R. W. Howard, D. W. Stanley-Samuelson, and R. D. Akre, "Biosynthesis and chemical mimicry of cuticular hydrocarbons from the obligate predator, *Microdon albicomatus* Novak (Diptera: Syrphidae) and its ant prey, *Myrmica incompleta* Provancher (Hymenoptera, Formicidae)," *Journal of the Kansas Entomological Society*, vol. 63, no. 3, pp. 437–443, 1990.

[19] R. D. Akre, G. Alpert, and T. Alpert, "Life cycle and behavior of *Microdon cothurnatus* in Washington (Diptera: Syrphidae)," *Journal of the Kansas Entomological Society*, vol. 46, no. 3, pp. 327–338, 1973.

[20] L. C. Forti, R. S. Camargo, S. S. Verza, A. P. P. Andrade, R. T. Fujihara, and J. F. S. Lopes, "*Microdon tigrinus* Curran, 1940 (Diptera, Syrphidae): populational fluctuations and specificity to the nest of *Acromyrmex coronatus* (Hymenoptera: Formicidae)," *Sociobiology*, vol. 50, no. 3, pp. 909–919, 2007.

[21] M. Carrera and K. Lenko, "Descrição de duas espécies novas de *Mixogaster* (Diptera, Syrphidae) e observações sôbre o inquilinismo de uma delas em ninhos de *Iridomyrmex humilis*, a 'formiga argentina'," *Studia Entomologica*, vol. 1, no. 3-4, pp. 465–486, 1958.

[22] U. Schmid, "*Microdon rhenanus* and *Microdon eggeri* var. *major* (Diptera: Syrphidae) revisited," *Volucella*, vol. 7, pp. 111–124, 2004.

[23] K. Schönrogge, B. Barr, J. C. Wardlaw et al., "When rare species become endangered: cryptic speciation in myrmecophilous

hoverflies," *Biological Journal of the Linnean Society*, vol. 75, no. 3, pp. 291–300, 2002.

[24] S. Bonelli, M. Witek, S. Canterino et al., "Distribution, host specificity, and the potential for cryptic speciation in hoverfly *Microdon myrmicae* (Diptera: Syrphidae), a social parasite of *Myrmica* ants," *Ecological Entomology*, vol. 36, no. 2, pp. 135–143, 2011.

[25] M. W. Gates and G. Pérez-Lachaud, "Description of *Camponotophilus delvarei*, gen. n. and sp. n. (Hymenoptera: Chalcidoidea: Eurytomidae), with discussion of diagnostic characters," *Proceedings of the Entomological Society of Washington*, vol. 114, no. 1, pp. 111–124, 2012.

[26] C. Hansson, J.-P. Lachaud, and G. Pérez-Lachaud, "Entedoninae wasps (Hymenoptera, Chalcidoidea, Eulophidae) associated with ants (Hymenoptera, Formicidae) in tropical America, with new species and notes on their biology," *ZooKeys*, vol. 134, pp. 65–82, 2011.

[27] E. I. Scott, "An account of the developmental stages of some aphidophagous Syrphidae (Dipt.) and their parasites (Hymenopt.)," *Annals of Applied Biology*, vol. 26, no. 3, pp. 509–532, 1939.

[28] H. V. Weems Jr., "Natural enemies and insecticides that are detrimental to beneficial Syrphidae," *The Ohio Journal of Science*, vol. 54, no. 1, pp. 45–54, 1954.

[29] G. E. Rotheray, "Host searching and oviposition behaviour of some parasitoids of aphidophagous Syrphidae," *Ecological Entomology*, vol. 6, no. 1, pp. 79–87, 1981.

[30] M. E. Schauff, "*Microdonophagus*, a new entedontine genus (Hymenoptera: Eulophidae) from Panama," *Proceedings of the Entomological Society of Washington*, vol. 88, no. 1, pp. 167–173, 1986.

[31] B. R. Subba Rao, "A redescription of *Exoristobia* Ashmead 1904 and description of two new species (Hymenoptera: Encyrtidae)," *Proceedings of the Royal Entomological Society of London B*, vol. 39, no. 7-8, pp. 109–113, 1970.

[32] W. M. Wheeler, "Neotropical ants collected by Dr. Elisabeth Skwarra and others," *Bulletin of the Museum of Comparative Zoölogy*, vol. 77, no. 5, pp. 159–240, 1934.

[33] M. Witek, D. Patricelli, L. P. Casacci, F. Barbero, E. Balletto, and S. Bonelli, "Notes on the biology and host ant specificity of the myrmecophilous syrphid fly *Microdon major* (Diptera: Syrphidae), a social parasite of *Formica* ants (Hymenoptera: Formicidae)," *Sociobiology*, vol. 57, no. 2, pp. 261–269, 2011.

[34] H. Lotfalizadeh, G. Delvare, and J.-Y. Rasplus, "Phylogenetic analysis of Eurytominae (Chalcidoidea: Eurytomidae) based on morphological characters," *Zoological Journal of the Linnean Society*, vol. 151, no. 3, pp. 441–510, 2007.

[35] O. Piel, "*Monema flavescens* Wkr. and its parasites (Lepidoptera, Heterogeneidae)," *Lingnan Journal of Science (supplement)*, vol. 12, pp. 173–202, 1933.

[36] H. Goulet and J. T. Huber, *Hymenoptera of the World: An Identification Guide to Families*, Research Branch, Agriculture Canada Publications, Ottawa, Canada, 1993.

[37] J. DiGiulio, "Family Eurytomidae," in *Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera)*, G. Gibson, J. Huber, and J. Woolley, Eds., pp. 477–497, NRC Research Press, Ottawa, Canada, 1997.

[38] M. D. Zerova, "Hymenoptera Parasitica. Chalcidoidea—Eurytomidae," *Fauna Ukraini*, vol. 11, no. 9, pp. 1–465, 1978.

[39] W. J. Phillips, "Report on *Isosoma* investigations," *Journal of Economic Entomology*, vol. 10, no. 1, pp. 139–146, 1917.

[40] W. J. Phillips, "*Eurytoma parva* (Girault) Phillips and its biology as a parasite of the wheat jointworm, *Harmolita tritici* (Fitch)," *Journal of Agricultural Research*, vol. 34, no. 8, pp. 743–758, 1927.

[41] M. Gates, J. Mena Correa, J. Sivinski, R. Ramírez-Romero, G. Córdova-García, and M. Aluja, "Description of the immature stages of *Eurytoma sivinskii* Gates and Grissell (Hymenoptera: Eurytomidae), an ectoparasitoid of *Anastrepha* (Diptera: Tephritidae) pupae in Mexico," *Entomological News*, vol. 119, no. 4, pp. 354–360, 2008.

[42] C. T. Brues, "*Conoaxima*, a new genus of the hymenopterous family Eurytomidae, with a description of its larva and pupa," *Psyche*, vol. 29, no. 4, pp. 153–158, 1922.

[43] J.-P. Lachaud and G. Pérez-Lachaud, "Diversity of species and behavior of hymenopteran parasitoids of ants, a review," *Psyche*, vol. 2012, Article ID 134746, 24 pages, 2012.

[44] J. T. Longino, "Taxonomy of the *Cecropia*-inhabiting *Azteca* ants," *Journal of Natural History*, vol. 25, no. 6, pp. 1571–1602, 1991.

[45] J. Settele and E. Kühn, "Insect conservation," *Science*, vol. 325, no. 5936, pp. 41–42, 2009.

[46] J. A. Thomas, D. J. Simcox, and R. T. Clarke, "Successful conservation of a threatened *Maculinea* butterfly," *Science*, vol. 325, no. 5936, pp. 80–83, 2009.

[47] K. Schönrogge, M. G. Gardner, G. W. Elmes et al., "Host propagation permits extreme local adaptation in a social parasite of ants," *Ecology Letters*, vol. 9, no. 9, pp. 1032–1040, 2006.

[48] D. P. Hughes, N. E. Pierce, and J. J. Boomsma, "Social insect symbionts: evolution in homeostatic fortresses," *Trends in Ecology and Evolution*, vol. 23, no. 12, pp. 672–677, 2008.

[49] J.-P. Lachaud, A. Lenoir, and V. Witte, "Ants and their parasites," *Psyche*, vol. 2012, Article ID 342157, 5 pages, 2012.

[50] G. Pérez-Lachaud, J. Noyes, and J.-P. Lachaud, "First record of an encyrtid wasp (Hymenoptera: Chalcidoidea) as a true primary parasitoid of ants (Hymenoptera: Formicidae)," *The Florida Entomologist*, vol. 95, no. 4, pp. 1066–1076, 2012.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 839181, 6 pages
http://dx.doi.org/10.1155/2013/839181

Review Article

Ant-Mimicking Spiders: Strategies for Living with Social Insects

Fadia Sara Ceccarelli

Department of Environmental Sciences, University of Basel, 4056 Basel, Switzerland

Correspondence should be addressed to Fadia Sara Ceccarelli; saracecca@hotmail.com

Received 8 February 2013; Revised 5 April 2013; Accepted 9 May 2013

Academic Editor: David P. Hughes

Mimicry is a fascinating topic, in particular when viewed in terms of selective forces and evolutionary strategies. Mimicry is a system involving a signaller, a signal receiver, and a model and has evolved independently many times in plants and animals. There are several ways of classifying mimicry based on the interactions and cost-benefit scenarios of the parties involved. In this review, I briefly outline the dynamics of the most common types of mimicry to then apply it to some of the spider-ant associative systems known to date. In addition, this review expands on the strategies that ant-associating (in particular ant-mimicking) spiders have developed to minimise the costs of living close to colonies of potentially dangerous models. The main strategy that has been noted to date is either chemical mimicry or actively avoiding contact with ants. If these strategies warrant protection for the spider (living close to potentially dangerous models), then the benefits of ant associations would outweigh the costs, and the association will prevail.

1. Introduction

The phenomenon of mimicry has intrigued numerous biologists, prompting studies from natural history to behaviour, ecology, evolution, and most recently genomics, to name but a few [1]. Perhaps mimicry so readily attracts attention because it is an evident example of natural selection in action. Mimicry—or the resemblance of one organism (or certain aspects of) to another, taxonomically unrelated one—almost always involves three parties: the signaller (mimic), the signal receiver (or operator), and the model. The mimics in these cases must have a selective advantage over nonmimics, and therefore the particular phenotype is fixed in these populations. The classification of mimicry largely depends on the functions of the parties involved and has, based on this scheme, been subdivided down to 40 theoretical classes, or types of mimicry [2], though the focus is generally on the most common types: Batesian, Müllerian, and aggressive mimicry.

Batesian mimicry, named after H. W. Bates, pioneer in the study of mimicry in Amazonian butterflies [3], is defined by a palatable mimic gaining protection from predators (the signal receiver in this case), by resembling a noxious or unpalatable model organism. In Müllerian mimicry, the line of "palatability" between mimic and model is less clear, with emphasis being placed on a certain phenotype of various organisms being reinforced and acting as a deterrent for predators. A third type of mimicry commonly encountered in nature is aggressive mimicry, so-called because the mimic, rather than gaining protection from potential predators, more easily gains access to resources or prey (sometimes the model itself) through its resemblance to another organism. Although many cases of mimicry can easily be categorised, sometimes an organism displays different strategies, either at the same time or at different stages of its life, such as the cuckoo which was found to be a Batesian mimic as an adult, and an aggressive mimic in other birds' nests [4].

In Batesian mimicry, the mimic is under predator-mediated selection thus resembling a noxious or unpalatable model, whereas traits of aggressive mimics are under pressure to deceive their prey. This means that the sensory channel of the receiver (be it visual, chemosensory, or other) greatly influences the evolution of the mimic [5]. In cases where learning by the signal receiver is involved, it is also important that the mimics do not outnumber the models and that both models and mimics live in sympatry [6]. Be it for protection from predators or access to resources, mimicry has arisen numerous times throughout animals and plants as a recurrent evolutionary stable strategy [6]. This is evidence for strong selection for traits associated with mimicry, where the fitness

of the mimic is expected to increase with a closer resemblance to the model [7, 8]. Studies based on theoretical population genetics have modelled Batesian mimicry traits and polymorphism within populations [9, 10]. The fact that Batesian mimicry may be a costly trait must also be considered together with an increased number of parameters such as the cognitive constraints of the signal receivers [11, 12]. Selection pressure on mimics to resemble a model very much depends on the visual system of the receiver [5]. In the particular case of Batesian mimicry, where mathematical models predict greater protection from predators with increasing resemblance to the model organism, the main question that arises is why are there still "imperfect" mimics or those that bear only a slight resemblance to any one model? One explanation given is that the term "imperfect" is subjective, dependent on the signal receiver; what may appear imperfect to a human observer may in fact be seen otherwise by potential predators [13]. Alternatively, an imperfect mimic may be an intermediate phenotype or one of polymorphism [14]. Certain conditions may relax the selection pressure towards a "perfect" phenotype, for example, if the model is very noxious [15] or if behavioural traits reenforce morphology [16]. The selection force towards one "perfect" phenotype is countered by polymorphism, which may arise due to kin selection [17], in some cases the potential cost of being too conspicuous [18] or through selection from receivers with opposing predatory preferences [19].

Mimicry occurs in all forms of terrestrial and aquatic plant and animal life [6]. For example, among vertebrates, marine fishes count with at least 98 cases of mimicry, including Batesian, Müllerian, aggressive, and social (or cases where the mimic aggregates with the school of models) [20]. Perhaps the most diverse and varied forms of mimicry can be found in arthropods, due to their impressive diversity resulting from relatively short generation times, which increase the recombination events, which in turn allow for more genetic diversity. Among terrestrial arthropods, ants are a common model system [21, 22]. Here, I intend to focus on an exceptional group of arthropods, namely, the spiders, and their varied forms of ant mimicry. Even though the majority of spiders are web builders [23], the most striking examples of ant associations can be found in cursorial spiders. Thorough and up-to-date reviews of ant-mimicry in spiders already exist [24–27], so my aim here is not to replicate the information found in these papers, but rather to focus on the various strategies that can be found in these spiders minimising the costs and maximising the benefits of living with or close to ants. I will do this by first talking briefly about ant association and then introducing various examples of benefits and costs to the spiders. Throughout, ant-mimicry will refer to cases of morphological and/or chemical mimicry and "ant associations" include mimics as well as spiders that do not mimic ants but nevertheless gain some advantage living close to ant colonies.

2. Ant Associations in Arthropods

Being social insects, ants form large colonies with numerous individuals, thus satisfying the condition of mimicry where any mimic should be at lower densities than the model [6]. For the purpose of Batesian mimicry, ants are also good model organisms because they are unpalatable for many other animals due to characteristics, or combinations thereof, such as formic acid, stings, strong mandibles that bite, and in general an aggressive nature [21, 22, 28]. So acquiring morphological and/or behavioural resemblance to ants confers a certain degree of protection from predation to otherwise palatable arthropods.

Morphological and/or behavioural resemblance to ants, also known as myrmecomorphy, has evolved at least 70 times in more than 2000 described species belonging to 54 arthropod families in groups such as spiders, plant bugs, and staphylinid beetles [21]. In spiders alone, myrmecomorphy can be found in numerous species belonging to 13 different families [24, 25]. Myrmecomorphic spiders have morphological and/or behavioural modifications that increase their resemblance to ants. These include a generally narrower body and longer legs compared to other spiders: at times a constricted carapace or abdomen giving the impression of a three- instead of two-segmented body. The cuticular surface of myrmecomorphic spiders is often strikingly similar to that of their model ant species as well, including hairs and coloration and fake eye spots. As spiders have four pairs of legs while ants have three and one pair of antennae, myrmecomorphic spiders often raise their first pair of legs and wave them as an "antennal illusion" [29, 30] and also carry out an up-and-down movement of the gaster, akin to some ants when they are recruiting nestmates [30–32].

The family of spiders with perhaps the most striking examples of myrmecomorphy is the jumping spiders (Araneae: Salticidae). Here again, myrmecomorphy has evolved independently various times [33], and the most speciose genus of myrmecomorphic salticids is *Myrmarachne*, which has more than 200 described species and many more undescribed [34].

Arthropods that are not morphological mimics of ants can nevertheless form close associations with colonies. These arthropods are generally referred to as myrmecophiles, and their association to ant colonies can vary in extent [24, 25]. The ecological advantage for myrmecophiles is that the nests of many ant species are relatively stable microhabitats where resources can be readily available, and a certain degree of protection is conferred as well [24, 25]. Some examples of this will be given in the following section.

3. Benefits of Ant Associations for Spiders

The fact that ant mimicry exists in such varied forms across many invertebrate taxa implies that the benefits must outweigh the costs. As social insects, ants form colonies, often containing thousands and in some cases millions of individuals [22], and in many cases their nests are sophisticated structures and spaces in the environment. This has advantages for invertebrates that associate so closely with ants that they actually live inside the ants' nests. The nest provides a stable environment, often with plenty of resources to feed on, be it other inquilines, materials the ants gathered or bred,

or the ants/larvae themselves [35]. For example, the linyphiid spider *Masoncus pogonophilus* feeds on collembolans that also live inside its host ant nests [36], while the salticid spider *Cosmophasis bitaeniata* enters ants' nests to feed on their larvae [37].

In the case of myrmecomorphic spiders, the main benefit is that they gain protection from ant-averse predators that would otherwise feed on them. Several experiments have been carried out to show that myrmecomorphic spiders are Batesian mimics because they gain protection from potential predators such as wasps [38], mantises [39], and other spiders [40–42] and that ant-aversion is even innate in some predators [39, 43]. Salticids as predators alone were suggested to be a driving force for myrmecomorphy in jumping spiders [44]. To date, there is little evidence that myrmecomorphy serves in protecting the spider directly from the ant, as the ants' primary sensory channel seems to be chemical [45]. On the other hand, most myrmecomorphs do not routinely prey on ants, although there have been cases reported where the myrmecomorphs do prey on ants [46–48].

Within Batesian ant-mimicking spiders, several alternative or supplementary strategies have been described that confer protection from potential predators. One of these strategies is transformational mimicry, meaning that the model mimics different species as it grows [49]. Several *Myrmarachne* species are transformational mimics, thus always being approximately the same size as their model ants [50]. Another strategy involves the common occurrence among males of several *Myrmarachne* species that have enlarged chelicerae (thought to be a sexually selected character [51]), a phenotype that could be seen as reducing their resemblance to ants. However, these males were found to be "compound mimics" resembling ants carrying a "parcel" in their mandibles [52]. Additionally, *Myrmarachne melanotarsa*, a spider unusual in that it lives in aggregations, resembles, as a group, a whole ant colony [53]. Selection has acted on these varied strategies found among myrmecomorphs, increasing their resemblance to ants, yet forces countering the selection of "perfect" resemblance to ants also exist, as polymorphism has been recorded in various *Myrmarachne* species [54, 55].

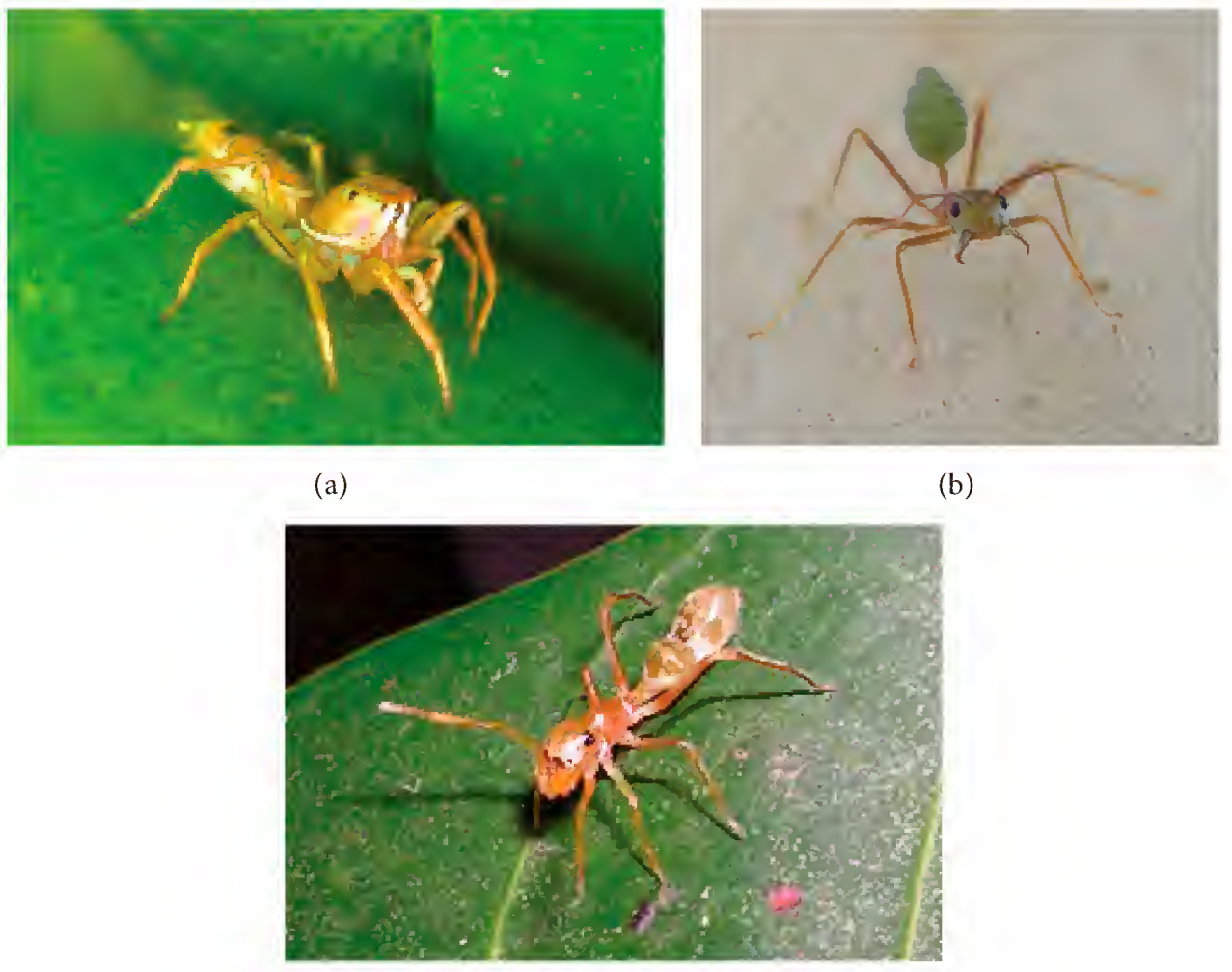
So the benefits for spiders of associating with ants come mainly in the form of increased chances of survival for the individuals. These increased survival chances are either due to an easier access to readily available resources or heightened protection from predators. If these benefits did not exist, selection would not have favoured the traits allowing these spiders to associate with ants. However, for the spiders there are not only benefits to these associations, but also costs. For the associations to persist in evolutionary terms, the benefits must still outweigh the costs, meaning that the costs are kept minimal. The next section deals with the spiders' strategies that minimise the various costs.

4. Minimising Costs of Ant Mimicry

The costs of ant mimicry for spiders come in varied forms. First of all, for myrmecomorphs there is the fact that morphological modifications, such as a restriction of the abdomen, mean that females can lay fewer eggs than non-myrmecomorphic spiders ([25] and references therein). A major problem that myrmecomorphic spiders face is that while their resemblance to ants confers protection from ant-averse predators, they are more prone to fall victims to predators that specialise on eating ants [19, 56]. To counter this problem, jumping spiders of the genus *Myrmarachne* have developed signals using their first pair of legs, aimed at deterring ant-eating salticids from attacking [57]. This "display posture" of holding the first pair of legs almost fully extended, elevated 45°, and held out to the side 45° [57] was also noted in other studies on *Myrmarachne* when the spiders were in the presence of ants [58], and it resembles the aggressive display posture of worker ants from certain species such as *Oecophylla smaragdina* (see Figure 1). This display posture, while being efficient in deterring salticid predators, seems to be adopted by *Myrmarachne* as a general measure when threatened, before fleeing, and may also affect ants—such as *O. smaragdina*—that have a more sophisticated visual system [59, 60].

Perhaps the biggest challenge for ant-associating spiders comes from living close to ant species, most of which would react aggressively towards inquilines or mimics themselves. In fact, spiders may easily be killed or injured by their own model [61]. The negative effects of ants on spiders are not only restricted to the individuals' survival, but also the spiders' reproductive success in some cases, in that they are less likely to mate if the ants are close by [62]. Certain spiders that have developed a close association with ants deploy chemical mimicry to be able to live among and at times exploit the ants [63]. *Cosmophasis bitaeniata* even acquires the hosts' cuticular hydrocarbons specific to the ant colony with which it lives [64], as the cuticular hydrocarbons are transferred to the spider while feeding on the ant larvae [65]. In the case of this spider, the host ant species, *Oecophylla smaragdina*, is particularly aggressive [59, 60, 66] (see Figure 1), and chemical mimicry is a form of protection. Through chemical mimicry, many nonant nestmates are able to enter ant colonies and take advantage of the ants and/or their mutualistic relationships [67]. Some myrmecophiles are small enough to live among the ants undetected without chemical mimicry [36], while others, such as *Gamasomorpha maschwitzi*, have alternative strategies to chemical mimicry which are to date poorly known but could consist of acoustical, behavioural, and/or morphological adaptations [68].

For those spiders that do not live in, or enter into the ants' nests, there does not seem to be as much danger as of being killed by an ant. However, for the myrmecomorphs that are Batesian mimics, the premise is to be in the model ants' vicinity, which nevertheless poses a considerable danger [61]. As there is no known case of chemical mimicry in myrmecomorphic spiders [69], their defence strategies need to rely on different approaches, which are mainly behavioural. It has long been observed that ant-associating spiders such as *Myrmarachne* generally avoid contact with ants [45, 58, 61, 70], and this holds true not only for myrmecomorphs, but also for aggressive mimics such as *C. bitaeniata*, despite its chemical protection [35, 58]. Upon seeing an ant approach, myrmecomorphic spider species of the genus *Myrmarachne*



(a) (b) (c)

FIGURE 1: Ant-associating salticids (a) *Cosmophasis bitaeniata*, chemical, aggressive myrmecophile and (c) *Myrmarachne smaragdina*, myrmecomorphic Batesian mimic, and their common model ant species (b) *Oecophylla smaragdina* in an aggressive display posture.

actively move away from the ant, regardless of the ant species, and contact occurs in fewer than 3% of the cases when the spiders react to the presence of the ants [58]. These spiders are able to distinguish between ants and conspecifics, due to their remarkable visual acuity [71]. They also react differently to ants depending on whether the ants are facing them, side-on, moving, or stationary but generally do not let the ant get closer than approximately 2 cm [58]. At times, however, contact is unavoidable, and the spiders flee even upon contact, only very rarely reacting aggressively, perhaps as a last resort [72]. Active avoidance of ants is common in myrmecomorphic spiders, and the behavioural reactions of myrmecomorphs towards sympatric ant species are different depending on the species of spiders (as was shown with *Myrmarachne*). Innate behavioural traits are different between species due to selection (as is the case in morphological traits). Aversion to ants is innate in arthropods such as mantises [39], and avoidance of ants could therefore also be an innate trait in myrmecomorphic jumping spiders such as *Myrmarachne*. If that is the case, the fact that each species of *Myrmarachne* reacts differently to the presence of ants suggests that these behavioural traits are under selection pressure [58].

5. Conclusions

There are advantages and disadvantages for ant-associating spiders related to living near or inside ant colonies. When looking purely at ant mimicry, it is clear that there is an arms race between the parties involved in terms of evolutionary costs and benefits. Varied strategies have evolved in ant-mimicking spiders allowing them to reap the benefits of resembling ants. In addition, these spiders have innate and/or learned behaviours that reduce the costs of having models that are often aggressive and a real danger to the spiders themselves. Despite the considerable studies that have been carried out recently, ant mimicry in spiders is definitely a topic which deserves more attention and in-depth studies. In particular, with the increasing use of genomics, it is possible to carry out studies relating the underlying genetic mechanisms to phenotypic adaptations to ant mimicry, as have been carried out by D. Charlesworth and B. Charlesworth [72] which would give even more insight into the evolution of mimicry.

Acknowledgments

The author thanks all the people who supported her during her time working on ant-mimicking spiders in North Queensland, in particular her supervisors Richard Rowe and Ross Crozier, and people from the Crozier Lab and the School of Marine and Tropical Biology, James Cook University, Townsville, for funding. The author also thanks two the anonymous referees for their comments on the paper.

References

[1] G. D. Ruxton, T. N. Sherratt, and M. Speed, *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry*, Oxford University Press, Oxford, UK, 2004.

[2] R. I. Vane-Wright, "A unified classification of mimetic resemblances," *Biological Journal of the Linnean Society*, vol. 8, no. 1, pp. 25–56, 1976.

[3] H. W. Bates, "Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae," *Transactions of the Linnean Society London*, vol. 23, no. 3, pp. 495–566, 1862.

[4] J. A. Welbergen and N. B. Davies, "A parasite in wolf's clothing: hawk mimicry reduces mobbing of cuckoos by hosts," *Behavioral Ecology*, vol. 22, no. 3, pp. 574–579, 2011.

[5] K. L. Cheney and N. J. Marshall, "Mimicry in coral reef fish: how accurate is this deception in terms of color and luminance?" *Behavioral Ecology*, vol. 20, part 3, pp. 459–468, 2009.

[6] W. Wickler, *Mimicry in Plants and Animals*, McGraw-Hill, New York, NY, USA, 1968.

[7] U. Nur, "Evolutionary rates of models and mimics in Batesian mimicry," *The American Naturalist*, vol. 104, no. 939, pp. 477–486, 1970.

[8] M. Edmunds, *Defence in Animals—A Survey of Anti-Predator Defences*, Longman, Harlow, UK, 1974.

[9] D. Charlesworth and B. Charlesworth, "Theoretical genetics of Batesian mimicry. I. Single locus models," *Journal of Theoretical Biology*, vol. 55, no. 2, pp. 283–303, 1975.

[10] C. Matessi and R. Cori, "Models of population genetics of Batesian mimicry," *Theoretical Population Biology*, vol. 3, no. 1, pp. 41–68, 1972.

[11] O. H. Holen and R. A. Johnstone, "The evolution of mimicry under constraints," *The American Naturalist*, vol. 164, no. 5, pp. 598–613, 2004.

[12] O. H. Holen and R. A. Johnstone, "Context-dependent discrimination and the evolution of mimicry," *The American Naturalist*, vol. 167, no. 3, pp. 377–389, 2006.

[13] W. Dittrich, F. Gilbert, P. Green, P. Mcgregor, and D. Grewcock, "Imperfect mimicry: a pigeon's perspective," *Proceedings of the Royal Society B*, vol. 251, no. 1332, pp. 195–200, 1993.

[14] M. Edmunds, "Why are there good and poor mimics?" *Biological Journal of the Linnean Society*, vol. 70, no. 3, pp. 459–466, 2000.

[15] T. N. Sherratt, "The evolution of imperfect mimicry," *Behvioral Ecology*, vol. 13, part 6, pp. 821–826, 2002.

[16] S. Pekár and M. Jarab, "Assessment of color and behavioral resemblance to models by inaccurate myrmecomorphic spiders (Araneae)," *Invertebrate Biology*, vol. 130, no. 1, pp. 83–90, 2011.

[17] R. A. Johnstone, "The evolution of inaccurate mimics," *Nature*, vol. 418, no. 6897, pp. 524–526, 2002.

[18] M. P. Speed and G. D. Ruxton, "Imperfect Batesian mimicry and the conspicuousness costs of mimetic resemblance," *The American Naturalist*, vol. 176, no. 1, pp. E1–E14, 2010.

[19] S. Pekár, M. Jarab, L. Fromhage, and M. E. Herberstein, "Is the evolution of inaccurate mimicry a result of selection by a suite of predators? A case study using myrmecomorphic spiders," *The American Naturalist*, vol. 178, no. 1, pp. 124–134, 2011.

[20] J. E. Randall, "A review of mimicry in marine fishes," *Zoological Studies*, vol. 44, no. 3, pp. 299–328, 2005.

[21] J. D. McIver and G. Stonedahl, "Myrmecomorphy: morphological and behavioral mimicry of ants," *Annual Review of Entomology*, vol. 38, pp. 351–379, 1993.

[22] B. Hölldobler and E. O. Wilson, *The Ants*, The Belknap Press of Harvard University Press, Cambridge, Mass, USA, 1990.

[23] R. F. Foelix, *Biology of Spiders*, Oxford University Press, Oxford, UK, 3rd edition, 2011.

[24] P. E. Cushing, "Myrmecomorphy and myrmecophily in spiders: a review," *Florida Entomologist*, vol. 80, no. 2, pp. 165–193, 1997.

[25] P.E. Cushing, "Spider-ant associations: an updated review of myrmecomorphy, myrmecophily, and myrmecophagy in spiders," *Psyche*, vol. 2012, Article ID 151989, 23 pages, 2012.

[26] R. R. Jackson and X. J. Nelson, "Specialised exploitation of ants (Hymenoptera: Formicidae) by spiders (Araneae)," *Myrmecological News*, vol. 17, pp. 33–49, 2012.

[27] X. J. Nelson and R. R. Jackson, "How spiders practice aggressive and Batesian mimicry," *Current Zoology*, vol. 58, part 4, pp. 620–629, 2012.

[28] M. A. Fürst, M. Durey, and D. R. Nash, "Testing the adjustable threshold model for intruder recognition on *Myrmica* ants in the context of a social parasite," *Proceedings of the Royal Society B*, vol. 279, pp. 516–522, 2012.

[29] J. Reiskind, "Ant-mimicry in Panamanian clubionid and salticid spiders (Araneae: Clubionidae, Salticidae)," *Biotropica*, vol. 9, no. 1, pp. 1–8, 1977.

[30] F. S. Ceccarelli, "Behavioral mimicry in *Myrmarachne* species (Araneae, Salticidae) from North Queensland, Australia," *Journal of Arachnology*, vol. 36, no. 2, pp. 344–351, 2008.

[31] R. R. Jackson, "The biology of ant-like jumping spiders: intraspecific interactions of *Myrmarachne lupata* (Araneae, Salticidae)," *Zoological Journal of the Linnean Society*, vol. 76, no. 4, pp. 293–319, 1982.

[32] J. L. Mercier, A. Lenoir, and A. Dejean, "Ritualised versus aggressive behaviours displayed by *Polyrhachis iaboriosa* (F. Smith) during intraspecific competition," *Behavioural Processes*, vol. 41, no. 1, pp. 39–50, 1997.

[33] M. C. Hedin and W. P. Maddison, "A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae)," *Molecular Phylogenetics and Evolution*, vol. 18, no. 3, pp. 386–403, 2001.

[34] J. Proszynski, "Monograph of Salticidae (Araneae) of the World," 2012, http://www.peckhamia.com/salticidae/.

[35] B. Hölldobler, "Communication between ants and their guests," *Scientific American*, vol. 224, pp. 86–93, 1971.

[36] P. E. Cushing, "Population structure of the ant nest symbiont *Masoncus pogonophilus* (Araneae: Linyphiidae)," *Annals of the Entomological Society of America*, vol. 91, part 5, pp. 626–631, 1998.

[37] R. A. Allan and M. A. Elgar, "Exploitation of the green tree ant, *Oecophylla smaragdina*, by the salticid spider *Cosmophasis bitaeniata*," *Australian Journal of Zoology*, vol. 49, no. 2, pp. 129–137, 2001.

[38] M. Edmunds, "Does mimicry of ants reduce predation by wasps on salticid spiders?" *Memoirs—Queensland Museum*, vol. 33, no. 2, pp. 507–512, 1993.

[39] X. J. Nelson, R. R. Jackson, D. Li, A. T. Barrion, and G. B. Edwards, "Innate aversion to ants (Hymenoptera: Formicidae) and ant mimics: experimental findings from mantises (Mantodea)," *Biological Journal of the Linnean Society*, vol. 88, no. 1, pp. 23–32, 2006.

[40] B. Cutler, "Reduced predation on the antlike jumping spider *Synageles occidentalis* (Araneae: Salticidae)," *Journal of Insect Behavior*, vol. 4, part 3, pp. 401–407, 1991.

[41] C. A. Durkee, M. R. Weiss, and D. B. Uma, "Ant mimicry lessens predation on a North American jumping spider by larger salticid spiders," *Environmental Entomology*, vol. 40, part 5, pp. 1223–1231, 2011.

[42] X. J. Nelson, "A predator's perspective of the accuracy of ant mimicry in spiders," *Psyche*, vol. 2012, Article ID 168549, 5 pages, 2012.

[43] X. J. Nelson and R. R. Jackson, "Vision-based innate aversion to ants and ant mimics," *Behavioral Ecology*, vol. 17, no. 4, pp. 676–681, 2006.

[44] J. N. Huang, R. C. Cheng, D. Li, and I. M. Tso, "Salticid predation as one potential driving force of ant mimicry in jumping spiders," *Proceedings of the Royal Society B*, vol. 278, no. 1710, pp. 1356–1364, 2011.

[45] B. Hölldobler, "Multimodal signals in ant communication," *Journal of Comparative Physiology A*, vol. 184, no. 2, pp. 129–141, 1999.

[46] K. Wing, "*Tutelina similis* (araneae: Salticidae): an ant mimic that feeds on ants," *Journal of the Kansas Entomological Society*, vol. 56, no. 1, pp. 55–58, 1983.

[47] P. S. Oliveira and I. Sazima, "The adaptive bases of ant-mimicry in a neotropical aphantochilid spider (Araneae: Aphantochilidae)," *Biological Journal of the Linnean Society*, vol. 22, no. 2, pp. 145–155, 1984.

[48] X. J. Nelson and R. R. Jackson, "Aggressive use of Batesian mimicry by an ant-like jumping spider," *Biology Letters*, vol. 5, no. 6, pp. 755–757, 2009.

[49] A. P. Mathew, "Transformational deceptive resemblance as seen in the life history of a plant bug (*Riptortus pedestris*), and of a mantis (*Evantissa pulchra*)," *Journal of the Bombay Natural History Society*, vol. 37, pp. 803–813, 1935.

[50] M. Edmunds, "On the association between *Myrmarachne* spp. (Salticidae) and ants," *Bulletin of the British Arachnological Society*, vol. 4, pp. 149–160, 1978.

[51] S. D. Pollard, "Consequences of sexual selection on feeding in male jumping spiders (Araneae: Salticidae)," *Journal of Zoology*, vol. 234, no. 2, pp. 203–208, 1994.

[52] X. J. Nelson and R. R. Jackson, "Compound mimicry and trading predators by the males of sexually dimorphic Batesian mimics," *Proceedings of the Royal Society B*, vol. 273, no. 1584, pp. 367–372, 2006.

[53] X. J. Nelson and R. R. Jackson, "Collective Batesian mimicry of ant groups by aggregating spiders," *Animal Behaviour*, vol. 78, no. 1, pp. 123–129, 2009.

[54] F. S. Ceccarelli and R. H. Crozier, "Dynamics of the evolution of Batesian mimicry: molecular phylogenetic analysis of ant-mimicking *Myrmarachne* (Araneae: Salticidae) species and their ant models," *Journal of Evolutionary Biology*, vol. 20, no. 1, pp. 286–295, 2007.

[55] X. J. Nelson, "Polymorphism in an ant mimicking jumping spider," *Journal of Arachnology*, vol. 38, no. 1, pp. 139–141, 2010.

[56] X. J. Nelson, D. Li, and R. R. Jackson, "Out of the frying pan and into the fire: a novel trade-off for batesian mimics," *Ethology*, vol. 112, no. 3, pp. 270–277, 2006.

[57] X. J. Nelson, R. R. Jackson, and D. Li, "Conditional use of honest signaling by a Batesian mimic," *Behavioral Ecology*, vol. 17, no. 4, pp. 575–580, 2006.

[58] F. S. Ceccarelli, *Dynamics of Salticid-Ant Mimicry Systems—A Study of Behavioural Ecology and Evolution in Two Genera of Ant-Associating Jumping Spiders*, Lambert Academic Publishing, Saarbrücken, Germany, 2010.

[59] B. Hölldobler, "Territorial behavior in the green tree ant (*Oecophylla smaragdina*)," *Biotropica*, vol. 15, no. 4, pp. 241–250, 1983.

[60] X. J. Nelson, R. R. Jackson, G. B. Edwards, and A. T. Barrion, "Living with the enemy: jumping spiders that mimic weaver ants," *Journal of Arachnology*, vol. 33, pp. 813–819, 2005.

[61] X. J. Nelson and R. R. Jackson, "The influence of ants on the mating strategy of a myrmecophilic jumping spider (Araneae, Salticidae)," *Journal of Natural History*, vol. 43, no. 11-12, pp. 713–735, 2009.

[62] R. A. Allan, R. J. Capon, W. V. Brown, and M. A. Elgar, "Mimicry of host cuticular hydrocarbons by salticid spider *Cosmophasis bitaeniata* that preys on larvae of tree ants *Oecophylla smaragdina*," *Journal of Chemical Ecology*, vol. 28, no. 4, pp. 835–848, 2002.

[63] M. A. Elgar and R. A. Allan, "Chemical mimicry of the ant *Oecophylla smaragdina* by the myrmecophilous spider *Cosmophasis bitaeniata*: is it colony-specific?" *Journal of Ethology*, vol. 24, no. 3, pp. 239–246, 2006.

[64] M. A. Elgar and R. A. Allan, "Predatory spider mimics acquire colony-specific cuticular hydrocarbons from their ant model prey," *Naturwissenschaften*, vol. 91, no. 3, pp. 143–147, 2004.

[65] R. H. Crozier, P. S. Newey, E. A. Schlüns, and S. K. A. Robson, "A masterpiece of evolution *Oecophylla* weaver ants (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 13, pp. 57–71, 2009.

[66] K. Dettner and C. Liepert, "Chemical mimicry and camouflage," *Annual Review of Entomology*, vol. 39, pp. 129–154, 1994.

[67] C. von Beeren, R. Hashim, and V. Witte, "The social integration of a myrmecophilous spider does not depend exclusively on chemical mimicry," *Journal of Chemical Ecology*, vol. 38, no. 3, pp. 262–271, 2012.

[68] S. Pekar and P. Jiros, "Do ant mimics imitate cuticular hydrocarbons of their models?" *Animal Behaviour*, vol. 82, no. 5, pp. 1193–1199, 2011.

[69] A. P. Mathew, "Observations on the habits of two spider mimics of the red ant, *Oecophylla smaragdina* (Fabr.)," *Journal of the Bombay Natural History Society*, vol. 52, pp. 249–263, 1944.

[70] X. J. Nelson, "Visual cues used by ant-like jumping spiders to distinguish conspecifics from their models," *Journal of Arachnology*, vol. 38, no. 1, pp. 27–34, 2010.

[71] F. S. Ceccarelli, "Contact between *Myrmarachne* (Araneae: Salticidae) and ants," *Bulletin of the British Arachnological Society*, vol. 14, part 2, pp. 54–58, 2007.

[72] D. Charlesworth and B. Charlesworth, "Mimicry: the hunting of the supergene," *Current Biology*, vol. 21, part 20, pp. R846–R848, 2011.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 238602, 9 pages
http://dx.doi.org/10.1155/2013/238602

Review Article

Nesting Associations without Interdependence: A Preliminary Review on Plesiobiosis in Ants

Orsolya Kanizsai, Gábor Lőrinczi, and László Gallé

Department of Ecology, University of Szeged, 52 Közép Fasor, Szeged 6726, Hungary

Correspondence should be addressed to Orsolya Kanizsai; orsi.kanizsai@gmail.com

Received 6 February 2013; Revised 24 April 2013; Accepted 9 May 2013

Academic Editor: David P. Hughes

Plesiobiosis, the most basic form of interspecific associations in ants, denotes occasional or regular nesting of heterospecific colonies of certain species pairs in close proximity to each other without biological interdependence. Plesiobionts differ from each other both in morphology and in behaviour (e.g., in their foraging strategies), and at least one of the plesiobiotic pair is a submissive species. Recent studies on plesiobiosis have revealed that *Formica fusca* and *Lasius flavus* are two of the most frequent plesiobionts. To date, at least 48 different plesiobiotic species pairs have been recorded from various habitat types of the Holarctic region. Two main habitat properties may play a role in the forming of plesiobiosis: the scarcity of suitable nesting sites as a forcing factor and the sufficient amount of food sources available, influencing the abundance of colonies. Thus, high colony density may contribute to the formation of such associations, resulting in (1) frequent nesting in each other's neighbourhood and (2) stronger intraspecific competition, which forces colonies into the vicinity of heterospecific nests. Plesiobiotic associations formed this way may promote persistent coexistence, leading to the formation of other types of interspecific associations (e.g., clepto- or lestobiosis).

1. Introduction

Various types of interspecific associations exist among ant species. These can be categorised on the basis of the degree of interactions between heterospecific colonies, ranging from simple cooccurrence with loose interaction to highly specialised social parasitism [1–3]. Following the suggestion by Wasmann [4] and Wheeler [5], Hölldobler and Wilson [6] distinguished two main types of associations between ant colonies, namely, "compound nests" and "mixed nests." Associations belonging to "mixed nests" mostly result from social parasitism, where one of the species (as a social parasite) depends on its partner, which represents the host. On the other hand, the association types of "compound nests" differ from each other in the degree of interspecific relations ranging from neutral associations through mutualism and commensalism to typical parasitism.

The vast majority of studies on interspecific associations in ants have focused on the forms of typical social parasitism (i.e., temporary parasitism, slavery, and inquilinism) [1, 3, 7–9] or on associations that belong to "compound nests" representing a higher degree of biological interdependence between heterospecific colonies (i.e., cleptobiosis, lestobiosis, xenobiosis, or parabiosis) ([10–20] etc.). However, few studies have dealt with plesiobiotic associations so far, and most of these reported only observations that might indicate the existence of such associations [5, 21–36].

Although numerous classifications exist for associations related to "compound nests" [2, 4–6, 22, 37], most of them are based on relatively few reports [2]. According to each of the classification systems, plesiobiosis is the most rudimentary form of heterospecific associations. This type of association occurs between species pairs that differ from each other in morphology, in behaviour, and in taxonomy, and it denotes nesting close to each other without biological interdependence. Owing to this close proximity, plesiobiotic partners share not only the nesting shelter, but the same microhabitat, and possibly the foraging area as well.

In this review our aim was to summarise the existing information on plesiobiosis, by listing and discussing (1) the recorded plesiobionts and plesiobiotic partner species and (2) the assumed background factors that may promote

the formation and persistence of plesiobiotic associations. Furthermore, we pose open questions to call attention to the importance of collecting data considering the mentioned ecological approaches.

2. General Categorization of Interspecific Associations in Ants

The general classification system of "compound nests" includes five different association types with increasing degree of interactions and biological interdependence between the associated heterospecific colonies. As mentioned above, the most basic form of these associations is plesiobiosis [5, 6, 22]. According to the classical definition, plesiobiotic partner colonies share the same microhabitat without further interactions [1, 5]. In the case of cleptobiosis and lestobiosis, one of the associated colonies gains benefit from being in the vicinity of the other colony. This can be through robbing the stored resources of the other colony, stealing food from returning foragers (cleptobiosis), or preying on the brood of the alien colony (lestobiosis), thereby reducing the costs of searching and handling of food [1, 6, 9, 10]. Parabiosis differs from the other types of "compound nests" since it is a mutualistic relationship between the associated colonies [1, 6]. In these cases, each species gains benefit from its partner (e.g., by protection from enemies or competitors, interspecific trail following, etc.), and these benefits overweigh the costs of the maintenance of the coexistence [11]. Although xenobiosis is considered as a type of "compound nests," it has more social parasitic features than the previous ones. Xenobiotic species (i.e., "guest ants") spend their life inside the nest of their host colony stealing food or inducing trophallaxis with host workers [9]; therefore, xenobiosis is a truly parasitic form of interspecific associations [1, 6, 9].

In typical social parasitic associations, individuals of different colonies mix inside the nest, and heterospecific brood is mostly cared for by host workers. These associations imply biological interdependence; that is, the parasite always depends on its host(s) [9]. The queens of temporary social parasitic species use their host colonies during colony foundation, and the mixed colony gradually develops to a pure, monospecific colony of the parasitic species [1]. In this case, the parasitic species depends on its host only during colony foundation [1, 6, 9, 12]. Unlike temporary social parasitism, slave-maker species depend on their hosts throughout their lives; that is, they are constrained to renew their labour force through robbing brood from host colonies in the course of slave-making raids [1, 6, 9]. The final and most extreme stage of social parasitism is inquilinism. Inquilinous species are the "ultimate social parasites," as they spend their entire life cycle inside the nest of their host colony. Most of these species lack the worker caste, and their queens invest their energy to produce only reproductive offspring [1, 6, 9].

3. Plesiobiotic Association

Regarding the lack of biotic interdependence between the associated colonies [1, 5, 6, 12, 30], plesiobiosis is considered the most rudimentary form of interspecific associations in ants. This relationship denotes the nesting of mostly two colonies of different species in the direct proximity of each other, which means that the plesiobiotic colonies occupy the same nesting shelter (e.g., in or under logs, stumps, rocks, etc.). On the basis of the currently available data on plesiobiotic associations, this close nesting can occur occasionally or regularly, depending on the species and/or habitat type (as discussed below). Although plesiobiotic nests are adjacent to one another in several cases, they always remain separate as individual units, and the members of heterospecific colonies do not mix [6]. Plesiobionts are potentially hostile to each other, and if the nest galleries accidentally break in, fighting and brood theft may occur [6, 28, 37]. As a rule, plesiobiotic partner species differ from each other morphologically (e.g., different body size) and/or behaviourally (e.g., different foraging strategies or competitive ability), and they belong to at least different genera [6]. These differences may promote the coexistence of associated colonies according to the "limiting similarity" hypothesis suggested by MacArthur and Levins [38]. Basically, the less similar the species are the more likely they occur together in a plesiobiotic relationship in order to avoid intraspecific competition.

4. A Synthesis of the Recorded Cases of Plesiobiosis

4.1. Plesiobionts and Plesiobiotic Partners. In Table 1, we list 49 species that have been observed so far in plesiobiotic associations. 29 of these belong to the subfamily Formicinae, 17 to Myrmicinae, and only 3 to Ponerinae. The four most frequent genera whose members established plesiobiotic relationships were *Formica* (11 species), *Camponotus* (9 species), *Lasius* (8 species), and *Myrmica* (4 species), well representing the general number of genera and species in the Holarctic [6].

Recent studies on plesiobiosis revealed that two species, *Formica fusca* (Linnaeus, 1758) and *Lasius flavus* (Fabricius, 1782), can be considered as two of the most frequent plesiobionts, on the basis of the total number of their so far known plesiobiotic partner species (Table 1).

Up to the present, at least 48 different plesiobiotic species pairs have been recorded from different habitats of the Holarctic region. Among these, *F. fusca* was involved in 12 cases (25%), *L. flavus* in 8 cases (16.3%), *Monomorium minimum* in 5 cases (10.2%), *M. rubra* and *Myrmecina americana* in 4 cases (8.16%), respectively, and *Pheidole picea* and *Lasius umbratus* in 3 cases (6.12%) each (Table 1). The total number of plesiobiotic associations—where the exact number of the observed cases was given—was 69, from which the two most frequent plesiobionts participated in 46 associations, *F. fusca* in 28 cases (60.9%) and *L. flavus* in 18 cases (39.1%) (Table 1). *F. fusca* established plesiobiotic associations with species belonging to 6 different genera of two subfamilies (Myrmicinae and Formicinae). Its typical plesiobiotic partners were *Myrmica* spp. (*M. rubra* and *M. ruginodis*), *Tetramorium* spp. (*T.* cf. *caespitum*), *Leptothorax* spp. (*L. acervorum*), *Lasius* spp. (*L. platythorax*, *L. niger*, and *L. flavus*), and *Camponotus* spp. (*C. vagus* and *C. herculeanus*). Plesiobiotic partners of *L. flavus* belonged to 3 different genera, *Formica* spp. (*F. fusca*, *F. cunicularia*, *F. fuscocinerea*, and *F. aquilonia*),

Table 1: Observed cases of plesiobiotic associations in ants.

No. of species pairs	Species pairs recorded in plesiobiotic associations	Country	Habitats	Location of nests/type of nesting shelter	No. of cases of plesiobiotic nests	Source
1	*Formica fusca, Myrmica rubra*	Finland; UK	Different successional series of rocky habitats; foreshore	In/under moss; under decaying wood; under stone	2; ?	Czechowski 2003, 2004 [32, 34] Morley 1945 [25]
2	*Formica fusca, Myrmica ruginodis*	Poland	Clearcut of managed forest	In tree stumps	1	Włodarczyk et al. 2009 [36]
3	*Formica fusca-Tetramorium caespitum*	Poland	Clearcut of managed forest	In tree stumps	3	Włodarczyk et al. 2009 [36]
4	*Formica fusca-Leptothorax acervorum*	Finland	Different successional series of rocky habitats	Mound of *F. lugubris*	1	Czechowski 2004 [34]
5	*Formica fusca-Lasius flavus*	Finland	Different successional series of rocky habitats	Under wood; in rock crevice; under stone	4	Czechowski 2004 [34]
6	*Formica fusca-Lasius platythorax*	Finland; Poland	Forest on rocks; clearcut of managed forest	In decaying wood; in tree stump	3	Włodarczyk et al. 2009 [36]
7	*Formica fusca-Lasius niger*	UK	Foreshore	Under stone	?	Morley 1945 [25]
8	*Formica fusca-Camponotus herculeanus*	Poland	Forest edge	Under wood	1	Czechowski 2005 [35]
9	*Formica fusca-Camponotus vagus*	Hungary	Pine and poplar forest patches	In/under wood	10	Kanizsai (unpubl.)
10	*Formica fusca-Formica lugubris*	Finland	Different successional series of rocky habitats	Mound of *F. lugubris*	1	Czechowski 2004 [34]
11	*Formica fusca-Formica aquilonia*	Finland	Forest on rocks	Mound of *F. lugubris*	1	Czechowski and Vepsäläinen 1999 [29]
12	*Formica fusca-Formica truncorum*	Finland	Different successional series of rocky habitats	In rock crevice	1	Czechowski 2004 [34]
13	*Lasius flavus-Formica cunicularia*	UK	Foreshore	Under stone	?	Morley 1945 [25]
14	*Lasius flavus-Formica aquilonia*	Finland	Different successional series of rocky habitats	Mound of *F. aquilonia*	1	Czechowski 2004 [34]
15	*Lasius flavus-Formica fuscocinerea*	Poland	Grassy mountain slope	Under stone	1	Czechowski & Czechowska 2000 [30]
16	*Lasius flavus-Tetramorium caespitum*	Finland	Different successional series of rocky habitats	Under stone	1	Czechowski 2004 [34]
17	*Lasius flavus-Myrmica scabrinodis*	UK	Foreshore	Under stone	?	Morley 1945 [25]
18	*Lasius flavus-Lasius niger*	Finland; UK	Rocky outcrop; shore meadow, foreshore	In rock crevice/under stone	12; ?	Czechowski 2004 [34], Morley 1945 [25]
19	*Lasius flavus-Lasius platythorax*	Finland	Different successional series of rocky habitats	In rock crevice/under stone/overgrown soil	3	Czechowski 2004 [34]
20	*Monomorium minimum-Pachycondyla harpax*	USA	?	?	?	Wheeler 1901 [5]
21	*Monomorium minimum-Pogonomyrmex barbatus*	USA	?	?	?	Wheeler 1901 [5]
22	*Monomorium minimum-Camponotus festinatus*	USA	?	?	?	Wheeler 1901 [5]
23	*Monomorium minimum-Camponotus sansabeanus*	USA	?	?	?	Wheeler 1901 [5]
24	*Monomorium minimum- Formica gnava*	USA	?	?	?	Wheeler 1901 [5]

Table 1: Continued.

No. of species pairs	Species pairs recorded in plesiobiotic associations	Country	Habitats	Location of nests/type of nesting shelter	No. of cases of plesiobiotic nests	Source
25	*Myrmecina americana-Myrmica pinetorum*	USA	?	In the sand	1	Wheeler 1905 [21]
26	*Myrmecina americana-Pheidole picea*	USA	?	?	?	Wheeler 1901 [5]
27	*Myrmecina americana-Ponera pennsylvanica*	USA	?	?	?	Wheeler 1901 [5]
28	*Myrmecina americana-Formica gnava*	USA	?	Under stone	1	Wheeler 1901 [5]
29	*Myrmica rubra-Lasius niger*	Finland	Shore meadow; at road	Under stone; between asphalt edge and grass	2	Czechowski 2004 [34]
30	*Myrmica rubra-Lasius platythorax*	Finland	Forest	In decaying wood	1	Czechowski 2004 [34]
31	*Myrmica rubra-Leptothorax muscorum*	Finland	Shore meadow	Under stone	1	Czechowski 2004 [34]
32	*Lasius umbratus-Formica sanguinea*	Poland	Clearings in a pine forest	In the sandy soil	1	Czechowski & Rotkiewicz 1997 [27]
33	*Lasius umbratus-Polyergus rufescens*	Poland	Clearings in a pine forest	In the sandy soil	1	Czechowski & Rotkiewicz 1997 [27]
34	*Lasius umbratus-Lasius sabularum*	Poland	Stand of oak trees	Under stone	1	Borowiec 2011 [40]
35	*Pheidole picea-Lasius minutus*	USA	Hardwood forest	In a stump	1	Gaige 1914 [23]
36	*Pheidole picea-Lasius nearcticus*	USA	Hardwood forest	Under rock	1	Gaige 1914 [23]
37	*Camponotus fallax-Lasius brunneus*	Poland	Urban park	In decaying wood	1	Czechowski 2004 [33]
38	*Camponotus herculeanus-Lasius platythorax*	Finland	?	In decaying wood	1	Czechowski 2004 [33]
39	*Camponotus yogi-Temnothorax andrei*	USA	Chaparral	In living stems of *Haplopappus pinifolius*	1	Creighton & Snelling 1966 [26]
40	*Camponotus modoc-Leptothorax calderoni*	USA	Pine forest	In log/in stump	?	Wheeler 1917 [24]
41	*Camponotus pennsylvanicus-Formica subaenescens*	USA	Hardwood forest	Under log	1	Gaige 1914 [23]
42	*Camponotus festinatus-Pachycondyla harpax*	USA	At road	Under stone	2	Wheeler 1901 [5]
43	*Camponotus sansabeanus-Pachycondyla harpax*	USA	?	?	?	Wheeler 1901 [5]
44	*Camponotus ligniperdus-Aphaenogaster subterranea*	Hungary	Pine forest	Under stone	3	Lőrinczi (unpubl.)
45	*Formica japonica-Tetramorium tsushimae*	Japan	Urban area	In the soil	1	Czechowski & Yamauchi 1998 [28]
46	*Formica rufa-Leptothorax muscorum*	Sweden	?	?	?	Wheeler 1901 [5]
47	*Myrmecina graminicola-Ponera coarctata*	Hungary	Pine forest	Under stone	2	Lőrinczi (unpubl.)
48	*Strumigenys pergandei-Formica* spp., and so forth	USA	?	In the soil	?	Wheeler 1905 [21]

Tetramorium spp. (*T.* cf. *caespitum*), and interestingly other members of the genus *Lasius* (*L. niger* and *L. platythorax*).

Although plesiobiotic partners usually belong to at least different genera, both *F. fusca* and *L. flavus* occurred in plesiobiosis with species of the same genera. These untypical associations were, however, mostly formed between species of different subgenera with different behavioural features. There was only one exception to this rule in which two species from the subgenus *Chthonolasius*, namely, *Lasius umbratus* and *Lasius sabularum* occurred in each other's close proximity, although the exact nature of this association is unknown [40]. Among the untypical plesiobiotic associations, the ones between *F. fusca* and wood ants (*Formica lugubris*, *F. aquilonia*, and *F. truncorum*) were the most peculiar cases considering the well known temporary social parasitic character of wood ants, whose young queens often use *F. fusca* as host for colony foundation [6]. Nevertheless, in one case *F. fusca* was observed to move into an uninhabited part of the nest mound of a *F. aquilonia* colony, which was possibly queenless, though this *F. fusca* colony still remained there after the reviving of the wood ants [29].

4.2. Background Factors and Driving Forces of Plesiobiosis

4.2.1. Role of Habitat Type and Food Supply. Plesiobiotic nests have been recorded from various habitat types, representing different stages of both primary succession and secondary succession. It is important to note, however, that a number of records on plesiobiosis were mere observations without any significant ecological information, for example, on habitat type, nesting site, and/or the number of observed cases of plesiobiotic pairs.

Many of the recorded plesiobiotic species pairs have been described in rocky habitats in Finland. The spectrum of study sites ranged from earlier stages of primary succession, such as open rocky outcrops and shore meadows, to mature pine forests, which represented the last successional stage of rocky habitats. According to this study, most of the plesiobiotic associations involving *Lasius* s. str. were observed in earlier stages of primary succession. This observation confirmed the hypothesis by Czechowski [31], stating that plesiobiosis is especially frequent in habitats lacking suitable nesting sites, and the scarcity of these is one of the main factors promoting the formation of plesiobiotic associations between ant colonies [34].

Another investigation was conducted in a sand dune complex in Finland, where only one plesiobiotic association was observed, which was between *F. fusca* and *M. rubra* [32]. The reason for this may be that each successional stage of the sand dunes represents more homogenous habitats and larger areas optimal for nesting than rocky habitats [32].

Species that prefer to inhabit stumps can be suitable objects for studying the effect of the amount of potential nesting sites on the frequency of plesiobiotic associations. Włodarczyk et al. [36], for instance, studied clearcuts in a managed forest in western Poland, where stumps that were left on clearcuts served as suitable nesting sites for several species. Although clearcuts represented the initial stage of secondary succession, the amount of potential nesting sites for ants preferring stumps was relatively high, and almost half of the available stumps were occupied by colonies of 9 different ant species [36]. Of the 512 stumps that were checked, five were inhabited by more than one ant species, representing plesiobiotic associations, with *F. fusca* as one of the partners in all cases (*F. fusca, Tetramorium caespitum* in three cases; *F. fusca, Myrmica ruginodis* in one case and *F. fusca, L. platythorax* in one case) [36]. Although clearcuts offered a high number of stumps suitable for nesting, the sparse vegetation cover provided poor trophic conditions for aphid-related ant species compared with forest patches [36], resulting in the presence of fewer species competing for the available nesting sites.

Investigations on plesiobiosis between *F. fusca* and *C. vagus* were conducted in patches of pine and poplar forests in central Hungary (Kanizsai, unpubl.). It was shown that both the density of nests and the number of plesiobiotic associations were influenced by the age of forest patches, and there were more plesiobiotic relationships in older patches than in younger ones. A possible explanation can be that the higher nest density of either species may have facilitated the formation of plesiobiotic associations in older patches.

4.2.2. Role of Nest Density and Intraspecific Competition. Two main habitat properties may contribute to the formation of plesiobiotic associations: the scarcity of suitable nesting sites as a forcing factor [34] and the sufficient amount of food sources available, which significantly influence the abundance and reproductivity of ant colonies [41]. When colony density is high, the depletion of food resources by neighbouring colonies may be more intensive, resulting in an increased mortality, especially in the case of incipient colonies [42]. According to former studies ([43] and references therein), the spacing pattern of the nests of *F. fusca* and *L. flavus* (the two most frequent plesiobionts) was, or tended to be regular, when the density of their colonies were high in a suitable habitat. Although competition can produce any type of spacing pattern [44], the regular spatial arrangement of conspecific nests may indicate an intensive intraspecific competition for the same resources [42, 45–49]. Owing to similar food requirements, intraspecific competition supposed to be stronger than interspecific competition [43, 48–50]. The regular dispersion of conspecific nests can reduce the overlapping of foraging areas, thereby minimising intraspecific competition [43, 46, 49]. To effectively utilise foraging areas, it can be advantageous in these cases to maximise the distance between conspecific colonies with similar food requirements and foraging ranges [48]. Thus, it is more favourable for colonies if their nearest neighbours are rather heterospecifics with less overlapping requirements, resulting in a kind of "dear enemy" effect. Therefore, strong intraspecific competition can also contribute to the formation of plesiobiotic associations.

4.2.3. Significance of Differences between Plesiobiotic Partners

Potential Role of Competition: Position of the Plesiobionts in the Interspecific Competitive Hierarchy. Recent studies have

revealed that *F. fusca* is one of the most frequent plesiobionts among the studied ants. Similarly to other common plesiobionts, *F. fusca* is also a submissive species in the three-level classification of the competitive hierarchy in ants [51, 52]. The submissive behaviour and the opportunistic character of this species can be considered as one of the main features that contribute to its frequent cooccurrence with other species in plesiobiotic associations. Although most of the plesiobiotic partners of *F. fusca* occupied a higher level in the interspecific competition hierarchy, it established plesiobiotic relationships with species that are also submissive (e.g., with *M. rubra*, *L. flavus*, and *Leptothorax acervorum*).

Being also submissive, *Myrmica* spp. are also able to coexist with aggressive ant species. For example, *M. ruginodis* and *M. scabrinodis* were observed to shift their foraging to periods with lower temperature. Accordingly, in areas where territorial competitors were also present, they visited baits at night instead [53].

In the case of the subterranean, cryptic species *L. flavus*, competitive ability may play a less significant role regarding the coexistence with other species. While the two above-mentioned plesiobionts are surface foragers, that is, they mostly search for food on or above the ground, the colonies of *L. flavus*, however, were found to be associated with various species of root aphids [54]. Thus, for subterranean *Cautolasius* species, the importance of vertical separation in foraging seems more significant than other mechanisms for reducing competition.

Contrary to the afore-mentioned species, several *Camponotus* species are typically regarded as encounter species that is, they defend not only their nests but the discovered resources as well [51, 52]; therefore only submissive species can be expected to be their plesiobiotic partners.

Conflict Avoidance: Differences in the Foraging Strategy of Plesiobiotic Partners and Resource Partitioning. As plesiobiotic partner colonies share the same microhabitat [1], they have overlapping foraging area and home ranges owing to the small distances between their associated nests. Accordingly, the probability of an encounter between the members of the two colonies increases as the distance between their nests decreases [55]. Due to the close neighbourhood of the associated colonies, they are expected to interact most intensely with each other. A common outcome of interspecific competition is the minimising of spatial and/or temporal overlapping during foraging, that is, differing from each other in their daily and/or seasonal activity, foraging area, or diet [56–59]. Beside partitioning spatially and/or temporally, different foraging strategies (e.g., individual searching, tandem running and other types of recruitment systems) may also contribute to the coexistence of different species [39, 60, 61]. Although body size can also influence the foraging range, the existence of food recruitment systems makes ants less constrained by their morphology than what can be seen in the case of other animals [60, 62, 63]; thereby, the effects of behavioural features seem more important than those of morphological ones. On the other hand, differences in body size can promote resource partitioning by reducing the overlap in resource use [64]. Although differences in body size cannot explain food-resource partitioning alone, these can still contribute to the formation of a number of plesiobiotic relationships.

5. Conclusions

On the basis of the above considerations, we define plesiobiosis as the occasional or regular nesting of heterospecific colonies of certain species in close proximity to each other without biological interdependence.

Based on the currently available data, members of the subfamily Formicinae establish plesiobiotic relationships the most frequently, and the most common plesiobionts among them seems to be *F. fusca*. The opportunistic and submissive behaviour of this species makes it a typical plesiobiont, and it is also a frequent host of both temporary social parasites and slave makers [6, 65].

As a rule, plesiobiosis can be formed between ant species that differ from each other in behaviour—primarily in their competitive ability—and in foraging strategies. Other subordinate species with different behaviour or species with higher competitive ability can also be potential partners as plesiobionts.

Beside the lack of suitable nesting sites, the appropriate amount of available food sources may also play a role in the formation of plesiobiosis, contributing to higher colony densities. The overlap in diet can enhance intraspecific competition, which may force colonies into the vicinity of heterospecific nests. Owing to higher colony density, nesting in each other's close neighbourhood will also occur more frequently. Plesiobiotic associations formed this way may promote a persistent coexistence in cases where the differences are considerable between the partners, which can lead to the formation of other types of interspecific associations with higher levels of biotic interactions.

It is important to note, that the currently available data concerning plesiobiosis are far from being representative. Only a couple of studies have dealt with this topic, and these are restricted to a small number of habitat types of few countries in the northern latitudes. Moreover, most of these studies reported only observations of plesiobiotic cases without additional ecological information, like the regularity of such associations between the species in question. Therefore, to get a more comprehensive picture about plesiobiosis, it would be essential to collect more and detailed data globally.

6. Open Questions

Regarding our present knowledge on plesiobiosis in ants, there are still many open questions that need to be answered, which are important for a better understanding of this kind of interspecific relationship.

(1) *Persistence of plesiobiosis.* Plesiobiosis can be formed occasionally between heterospecific colonies, but we still do not know how persistent these associations are. Although ant colonies have typically been treated as spatially fixed entities, inhabiting a given nesting site permanently, it seems that periodic nest relocation is an important aspect of the behaviour of

many ant species [66–68]. It is also uncertain what effects may trigger the disaggregation of plesiobiotic colonies and force the relocation of one of the associated plesiobionts.

(2) *The role of nesting shelters* and *"ecosystem engineering."* It also provides a basis for further investigation, to what extent the type of nesting shelters (e.g., logs, stumps, and rocks) promotes the formation of plesiobiotic associations and how the already established colonies facilitate the settlement of colonies due to their nest constructions. In temperate regions, a large number of species occupy dead logs and stumps or nest in the soil under rocks [6]. Due to their thermal properties, colonies occupying these shelters are allowed to enter to colony growth stage earlier and they are less vulnerable to unsuitable humidity and temperature values. These beneficial conditions can lead to the joint nesting of two or more species in or under the same shelter, especially if the number of suitable nesting sites is low. For example, the nest mounds of wood ants may provide suitable nesting sites for other species owing to their unique microhabitat conditions [69]. This may serve as an explanation for the untypical plesiobiotic associations observed between *F. fusca* and the members of *Formica* s. str., where the former species frequently settles into the uninhabited parts of the nest mounds of wood ants [29]. Similarly, many *Camponotus* species create their nest galleries in trunks and stumps [70–72], which may promote the establishment of colonies of other species in these microhabitats. Owing to this "ecosystem engineering," plesiobiotic associations may develop from an occasional to a regular relationship even without direct interactions between the associated colonies.

(3) *The "close" proximity of heterospecific colonies.* Former definitions of plesiobiosis emphasise the importance of the close proximity of plesiobiotic colonies, though it is not clear how close this proximity should be or whether these colonies should use the same nesting shelter. In Table 1 we listed only those cases where the plesiobiotic colonies occupied the same nest (i.e., they were under the same stone or in the same log). It is a question, however, whether the frequent neighbouring arrangement of the nests of certain species pairs (when their nests do not necessarily border on one another) can be considered as a plesiobiotic relationship.

(4) *Plesiobiotic associations of arboreal species.* Most of the recorded cases of plesiobiotic associations are between species that inhabit nests located on or under the ground surface. Arboreal species, however, are also known to frequently create their nests in the vicinity of each other on the same tree, as it was, for instance, observed in the case of *Camponotus fallax*, *Lasius brunneus*, and *Temnothorax affinis* [73]. Actually, it was demonstrated that the former two species can occur in a plesiobiotic relationship [33]. It is an interesting question how frequently arboreal species nest in one another's neighbourhood, and to what extent these cases can be considered as plesiobiosis.

Acknowledgments

The authors thank Jean-Paul Lachaud, Alan Lenoir, and David P. Hughes for giving them the opportunity to review the topic in this paper. They are also grateful to Wojciech Czechowski, who kindly provided them with his papers in this topic, and to Csaba Tölgyesi for his suggestions on linguistic issues.

References

[1] A. Lenoir, P. D'Ettorre, C. Errard, and A. Hefetz, "Chemical ecology and social parasitism in ants," *Annual Review of Entomology*, vol. 46, pp. 573–599, 2001.

[2] E. Kaufmann, A. K. F. Malsch, M. Erle, and U. Maschwitz, "Compound nesting of *Strumigenys* sp. (Myrmicinae) and *Diacamma* sp. (Ponerinae), and other nesting symbioses of myrmicine and ponerine ants in Southeast Asia," *Insectes Sociaux*, vol. 50, no. 1, pp. 88–97, 2003.

[3] M. H. Huang and A. Dornhaus, "A meta-analysis of ant social parasitism: host characteristics of different parasitism types and a test of Emery's rule," *Ecological Entomology*, vol. 33, no. 5, pp. 589–596, 2008.

[4] E. Wasmann, *Die zusammengesetzen Nester und gemischten Kolonien der Ameisen*, Aschendorffschen Buchdruckerei, Münster, Germany, 1891.

[5] W. M. Wheeler, "The compound and mixed nests of American ants," *The American Naturalist*, vol. 35, part 2-3, pp. 513–539, 791–818, 1901.

[6] B. Hölldobler and E. O. Wilson, *The Ants*, Belknap Press, Cambridge, UK; Harvard University Press, Cambridge, Mass, USA, 1990.

[7] A. Tinaut, F. Ruano, and M. D. Martínez, "Biology, distribution and taxonomic status of the parasitic ants of the Iberian Peninsula (Hymenoptera: Formicidae, Myrmicinae)," *Sociobiology*, vol. 46, no. 3, pp. 449–489, 2005.

[8] W. Czechowski, "The route of *Formica polyctena* Först. as a factor promoting emancipation of *Formica fusca* L. slaves from colonies of *Polyergus rufescens* (Latr.) (Hymenoptera: Formicidae)," *Polish Journal of Ecology*, vol. 54, no. 1, pp. 159–162, 2006.

[9] A. Buschinger, "Social parasitism among ants: a review (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 12, pp. 219–235, 2009.

[10] F. J. Richard, A. Dejean, and J. P. Lachaud, "Sugary food robbing in ants: a case of temporal cleptobiosis," *Comptes Rendus—Biologies*, vol. 327, no. 5, pp. 509–517, 2004.

[11] F. Menzel and N. Blüthgen, "Parabiotic associations between tropical ants: equal partnership or parasitic exploitation?" *Journal of Animal Ecology*, vol. 79, no. 1, pp. 71–81, 2010.

[12] F. Menzel, *Mechanisms and adaptive significance of interspecific associations between tropical ant species [Ph.D. thesis]*, Julius-Maximilans-Universität Würzburg, Würzburg, Germany, 2009.

[13] A. Buschinger, A. Francoeur, and K. Fischer, "Functional monogyny, sexual behaviour, and karyotype of the guest ant, *Leptothorax provancheri* Emery (Hymenoptera, Formicidae)," *Psyche*, vol. 87, no. 1-2, pp. 1–12, 1980.

[14] J. Orivel, C. Errard, and A. Dejean, "Ant gardens: interspecific recognition in parabiotic ant species," *Behavioral Ecology and Sociobiology*, vol. 40, no. 2, pp. 87–93, 1997.

[15] C. Errard, D. Fresneau, J. Heinze, A. Francoeur, and A. Lenoir, "Social organization in the guest-ant *Formicoxenus provancheri*," *Ethology*, vol. 103, no. 2, pp. 149–159, 1997.

[16] U. Maschwitz, C. Go, W. H. O. Dorow, A. Buschinger, and R. J. Kohout, "*Polyrhachis loweryi* (Formicinae): a guest ant

parasitizing *Rhytidoponera* sp. (Ponerinae) in Queensland, Australia," *Insectes Sociaux*, vol. 50, no. 1, pp. 69–76, 2003.

[17] A. Vantaux, A. Dejean, A. Dor, and J. Orivel, "Parasitism versus mutualism in the ant-garden parabiosis between *Camponotus femoratus* and *Crematogaster levior*," *Insectes Sociaux*, vol. 54, no. 1, pp. 95–99, 2007.

[18] F. Menzel, K. E. Linsenmair, and N. Blüthgen, "Selective interspecific tolerance in tropical *Crematogaster-Camponotus* associations," *Animal Behaviour*, vol. 75, no. 3, pp. 837–846, 2008.

[19] F. Menzel, T. Schmitt, and N. Blüthgen, "Intraspecific nestmate recognition in two parabiotic ant species: acquired recognition cues and low inter-colony discrimination," *Insectes Sociaux*, vol. 56, no. 3, pp. 251–260, 2009.

[20] F. Menzel, M. Staab, A. C. Chung, G. Gebauer, and N. Blüthgen, "Trophic ecology of parabiotic ants: do the partners have similar food niches?" *Austral Ecology*, vol. 37, pp. 537–546, 2011.

[21] W. M. Wheeler, "An annotated list of the ants of New Jersey," *Bulletin American Museum of Natural History*, vol. 21, pp. 371–403, 1905.

[22] W. M. Wheeler, *Ants: Their Structure, Development and Behavior*, Columbia University Press, New York, NY, USA, 1910.

[23] F. M. Gaige, "Results of the mershon expedition to the Charity Islands, Lake Huron," *Occasional Papers of the Museum of Zoology*, vol. 5, pp. 1–29, 1914.

[24] W. M. Wheeler, "The mountain ants of Western North America," *Proceedings of the American Academy of Arts and Sciences*, vol. 52, pp. 457–569, 1917.

[25] D. W. Morley, "Observations on some plesiobiotic colonies of ants (Hymenoptera), with notes on some other mixtobiotic colonies," *Proceedings of the Royal Entomological Society of London*, vol. 20, pp. 1–4, 1945.

[26] W. M. S. Creighton and R. R. Snelling, "The rediscovery of *Camponotus* (*Myrmaphaenus*) *yogi* Wheeler (Hymenoptera: Formicidae)," *Psyche*, vol. 73, no. 3, pp. 187–195, 1966.

[27] W. Czechowski and W. Rotkiewicz, "Common activities of female sexuals of *Lasius Umbratus* (nyl.) and *Formica sanguinea* Latr. or *Polyergus rufescens* (Latr.) on nests of the dulotic species (hymenoptera, formicidae)," *Annales Zoologici*, vol. 47, no. 3-4, pp. 465–467, 1997.

[28] W. Czechowski and K. Yamauchi, "Plesiobiosis of *Formica japonica* Motsch. and *Tetramorium* sp. (Hymenoptera: Formicidae)," *Annals of the Upper Silesian Museum (Entomology)*, vol. 8-9, pp. 99–101, 1998.

[29] W. Czechowski and K. Vepsäläinen, "Plesiobiosis between *Formica fusca* L. and *Formica aquilonia* Yarr. (Hymenoptera, Formicidae)," *Annales Zoologici*, vol. 49, no. 1-2, pp. 125–127, 1999.

[30] W. Czechowski and W. Czechowska, "*Formica cinerea fuscocinerea* For. in the Pieniny Mts, its untypical habitat and plesiobiosis with *Lasius flavus* (F.) (Hymenoptera, Formicidae)," *Fragmenta Faunistica*, vol. 43, pp. 131–133, 2000.

[31] W. Czechowski, "Plesiobiosis between *Formica fusca* L. and wood ants (Hymenoptera, Formicidae) as a sign of nest competition," *Przegląd Zoologiczny*, vol. 46, pp. 81–86, 2002.

[32] W. Czechowski, "Plesiobiosis between *Formica fusca* L. and *Myrmica rubra* (L.) (Hymenoptera, Formicidae)," *Przegląd Zoologiczny*, vol. 47, pp. 115–118, 2003.

[33] W. Czechowski, "Plesiobiosis between dendrophilous ants: *Camponotus fallax* (F.) and *Lasius brunneus* (Latr.) (Hymenoptera, Formicidae)," *Przegląd Zoologiczny*, vol. 48, pp. 201–205, 2004.

[34] W. Czechowski, "Scarcity of sites suitable for nesting promotes plesiobiosis in ants (Hymenoptera: Formicidae)," *Entomologica Fennica*, vol. 15, no. 4, pp. 211–218, 2004.

[35] W. Czechowski, "Nest competition between *Camponotus vagus* (Scopoli, 1763) and *Camponotus herculeanus* (Linnaeus, 1758) (Hymenoptera: Formicidae) in Białowieża Forest (Poland)," *Myrmecologische Nachrichten*, vol. 7, pp. 43–45, 2005.

[36] T. Włodarczyk, M. Zmihorski, and A. Olczyk, "Ants inhabiting stumps on clearcuts in managed forest in Western Poland," *Entomologica Fennica*, vol. 20, no. 2, pp. 121–128, 2009.

[37] E. O. Wilson, *The Insect Societies*, The Belknap Press of Harvard University, Cambridge, UK, 1971.

[38] R. H. MacArthur and R. Levins, "The limiting similarity, convergence and divergence of coexisting species," *The American Naturalist*, vol. 101, pp. 377–385, 1967.

[39] O. Kanizsai, "The possible role of the different foraging behaviour of *Formica fusca* and *Camponotus vagus* (Hymenoptera: Formicidae) in promoting their plesiobiotic associations," *Acta Scientiarum Transylvanica*, vol. 20, no. 2, pp. 5–20, 2012.

[40] M. L. Borowiec, "First records of *Lasius sabularum* (BONDROIT, 1918) in Poland (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 14, pp. 137–140, 2011.

[41] R. J. Deslippe and R. Savolainen, "Role of food supply in structuring a population of Formica ants," *Journal of Animal Ecology*, vol. 63, no. 4, pp. 756–764, 1994.

[42] R. T. Ryti and T. J. Case, "Overdispersion of ant colonies: a test of hypotheses," *Oecologia*, vol. 69, no. 3, pp. 446–453, 1986.

[43] S. C. Levings and J. F. A. Traniello, "Territoriality, nest dispersion, and community structure in ants," *Psyche*, vol. 88, no. 3-4, pp. 265–319, 1981.

[44] E. S. Adams and W. R. Tschinkel, "Spatial dynamics of colony interactions in young populations of the fire ant *Solenopsis invicta*," *Oecologia*, vol. 102, no. 2, pp. 156–163, 1995.

[45] R. A. Bernstein, "Foraging strategies of ants in response to variable food density," *Ecology*, vol. 56, pp. 213–219, 1975.

[46] R. A. Bernstein and M. Gobbel, "Partitioning of space in communities of ants," *Journal of Animal Ecology*, vol. 48, pp. 931–942, 1979.

[47] S. C. Levings and N. R. Franks, "Patterns of nest dispersion in a tropical ground ant community," *Ecology*, vol. 63, no. 2, pp. 238–344, 1982.

[48] R. T. Ryti and T. J. Case, "Spatial arrangement and diet overlap between colonies of desert ants," *Oecologia*, vol. 62, no. 3, pp. 401–404, 1984.

[49] J. H. Cushman, G. D. Martinsen, and A. I. Mazeroll, "Density- and size-dependent spacing of ant nests: evidence for intraspecific competition," *Oecologia*, vol. 77, no. 4, pp. 522–525, 1988.

[50] L. Gallé, "Study of ant populations in various grassland ecosystems," *Acta Biologica Szegediensis*, vol. 18, pp. 159–164, 1972.

[51] K. Vepsäläinen and B. Pisarski, "Assembly of island ant communities," *Annales Zoologici Fennici*, vol. 19, no. 4, pp. 327–335, 1982.

[52] O. Alinvi, J. Bohlin, and J. P. Ball, "Interspecific competition among ants in the boreal forest: testing predictions from a linear hierarchical competition model," *Insectes Sociaux*, vol. 55, no. 1, pp. 1–11, 2008.

[53] K. Vepsäläinen and R. Savolainen, "The effect of interference by formicine ants on the foraging of Myrmica," *Journal of Animal Ecology*, vol. 59, no. 2, pp. 643–654, 1990.

[54] N. Waloff and R. E. Blackith, "The growth and distribution of the mounds of *Lasius flavus* (Fabricius) (Hym: Formicidae) in Silwood Park, Bekshire," *Journal of Animal Ecology*, vol. 31, pp. 421–437, 1962.

[55] D. M. Gordon and A. W. Kulig, "Founding, foraging, and fighting: colony size and the spatial distribution of harvester ant nests," *Ecology*, vol. 77, no. 8, pp. 2393–2409, 1996.

[56] J. H. Fellers, "Daily and seasonal activity in woodland ants," *Oecologia*, vol. 78, no. 1, pp. 69–76, 1989.

[57] X. Cerdá, J. Retana, and A. Manzaneda, "The role of competition by dominants and temperature in the foraging of subordinate species in Mediterranean ant communities," *Oecologia*, vol. 117, no. 3, pp. 404–412, 1998.

[58] X. Cerdâ, J. Rctana, and S. Cros, "Prey size reverses the outcome of interference interactions of scavenger ants," *Oikos*, vol. 82, no. 1, pp. 99–110, 1998.

[59] M. Albrecht and N. J. Gotelli, "Spatial and temporal niche partitioning in grassland ants," *Oecologia*, vol. 126, no. 1, pp. 134–141, 2001.

[60] J. F. A. Traniello, "Foraging strategies of ants," *Annual Review of Entomology*, vol. 34, pp. 191–210, 1989.

[61] I. Perfecto, "Foraging behavior as a determinant of asymmetric competitive interaction between two ant species in a tropical agroecosystem," *Oecologia*, vol. 98, no. 2, pp. 184–192, 1994.

[62] C. R. Carroll and D. H. Janzen, "Ecology of foraging by ants," *Annual Review of Ecology and Systematics*, vol. 4, pp. 231–257, 1973.

[63] J. F. A. Traniello, "Comparative foraging ecology of north temperate ants: the role of worker size and cooperative foraging in prey selection," *Insectes Sociaux*, vol. 34, no. 2, pp. 118–130, 1987.

[64] A. E. Chew and R. M. Chew, "Body size as a determinant of small-scale distributions of ants in evergreen woodland south-eastern Arizona," *Insectes Sociaux*, vol. 27, no. 3, pp. 189–202, 1980.

[65] W. Czechowski and B. Markó, "Uncomfortable protection: *Formica polyctena* Först. shelters *Formica fusca* L. from *Formica sanguinea* Latr. (Hymenoptera: Formicidae)," *Annales Zoologici*, vol. 56, no. 3, pp. 539–548, 2006.

[66] J. Smallwood, "Nest relocations in ants," *Insectes Sociaux*, vol. 29, no. 2, pp. 138–147, 1982.

[67] D. M. Gordon, "Nest relocation in harvester ants," *Annals of the Entomological Society of America*, vol. 85, pp. 44–47, 1992.

[68] T. P. McGlynn, R. A. Carr, J. H. Carson, and J. Buma, "Frequent nest relocation in the ant *Aphaenogaster araneoides*: resources, competition, and natural enemies," *Oikos*, vol. 106, no. 3, pp. 611–621, 2004.

[69] J. Frouz and V. Jilková, "The effect of ants on soil properties and processes (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 11, pp. 191–199, 2008.

[70] C. J. Sanders, "The distribution of carpenter ant colonies in the spruce-fir forests of Northwestern Ontario," *Ecology*, vol. 51, no. 5, pp. 865–873, 1970.

[71] Y. Chen, L. D. Hansen, and J. J. Brown, "Nesting sites of the carpenter ant, *Camponotus vicinus* (Mayr) (Hymenoptera: Formicidae) in Northern Idaho," *Environmental Entomology*, vol. 31, no. 6, pp. 1037–1042, 2002.

[72] W. Czechowski, A. Radchenko, W. Czechowska, and K. Vepsäläinen, *The Ants of Poland with Reference to the Myrmecofauna of Europe*, Museum and Institute of Zoology, Polish Academy of Sciences, Warszawa, Poland, 2012.

[73] L. Gallé, É. Kovács, and K. Margóczi, "Ants on trees: an example of metacommunities in extremely small patches," in *Proceedings of the 7th European Ecological Congress*, p. 85, Budapest, Hungary, 1995.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 573541, 11 pages
http://dx.doi.org/10.1155/2013/573541

Research Article

Discrimination of the Social Parasite *Ectatomma parasiticum* by Its Host Sibling Species (*E. tuberculatum*)

Renée Fénéron,[1] Chantal Poteaux,[1] Marie Boilève,[1] Jorge Valenzuela,[2] and Fabrice Savarit[1]

[1] *Laboratoire d'Ethologie Expérimentale et Comparée, EA 4443, Université Paris 13, Sorbonne Paris Cité, 99 avenue J.-B. Clément, 93430 Villetaneuse, France*
[2] *Departamento de Entomologia, Instituto de Ecologia, Antigua Carretera a Coatepec Km 2.5, A. 63, 91000 Xalapa, Ver, Mexico*

Correspondence should be addressed to Renée Fénéron; feneron@leec.univ-paris13.fr

Received 6 March 2013; Revised 30 April 2013; Accepted 15 May 2013

Academic Editor: Jean-Paul Lachaud

Among social parasites, workerless inquilines entirely depend on their host for survival and reproduction. They are usually close phylogenetic relatives of their host, which raises important questions about their evolutionary history and mechanisms of speciation at play. Here we present new findings on *Ectatomma parasiticum*, the only inquiline ant described in the Ectatomminae subfamily. Field data confirmed its rarity and local distribution in a facultative polygynous population of *E. tuberculatum* in Mexico. Genetic analyses demonstrated that the parasite is a sibling species of its host, from which it may have diverged recently. Polygyny is suggested to have favored the evolution of social parasite by sympatric speciation. Nevertheless, host workers from this population were able to discriminate parasites from their conspecifics. They treated the parasitic queens either as individuals of interest or as intruders, depending on their colonial origin, probably because of the peculiar chemical profile of the parasites and/or their reproductive status. We suggest that *E. parasiticum* could have conserved from its host sibling species the queen-specific substances that produce attracting and settling effect on workers, which, in return, would increase the probability to be detected. This hypothesis could explain the imperfect social integration of the parasite into host colonies.

1. Introduction

Parasitism is found at all levels of biological organization from genes to societies. Social parasites are specialized in exploiting the social living conditions of one or several species [1]. They have evolved manifold in social Hymenoptera, especially in ants where they occur with a huge diversity [1–3]. Parasitic ants can take advantage of the host-colony resources only during the phase of colony founding (temporary social parasitism) or throughout their life cycle, either by raiding host brood and then enslaving workers (slave-making) or by cohabiting in the nest alongside the host queens (inquilinism) [1–3]. In the most derived form, inquilines have developed a set of adaptations such as the loss of the worker caste and a reduced body size (the "inquiline syndrome" [4]).

Typically, social parasites and their respective hosts are close phylogenetic relatives. This trend has been formalized as Emery's rule and generalized in two versions [5, 6]. In the strict version, the parasite is a sibling species of its host; in the loose version, the parasite and the host are nonsiblings but belong to the same or a closely related genus. Some empirical studies support the strict version of Emery's rule hypothesis (see, e.g., [7, 8]). This has major evolutionary implications since it may argue for sympatric speciation. Indeed, although still in debate, it has been repeatedly suggested that inquilines may have diverged from their sister host species (or from a common ancestor) through intraspecific parasitism [1, 6, 9, 10]. Reproductive isolation in sympatry has been probably facilitated by the social biology and ecology of the host ant species. In particular, polygyny and later miniaturization of polygynous queens are considered as prerequisites for this scenario, as it is assumed for some *Myrmica* [7, 11, 12] and *Acromyrmex* [8]. It could also be the case for *Ectatomma tuberculatum* [13], but not for all cases of reduced-size queens (see e.g., [14, 15]). Beyond the species model, understanding the evolutionary processes and ecological constraints that could lead to speciation and promote the emergence of social parasitism is thus of a high relevance for evolutionary biologists.

Association between species requires well-matched communication systems. Cuticular hydrocarbons, a blend of surface chemicals, are involved in multiple levels of recognition in ants [16, 17]. They are shared between all colony members thus acting as nestmate recognition cues, and they also provide information on certain individuals inside the colony thus potentially signaling age, caste, or fertility [18, 19]. Inquilines that invade established host colonies to be adopted therein have to overcome the colony-specific barriers [1]. To this end, they can mimic the chemical profiles of their hosts. We refer to "chemical mimicry" following Von Beeren et al. [20] (see also [21]) when social parasites either express no identification cues, produce, or acquire host-specific chemical cues from the host individuals and nest materials [3, 22, 23]. In addition, specific chemicals such as appeasing or propaganda signals can be released by the parasites during host-colony invasion [23]. More generally, chemical strategies can also be combined with behavioral adaptations, for example, to promote colony odor transfer [24, 25].

Workerless inquilines are scarce in ants, and most of them are confined to the Formicinae and Myrmicinae subfamilies. *Ectatomma parasiticum* is the only parasitic species described in the Ectatomminae subfamily [26] and among the rare inquilines from the tropics. It was found to be associated with its host ant, *E. tuberculatum*, in one Mexican population, and to possess several parasitic life-history traits, such as the miniaturization of the queen [13, 27]. However, previous observations have shown that some parasitic queens were attacked by the host workers into their own colony, suggesting a probable failure in their social integration [25]. This could be due to an imperfect chemical mimicry as a result of coevolutionary processes [28].

To get a broader knowledge of the relationship between the parasite and its host, we present here up-to-date field, genetic, and behavioral data in these ants. First, we characterized the population of *E. parasiticum* by compiling data from all our field collection trips in the site of Apazapan. Second, we performed new genetic analyses including data from other Mexican populations (from Chiapas) but presenting neither polygyny nor social parasitism in order to refine phylogenetic relationships of *E. parasiticum* and *E. tuberculatum*. Finally, we conducted discrimination tests to determine the extent to which the host species is able to recognize its social parasite. If chemical mimicry is effective, the parasites should be either undetected by any host, or treated as nestmates by hosts of their own colony and as intruders by hosts of all other colonies. In case of an imperfect chemical mimicry, as suggested in *E. parasiticum* [28], we expected to find some differences from these patterns of responses.

2. Material and Methods

2.1. Studied Sites and Colonies. A total of 98 colonies of *E. tuberculatum* were collected in the population of Apazapan, Veracruz State, Mexico (19°19′38″ N; 96°43′21″ W, 300 m above sea level) during six field trips between September 1999 and November 2011. They were sampled from three sites (referred as Apz1, Apz2, and Apz3) about 500 m apart and covering a surface area of about 10 hectares each. These sites are remnants of tropical dry forest [29] and are characterized by a warm and subhumid climate, with heavy rains in early and late summer, sparse rains in winter, and a dry period in the middle of summer [30]. In addition, four colonies were collected in 2007 around Tapachula, Chiapas State, Mexico (14°54′00″ N; 92°15′60″ W), and were used for genetic and behavioral analyses.

After nest collection, colonies were carried to the laboratory to both check for the presence of the social parasite and count the number of *E. tuberculatum* queens and workers. Queenless colonies having less than 40 workers were excluded from the analysis, as considered to be not entirely collected. Ninety colonies were transported to the LEEC in Paris where they were reared in an experimental room (T = 28 ± 2°C, 60%–80% of relative hygrometry, light-dark cycle = 12 h : 12 h). They were housed in plaster nests each connected to a foraging area where food and water were provided. They were fed on the same diet composed of honey-apple mixture, mealworms, and crickets. Groups of ants were sampled in the field and from the rearing colonies, and they were preserved in 95°C alcohol for further genetic analysis.

2.2. Genetic Analysis. Previous sequences of a fragment of the cytochrome b region (cyt b) of the mitochondrial genome were published in Hora et al. [13]: twenty-seven individuals (9 parasites, 5 queens, and 13 workers of *E. tuberculatum*) from seven parasitized colonies of Apazapan were sequenced for a 750-base pair of cyt b (using the set of primers CB1 and tRS designed from *Apis*, according to standard conditions of amplification, [31]). We compared them with the sequences of individuals from two other Mexican nonparasitized populations (5 individuals from Tapachula (GenBank AF452379) and five from Tuxtla (AF452380)) together with 5 individuals from a Brazilian population (Bahia, AF452381). Purified PCR fragments were sequenced using an ABI 370 automated sequencer and a dye terminator cycle sequencing kit. All sequences were unambiguously aligned using the algorithm CLUSTAL W [32], and checked by eye, on the sequence of *Rhytidoponera victoriae* present in GenBank (U75350). Distances between sequences were calculated according to Jukes and Cantor [33]. A neighbor-joining (NJ) tree based on these distances was constructed using MEGA 5.1 [34], and nodes support was assessed by conducting 1000 bootstrap replicates.

2.3. Behavioral Experiments

2.3.1. Description of the Discrimination Test. These experiments investigated whether *E. tuberculatum* workers distinguish the social parasites from their conspecifics, from either their own colony or another one. For this we performed discrimination tests where a single host worker faced two stimuli-ants in a neutral arena (Figure 1(a)). The test was modified from Fénéron [35] by using only two (instead of four) categories of stimuli-ants and confronting the workers to stimuli-ants issued from the same parasitized colony. This

(a) (b)

FIGURE 1: The experimental device used for discrimination tests. (a) Overview of the device composed of a round plastic box (11.8 cm diameter) and two fixation systems. The test-worker faced two immobilized stimuli-ants, here a parasite and a conspecific queen. (b) Detailed view of the fixation system on which a queen was immobilized.

allowed us to measure the differential behaviors towards stimuli-ants, while the confounding effects of the stimuli-ants' responses were minimized.

During each test, one parasite and one host were used as stimuli-ants, both from the same colony collected in the Apz1 site. Stimuli-ants were kept alive but immobilized by a thread over the petiole (Figure 1(b)). The test-workers came from different colonial origin as mentioned in the next section. They were sampled from the foraging area by selecting workers that behaved aggressively towards entomological pliers. Foragers are both discriminating and aggressive towards nonnestmate conspecific ants [35], and they are then supposed to be able to reject the parasite. Each test-worker was used only once, but stimuli-ants could be used for several consecutive trials.

After the stimuli-ants have been carefully immobilized, the test-worker was introduced into a glass cylinder in the middle of the arena and was allowed to calm down for about 1 min. The cylinder was then gently removed and the test video-recorded for 5 min (SONY DCR-SR58 camera). After each test the edges of the arena were cleaned with alcohol and the filter paper covering the arena surface was changed to remove any potential chemical marking. The behaviors of the test-workers towards the two stimuli-ants were quantified by scan sampling the video every 5 s (60 scans per individual). Videos were analyzed blindly with respect to the colonial origin of the test-workers.

2.3.2. Conducted Discrimination Tests. Two experiments were conducted. In the first one, the *E. tuberculatum* test-workers faced one parasitic queen and one host worker from the same colony of the Apz1 site. Different tests were defined according to the colonial origin of the test-workers. The tests were (1) homocolonial when the test-workers were the nestmates of the stimuli-ants (Apz1H) and allocolonial in all other cases, (2) nonnestmates from parasitized colonies of the Apz1 site (Apz1P), (3) nonnestmates from nonparasitized colonies of the Apz1 site (Apz1NP), (4) nonnestmates from a different and nonparasitized site (Apz2), and (5) nonnestmates from the nonparasitized population of Tapachula (Tap). A total of 124 tests were performed (22–31 replicates per condition; 8 colonies). Eleven tests were stopped before the 5 min period due to a strong attack against one of the stimuli-ants (i.e., instantaneous and continuous biting over more than 15 s and stinging attempt), and insects were pulled apart. These tests were excluded from the analysis of the behavioral scans.

In the second experiment, we used the same protocol but the test-workers faced one parasitic queen and one host queen from the same colony. In order to prevent *E. tuberculatum* queens from being injured, we carried out only the three types of tests expected to be less aggressive: Apz1H, Apz1P, Apz1NP. A total of 57 tests were performed (12–27 replicates per condition; 4 colonies).

2.3.3. Behaviors and Data Analysis. The behaviors displayed towards the stimuli-ants were recorded and categorized as agonistic acts (i.e., escaping, threatening with wide open mandibles, and biting), antennation (i.e., antennal contact on any part of the ant's body), and immobility close to an ant (i.e., standing motionless less than 2 cm away from a stimulus-ant). The latter usually followed antennation and was interpreted as an attracting and settling effect [36].

For each experiment and each type of test, the proportions of tests including aggression, that is, in which at least one agonistic behavior was directed towards the parasite or the conspecific individual, were calculated. They were compared between the types of test for the parasite and the conspecific individual separately using Pearson's exact Chi-Square tests applied to raw data. The behaviors directed

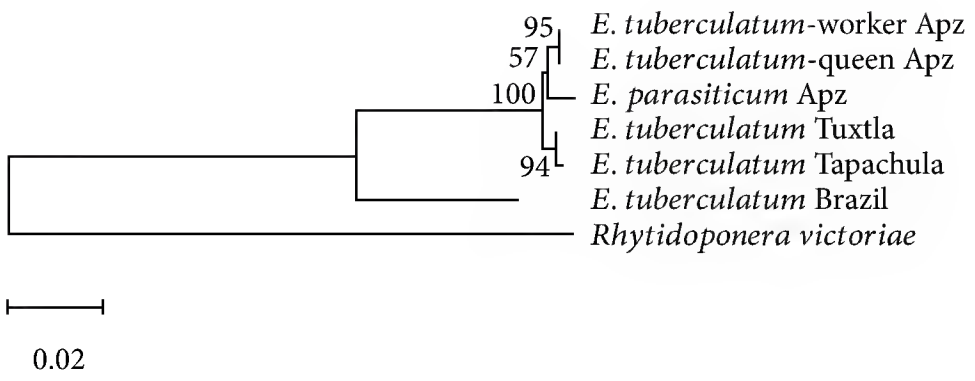


FIGURE 2: Neighbor-joining tree for the different populations. Bootstrap values (1000 replicates) are shown for each node.

toward the parasite and the conspecific individual were quantified as percentages of scans for each test-worker and were compared with Permutation tests for paired samples using the exact method. All statistical analyses were performed using the StatXact-8 software.

3. Results

3.1. Field Study. Details of the different collections in Apazapan are presented in Table 1. Adult parasites were found only during two out of six field trips, and only in the Apz1 site (but sampling effort was scarce in Apz3). In these cases, alate and dealate parasites were abundant since they were present in 15 out of the 24 collected colonies (63%), and they included a median of 3 alate parasites (range: 0–17) and of 1 dealate parasite (0–5) per colony. In addition, some parasites emerged during March-April 2009 in the laboratory from three colonies collected in January 2009, implying that the parasite was still present in this site at this date.

In the Apazapan population, 26 out of the 98 colonies of E. tuberculatum (27%) were polygynous, with a median of two queens (2–8). However, neither the number of host queens (median (and range): 1 (0–3) in the parasitized colonies; 1 (0–8) in nonparasitized colonies, respectively; Permutation tests for independent samples: $P = 0.48$) nor the number of host workers (121 (12–428) in the parasitized colonies; 178 (22–383) in nonparasitized colonies, respectively; $P = 0.43$) was found to differ between parasitized colonies and nonparasitized colonies of the same site (see Supplementary Material available online at http://dx.doi.org/10.1155/2013/573541). This showed that host colony size may not limit successful invasion of the parasite and that the parasite did not select specifically populous colonies, or polygynous colonies. The nest distribution of E. tuberculatum was patchy, with a distance between nests from 0.6 to 15 m, and we often found several colonies parasitized in the same patch.

3.2. Genetic Analysis. Intracolonial variation in Apazapan was constituted by two haplotypes, which discriminate *E. parasiticum* from the group composed of host workers and queens from the same colony (Figure 2). There was no haplotype polymorphism between Apazapan colonies, except between the parasite and its host. The two haplotypes diverged by seven variable sites, all of them being transitions, with a nucleotide sequence difference of 0.95%.

Biogeographic variation between *E. tuberculatum* colonies was quite low, with only 6 polymorphic sites discriminating Apazapan from Tapachula (5 transitions and 1 transversion, 0.81%) whereas the parasite diverged from Tapachula colonies by 9 variable sites (8 transitions and 1 transversion, 1.08%).

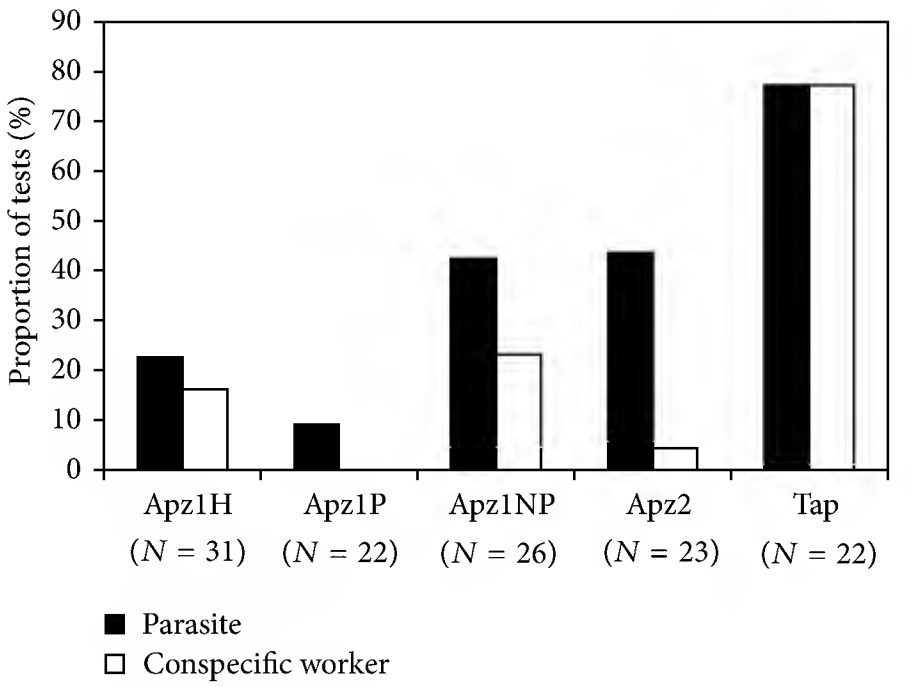


FIGURE 3: Proportions of tests including aggression towards the social parasite or the conspecific worker according to the type of tests. Apz1H = homocolonial tests, Apz1P = tests between nonnestmates from parasitized colonies, Apz1NP = tests between nonnestmates from parasitized and nonparasitized colonies of the same site, Apz2 = tests between sites, Tap = tests between populations, and N = number of tests.

3.3. Behavioral Experiments

3.3.1. Discrimination Tests between a Parasitic Queen and a Conspecific Worker. The proportions of tests including at least one aggression towards the parasite differed among the type of tests (Figure 3; Pearson's exact Chi-Square test, $P < 0.001$). These proportions were higher in nonparasitized colonies than in parasitized colonies within the Apazapan population ($P = 0.026$), and they reached a maximum level between populations ($P < 0.001$). By contrast, the proportion of tests including aggression against the conspecific workers remained low, except between populations ($P < 0.001$).

Agonistic acts were rare and not specifically directed towards the parasite in homocolonial tests (Apz1H) and allocolonial tests between parasitized colonies (Apz1P) (Figure 4(a)). By contrast, the tests using nonparasitized colonies showed aggression against the parasite, but the difference was significant only between sites (Apz2). In the two other conditions, the rate of aggression was probably underestimated due to strong attacks which put an end to some tests and excluded them from the statistical analysis. This could explain the absence of significant difference for Apz1NP as 2 out of 26 tests were stopped due to a strong aggression against the parasite, but not for Tap as 9 out of 22 tests were stopped but equally distributed across both species (i.e., 4 against the parasite and 5 against the conspecific).

Antennation was much more frequent towards the parasite than the conspecific worker whatever the tests within the Apazapan population, showing a clear discrimination (Figure 4(b)). This was not the case for the tests between populations where the rate of antennation remained low.

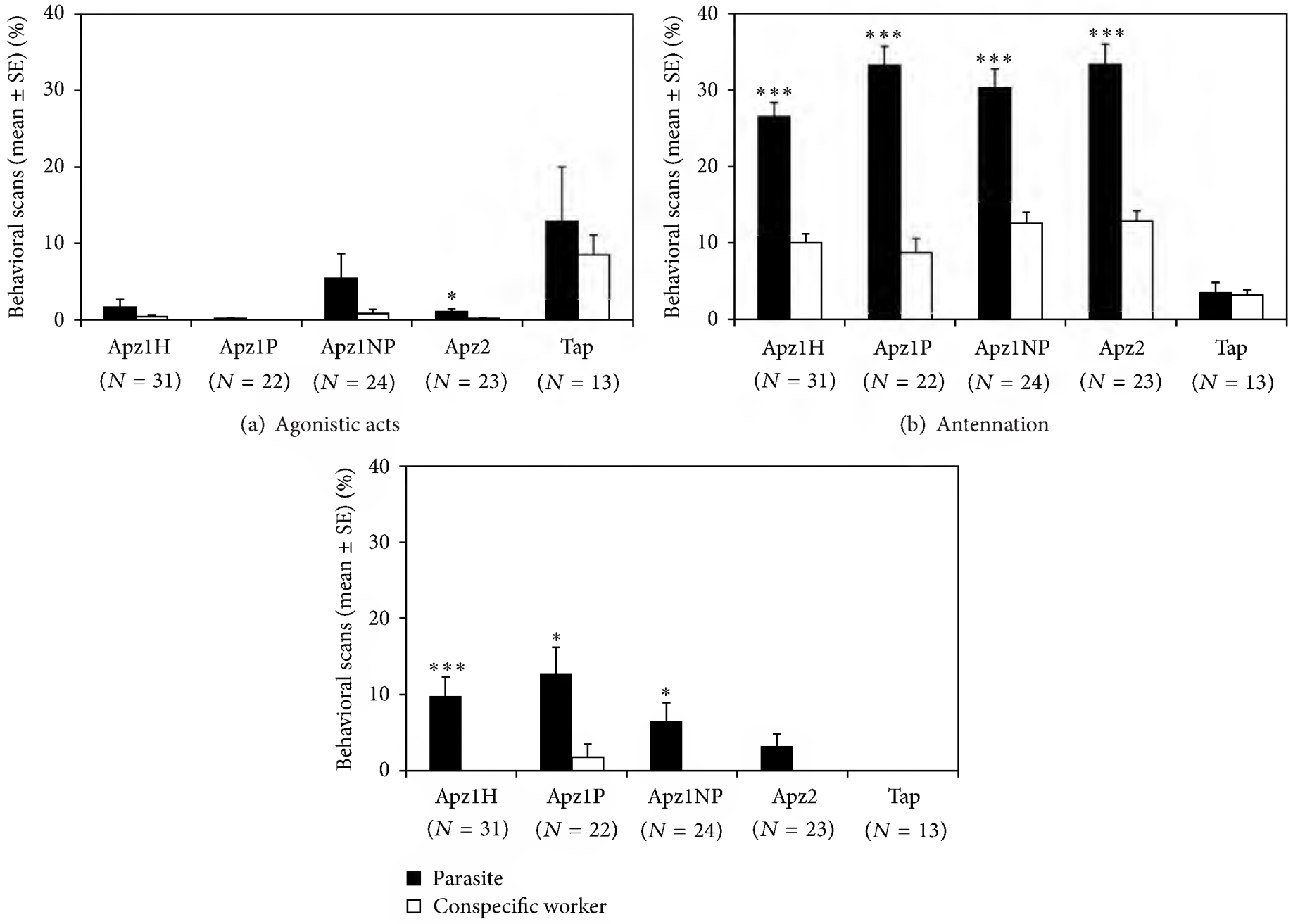


FIGURE 4: Comparison of the behavioral reactions towards the social parasite (black bars) and the conspecific worker (white bars) in the different types of tests (see Figure 3 for the abbreviations). Pairwise comparisons were made with Permutation tests: $^{*}P < 0.05$, $^{***}P < 0.001$. N = number of tests.

Similarly, although at a lesser rate, workers stayed more often motionless near a parasite than a conspecific worker, but the difference was not significant in the tests between sites and never occurred with the Tapachula population (Figure 4(c)).

3.3.2. Discrimination Tests between a Parasitic Queen and a Conspecific Queen. In this experimental condition, only a few tests included at least one aggression (Figure 5), and no difference between the types of tests was found for the parasitic queen (Pearson's exact Chi-Square test, $P = 0.21$) and the conspecific queen ($P = 0.66$). When occurring, the rate of aggression was low and similar towards both queens (Figure 6(a)). However, the parasite was discriminated through a lesser rate of antennation and immobility compared with the conspecific queen (Figures 6(b) and 6(c)). All differences were statistically significant, except for antennation between nestmates.

4. Discussion

4.1. Field Study. Field data confirmed and strengthened our previous reports [13, 27] that, unlike the host species [37], the social parasite *E. parasiticum* is rare and very local in occurrence. Along with its patchy distribution, this suggests a short-range dispersal of the species. Moreover, we showed a change in abundance of the parasite over the time. This could be due to not only its rarity, but also its vulnerability to environmental conditions. Unfortunately climatic data were not available for the whole period, but it seems that the successful collections of the parasites in 1999 and 2000 were preceded by rainy periods, and the unsuccessful one in 2002 was characterized by a long dry period.

Furthermore, environmental constraints, along with genetic factors, are known to explain variation in reproductive strategies [39, 40]. Our data confirm that the colonies of *E. tuberculatum* exhibit a facultative polygyny in the Apazapan population with queens being functionally reproductive [13, 25]. By comparison, in the whole Soconusco region including Tapachula, only three out of 253 colonies collected (1%) were polygynous, including only two queens, and the parasite was never found [38]. A polygynous social organization, by readoption of daughter queens, seems to be the rule in *E. tuberculatum* in Brazil, where 49% of the nests exhibited at least two reproductive queens (2–14 queens per nest, $n = 165$, recalculated from Hora et al. [13] and Zinck et al. [41]). The social organization

Table 1: Composition of the *Ectatomma tuberculatum* colonies sampled in three sites of Apazapan between September 1999 and November 2011.

Date	Site Apz1				Site Apz2				Site Apz3			
	N of colonies			*N* of workers	*N* of colonies			*N* of workers	*N* of colonies			*N* of workers
	Collected	Parasitized	Polygynous	Median (range)	Collected	Parasitized	Polygynous	Median (range)	Collected	Parasitized	Polygynous	Median (range)
September 1999	3	3	1	51 (12–120)	—	—	—	—	—	—	—	—
June-July 2000	21	12	3	118 (15–428)	—	—	—	—	—	—	—	—
March-April 2002	19	0	4	178 (64–358)	7	0	0	146 (55–243)	6	0	3	139 (51–225)
January 2009	8	0 (3*)	4	263 (163–341)	15	0	5	198 (46–287)	—	—	—	—
September 2010**	—	—	—	—	8	0	2	68 (47–150)	—	—	—	—
November 2011	4	0	0	162 (95–383)	4	0	2	242 (74–254)	3	0	2	94 (57–148)
Total	55	18	12	157 (12–428)	34	0	9	146.5 (46–287)	9	0	5	99 (51–225)

—: no sampled data; *emergence of parasites from larvae collected in field; **data from Pérez-Lachaud et al. [38].

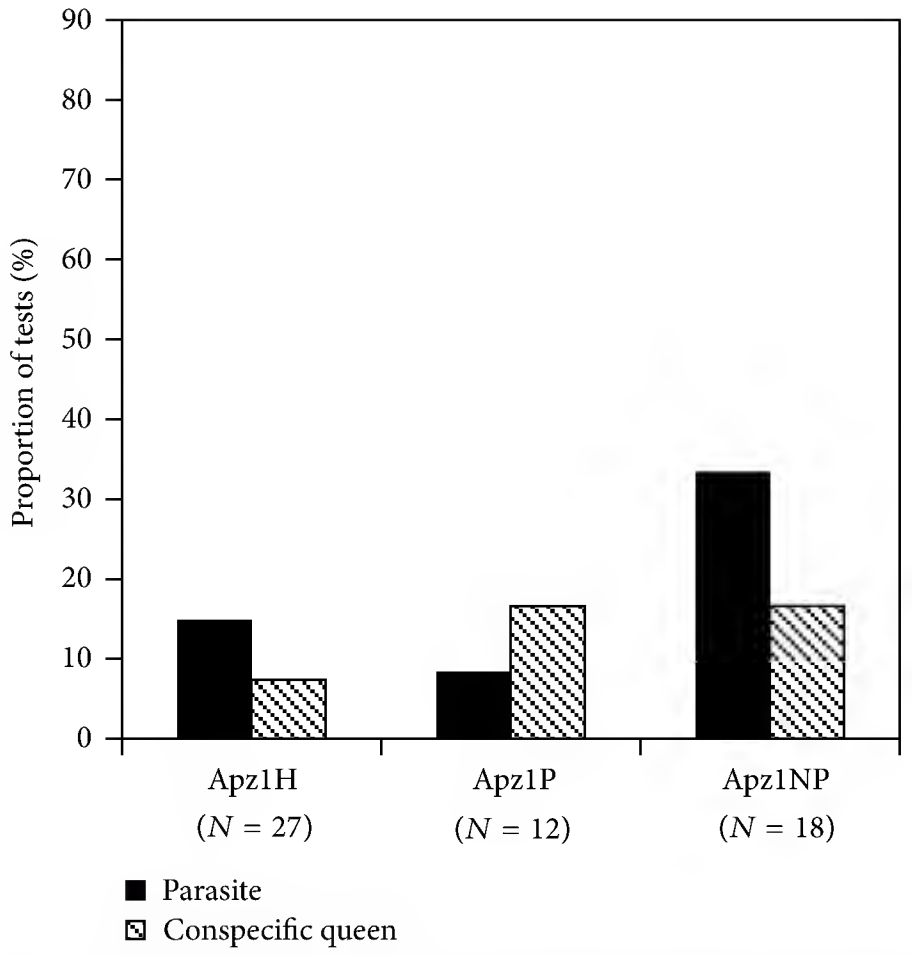


FIGURE 5: Proportions of tests including aggression towards the social parasite or the conspecific queen according to the type of tests (see Figure 3 for the abbreviations). N = number of tests.

in Brazil is characterized by a polydomous structure and reproduction by nest budding, both characteristics increasing the size of *E. tuberculatum* colonies territory, and therefore insuring the ecological dominance of the species [42, 43]. In the case of Apazapan in Mexico, nest distribution of *E. tuberculatum* is also patchy, but the soil is highly rocky, the stone often forming a horizontal homogeneous layer that limits abilities for queens to found new nests. The nest site limitation, plus other ecological factors yet unidentified, might have constrained polygyny, as already suggested for the *E. tuberculatum* population of Apazapan [38] and shown in other ant species [44]. In a second step, polygyny might have favored the selection of selfish reproductive strategies and then the evolution of social parasite by sympatric speciation [1, 6, 10].

4.2. Genetic Analysis. The node built from cytochrome b sequences was poorly supported between *E. tuberculatum* and *E. parasiticum*. Investigation in both other genes sequencing and more populations are needed to resolve this divergence. However, the low levels of divergence between *E. parasiticum* and its host combined with the observed geographic variation are consistent with the strict acceptation of Emery's rule [5] and support the hypothesis of a recent divergence between *E. tuberculatum* and its parasite. *Ectatomma parasiticum* might have evolved by sympatric speciation from its host species in Apazapan, due to a previous evolution of *E. tuberculatum* to polygyny (polygyny syndrome [45]) and environmental conditions. Miniaturization of queens was linked to social parasitism in several ant species (see [46, 47], and also see, e.g., [14, 15]). Convergent arguments from field studies and laboratory experiments suggest that assortative mating through direct mate choice, or through choice of different mating habitat between miniaturized and large queens, led divergent selection up to sympatric speciation [7].

4.3. Discrimination Ability and Social Tolerance. Our results show that *E. tuberculatum* host workers were able to distinguish the social parasites *E. parasiticum* from their conspecifics. Such discrimination occurred only within the parasitized population (Apazapan) and was inferred from differential responses in antennation and immobility, and in some cases in aggression. By contrast, workers from the nonparasitized, monogynous, and geographically distant population (Tapachula) attacked vigorously both parasitic and conspecific ants, considering both as intruders.

When confronted to *E. tuberculatum* workers from its own colony, the parasitic queen was more antennated and more attractive than a nestmate worker, but less attractive than a nestmate queen. The parasite was thus perceived as a distinct entity, even by the members of its own host colony. This is unusual because inquiline species are expected either to avoid any detection or to be treated as a nestmate, depending on the chemical strategy (see e.g., [24, 48] in ants, [49], in bumblebees, and [50] in wasps). Because our test was independent of the stimuli-ants' behaviors, such discrimination was supposed to be primarily based on chemicals, even if differences in size could also be detected. This is congruent with recent chemical analyses showing that *E. parasiticum* was chemically distinct from its host species [28]. In particular, the parasite had reduced amounts of cuticular hydrocarbons, and it differed from its host in the relative composition of some of these compounds. This is also consistent with behavioral observations in a more natural context, as some parasites were specifically antennated or attacked by the host workers within their colony [25].

Allocolonial tests within the Apazapan population showed that workers responded differentially towards parasites and conspecific nonnestmates, either workers or queens. Both parasitic and conspecific queens from another colony were considered as individuals of interest, as they elicited intense antennal inspection. It could be a result of novelty due to the detection of unfamiliar odors. These odors, however, could not be exclusively colony specific as nonnestmate workers of *E. tuberculatum* were treated differently from conspecific nonnestmate queens. Because antennation and immobility were mostly associated with the presence of *E. tuberculatum* queens, we supposed that workers were attracted to queen-specific substances. Queen pheromones are known to produce an attracting and settling effect on workers and cause the retinue behavior in ants [36, 51] and honeybees [52]. In ants, this effect can be elicited by surface molecules probably linked to fertility signals and esters from Dufour's gland secretion [2, 18, 53]. The hydrocarbon cuticular profile of *E. tuberculatum* queens differed from that of workers [28, 54] and virgin queens [55]. Some alkanes have been proposed as fertility signals in this species [55], but we also found esters on the queens' cuticle that could be involved as well [28]. The lesser amount of these compounds on the parasite's cuticle compared with conspecific queen could explain the lower effect of attractiveness on *E. tuberculatum* workers.

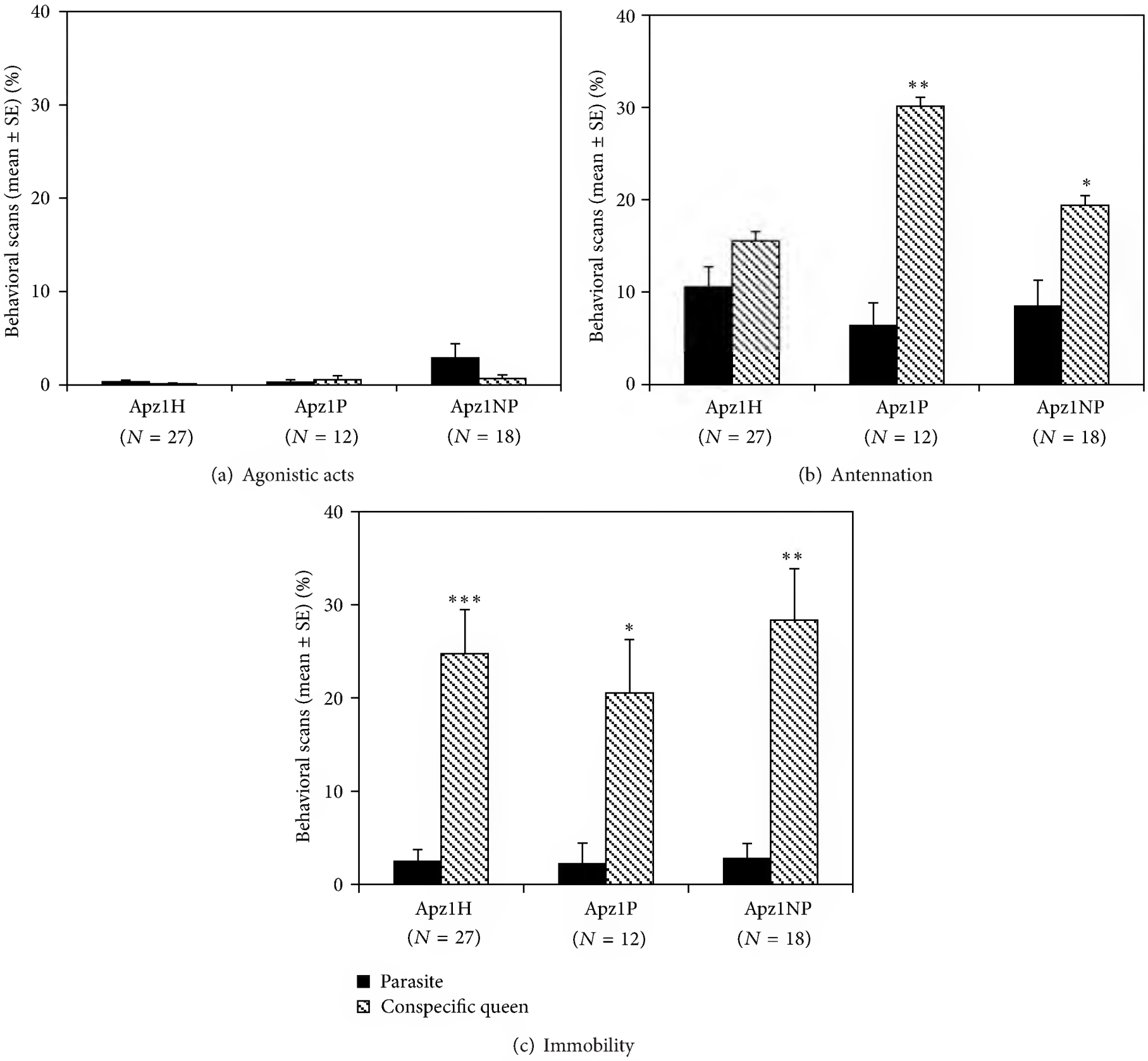


FIGURE 6: Comparison of the behavioral reactions towards the social parasite (black bars) and the conspecific queen (dashed bars) in the different types of tests (see Figure 3 for the abbreviations). Pairwise comparisons were made with Permutation tests: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. N = number of tests.

However, workers from nonparasitized and distant colonies in the Apazapan population were less attracted by the parasite and some of them attacked it, thus considering it as an intruder. Aggression means a possible rejection that could explain why some colonies were parasitized and the others not. The level of aggression, however, remained low. This could be partly due to the experimental device, as the neutral arena and the immobilization of stimuli-ants are known to limit aggressive reactions [35]. But more likely, because strong aggression between nonnestmate workers of *E. tuberculatum* from the monogynous population of Tapachula was observed using the same discrimination test ([56] and this paper), it could be associated to life-history traits specific to individuals from the Apazapan population. Polygyny by mixing odors from individuals of different genetic lineages (Gestalt model [57]) may affect recognition systems. It is likely to increase the tolerance threshold of the workers within colonies and to reduce the variation in chemical cues between colonies, resulting in a lower level of aggression between nonnestmates at a population level ([39, 58], but see [59]). Both of these features may have facilitated the exploitation of the host by a social parasite [3].

5. Conclusions

Ectatomma parasiticum shared several life-history traits with other workerless inquiline ants [1, 3]: rarity, local distribution, variation in abundance, limited dispersal, intracolonial mating, queen miniaturization, morphological similarity with its host, and quasiexclusive production of sexuals ([13, 28] and this paper). Some of these parasitic traits, the polygynous population of the host, and the association between sibling

species are arguments which may support the hypothesis of sympatric speciation. Despite a possible recent divergence of the social parasite from its host, we showed that *E. parasiticum* could be discriminated by its host, and then potentially rejected. Nevertheless, most parasites elicited interest and attractiveness from the host, probably because of their peculiar chemical profile (a weak chemical signature) and/or their reproductive status. We suggest that *E. parasiticum* could have conserved from its host sibling species the queen-specific substances that produce attracting and settling effect on workers, then making the exploitation of the host easier. However, recognition in ants is a multi-component system which encodes different types of information [17, 18], but not independently of one another. For example, it has been recently suggested that fertility signal interferes with the production or the perception of colony-specific cues in *Camponotus floridanus* [60]. In case of *E. parasiticum*, host worker attractiveness due to the queen-specific substance could, in return, increase the probability to be detected as carrying distinct recognition cues, and then to be attacked by the most discriminating host workers. This hypothesis would explain why the social integration of the parasite into host colonies is imperfect [25]. Which peculiar compounds or class of compounds are involved in each recognition level remains to be clarified. Further experiments by manipulating queen odors are needed that should also enlighten the function of queen chemicals in social insects, in general.

Acknowledgments

The authors would like to thank Nicolas Châline, Stéphane Chameron, Jean-Paul Lachaud, and three anonymous reviewers for providing critical comments on the manuscript and improving English. The authors are particularly grateful to Mario Favila, the INECOL staff, and students for helping during the fieldwork in Mexico, and also grateful to Dominique Fresneau and Riviane R. da Hora who collected the colonies from 1999 to 2002. The authors are grateful to Marie-Claire Malherbe and Marjorie Labédan as well for rearing the colonies in the laboratory. This work was partly financed by the CREI of the Université Paris 13 (no. 900-RELI-247) and by the ANR (no. ANR-09JCJC-0031).

References

[1] A. Buschinger, "Social parasitism among ants: a review (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 12, pp. 219–235, 2009.

[2] B. Hölldobler and E. O. Wilson, *The Ants*, Springer, Berlin, Germany, 1990.

[3] D. R. Nash and J. J. Boomsma, "Communication between hosts and social parasites," in *Sociobiology of Communication: An Interdisciplinary Perspective*, P. d'Ettorre and D. Hughes, Eds., pp. 55–79, Oxford University Press, Oxford, UK, 2008.

[4] E. O. Wilson, *The Insect Societies*, Belknap Press of Havard University Press, Cambridge, Mass, USA, 1971.

[5] G. Le Masne, "Recherches sur les fourmis parasites. *Plagiolepis grassei* et l'évolution des *Plagiolepis* parasites," *Comptes rendus de l'Académie des Sciences*, vol. 243, pp. 673–675, 1956.

[6] A. F. G. Bourke and N. R. Franks, "Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants," *Biological Journal of the Linnean Society*, vol. 43, no. 3, pp. 157–178, 1991.

[7] R. Savolainen and K. Vepsäläinen, "Sympatric speciation through intraspecific social parasitism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 12, pp. 7169–7174, 2003.

[8] S. Sumner, D. K. Aanen, J. Delabie, and J. J. Boomsma, "The evolution of social parasitism in *Acromyrmex* leaf-cutting ants: a test of Emery's rule," *Insectes Sociaux*, vol. 51, no. 1, pp. 37–42, 2004.

[9] J. A. Smith, S. M. Tierney, Y. C. Park, S. Fuller, and M. P. Schwarz, "Origins of social parasitism: the importance of divergence ages in phylogenetic studies," *Molecular Phylogenetics and Evolution*, vol. 43, no. 3, pp. 1131–1137, 2007.

[10] A. Buschinger, "Sympatric speciation and radiative evolution of socially parasitic ants—heretic hypotheses and their factual background," *Zeitschrift für Zoologische Systematik und Evolutionsforschung*, vol. 28, no. 4, pp. 241–260, 1990.

[11] K. Vepsäläinen, J. R. Ebsen, R. Savolainen, and J. J. Boomsma, "Genetic differentiation between the ant *Myrmica rubra* and its microgynous social parasite," *Insectes Sociaux*, vol. 56, no. 4, pp. 425–437, 2009.

[12] G. Jansen, R. Savolainen, and K. Vepsäläinen, "Phylogeny, divergence-time estimation, biogeography and social parasite-host relationships of the Holarctic ant genus *Myrmica* (Hymenoptera: Formicidae)," *Molecular Phylogenetics and Evolution*, vol. 56, no. 1, pp. 294–304, 2010.

[13] R. R. Hora, C. Doums, C. Poteaux et al., "Small queens in the ant *Ectatomma tuberculatum*: a new case of social parasitism," *Behavioral Ecology and Sociobiology*, vol. 59, no. 2, pp. 285–292, 2005.

[14] J. P. Lachaud, A. Cadena, B. Schatz, G. Pérez-Lachaud, and G. Ibarra-Núñez, "Queen dimorphism and reproductive capacity in the ponerine ant, *Ectatomma ruidum* Roger," *Oecologia*, vol. 120, no. 4, pp. 515–523, 1999.

[15] J. C. Lenoir, J. P. Lachaud, A. Nettel, D. Fresneau, and C. Poteaux, "The role of microgynes in the reproductive strategy of the neotropical ant *Ectatomma ruidum*," *Naturwissenschaften*, vol. 98, no. 4, pp. 347–356, 2011.

[16] R. W. Howard and G. J. Blomquist, "Ecological, behavioral, and biochemical aspects of insect hydrocarbons," *Annual Review of Entomology*, vol. 50, pp. 371–393, 2005.

[17] P. D'Ettorre, "Multiple levels of recognition in ants: a feature of complex societies," *Biological Theory*, vol. 3, no. 2, pp. 108–113, 2008.

[18] A. Hefetz, "The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae)—interplay of colony odor uniformity and odor idiosyncrasy. A review," *Myrmecological News*, vol. 10, pp. 59–68, 2007.

[19] P. D'Ettorre and A. Lenoir, "Nestmate recognition," in *Ant Ecology*, L. Lach, C. L. Parr, and K. L. Abbott, Eds., pp. 194–209, Oxford University Press, Oxford, UK, 2010.

[20] C. Von Beeren, S. Pohl, and V. Witte, "On the use of adaptive resemblance terms in chemical ecology," *Psyche*, vol. 2012, Article ID 635761, 7 pages, 2012.

[21] K. Dettner and C. Liepert, "Chemical mimicry and camouflage," *Annual Review of Entomology*, vol. 39, pp. 129–154, 1994.

[22] A. Lenoir, P. D'Ettorre, C. Errard, and A. Hefetz, "Chemical ecology and social parasitism in ants," *Annual Review of Entomology*, vol. 46, pp. 573–599, 2001.

[23] T. Akino, "Chemical strategies to deal with ants: a review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods," *Myrmecological News*, vol. 11, pp. 173–181, 2008.

[24] N. Franks, M. Blum, R. K. Smith, and A. B. Allies, "Behavior and chemical disguise of cuckoo ant *Leptothorax kutteri* in relation to its host *Leptothorax acervorum*," *Journal of Chemical Ecology*, vol. 16, no. 5, pp. 1431–1444, 1990.

[25] R. R. Hora, R. Blatrix, D. Fresneau, and R. Fénéron, "Social interactions between an inquiline ant, *Ectatomma parasiticum*, and its host, *Ectatomma tuberculatum* (Formicidae, Ectatomminae)," *Journal of Ethology*, vol. 27, no. 2, pp. 285–288, 2009.

[26] R. M. Feitosa, R. R. Hora, J. H. C. Delabie, J. Valenzuela, and D. Fresneau, "A new social parasite in the ant genus *Ectatomma* F. Smith (Hymenoptera, Formicidae, Ectatomminae)," *Zootaxa*, no. 1713, pp. 47–52, 2008.

[27] R. R. Da Hora, R. Fénéron, J. Valenzuela, M. E. Favila, and D. Fresneau, "Queen-size dimorphism in the ant *Ectatomma tuberculatum* (Hymenoptera: Formicidae: Ponerinae)," *Sociobiology*, vol. 38, no. 3, pp. 407–420, 2001.

[28] F. Savarit and R. Fénéron, "Imperfect chemical mimicry explains the imperfect social integration of the inquiline ant, *Ectatomma parasiticum*," in preparation.

[29] C. G. Castillo, "Ecología del paisaje del municipio de jalcomulco," in *Faculdad de Ciencias*, UNAM, Veracruz MSc, 1995.

[30] E. Garcia, "Modificaciones al sistema de clasificación climática de Köppen," in *Instituto de Geografía*, Universidad Nacional Autónoma de México, México D.F., 1973.

[31] R. H. Crozier and Y. C. Crozier, "The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization," *Genetics*, vol. 133, no. 1, pp. 97–117, 1993.

[32] J. D. Thompson, D. G. Higgins, and T. J. Gibson, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice," *Nucleic Acids Research*, vol. 22, no. 22, pp. 4673–4680, 1994.

[33] T. H. Jukes and C. R. Cantor, "Evolution of protein molecules," in *Mammalian Protein Metabolism*, H. N. Munro, Ed., pp. 21–132, Academic Press, New York, NY, USA, 1969.

[34] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar, "Mega5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods," *Molecular Biology and Evolution*, vol. 28, no. 10, pp. 2731–2739, 2011.

[35] R. Fénéron, "A new ethological test to study nestmate recognition in adult ants," *Insectes Sociaux*, vol. 43, no. 2, pp. 131–135, 1996.

[36] B. Hölldobler, C. Peeters, and M. Obermayer, "Exocrine glands and the attractiveness of the ergatoid queen in the ponerine ant *Megaponera foetens*," *Insectes Sociaux*, vol. 41, no. 1, pp. 63–72, 1994.

[37] N. A. Weber, "Two common ponerine ants of possible economic significance, *Ectatomma tuberculatum* (Olivier) and *E. ruidum* Roger," *Proceedings of the Entomological Society of Washington*, vol. 48, no. 1, pp. 1–16, 1946.

[38] G. Pérez-Lachaud, J. E. Valenzuela, and J.-P. Lachaud, "Is increased resistance to parasitism at the origin of polygyny in a mexican population of the ant *Ectatomma tuberculatum* (Hymenoptera: Formicidae)?" *The Florida Entomologist*, vol. 94, no. 3, pp. 677–684, 2011.

[39] L. Keller, Ed., *Queen Number and Sociality in Insects*, Oxford University Press, Oxford, UK, 1993.

[40] J. Heinze, "Social plasticity: ecology, genetics and the structure of ant societies," in *Ecology of Social Evolution*, J. Korb and J. Heinze, Eds., pp. 129–150, Springer, Berlin, Germany, 2008.

[41] L. Zinck, P. Jaisson, R. R. Hora, D. Denis, C. Poteaux, and C. Doums, "The role of breeding system on ant ecological dominance: genetic analysis of *Ectatomma tuberculatum*," *Behavioral Ecology*, vol. 18, no. 4, pp. 701–708, 2007.

[42] D. Leston, "A neotropical ant mosaic," *Annals of the Entomological Society of America*, vol. 71, no. 4, pp. 649–653, 1978.

[43] J. D. Majer, J. H. C. Delabie, and M. R. B. Smith, "Arboreal ant community patterns in Brazilian cocoa farms," *Biotropica*, vol. 26, no. 1, pp. 73–83, 1994.

[44] T. McGlynn, "Serial monodomy in the gypsy ant, *Aphaenogaster araneoides*: does nest odor reduction influence colony relocation?" *Journal of Insect Science*, vol. 10, article 195, 2010.

[45] L. Keller, "Social life: the paradox of multiple-queen colonies," *Trends in Ecology and Evolution*, vol. 10, no. 9, pp. 355–360, 1995.

[46] P. Nonacs and J. E. Tobin, "Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera," *Evolution*, vol. 46, no. 6, pp. 1605–1620, 1992.

[47] S. Aron, L. Passera, and L. Keller, "Evolution of social parasitism in ants: size of sexuals, sex ratio and mechanisms of caste determination," *Proceedings of the Royal Society B*, vol. 266, no. 1415, pp. 173–177, 1999.

[48] D. Lambardi, F. R. Dani, S. Turillazzi, and J. J. Boomsma, "Chemical mimicry in an incipient leaf-cutting ant social parasite," *Behavioral Ecology and Sociobiology*, vol. 61, no. 6, pp. 843–851, 2007.

[49] S. Dronnet, X. Simon, J. C. Verhaeghe, P. Rasmont, and C. Errard, "Bumblebee inquilinism in *Bombus (Fernaldaepsithyrus) sylvestris* (Hymenoptera, Apidae): behavioural and chemical analyses of host-parasite interactions," *Apidologie*, vol. 36, no. 1, pp. 59–70, 2005.

[50] M. F. Sledge, F. R. Dani, R. Cervo, L. Dapporto, and S. Turillazzi, "Recognition of social parasites as nest-mates: adoption of colony-specific host cuticular odours by the paper wasp parasite *Polistes sulcifer*," *Proceedings of the Royal Society B*, vol. 268, no. 1482, pp. 2253–2260, 2001.

[51] C. W. Rettenmeyer, H. Topoff, and J. Mirenda, "Queen retinues of army ants (Hymenoptera, Formicidae, Ecitoninae)," *Annals of the Entomological Society of America*, vol. 71, no. 4, pp. 519–528, 1978.

[52] K. N. Slessor, M. L. Winston, and Y. Le Conte, "Pheromone communication in the honeybee (*Apis mellifera* L.)," *Journal of Chemical Ecology*, vol. 31, no. 11, pp. 2731–2745, 2005.

[53] T. Monnin, "Chemical recognition of reproductive status in social insects," *Annales Zoologici Fennici*, vol. 43, no. 5-6, pp. 515–530, 2006.

[54] L. Zinck, D. Denis, R. R. Hora et al., "Behavioral and chemical correlates of long-term queen adoption in the facultative polygynous ant *Ectatomma tuberculatum*," *Journal of Insect Behavior*, vol. 22, no. 5, pp. 362–374, 2009.

[55] R. R. Hora, A. Ionescu-Hirsh, T. Simon et al., "Postmating changes in cuticular chemistry and visual appearance in *Ectatomma tuberculatum* queens (Formicidae: Ectatomminae)," *Naturwissenschaften*, vol. 95, no. 1, pp. 55–60, 2008.

[56] R. Fénéron, E. Nowbahari, and F. Dutrou, "Reconnaissance intercoloniale et niveau d'agression chez la fourmi Ponérine, *Ectatomma tuberculatum*," *Actes Colloques Insectes Sociaux*, vol. 12, pp. 33–36, 1999.

[57] R. H. Crozier and M. W. Dix, "Analysis of two genetic models for the innate components of colony odor in social Hymenoptera," *Behavioral Ecology and Sociobiology*, vol. 4, no. 3, pp. 217–224, 1979.

[58] L. Sundström, "Queen acceptance and nestmate recognition in monogyne and polygyne colonies of the ant *Formica truncorum*," *Animal Behaviour*, vol. 53, no. 3, pp. 499–510, 1997.

[59] S. J. Martin, H. Helanterä, K. Kiss, Y. R. Lee, and F. P. Drijfhout, "Polygyny reduces rather than increases nestmate discrimination cue diversity in *Formica exsecta* ants," *Insectes Sociaux*, vol. 56, no. 4, pp. 375–383, 2009.

[60] D. Moore and J. Liebig, "Mixed messages: fertility signaling interferes with nestmate recognition in the monogynous ant *Camponotus floridanus*," *Behavioral Ecology and Sociobiology*, vol. 64, no. 6, pp. 1011–1018, 2010.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 926572, 14 pages
http://dx.doi.org/10.1155/2013/926572

Review Article

A Review of the Biology of Eucharitidae (Hymenoptera: Chalcidoidea) from Argentina

Javier Torréns

CRILAR-CONICET, Entre Ríos y Mendoza, 5301 Anillaco, La Rioja, Argentina

Correspondence should be addressed to Javier Torréns; jtorrens@crilar-conicet.gob.ar

Received 3 March 2013; Revised 30 April 2013; Accepted 12 May 2013

Academic Editor: Jean-Paul Lachaud

All the members of Eucharitidae are parasitoid of ants. Argentina has 14 genera and 41 species, but little is known about their biology. Herein are provided new data for host associations (host ant and/or host plant) of *Galearia latreillei*, *Kapala* spp., *Latina rugosa*, *Orasema aenea*, and *Orasema* sp. A revision of the most relevant biological aspects of *Dicoelothorax platycerus*, *Latina rugosa*, *Neolirata alta*, *N. daguerrei*, *Lophyrocera variabilis*, *Orasema argentina*, *O. salebrosa*, *O. simplex*, *O. susanae*, *O. worcesteri*, and *O. xanthopus* is included. New records of *K. sulcifacies*, *Lo. plagiata*, and *Ob. semifumipennis* in Argentina are presented. *Galearia proseni* is synonymized with *G. latreillei*.

1. Introduction

Eucharitidae parasitize the immature stages of Formicidae and are among the most diverse hymenopteran parasitoids of eusocial insects [1–8]. Females are oviparous and proovigenic and lay their eggs inside or on plant tissues, either individually or in masses. They oviposit away from the host, with the active first instar larva (planidium) responsible for getting into the ant nest through various associations with foraging adult ants [9]. Once in contact with the larval ant host, the planidium either remains as an external parasite or burrows into the host. Upon pupation of the host, the larva migrates to the ventral region of the thorax, just posterior to the legs of the newly formed pupa, then resumes development through two additional instars [10, 11]. The adults emerge and leave the nest on their own or may be carried by the ants and deposited in the accumulation of colony waste [10, 12, 13].

Eucharitidae are present in all zoogeographic regions but most abundant in the tropics [8]. Fifty-four genera and about 420 species worldwide have been described. In Argentina, 14 genera and 41 species have been reported [8, 14–16].

Eucharitidae were well studied in a series of early taxonomic papers by Gemignani [17–19]; however, very little information was provided on their biology. This paper reviews our current understanding and contributes new data for some of the Argentinean species.

2. Materials and Methods

Females were collected by sweep netting and provided twigs with leaves, fruits, and flowers of different species of plants in 10×3.5 cm plastic tubes to monitor oviposition habits. Host plants with eggs extracted from the field or oviposited by the captive females were placed into a cylindrical glass container of 10×10 cm with dampened cotton until emergence of the first instar (planidium).

Ant nests with adults, brood, and debris were collected into plastic containers. Adults and immature stages were then sorted from the debris, examined for parasitism, and in some cases returned to the containers to allow further development of immature ones. The immature stages were examined once daily until all parasitoids or ants emerged from the cocoons. In the cases where both parasitoid sexes emerged, they were put together in a cylindrical glass container of 10×10 cm containing different types of plants to allow for oviposition after mating.

A Leica MZ12 stereomicroscope was used for observations. Images were obtained using GT-Vision Ento-Vision software operating on a Leica M16 zoom lens linked to a JVC KY-F75U 3-CCD digital video camera or Leica Application Suite (version 3.5.0) software operating on a Leica MZ12 linked to a Leica DFC295 digital video camera. Images were

enhanced with Corel Photopaint and Corel Draw (version 15). Some images were processed using Deep Focus (Stuart Ball).

The biogeographical distribution and classification of ecoregions in Argentina was taken from Morrone [20] and Bertonatti and Corcuera [21]. Geographic coordinates for eucharitid localities were estimated using Google Earth (version 6.2.2.6613).

3. Genera and Species of Eucharitidae from Argentina

Two of the four subfamilies of Eucharitidae are represented in Argentina, Oraseminae and Eucharitinae (Table 1). Oraseminae is represented only by *Orasema* Cameron. The Eucharitinae are comprised of 12 genera of Eucharitini with a dubious record of *Psilocharis* Heraty (Psilocharitini) from Déan Funes (Córdoba) [5].

3.1. Dicoelothorax Ashmead. This genus includes two species distributed in the Neotropical region: *D. parviceps* Cameron (Argentina, Brazil, Colombia, and Guyana) and *D. platycerus* Ashmead (Argentina, Bolivia, and Brazil) (Figures 1(a) and 1(b)) [8, 14, 22]. Biological information is only available for *D. platycerus* [22].

3.1.1. Dicoelothorax platycerus Ashmead. Habitat and location are as follows. Specimens were collected in San Vicente (Tucumán). The vegetation of this region is characterized by dry forests, dominated by deciduous, spiny, and small-leaves plants typical of the Chaco ecoregion [40] (Figures 1(c) and 1(d)). The host plant, *Pseudabutilon virgatum* (Cav.) (Malvaceae), is a ligneous shrub that occurs throughout the area and persists year round.

Life history and host ants are next. A single gravid female oviposited about 40 eggs per 1 mm^2 on the underside of leaves (Figure 1(e)), and eggs hatched within 10 days. First instars (planidia) (Figure 1(f)) are mobile and have a propensity to jump; larvae presumably attach phoretically to foraging ants under the host plant and get carried back to the ant nest where they attack the ant larvae [3]. Of two pupae of *D. platycerus* obtained from the host ants nest one male emerged 12 days after the nest was excavated, whereas the other pupa (female) did not emerge (Figure 2(d)).

Ectatomma brunneum F. Smith (Ectatomminae) workers were observed and sampled from under the plants with *Dicoelothorax*. Of three ant nests found, immature ones were in two of them (H1 and H2). The disposition of chambers and general structure of nests are similar to those observed by Lapola et al. [41] (Figures 1(g) and 2(a)). Nest H1 contained 17 cocoons and 2 ant larvae, and nest H2 had 97 ant larvae and no cocoons. The percentage of parasitism ranged from 6.2% in H2 to 21% in H1. Of the 17 cocoons (H1) recovered, there were two cocoons each with one pupae of *D. platycerus* (1 female and 1 male) and 2 ant prepupae parasitized by second instars of *D. platycerus* (Figure 2(b)). In nest H2, 6 of the larvae were parasitized by externally located planidia (Figure 2(c)).

3.2. Galearia Brullé. The genus is comprised of two species, *G. latreillei* (Guérin-Méneville) and *G. proseni* Gemignani. Heraty [8] argued that the Argentinean male described as *G. proseni* by Gemignani [19] was likely the male of *G. latreillei* (Figures 2(e) and 2(f)). Based on the morphological similarity of a reared male with *G. proseni* (=*Pseudokapala proseni*), and its subsequent mating with a female of *G. latreillei*, *I* infer that the suggestion by Heraty is correct and propose here a new synonymy of *G. proseni with G. latreillei*. The one species has a widespread Neotropical distribution, being present in Argentina, Bolivia, Brazil, and Venezuela [8, 14].

The only known biological record was from Gemignani [17] in which he mentioned that an adult of *G. latreillei* (=*Thoracantha latreillei*) was collected from the waste pile of a nest of *Pogonomyrmex cunicularius* Mayr (=*P. carnivora*), but this ant association is likely invalid [8].

Galearia latreillei was collected in northcentral and northwestern Argentina, and information on life history, immature stages, and host association is included.

3.2.1. Galearia latreillei (Guérin-Méneville). Habitat and locations are as follows. Specimens were collected in Cabeza de Buey (Salta), Campo Gallo, Suncho Corral, and Tintina (Santiago del Estero). The Cabeza de Buey locality consists of mixed yunga (humid mountain forest) and xeric lowland Chaco vegetation. In the two localities in Santiago del Estero, located in the center and north of the province, the vegetation is typical of the chaco ecoregion (Figure 3(a)). The host plant, *Sida cordifolia* L. (Malvaceae), is a perennial, herbaceous plant with stems that are yellow-green, hairy, long, and slender, and their leaves are oblong-ovate, covered with hairs (Figure 3(b)).

Life history and host ants are next. Both sexes of *G. latreillei* were obtained from a nest of *Ectatomma brunneum*. The adult wasps were together for two days before mating occurred. The female then oviposited about 400 eggs that were dispersed among the spicules forming the pubescence on the stem of *S. cordifolia* near to the leaves or in the underside of leaves near the base (Figure 3(c)). Eggs hatched within 11 days. The planidia were very mobile and had a propensity to jump.

Nests of *Ectatomma brunneum* were excavated from near to the host plant, with immature ones found at a depth of 6 to 8 cm. From 50 cocoons, we extracted 10 pupae of *G. latreillei*. One male and one female emerged about 4 days after the nest was excavated, whereas the other pupae did not emerge (Figure 3(f)). Three other cocoons yielded one second-instar and two third-instars (Figures 3(d) and 3(e)). Of the 50 cocoons recovered, 13 were attacked giving a percentage parasitism of 26%.

Discussion. Ectatomma brunneum has also been reported as the ant host for *Dicoelothorax platycerus* [22] and for an unidentified species of *Kapala* Cameron (Eucharitidae: Eucharitini) in French Guiana [42]. Similarly, another species of the same ant genus, *E. tuberculatum* (Olivier), is known to be attacked by three different eucharitid genera, *Dilocantha* Shipp, *Isomerala* Shipp, and *Kapala* [43].

Table 1: List of species of Eucharitidae in Argentina. Known biology is indicated for host ants (HAs), host plants (HPs), or immature stages (ISs).

Subfamilies/tribes/genera	Species	Biology	References
Eucharitinae			
Psilocharitini			
Psilocharis Heraty	*Psilocharis* sp.*	?	—
Eucharitini			
Colocharis Heraty	*Colocharis hungi* Torréns	?	—
Dicoelothorax Ashmead	*Dicoelothorax parviceps* Cameron	?	—
	Dicoelothorax platycerus Ashmead	HP, HA, IS	[22]
Dilocantha Shipp	*Dilocantha bennetti* Heraty	?	—
	Dilocantha flavicornis (Walker)	?	—
Galearia Brullé	*Galearia latreillei* (Guérin-Méneville)	HP, HA, IS	***
Kapala Cameron	*Kapala argentina* Gemignani	?	—
	Kapala chacoensis Gemignani	?	—
	Kapala furcata (Fabricius)	HP	[2, 3]
	Kapala splendens Ashmead	?	—
	Kapala sulcifacies (Cameron)**	HP, IS	[23, 24]
Latina Koçak & Kemal	*Latina bonariensis* (Gemignani)	?	—
	Latina rugosa (Torréns, Heraty & Fidalgo)	HP, HA, IS	[25], HA***
	Latina vianai (Gemignani)	?	—
Lophyrocera Cameron	*Lophyrocera daguerrei* (Gemignani)	?	—
	Lophyrocera plagiata (Walker)**	?	—
	Lophyrocera variabilis Torréns, Heraty & Fidalgo	HP, HA, IS	[26]
Neolirata Torréns & Heraty	*Neolirata alta* (Walker)	HP, IS	[15]
	Neolirata daguerrei (Gemignani)	HP, IS	[15]
	Neolirata furcula Torréns & Heraty	?	—
Obeza Heraty	*Obeza maculata* (Westwood)	?	—
	Obeza nigriceps (Ashmead)	?	—
	Obeza semifumipennis (Girault)**	?	—
Parakapala Gemignani	*Parakapala decarloi* Gemignani	?	—
Pseudochalcura Ashmead	*Pseudochalcura alba* Heraty & Heraty	?	—
	Pseudochalcura americana (Howard)	?	—
	Pseudochalcura frustrata Heraty	?	—
	Pseudochalcura pauca Heraty	?	—
	Pseudochalcura prolata Heraty	?	—
Thoracantha Latreille	*Thoracantha spegazzinii* (Gemignani)	HP	[17]
	Thoracantha striata Perty	HP, IS	[8]
Oraseminae			
Orasema Cameron	*Orasema aenea* Gahan	HP, HA, IS	***
	Orasema argentina Gemignani	HA	[6, 17]
	Orasema deltae Gemignani	?	—
	Orasema freychei (Gemignani)	?	—
	Orasema gemignanii De Santis	?	—
	Orasema salebrosa Heraty	HA	[11, 27]
	Orasema simplex Heraty	HA, HP	[11, 28, 29]
	Orasema susanae Gemignani	HA	[6]
	Orasema vianai Gemignani	?	—
	Orasema worcesteri (Girault)	HA	[17]
	Orasema xanthopus (Cameron)	HP, HA, IS	[6, 11, 27, 28, 30–39]

Abbreviations: *doubtful record [5]; **new record of presence in Argentina; ***new biological record.

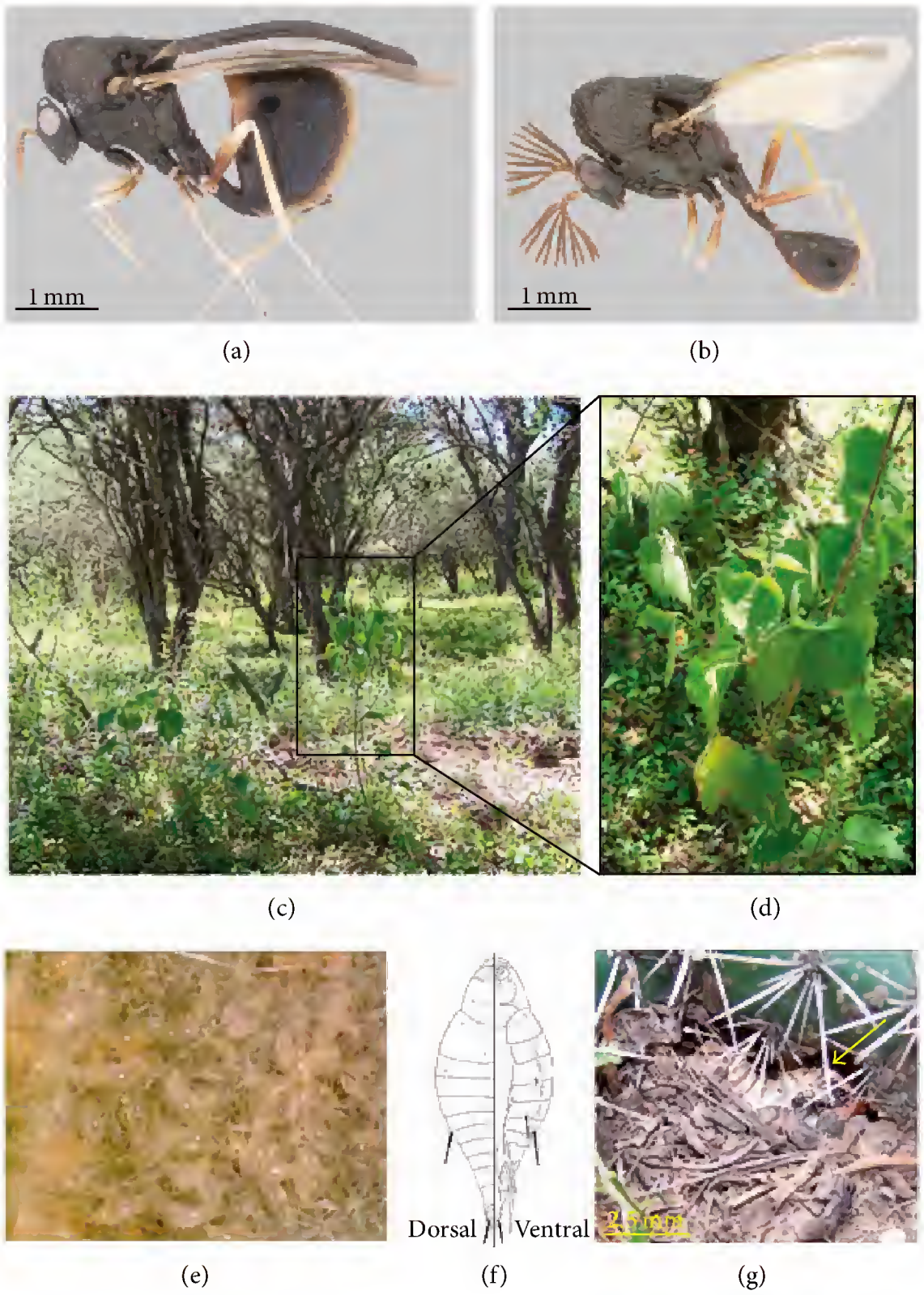


FIGURE 1: *Dicoelothorax platycerus*: (a) habitus (female); (b) habitus (male). Biology and immature stage of *D. platycerus*: (c) habitat; (d) *Pseudabutilon virgatum*; (e) underside of leaf of *P. virgatum* with eggs; (f) planidium (dorsal and ventral); (g) nest entrance of *Ectatomma brunneum* (opening indicated). Figures extracted from [22].

3.3. Kapala Cameron. *Kapala* includes 16 species, but there are many undescribed species in the Neotropical region. It is widespread and diverse in both the Nearctic and Neotropical regions and also includes one widespread afrotropical species, *Kapala ivorensis* Risbec [8].

In Argentina, 5 species were recorded: *K. argentina* Gemignani, *K. chacoensis* Gemignani, *K. furcata* (Fabricius), *K. splendens* Ashmead, and *K. sulcifacies* (Cameron) [8, 14]. Partial biological information is available for *K. furcata* and *K. sulcifacies* (summarized later). New data is also added for two unidentified species.

3.3.1. Kapala furcata (Fabricius). This species was observed ovipositing on floral buds of *Mikania* sp. (Asteraceae) [2] that were infested with aphids [3].

3.3.2. Kapala sulcifacies (Cameron). This species has been reported as ovipositing in floral buds of *Cordia curassavica* (Jacq.) Roem. & Schult. (Boraginaceae) (=*Cordia macrostachya*), *Gossypium hirsutum* L. (Malvaceae), and in a flowering asclepiad [23], with eggs laid in clusters of 200–300 eggs [24].

3.3.3. Kapala spp. A species sampled in Campo Gallo (Santiago del Estero) oviposited into flower buds of *Sphaeralcea bonariensis* (Cav.) Griseb. (Malvaceae), with the planidia emerging 9 days after oviposition. Another species was collected in Rosario de la Frontera (Salta) over an unidentified Sapindaceae, but no oviposition was observed.

3.4. Latina Koçak and Kemal. *Latina* (=*Laurella* Heraty) includes four species distributed in the Neotropical

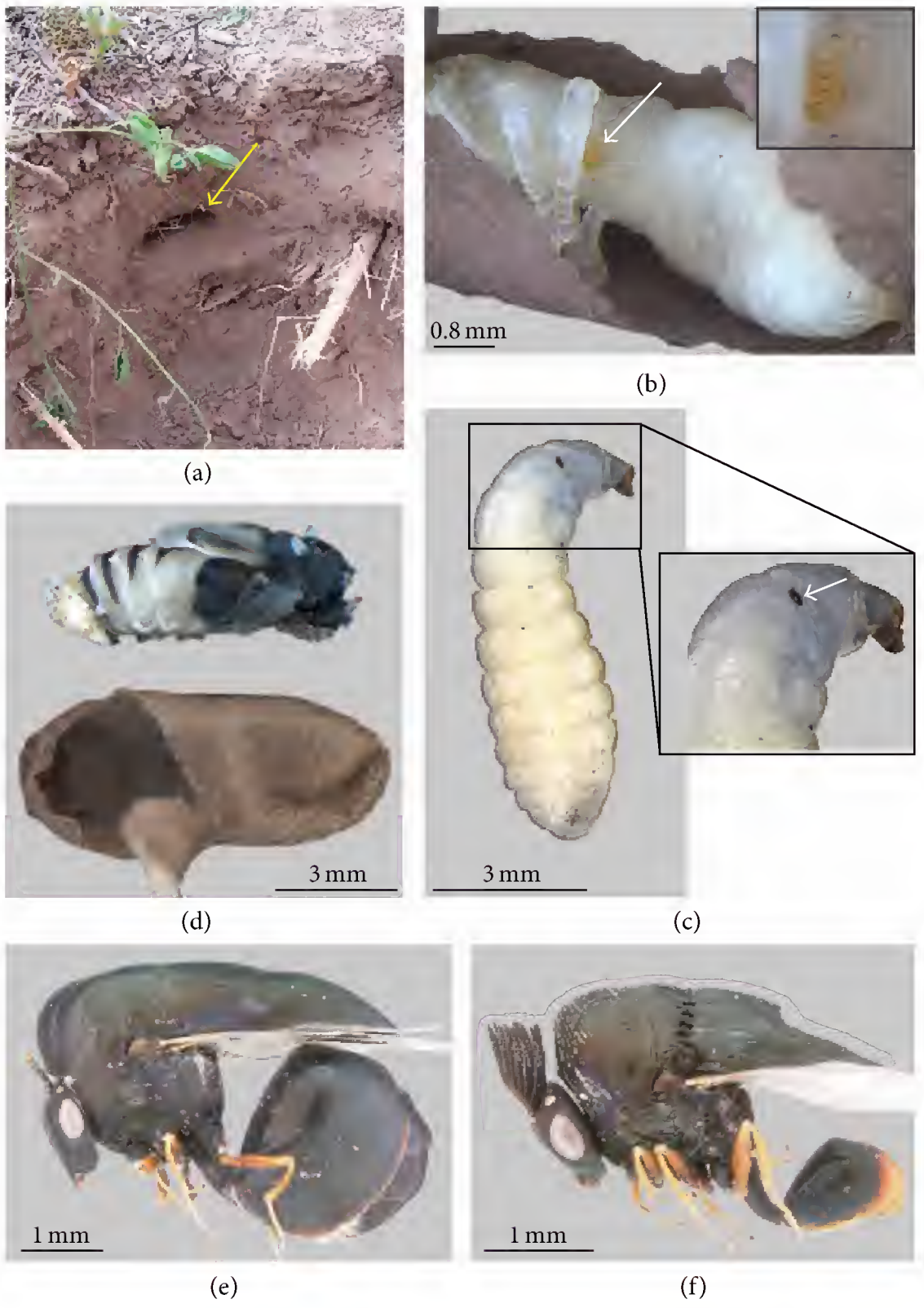


FIGURE 2: Biology and immature stage of *D. platycerus*: (a) brood chamber (indicated); (b) prepupa parasitized (2nd instar larva indicated and magnified); (c) ant larva parasitized (attached planidium magnified and indicated); (d) pupa extracted with ant cocoon (female, lateral). *Galearia latreillei*: (e) habitus (female); (f) habitus (male). Figures 2(a), 2(b), 2(c), and 2(d) are extracted from [22].

region: *Latina bonariensis* (Gemignani) (Argentina), *L. guriana* (Heraty) (Venezuela), *L. rugosa* (Torréns, Heraty and Fidalgo) (Argentina) (Figures 4(a) and 4(b)), and *L. vianai* (Gemignani) (Argentina) [8, 25].

Latina rugosa was collected in northwestern Argentina and the taxonomic and biological aspects provided by Torréns, Heraty, and Fidalgo [25].

3.4.1. Latina rugosa (Torréns, Heraty, and Fidalgo). Habitat and location are as follows. Specimens were collected at Rosario de la Frontera (Salta); the collection site was a forest of *Piptadenia macrocarpa* Benth. (Cebil Colorado) (Fabaceae). The vegetation of this region corresponds to the Yungas and Chaco ecoregions [40]. The host plants, *Serjania glabrata* (Sapindaceae), are perennial shrubs with pubescent and serrated leaves, with the plants dispersed between trees in the collection area (Figures 4(c) and 4(d)).

Life history and host ants are next. Adults of *L. rugosa* were collected in the same location, mainly close to or on the host plant. A single gravid female oviposited about 25 eggs per 1 mm^2 on the underside of leaves (Figure 4(d)). Eggs hatched within 6 days. The planidia (Figure 4(e)) were mobile and able to jump.

Odontomachus chelifer (Latreille) (Ponerinae) workers were observed and collected under the host plants from which *L. rugosa* were collected. One *O. chelifer* nest was identified only by a small ground opening. The ant nests were excavated and the cocoons and ants larvae extracted at a depth of 16 cm; however, the nest appeared to be much deeper, and it was difficult to tell whether the entire brood was extracted. Of the five ant larvae extracted, one had three

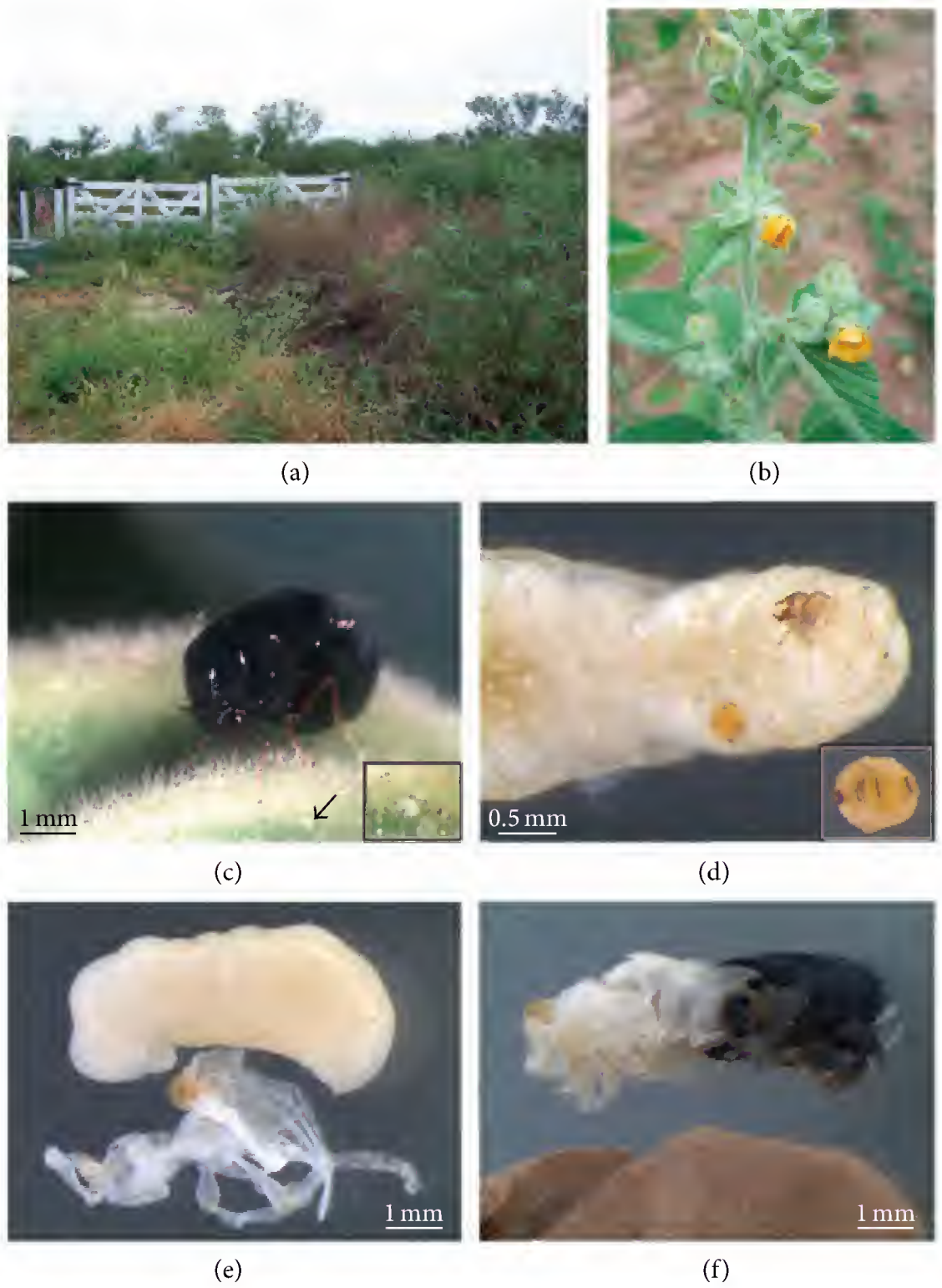


FIGURE 3: Biology and immature stage of *Galearia latreillei*: (a) habitat; (b) *Sida cordifolia*; (c) female of *G. latreillei* ovipositing on leaf of *S. cordifolia* (eggs indicated and magnified); (d) prepupa parasitized (1st instar larva indicated and magnified); (e) third instar (with remains of ant pupa); (f) pupa extracted from ant cocoon (male, lateral).

planidia externally attached (Figure 4(f)), while of the 19 cocoons only one planidium was found attached externally to a prepupa. From this sample, the percentage of parasitism was 8.3% of 24 immature ones.

Discussion. Data presented here confirm the ant host association of *Latina rugosa* as *Odontomachus chelifer*. This ant genus is also the host of other eucharitids genera as *Ancylotropus* Cameron, *Chalcura* Kirby, *Schizaspidia* Westwood, and *Kapala* Cameron [8, 42].

3.5. Lophyrocera Cameron. *Lophyrocera* Cameron includes seven species distributed across South and Central America and the western United States (Neotropical and Nearctic): *L. apicalis* Ashmead (USA), *L. daguerrei* (Gemignani) (Argentina), *L. chilensis* (Brèthes) (Chile), *L. plagiata* (Walker) (Argentina and Brazil), *L. pretendens* (Walker) (Brazil), *L. stramineipes* Cameron (Panama), and *L. variabilis* Torréns et al. (Argentina) (Figures 5(a)–5(c)) [8, 14, 26].

Lophyrocera variabilis was collected in northwestern Argentina, with information available on life history, immature stages, and host association [26].

3.5.1. Lophyrocera variabilis Torréns, Heraty, and Fidalgo. Habitat and location are as follows. The habitat consists of mixed Yungas and Chaco vegetation in Los Chorrillos (Tucumán) (Figure 5(d)). The host plant, *Vassobia breviflora* (Sendtn) Hunz. (Solanaceae), common name "Chalchal de la gallina", is a spiny shrub with globe-shaped fruits, which are red in color when mature (Figure 5(e)) [44].

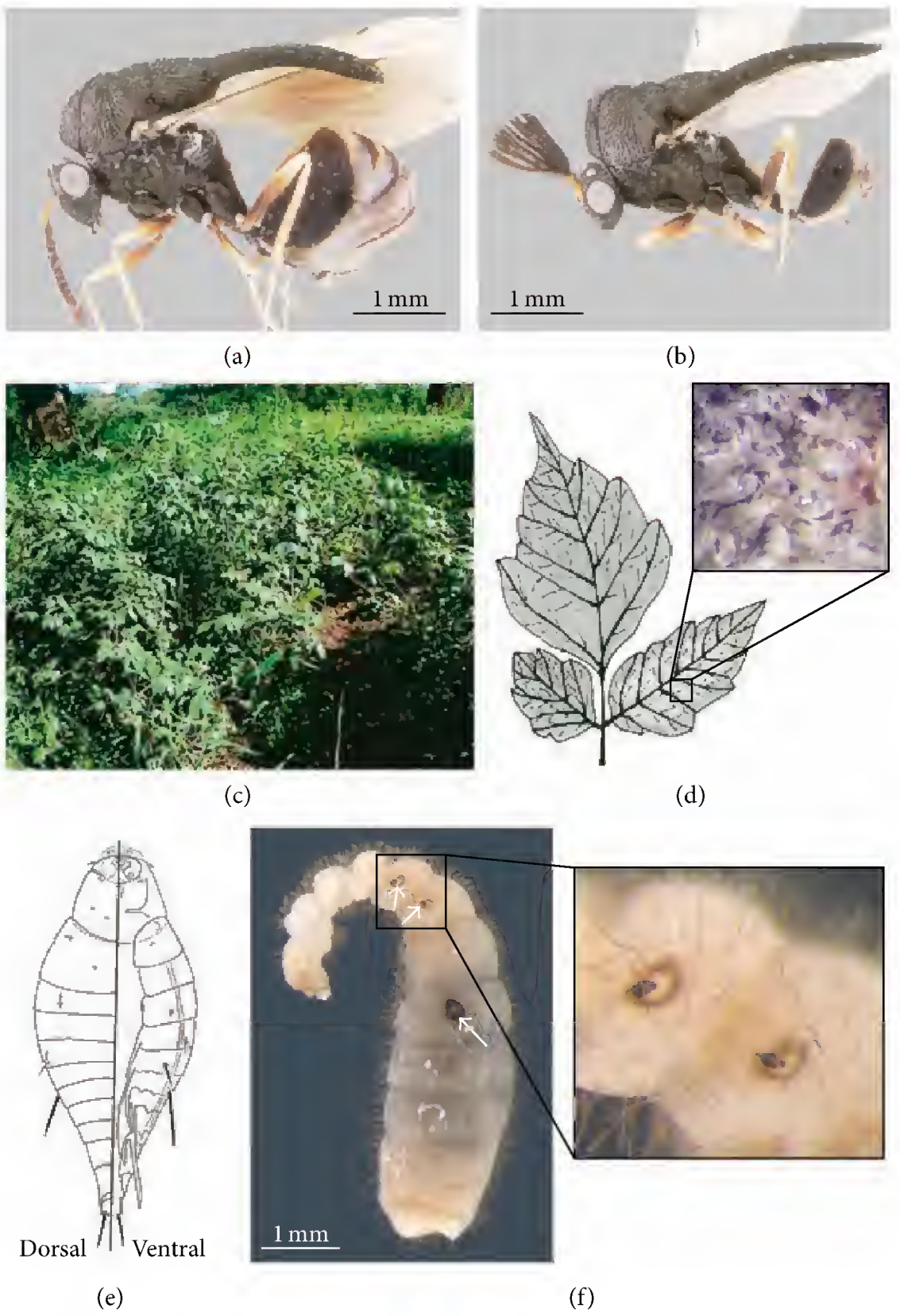


FIGURE 4: *Latina rugosa*: (a) habitus (female); (b) habitus (male). Biology and immature stage of *L. rugosa*: (c) habitat; (d) underside of leaf of *Serjania glabrata* with eggs (eggs represented in white area and magnified); (e) planidium (dorsal and ventral view); (f) ant larva parasitized (attached planidia magnified and indicated). Figures 4(d) and 4(e) are extracted from [25].

Life history and host ants are next. Females were observed ovipositing in the immature (green) fruit of *V. breviflora*, with eggs deposited in small masses within the fruit (Figure 5(f)). Only undeveloped eggs were obtained from immature fruits while mature fruits taken from the ground had mature eggs and larvae. The planidia (Figure 5(g)) crawl and do not have the ability to jump.

In the field, a species of *Camponotus* Mayr (Formicinae: Camponotini) visited and foraged below the host plant. *Camponotus* are known to collect fruit pulp and small seeds [45], and a direct interaction of foragers with the ripe fruit and planidia is very likely, as proposed for *Pseudochalcura* [9].

Nests of *Camponotus* were located under host plants or within a few meters (Figure 6(a)). In total, 35 *Lophyrocera* pupae were found in 7 of the 13 nests excavated, and of these, three had two pupae of *L. variabilis* in the same cocoon (Figure 6(b)). No larvae were found. The parasitism rate ranged from 0 to 6.21%.

3.6. Neolirata Torréns and Heraty. This genus includes three species distributed in the Neotropical region: *N. alta* (Walker) (Argentina, Brazil, and Uruguay) (Figure 6(c)), *N. daguerrei* (Gemignani) (Argentina, and Brazil) (Figures 7(a) and 7(b)), and *N. furcula* Torréns and Heraty (Brazil) [15].

Neolirata alta and *N. daguerrei* were collected in northwestern Argentina, and their taxonomic and biological information is given in Torréns and Heraty [15].

3.6.1. Neolirata alta (Walker). Habitat and location are as follows. Specimens were collected in Los Baños and Rosario de la Frontera (Salta) and Tapia and San Vicente (Tucumán). In Los Baños, the vegetation corresponds to the transition

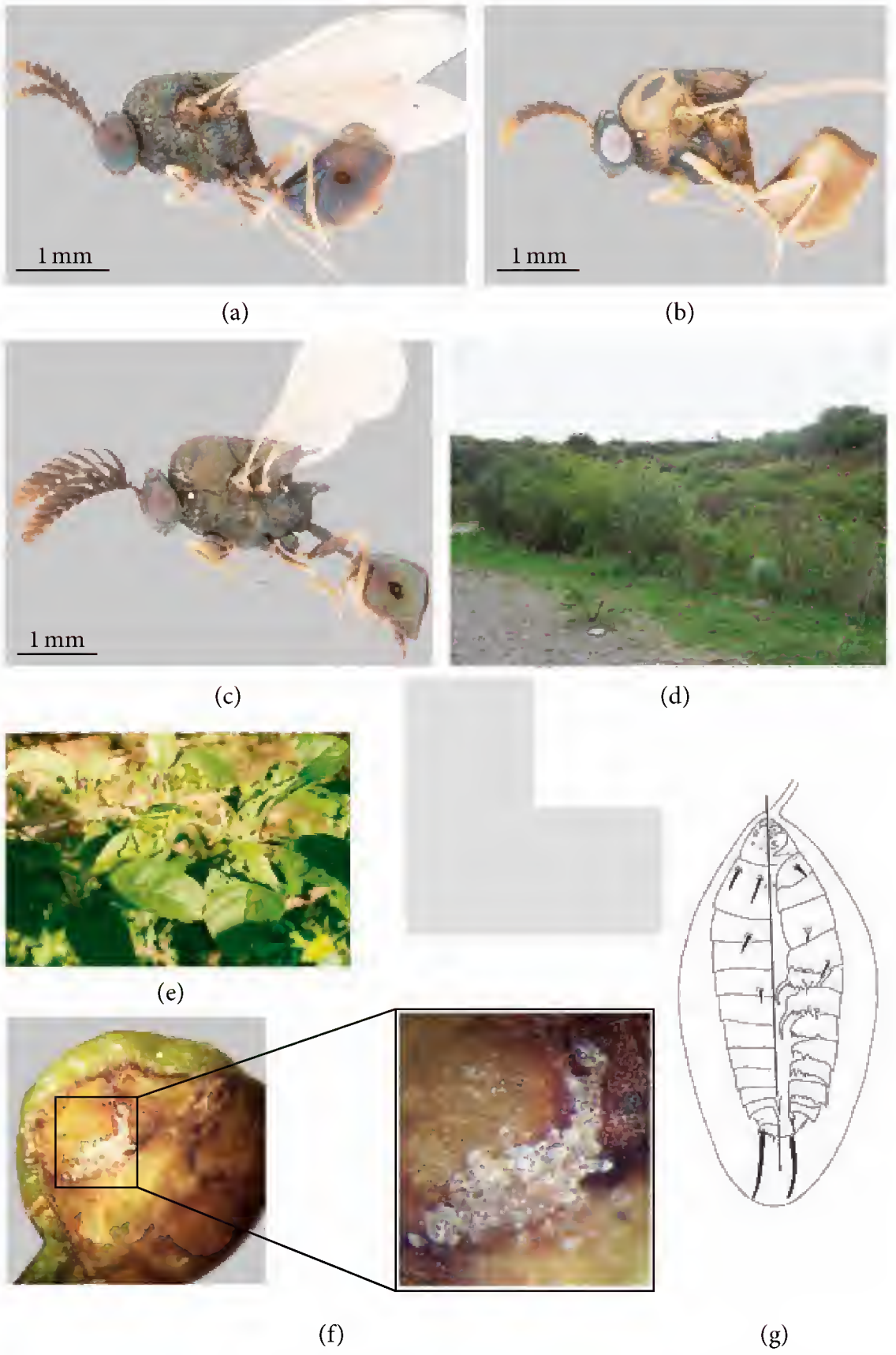


FIGURE 5: *Lophyrocera variabilis*: (a) and (b) habitus (female); (c) habitus (male). Biology and immature stages of *L. variabilis*: (d) habitat; (e) *Vasobia breviflora*; (f) saggittal section of unripe fruits of *V. breviflora* with eggs (egg mass magnified); (g) planidium (before hatching; dorsal and ventral view). Figures 5(a)–5(c), 5(f), and 5(g) are extracted from [26].

Yungas and Chaco ecoregions, while the others are typical of the Chaco ecoregion (Figure 6(d)). The host plant, *Pseudabutilon virgatum*, was widely distributed in all four areas (Figure 6(e)).

Life history and host ants are next. The female oviposited about 32 eggs per mm^2 at random between the spicules on the underside of a leaf (Figure 6(e)). Eggs hatched within 14 days. The planidia (Figure 6(f)) were mobile and have the ability to jump.

The host ant remains unknown. A few meters from where the female was collected in San Vicente (Tucumán), there was a nest of *Ectatomma brunneum*. This nest was excavated, but no immature stages were found.

3.6.2. Neolirata daguerrei (Gemignani). Habitat and location are as follows. Most specimens were collected in Tapia (Tucumán) (Figure 7(c)); the vegetation corresponds to the Chaco ecoregion [40]. The host plant, *Urvillea chacoensis* Hunz. (Sapindaceae), is a climbing vine distributed throughout the collection area; its leaves are marginally serrate and pubescent [46] (Figure 7(d)).

Life history and host ants are next. Females were observed ovipositing on the underside of leaves of *U. chacoensis*. A single gravid female oviposited about 28 eggs per mm^2 (Figure 7(d)). Eggs hatched within 9 days (Figure 7(e)). Planidia (Figure 7(f)) are very mobile and jump.

Host ant unknown.

3.7. Orasema Cameron. *Orasema* is composed of 57 species, but many are still undescribed. Their distribution is Neotropical, Nearctic, and Paleotropical [8]. In Argentina, *Orasema* is widely distributed, with 11 species documented:

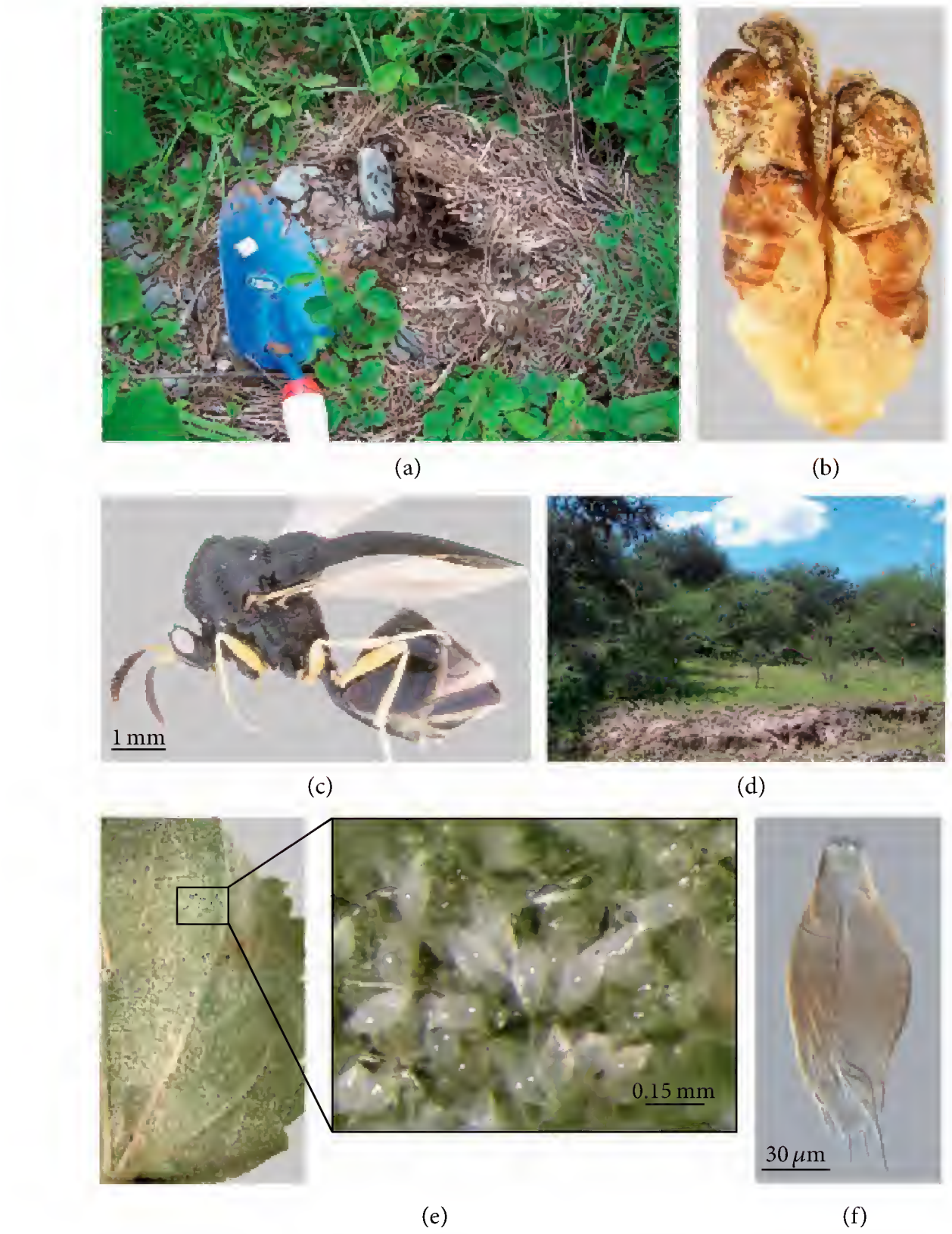


FIGURE 6: Biology and immature stage of *Lophyrocera variabilis*: (a) ant nest of *Camponotus* sp.; (b) two *Lophyrocera* pupae extract from same ant cocoon (females). *Neolirata alta*: (c) habitus (female). Biology and immature stage of *N. alta*: (d) habitat; (e) underside of leaf of *P. virgatum* with eggs (magnified area with eggs); (f) planidia. Figure 6(b) is extracted from [26], and Figures 6(c), 6(d), 6(e), and 6(f) are extracted from [15].

O. aenea Gahan, *O. argentina* Gemignani, *O. deltae* Gemignani, *O. freychei* (Gemignani), *O. gemignanii* De Santis, *O. salebrosa* Heraty, *O. simplex* Heraty, *O. susanae* Gemignani, *O. vianai* Gemignani, *O. worcesteri* (Girault), and *O. xanthopus* (Cameron).

Several authors have studied the biological aspects of *Orasema* [5, 7, 11, 27–29, 47–56]. Among the genera of ants recorded as attacked by *Orasema* are *Formica* Linnaeus, *Monomorium* Mayr, *Pheidole* Westwood, *Solenopsis* Westwood, *Temnothorax* Mayr, *Tetramorium* Mayr, *Wasmannia* Forel, and a dubious case of *Eciton* Latreille [5–8, 11, 27, 47, 54]. Immature stages were described by several authors [5–8, 11, 24, 27, 47, 48, 53, 54, 56, 57].

Herein are summarized the most relevant data on the biology of the species found in Argentina, with new data for *O. aenea* and some records of an unidentified species.

3.7.1. Orasema aenea Gahan. Habitat and location are as follows. Specimens of *O. aenea* (Figures 8(a) and 8(b)) were collected in Caimancito (Jujuy). The vegetation and geographic location corresponds to the foothills of the Yungas ecoregion. The host plant, *Tecoma stans* (L.) Juss. ex Kunth (Bignoniaceae) (common name, Guarán amarillo), is a shrub or small tree that grows 3–6 m tall, with leaves decussate with elliptic-lanceolate and serrated edges, and it blooms from August to October [40] (Figure 8(c)).

Life history and host ants are next. Females were observed ovipositing on the undersides of leaves of *T. stans* by creating an incision and laying a single egg in short linear rows (Figures 8(d) and 8(e)). Eggs hatched within 9 days. Planidia (Figure 8(f)) crawl and leave the incision but do not have the ability to jump.

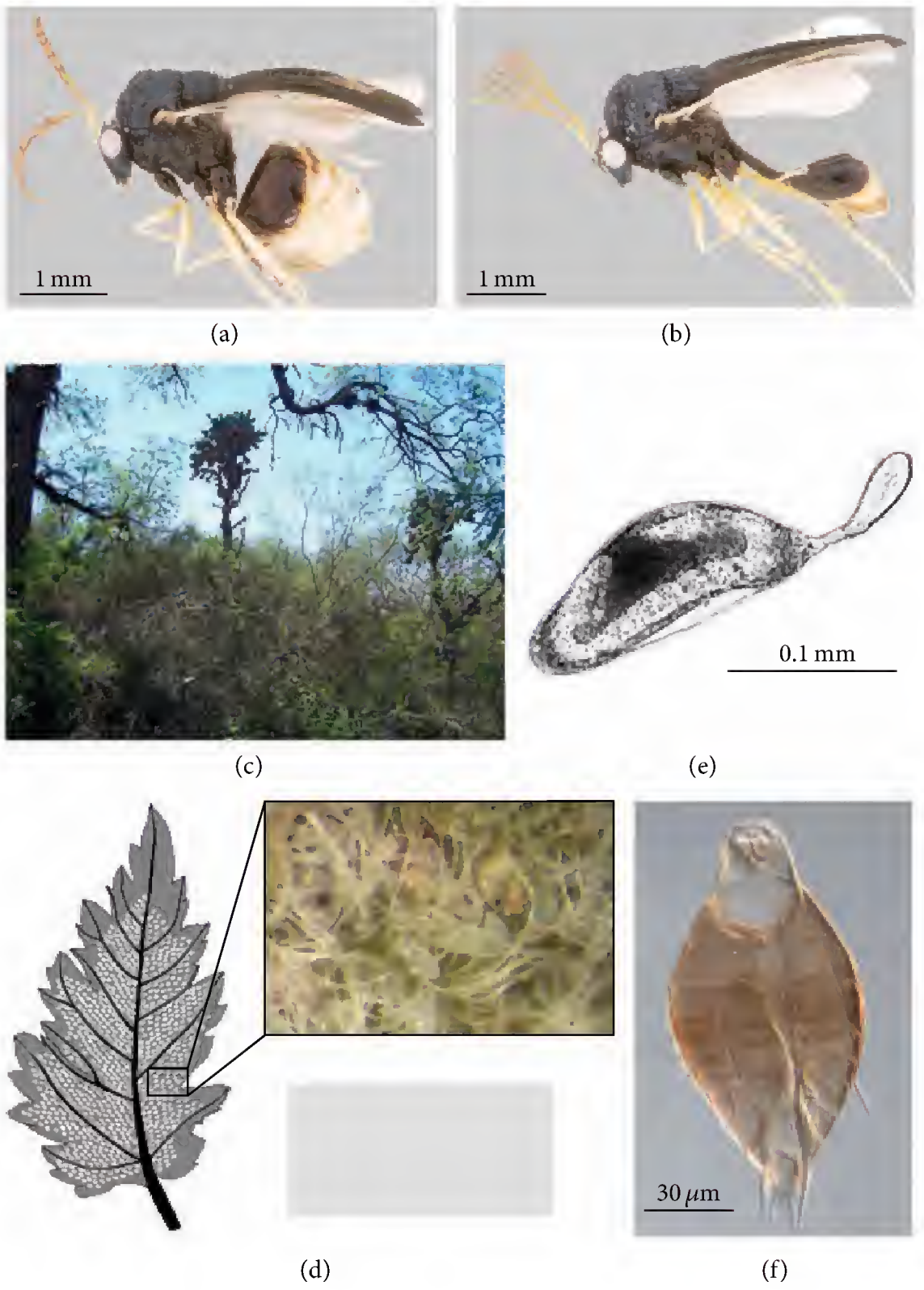


FIGURE 7: *Neolirata daguerrei*: (a) habitus (female); (b) habitus (male). Biology and immature stage of *N. daguerrei*: (c) habitat; (d) underside of leaf of *Urvillea chacoensis* with eggs (eggs represented in white area and magnified); (e) egg; (f) planidia. Figures extracted from [15].

Although host ants were not located in the area, the host has been reported as *Solenopsis quinquecuspis* Forel (Myrmicinae) [27].

Discussion. Plants used for oviposition also include *Ilex paraguayensis* A.St.-Hil. (Aquifoliaceae) (Yerba Mate) and *Olea europaea* L. (Oleaceae) (Olive) for which *Orasema* is considered as a potential pest [50, 57]. *Orasema aenea* was found on both *T. stans* and *Vaccinium corymbosum* L. (Ericaceae) (blueberry), with the latter association recorded by Varone and Briano [29].

3.7.2. Orasema argentina Gemignani. It is associated with *Pheidole nitidula* Emery (Myrmicinae) [6, 17].

3.7.3. Orasema salebrosa Heraty. It is associated with *Solenopsis invicta* Buren, *S. macdonaghi* Santschi and *S. richteri* Forel (Myrmicinae) [11, 27].

3.7.4. Orasema simplex Heraty. It is associated with *Solenopsis richteri*, *S. invicta*, and *S. quinquecuspis* (Myrmicinae) [11, 27]. Varone and Briano reported *Zea mays* L., *Glycine max* L., *Vinca rosae* L., *Citrus limon* (L.) Burn, *Capsicum annuum* L., *Smilax campestris* Griseb, *Paspalum unispicatum* (Scribn. & Merr.) Nash, *P. denticulatum* Trin., *P. notatum* Fluegge, *P. dilatatum* Poir, *Grindelia pulchella* Dann., *Stevia aff. entreriensis* Hieron, *Eupatorium aff. laevigatum* L., *Sesbania virgata* (Cav.) Pers., *Asclepias curassavica* L., Verbena *montevidensis* Spreng., *Sida rhombifolia* L., and *Stemodia aff. lanceolata* Benth. with oviposition marks of *Orasema simplex* in nonchoice laboratory tests and in field surveys [29].

3.7.5. Orasema susanae Gemignani. It is associated with *Pheidole* near *tetra* Creighton [6].

3.7.6. Orasema worcesteri (Girault). It is associated with *Pheidole radoszkowskii* Mayr (Myrmicinae) (=*P. nitidula*) [17].

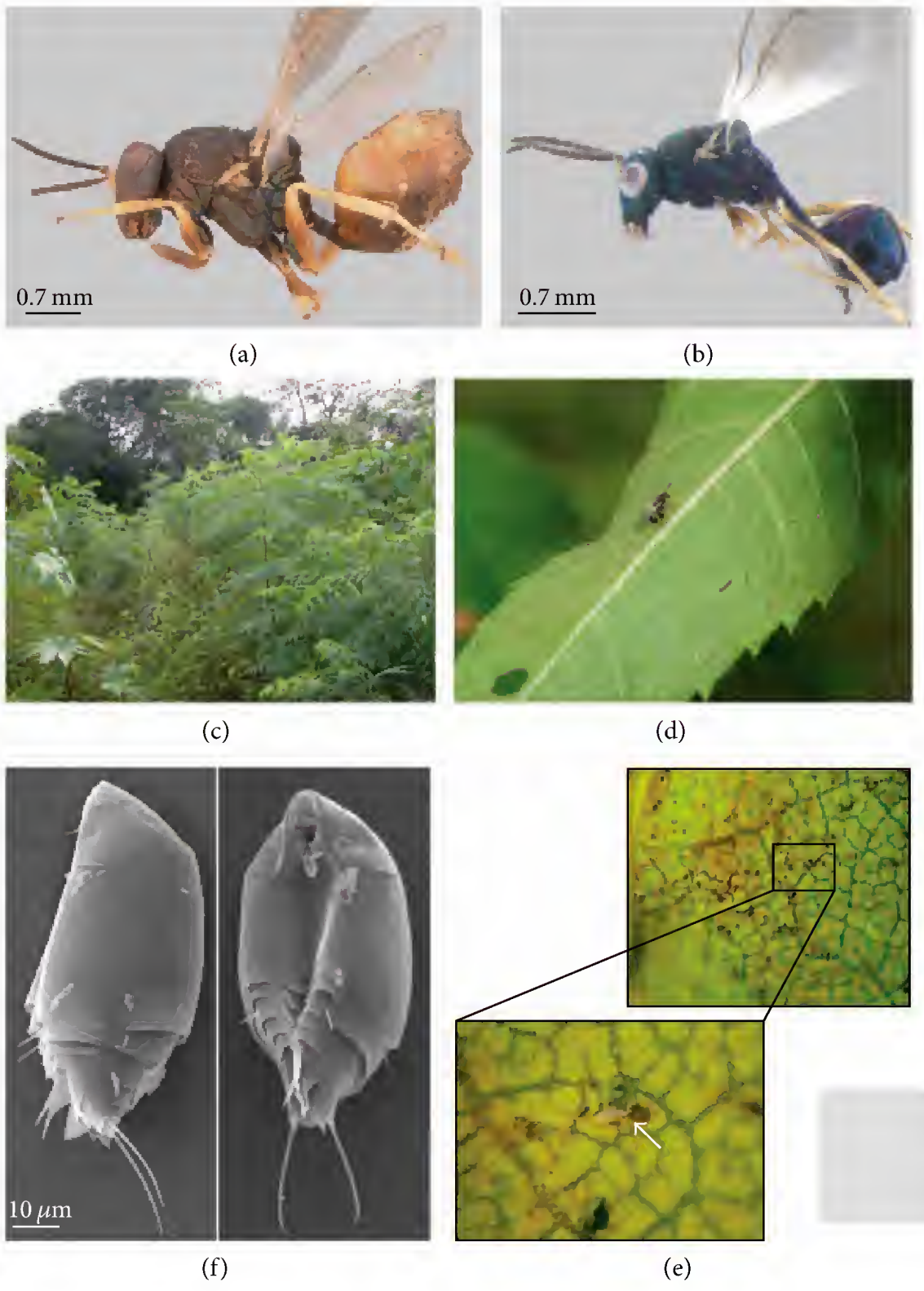


FIGURE 8: *Orasema aenea*: (a) habitus (female); (b) habitus (male). Biology and immature stage of *O. aenea*: (c) habitat; (d) female of *O. aenea* ovipositing on leaf of *Tecoma stans*; (e) underside of leaf of *T. stans* with incisions (magnified area with egg extracted from incision); (f) planidia (lateral and ventral).

3.7.7. Orasema xanthopus (Cameron). Various aspects related to its biology were recorded by several authors [11, 28, 30–39]. *Orasema xanthopus* is associated with several species of *Solenopsis*, such as *S. invicta* [11, 27, 32–35, 39, 58], *S. quinquecuspis* [27], *S. richteri* [33], and the *S. saevissima* (Smith) complex [6, 11, 35].

3.7.8. Orasema sp. Several females were collected in Villa Vil (Catamarca) ovipositing into the stem tissue below the flower buds and along the petiole and midrib of leaves of *Lantana xenica* Moldenke (Verbenaceae).

3.8. Thoracantha Latreille. This genus is comprised of three species, *Thoracantha anchura* Walker (Brazil), *T. spegazzinii* (Gemignani) (Argentina), and *T. striata* Perty (Argentina and Brazil) [8].

3.8.1. Thoracantha spegazzinii (Gemignani). A single female was collected on a flower of a Malvaceae. This data was included on the holotype label but not used in the original description of the species by Gemignani [17].

3.8.2. Thoracantha striata Perty. Heraty observed females ovipositing in patches on the underside of leaves of *Lantana* sp. (Verbenaceae); oviposition took place over 1-2 hours. Eggs and planidia were obtained [8].

4. Conclusion

Eucharitidae are found in almost all biogeographic regions in northern Argentina (Figure 9). Most genera are distributed in the Chaco ecoregion and the transition between Chaco and Yungas, but more surveys are necessary in the Monte and Pampa ecoregions, and in those it was areas never

FIGURE 9: Distribution of genera of Eucharitidae in Argentina.

surveyed for eucharitids including Central Patagonia and Prepuna. Herein we presented a new record for *Kapala sulcifacies* (Cameron) from Salta (Rosario de la Frontera, 20/03/2003; one female and two males deposited in Instituto Fundación Miguel Lillo, Tucumán, Argentina), *Lophyrocera plagiata* (Walker) from Misiones (Mado, Puerto Magdalena, 23/10/1964; one female deposited in American Museum of Natural History, NY, USA) and *Obeza semifumipennis* (Girault) from Formosa (Pirané, 31/12/1948; two males deposited in Instituto Fundación Miguel Lillo, Tucumán, Argentina).

Although we have detailed information for most genera, little or nothing is known about the biology of many species. Host relationships were summarized by Heraty [8], Lachaud and Pérez-Lachaud [59], and Lachaud et al. [42]. Herein we presented a new host association for *Galearia latreillei* from *Ectatomma brunneum* and confirm the association of *Latina rugosa* with *Odontomachus chelifer* suggested by Torréns et al. [25]. Of the remaining genera present in Argentina, ant host relationships can be inferred from species found elsewhere in South and Central America. Generally, it is expected that in Argentina, *Orasema* (Oraseminae) are exclusively found on Myrmicinae, the genera *Lophyrocera*, *Obeza*, and *Pseudochalcura* attack Camponotini (Formicinae), and the remaining genera in the *Kapala* clade all attack either Ectatomminae or Ponerinae [8, 9, 11, 12, 23, 26, 27, 29, 42, 43, 59].

Eucharitidae utilize a variety of distinct methods for oviposition. Oraseminae oviposit into incisions made in leaf tissues [2, 5, 49, 52]. Damage to the leaves can be caused by scaring of the plant tissue [7] or through secondary infections caused by the punctures [50]. Because of this, *Orasema* have been considered as potential pests of banana, citrus, olive, tea, and yerba mate [30, 31, 48, 50, 52, 53]. However, they are never regarded as a continuing pest problem. In contrast, Eucharitinae oviposit either on the undersides of the leaves, into fruits or into the bracts of flower buds, without causing cosmetic damage to the plants. However, as parasitoids of *Ectatomma*, they might have a negative impact on ants that are potential biological control agents [60]. Importantly, various details of the oviposition behavior, plant and ant host choice, behavior of the planidia both within and outside of the nest, and development within the nest are all key pieces of information to provide a better understanding of how the eucharitids gain access and specialize on their particular ant host group.

Acknowledgments

The author would like to thank J. M. Heraty and P. Fidalgo for their help in all his studies and Elizabeth Murray for constructive comments and suggestions of this paper. This investigation was made possible through funding by the Projects PIP6361 from CONICET and PICT 01238 provided by Agencia Nacional de Promoción Científica y Tecnológica. He dedicates this paper to the memory of Emma "Chongui" Wuscovi who supported him at all times in his Ph.D. degree.

References

[1] C. P. Clausen, "The immature stages of the Eucharidae," *Proceedings of the Entomological Society of Washington*, vol. 42, no. 8, pp. 161–170, 1940.

[2] C. P. Clausen, "The oviposition habits of the Eucharidae (Hymenoptera)," *Journal of the Washington Academy of Sciences*, vol. 30, no. 12, pp. 504–516, 1940.

[3] C. P. Clausen, "The habits of the Eucharidae," *Psyche*, vol. 48, pp. 57–69, 1941.

[4] J. M. Heraty, "A revision of the Nearctic Eucharitinae (Hymenoptera: Chalcidoidea: Eucharitidae)," *Proceedings of the Entomological Society of Ontario*, vol. 85, pp. 61–103, 1985.

[5] J. M. Heraty, "Classification and evolution of the Oraseminae in the Old World, with revisions of two closely related genera of Eucharitinae (Hym: Eucharitidae)," *Life Sciences Contributions (Royal Ontario Museum)*, vol. 157, pp. 1–174, 1994.

[6] J. M. Heraty, "Biology and importance of two eucharitid parasites of *Wasmannia* and *Solenopsis*," in *Exotic Ants: Biology, Impact and Control of Introduced Species*,, D. Williams, Ed., pp. 104–120, Westview Press, Boulder, Colo, USA, 1994.

[7] J. M. Heraty, "Phylogenetic relationships of Oraseminae (Hymenoptera: Eucharitidae)," *Annals of the Entomological Society of America*, vol. 93, no. 3, pp. 374–390, 2000.

[8] J. M. Heraty, "A revision of the genera of Eucharitidae (Hymenoptera: Chalcidoidea) of the world," *Memoirs of the American Entomological Institute*, vol. 68, pp. 1–367, 2002.

[9] J. M. Heraty and K. N. Barber, "Biology of *Obeza floridana* (Ashmead) and *Pseudochalcura gibbosa* (Provancher) (Hymenoptera: Eucharitidae)," *Proceedings of the Entomological Society of Washington*, vol. 92, no. 2, pp. 248–258, 1990.

[10] C. P. Clausen, "The biology of *Schizaspidia tenuicornis* Ashm., a eucharid parasite of *Camponotus*," *Annals of the Entomological Society of America*, vol. 16, pp. 195–217, 1923.

[11] J. M. Heraty, D. P. Wojcik, and D. P. Jouvenaz, "Species of *Orasema* parasitic on the *Solenopsis saevissima*-complex in South America (Hymenoptera: Eucharitidae, Formicidae)," *Journal of Hymenoptera Research*, vol. 2, no. 1, pp. 169–182, 1993.

[12] G. Pérez-Lachaud, J. M. Heraty, A. Carmichael, and J.-P. Lachaud, "Biology and behavior of Kapala (Hymenoptera: Eucharitidae) attacking *Ectatomma*, *Gnamptogenys*, and *Pachycondyla* (Formicidae: Ectatomminae and Ponerinae) in Chiapas, Mexico," *Annals of the Entomological Society of America*, vol. 99, no. 3, pp. 567–576, 2006.

[13] S. C. Buys, R. Cassaro, and D. S. Alomon, "Biological observations on *Kapala* Cameron 1884 (Hymenoptera Eucharitidae) in parasitic association with *Dinoponera lucida* Emery 1901 (Hymenoptera Formicidae) in Brazil," *Tropical Zoology*, vol. 23, no. 1, pp. 29–34, 2010.

[14] J. Torréns, *Estudios de Biología y Taxonomía de Eucharitidae (Hymenoptera: Parasitica) de Argentina, parasitoides de Formicidae (Hymenoptera: Aculeata) [Ph.D. thesis]*, Universidad Nacional de Tucumán, Tucumán Province, Argentina, 2010.

[15] J. Torréns and J. M. Heraty, "A new genus of Eucharitidae (Hymenoptera: Chalcidoidea), with notes on life history and immature stages," *Zootaxa*, vol. 3630, no. 2, pp. 347–358, 2013.

[16] J. Torréns, "Primer registro de *Colocharis* (Chalcidoidea: Eucharitidae) en la Argentina y descripción de una nueva especie," *Revista de la Sociedad Entomológica Argentina*, vol. 71, no. 3-4, pp. 215–218, 2012.

[17] E. V. Gemignani, "La familia " Eucharidae" (Hymenoptera: Chalcidoidea) en la República Argentina," *Anales del Museo Nacional de Historia Natural*, vol. 37, pp. 477–493, 1933.

[18] E. V. Gemignani, "Nueva nota sobre la familia Eucharidae (Hymenoptera: Chalcidoidea)," *Anales del Museo Nacional de Historia Natural*, vol. 39, pp. 159–166, 1937.

[19] E. V. Gemignani, "Nuevas especies de la familia Eucharidae (Insecta, Hymenop. Chalcid.)," *Comunicaciones del Museo Argentino de Ciencias Naturales, "Bernardino Rivadavia"*, vol. 1, pp. 1–15, 1947.

[20] J. J. Morrone, *"Biogeografía de América Latina y el Caribe*, vol. 3 of *M&T-Manuales & Tesis SEA*, Sociedad Entomológica Aragonesa, Zaragoza, Spain, 2001.

[21] C. Bertonatti and J. Corcuera, *Situación Ambiental Argentina*, Fundación Vida Silvestre Argentina, Buenos Aires, Argentina, 2000.

[22] J. Torréns and J. M. Heraty, "Description of the species of *Dicoelothorax* Ashmead (Chalcidoidea, Eucharitidae) and biology of *D. platycerus* Ashmead," *ZooKeys*, vol. 165, pp. 33–46, 2012.

[23] J. M. Heraty and J. B. Woolley, "Separate species or polymorphism: a recurring problem in Kapala (Hymenoptera: Eucharitidae)," *Annals of the Entomological Society of America*, vol. 86, no. 5, pp. 517–531, 1993.

[24] J. M. Heraty and D. C. Darling, "Comparative morphology of the planidial larvae of Eucharitidae and Perilampidae (Hymenoptera: Chalcidoidea)," *Systematic Entomology*, vol. 9, no. 3, pp. 309–328, 1984.

[25] J. Torréns, J. M. Heraty, and P. Fidalgo, "Biology and description of a new species of Laurella Heraty (Hymenoptera: Eucharitidae) from Argentina," *Proceedings of the Entomological Society of Washington*, vol. 109, no. 1, pp. 45–51, 2007.

[26] J. Torrens, J. M. Heraty, and P. Fidalgo, "Biology and description of a new species of *Lophyrocera* Cameron (Hymenoptera: Eucharitidae) from Argentina," *Zootaxa*, no. 1871, pp. 56–62, 2008.

[27] L. Varone, J. M. Heraty, and L. A. Calcaterra, "Distribution, abundance and persistence of species of *Orasema* (Hym: Eucharitidae) parasitic on fire ants in South America," *Biological Control*, vol. 55, no. 1, pp. 72–78, 2010.

[28] R. K. Vander Meer, D. P. Jouvenaz, and D. P. Wojcik, "Chemical mimicry in a parasitoid (Hymenoptera: Eucharitidae) of fire ants (Hymenoptera: Formicidae)," *Journal of Chemical Ecology*, vol. 15, no. 8, pp. 2247–2261, 1989.

[29] L. Varone and J. Briano, "Bionomics of *Orasema simplex* (Hymenoptera: Eucharitidae), a parasitoid of *Solenopsis* fire ants (Hymenoptera: Formicidae) in Argentina," *Biological Control*, vol. 48, no. 2, pp. 204–209, 2009.

[30] A. Tocchetto, "Bicho costureiro," *Revista Agronomica, Porto Alegre*, vol. 6, pp. 587–588, 1942.

[31] F. S. Roberts, "Insects affecting banana production in central America," in *Proceedings of the 10th International Congress of Entomology*, vol. 3, pp. 411–415, 1958.

[32] A. Silveira-Guido, "Investigations on the biology and biological control of the fire ant, *Solenopsis saevissima richteri* Forel in Uruguay," Annual Report, Departamento de Sanidad Vegetal, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay, 1962.

[33] A. Silveira-Guido, P. San-Martin, C. Crisci-Pisano, and J. Carbonnell-Bruhn, "Investigations on the biology and biological control of the fire ant, *Solenopsis saevissima richteri* Forel in Uruguay," Third Report, Departamento de Sanidad Vegetal, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay, 1964.

[34] R. N. Williams, "Insect natural enemies of fire ants in South America with several new records," in *Proceedings of the Tall Timbers Conference on Ecology, Animal Control and Habitat Management*, vol. 7, pp. 123–134, 1980.

[35] R. N. Williams and W. H. Whitcomb, "Parasites of fire ants in South America," in *Proceedings of the Tall Timbers Conference on Ecology, Animal Control and Habitat Management*, vol. 5, pp. 49–59, 1973.

[36] D. P. Wojcik, "Status and search for natural control agents in South America," in *Proceedings of the Annual Fire Ant Conference*, vol. 5, pp. 29–37, 1986.

[37] D. P. Wojcik, "Survey for biocontrol agents in Brazil: a final report, with comments on preliminary research in Argentina,"

in *Proceedings of the Imported Fire Ant Conference*, pp. 50–62, Athens, Ga, USA, 1988.

[38] D. P. Wojcik, "Behavioural interactions between ants and their parasites," *The Florida Entomologist*, vol. 72, no. 1, pp. 43–51, 1989.

[39] D. P. Wojcik, D. P. Jouvenaz, W. A. Banks, and A. C. Pereira, "Biological control agents of fire ants in Brazil," in *Chemistry and Biology of Social Insects*, J. Eder and H. Rembold, Eds., pp. 627–628, J. Peperny, München, Germany, 1987.

[40] A. P. L. Digilio and P. R. Legname, "Los árboles indígenas de la provincia de Tucumán," *Opera Lilloana*, vol. 15, pp. 1–283, 1966.

[41] D. M. Lapola, W. F. Aantonalli Jr., and E. Giannotti, "Arquitetura de ninhos da formiga neotropical *Ectatomma brunneum* F. Smith, 1858 (Formicidae: Ponerinae) em ambientes alterados," *Revista Brasileira de Zoociências*, vol. 5, no. 2, pp. 177–188, 2003.

[42] J.-P. Lachaud, P. Cerdan, and G. Pérez-Lachaud, "Poneromorph ants associated with parasitoid wasps of the genus Kapala Cameron (Hymenoptera: Eucharitidae) in French Guiana," *Psyche*, vol. 2012, Article ID 393486, 6 pages, 2012.

[43] G. Pérez-Lachaud, J. A. López-Méndez, and J. P. Lachaud, "Eucharitid parasitism of the Neotropical ant *Ectatomma tuberculatum*: parasitoid co-occurrence, seasonal variation, and multiparasitism," *Biotropica*, vol. 38, no. 4, pp. 574–576, 2006.

[44] J. Pensiero, J. D. Muñoz, and V. Martinez, "Alternativas de sustentabilidad del bosque nativo del Espinal. Área Etnobotánica," Proyecto de Investigación Aplicada a los Recursos Forestales Nativos (PIARFON), January 2013, http://www.ambiente.gov.ar/?idarticulo=949.

[45] D. W. Davidson, "Ecological studies of neotropical ant gardens," *Ecology*, vol. 69, no. 4, pp. 1138–1152, 1988.

[46] M. S. Ferrucci, "Sapindaceae Juss. Guías ilustradas de clases," *Aportes Botánicos de Salta. Serie Flora*, vol. 7, no. 4, pp. 1–44, 2004.

[47] W. M. Wheeler, "The polymorphism of ants, with an account of some singular abnormalities due to parasitism," *Bulletin of the American Museum of Natural History*, vol. 23, pp. 1–93, 1907.

[48] G. C. Wheeler and E. W. Wheeler, "New hymenopterous parasites of ants (Chalcidoidea: Eucharidae)," *Annals of the Entomological Society of America*, vol. 30, no. 1, pp. 163–175, 1937.

[49] H. L. Parker, "Oviposition habits and early stages of *Orasema* sp.," *Proceedings of the Entomological Society of Washington*, vol. 44, no. 7, pp. 142–145, 1942.

[50] J. C. Nicolini, "La avispa costurera y la tuberculosis del olivo," *Revista Agronomica, Porto Alegre*, vol. 20, pp. 35–36, 1950.

[51] A. F. van Pelt, "*Orasema* in nests of *Pheidole dentata* Mayr (Hymenoptera: Formicidae)," *Entomological News*, vol. 61, no. 6, pp. 161–163, 1950.

[52] G. M. Das, "Preliminary studies on the biology of *Orasema assectator* Kerrich (Hym., Eucharitidae), parasitic on *Pheidole* and causing damage to leaves of tea in Assam," *Bulletin of Entomological Research*, vol. 54, no. 3, pp. 373–379, 1963.

[53] G. J. Kerrich, "Descriptions of two species of Eucharitidae damaging tea, with comparative notes on other species (Hym., Chalcidoidea)," *Bulletin of Entomological Research*, vol. 54, no. 3, pp. 365–372, 1963.

[54] J. B. Johnson, T. D. Miller, J. M. Heraty, and F. W. Merickel, "Observations on the biology of two species of *Orasema* (Hym.: Eucharitidae)," *Proceedings of the Entomological Society of Washington*, vol. 88, no. 3, pp. 542–549, 1986.

[55] B. Carey, K. Visscher, and J. Heraty, "Nectary use for gaining access to an ant host by the parasitoid *Orasema simulatrix* (Hymenoptera, Eucharitidae)," *Journal of Hymenoptera Research*, vol. 27, pp. 47–65, 2012.

[56] J. M. Heraty, *Classification and evolution of the Oraseminae (Hymenoptera: Eucharitdae) [Ph.D. thesis]*, Texas A&M University, College Station, Tex, USA, 1990.

[57] A. B. Gahan, "A contribution to the knowledge of the Eucharidae (Hymenoptera: Chalcidoidea)," *Proceedings of the United States National Museum*, vol. 88, pp. 425–458, 1940.

[58] L. de Santis, "Una nueva especie de "*Orasema*" del Uruguay (Hymenoptera: Eucharitidae)," *Revista Sociedad Entomológica Argentina*, vol. 7, pp. 1–3, 1968.

[59] J.-P. Lachaud and G. Pérez-Lachaud, "Diversity of species and behavior of hymenopteran parasitoids of ants: a review," *Psyche*, vol. 2012, Article ID 134746, 24 pages, 2012.

[60] J.-P. Lachaud and G. Pérez-Lachaud, "Impact of natural parasitism by two eucharitid wasps on a potential biocontrol agent ant in southeastern Mexico," *Biological Control*, vol. 48, no. 1, pp. 92–99, 2009.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 541804, 7 pages
http://dx.doi.org/10.1155/2013/541804

Review Article

Rossomyrmex, the Slave-Maker Ants from the Arid Steppe Environments

F. Ruano,[1] O. Sanllorente,[1,2] A. Lenoir,[3] and A. Tinaut[1]

[1] *Departamento de Zoología, Universidad de Granada, 18071 Granada, Spain*
[2] *Departamento de Biología Experimental, Facultad de Ciencias Experimentales, Universidad de Jaén, Campus Las Lagunillas s/n, 23071 Jaén, Spain*
[3] *Institut de Recherche sur la Biologie de l'Insecte, IRBI-UMR CNRS 7261, Faculté des Sciences et Techniques, Université François Rabelais, 37200 Tours, France*

Correspondence should be addressed to F. Ruano; fruano@ugr.es

Received 8 March 2013; Accepted 9 May 2013

Academic Editor: David P. Hughes

The host-parasite genera *Proformica-Rossomyrmex* present four pairs of species with a very wide range of distribution from China to Southeastern Spain, from huge extended plains to the top of high mountains. Here we review (1) the published data on these pairs in comparison to other slave-makers; (2) the different dispersal ability in hosts and parasites inferred from genetics (chance of migration conditions the evolutionary potential of the species); (3) the evolutionary potential of host and parasite determining the coevolutionary process in each host-parasite system that we treat to define using cuticular chemical data. We find a lower evolutionary potential in parasites than in hosts in fragmented populations, where selective pressures give advantage to a limited female parasite migration due to uncertainty of locating a host nest. A similar evolutionary potential is detected for hosts and parasites when the finding of host nests is likely (i.e., in continuous and extended populations). Moreover, some level of local adaptation at CHC profiles between host and parasite exists independently of the kind of geographic distribution and the ability of dispersal of the different populations. Similarity at CHC profiles appears to be a trait imposed by natural selection for the interaction between hosts and slave-makers.

1. Introduction

Slave-making ants are a type of permanent social parasites (thus depending on enslaved hosts ants throughout their whole live) whose newly mated queens need to usurp a host nest in order to initiate a new parasite colony. Then the host brood will turn into slaves working for the parasite species while parasite workers only concentrate on replenishing the labour force from neighboring host nests, a process called slave raiding (see reviews [1–4]).

The slave-maker style of life imposes selection pressures to both parts, as frequent slave raids strongly affect host populations and on the other hand, invading a host nest by parasite queens is determinant for their survival (see [2, 5, 6]). In this sense the study of host-parasite systems allows the study of coevolutionary strategies.

Within the subfamily Formicinae only two genera fit the previous definition of slave-makers: *Polyergus* and *Rossomyrmex* [5–7]. The species of the *Formica sanguinea* group are facultative slave-makers [8, 9]. Thus, in relation with the obligate slave-maker genera most of the published studies are focused on *Polyergus* biology (e.g., [10–15]) whereas the genus *Rossomyrmex* has received little attention, probably due to its geographic distribution and biology. However, this genus presents unique raiding [7, 16] and mating [17] behaviors in ants (for a comparison with other Formicini genera see Table 1) that make its study very interesting from an evolutionary point of view.

To date there are four species of the slave-making ants *Rossomyrmex* and, to our knowledge, each parasite species has a single host from the genus *Proformica*, thus forming unique coevolving pairs: *R. proformicarum* Arnoldi 1928—*P. epinotalis* Kuznetsov-Ugamsky 1927 from Caucasus and Volga plains (Russia), *R. quandratinodum* Xia and Zeng 1995—*P.* sp. (Kazakhstan and China), and *R. anatolicus* Tinaut 2007—*P. korbi* Emery 1909 (from Turkey). These Asian

Table 1: Some traits about the biology of the three Formicini slave-making genera.

	Rossomyrmex	*Polyergus*	*F. sanguinea* group
Parasitism	Obligate	Obligate	Facultative
Recruitment	Transport of workers to the target nest	Group recruitment	Group recruitment
Raiding	(i) No use of semiochemicals (ii) Rare fights (iii) Host-nest exploitation extended in time (2 days) (iv) Not reraiding on the same nest (v) Average 2 raids/year (vi) Slaves do not participate	(i) Alarm semiochemicals (ii) Some fights (iii) Intense and quick host-nest exploitation (<1 h) (iv) Reraiding on the same nest (v) Maximum 50 raids/year (vi) Slaves do not participate	(i) No use of semiochemicals (ii) Intense fights (iii) Intense host-nest exploitation (several hours) (iv) Reraiding on the same nest (v) More than 26 raids/year (vi) Slaves participate
Mating	(i) Sexual calling (ii) Return to the mother nest after mating (iii) Polygamous male (iv) Single female mating: monandry (with some exceptions)	(i) Mating on the ground or even during raids (ii) Variable. Return to the mother nest after mating, fly away (iii) ? (iv) Single female mating: monandry (with some exceptions)	(i) Nuptial flight, intranidal mating (ii) Return to the own or conspecific nest after mating (iii) ? (iv) Multiple mating: polyandry
Sex allocation	Female biased	?	Female biased
Foundation	(i) Usurpation (ii) New queen enters a host nest alone (iii) repellent substance from Dufour's gland (Tetradecanal)	(i) Usurpation (ii) New queen enters a host nest during a raid (iii) Appeasement substance from Dufour's gland (decyl butanoate)	(i) Variable (adoption, usurpation) (ii) New queen enters a host nest during a raid (iii) Substances from Dufour's gland of unknown effect (n-decyl acetates)

Figure 1: Distribution of the studied species: Spain (with three *Rossomyrmex minuchae* populations: SN = Sierra Nevada, SG = Sierra de Gador, and SF = Sierra de Filabres), Turkey (with two *R. anatolicus* populations: BB = Belembaçi Beli, ZT = Ziyaret Tepesi), and Kazakhstan (one *R. quandratinodum* population: CC = Charyn Canyon) (from [20]).

parasite-host pairs live mostly in extended plains whereas the Spanish pair *R. minuchae* Tinaut 1981—*P. longiseta* Collingwood 1978 inhabits the top of three high mountains in southern Spain (Figure 1). Despite this apparent difference in habitat (extended plains versus high mountains), the abiotic conditions are quite similar and are consistent with a typical arid steppe [7, 18, 19]. However, the main difference comes from the fact that the Spanish populations are small and are geographically isolated from each other [20].

The most studied pair is *R. minuchae-P. longiseta*, and in the last years we obtained data on Asian *R. anatolicus-P. korbi* and *R. quandratinodum-P.* sp. pairs. Dispersal ability of hosts and parasites and how this trait conditions the genetics and distribution of the species and its coevolution are principal goals of many of the articles recently published in slave-making ants.

2. A Singular Biology

The reproductive behavior of slave-making ants usually consists in synchronous emergence of sexuals followed by a nuptial flight and the invasion of a host nest [21], but also in some cases females display a mating call around the natal nest to attract males and immediately after mating search for a host nest to usurp (e.g., [2]). However, the reproductive strategy of *Rossomyrmex* greatly differs from the one described above. Males and females emerge from the natal nest at a different time during the day and males always fly away short after their emergence. Virgin females of *Rossomyrmex* show a typical mating call behavior near the natal nest but due to the scarce number of nests and that sexuals are not produced every year in all nests, some females remain virgin and cannot produce new nests despite performing sexual calling chorus for several days [17]. When a male arrives at a female-calling nest, he will mate to as many females as possible, being one of the few cases known of polygamous males in ants [17, 22], especially when mating occurs out of the nest. In contrast, females are strictly monandrous although there are some reported cases of multiply mated queens [20]. Females recently mated always run to hide in their natal nest after the first copulation and do not seek for subsequent mating [17]. This reproductive behavior seems to be constrained by

the low production of sexuals, especially males (which gives advantage to female-calling behavior rather than nuptial flights and multiple mating by males).

Newly mated queens search for a host nest to invade and they are unchallenged by host workers and queens thanks to the repellent effect of the Dufour's gland that they have highly inflated before the usurpation. After taking over the host nest by killing the resident queens, the size of this gland decreases [23]. This strategy to invade a host nest contrasts with other extended strategy consisting in newly mated queens embarking in a slave raid with workers, which would facilitate the penetration of the host nest immersed in chaos [2, 3, 9].

As stated before, parasitized nests need to replenish the host workers periodically and this is achieved by raiding. The normal process is that after finding a new host nest to invade, the parasite worker marks the way to its nest with pheromones and afterwards fellow slave-makers are attracted in few seconds. Then they go quickly to the targeted host nest, attack it, and carry as many larvae and pupae as possible and return to their nest following the same trail marked by the pheromone [14]. Workers of the attacked nest can fight or flee although in *Proformica* the most common behaviour is flight probably because hosts always lose fights [24]. Interestingly, *Rossomyrmex* is the only reported slave-maker that exclusively uses adult transport and single recruitment chain instead of pheromones during raids [7, 16, 19], a behavior probably constrained by the arid habitat: raids take place in early summer when soil surface temperature can reach up to 30°C, a temperature for which pheromones would quickly evaporate [6, 25]. This condition imposes that *Rossomyrmex* raids appears as less efficient than those carried out with pheromones; this together with the usually flee behavior of the *Proformica* hosts [19] permits the survival of several attacked nests [24]. Finally, another important difference in the raiding behavior of *Rossomyrmex* is that the return to the parasite nest with the robbed brood takes place at the following day of the assault instead of later in the same day [7].

3. Dispersal Abilities Evidence and Evolutionary Potential Inferred from Genetics

In the *Proformica-Rossomyrmex* system, dispersal ability is quite different for host and parasite species. The ant genus *Proformica* is generally polygynous (multiple queen colonies) with wingless queens that found new nests by budding [26]; therefore they are likely to show restricted dispersal and strong population structure. The genus *Rossomyrmex* is monogynous (single queen colonies), with both sexes winged and show independent colony founding [17, 27, 28]. In the species studied we can distinguish between *R. minuchae*, living on the top of three different mountains and the Asian species living in continuous plains, without apparent geographical barriers.

Dispersal is a crucial life-history trait determining genetic variability and sometimes the survival of entire populations [29]. The coevolutionary trajectories of hosts and parasites are mostly affected by the difference in migration [30], so that if the migration rate of the parasite is lower than that of the host, the host is expected to present stronger local adaptation to the parasite than vice versa [31, 32]. Population genetics theory states that genetic diversity is positively correlated with population size and this, in turn, is reduced as a consequence of the habitat fragmentation [33].

In agreement with this, *R. anatolicus* from Turkey shows higher levels of microsatellite variation than *R. minuchae* but lower population differentiation (even 425 km distant) than in the Spanish species, whose genetic differences among populations were highly significant [20]. Likewise *R. anatolicus* presents a lack of mitochondrial haplotype variation (for cytochrome oxidase c gene), confirming a continuous distribution of the species in the Turkish extended steppe. In contrast, the Spanish *R. minuchae* populations presented a highly significant population differentiation for this trait, clearly separated in different high mountains, but with very low and nonsignificant within population differences [34]. These results from microsatellites and mitochondrial COI likely reflect a history of long-term fragmentation for *R. minuchae*, compared to a more continuous distribution for *R. anatolicus*.

On the other hand, relative levels of gene flow and population sizes of hosts and parasites determine their coevolutionary potential and are therefore among the main determinants of the coevolutionary dynamics. Parasites have usually been predicted to have an evolutionary advantage, leading the coevolutionary process [35, 36], although in some studies a similar evolutionary potential for hosts and parasites has been described [37], or even lower for parasites than for hosts [38].

In the Spanish *R. minuchae-P. longiseta* parasite-host system the estimates of gene flow for both species resulted in great differences, being in the host an order of magnitude higher [39]. Therefore there is a good probability that these estimates indicate a higher migration rate for the host species (despite females being wingless) than the parasite, which would be interpreted as to they are more prone for local adaptation due to a higher evolutionary potential than in the parasite, as occurred in other slave-maker ants [38]. The existence of this disequilibrium suggests that natural selection can act favoring low dispersal in slave-making ants living in fragmented habitats. In this case a short range dispersal can be selective for ensuring the possibility of finding a host nest in the same population, with an appropriate density, and in which hosts can be locally adapted to the parasite (more similar CHCs ensuring tolerance) [24]. In fact, adaptation of the parasite to the host is the result of the strength of natural selection and the evolutionary potential of the parasite [35].

In contrast to this result, we did not find significant differences in genetic diversity and population differentiation for *R. anatolicus* with a mean gene diversity of 0.657 ± 0.07 (SE) [20], similar to that of its host *P. korbi* (0.70 ± 0.06) (unpublished). In the Asian extended plains host and parasite showed a similar dispersal ability and evolutionary potential, as a result of a continuous host distribution not offering obstacles to the spread of the parasite.

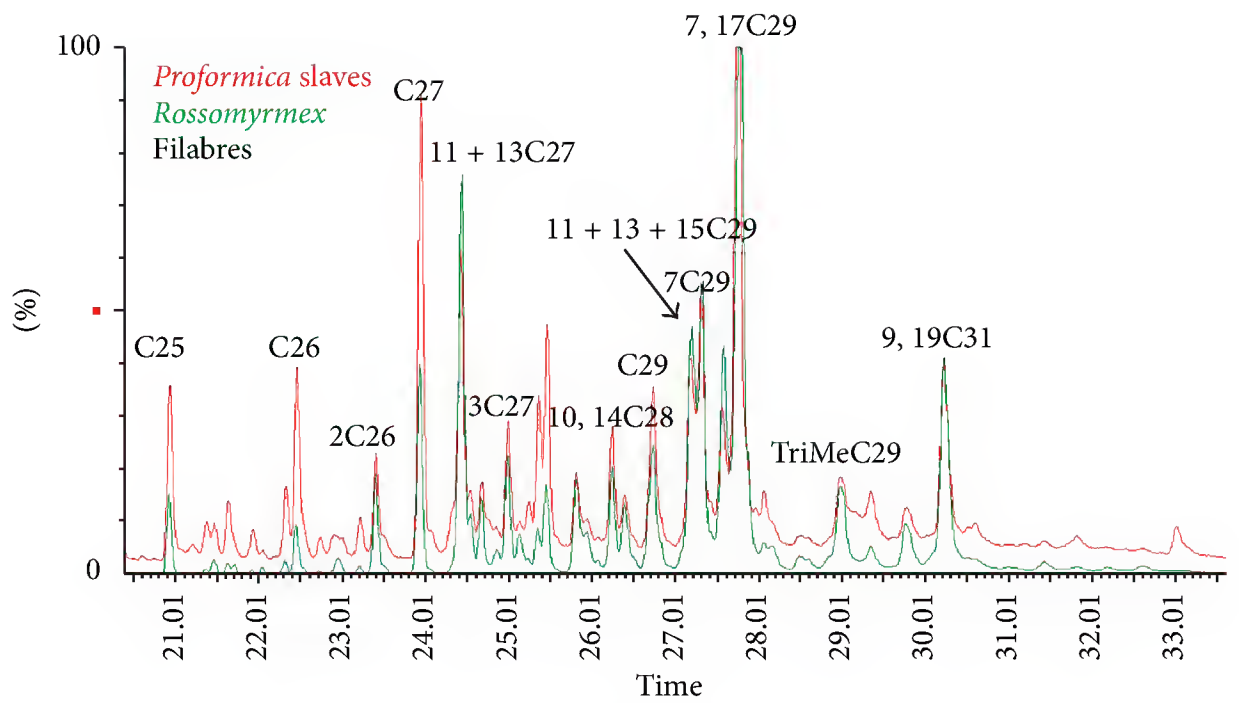


Figure 2: CHCs profiles of *R. minuchae* and *P. longiseta* (Sierra de Filabres population). The profiles are superposed to show the similarity between the host and parasite with some differences, for example, in alkanes C25, C26, and C27.

4. Cuticular Hydrocarbons as a Tool to Study Coevolution

Nestmate recognition is a key trait in social insect organization, which is essential to avoid parasitism, predation, and competition [5]. In this sense, cuticular hydrocarbons (CHCs) have been demonstrated to play a main role in nestmate recognition [40] and usually each ant species has its own chemical profile [41, 42]. Social parasites such as slave-makers are able to cheat their hosts chemically by actively acquiring or evolving similar cuticular profiles of their hosts (see [43]) in order to favor social integration in the nest and avoid aggression [44]. Hence, chemical distances between CHC profiles are a useful trait to study local host-parasite coevolution and adaptation, as a measure of recognition ability and potential aggression between host and parasite [24, 39, 45, 46].

R. minuchae and its *host P. longiseta* have exactly the same cuticular hydrocarbons, as predicted in a host-parasite acceptance in the same nest. However, small quantitative differences between host and parasite profiles indicate that they are able to recognize each other (Figure 2). Combined chemical and behavioral studies conducted in the *R. minuchae-P. longiseta* system showed that sympatric hosts were chemically closer to the parasites than to allopatric hosts despite being from the same species. This result was also supported by a reduced aggression between sympatric parasites and hosts compared to allopatric hosts [24]. Hosts that better match the chemical profile of the parasite have a higher survival chance during raids. This possibility comes from the fact that slave-makers would not benefit from a less virulent behavior (given that they always win the fights) if host densities are constantly high [30], as it is the case of *P. longiseta* [47]. Contrarily, in other host-parasite systems involving phylogenetically distant species (*Maculinea-Myrmica* species [45]), the coevolutionary outcome for host species is diverging CHCs. For *Myrmica* hosts, nests that detect the parasite have a differential survival, being clearly advantageous.

It has been proved that the differences between the CHC profiles of the host and parasite, which may be responsible for the tolerance towards the parasite, varied between the Spanish *P. longiseta-R. minuchae* populations, suggesting, at a regional level, a selection mosaic of coevolution [39]. Each host-parasite Spanish population is in a different coevolutionary time, as evidenced by the different CHC distances (Nei distances, [48]) between parasites and hosts in each population. This situation probably produces different host strategies to minimize the effects of parasitism on fitness: from resistance, in species or populations with more separated host-parasite CHC, to tolerance, in those with closest host-parasite CHC [39].

For the Asian host-parasite systems, different profiles appeared in the various parasite species (Figure 3). As for the chemical congruence between host and parasite, *R. quandratinodum* and *P.* sp. present the highest cuticular distances that would indicate the highest level of host-parasite aggressiveness [34]. This is also supported by the significantly lower proportion of slaves in *R. quandratinodum* nests compared to the other species (see [49]) and the aggressive behavior observed by the authors in the laboratory. In contrast, *R. anatolicus* and *P. korbi* seem to be the most similar chemically [34] and locally adapted, showing host and parasite with a similar evolutionary potential; therefore this host species should be the least aggressive.

This finding supports that population isolation is not strictly necessary for coevolution meanwhile dispersal may favor local adaptation in broadly distributed species by incorporating genetic variability and more chances to a local adaptation [5, 36, 50]. Nevertheless, some level of local adaptation at CHC profiles between host and parasite exists independently of the kind of geographic distribution (continuous or fragmented) and the ability of dispersal of the different populations. Similarity at CHC profiles appears to be

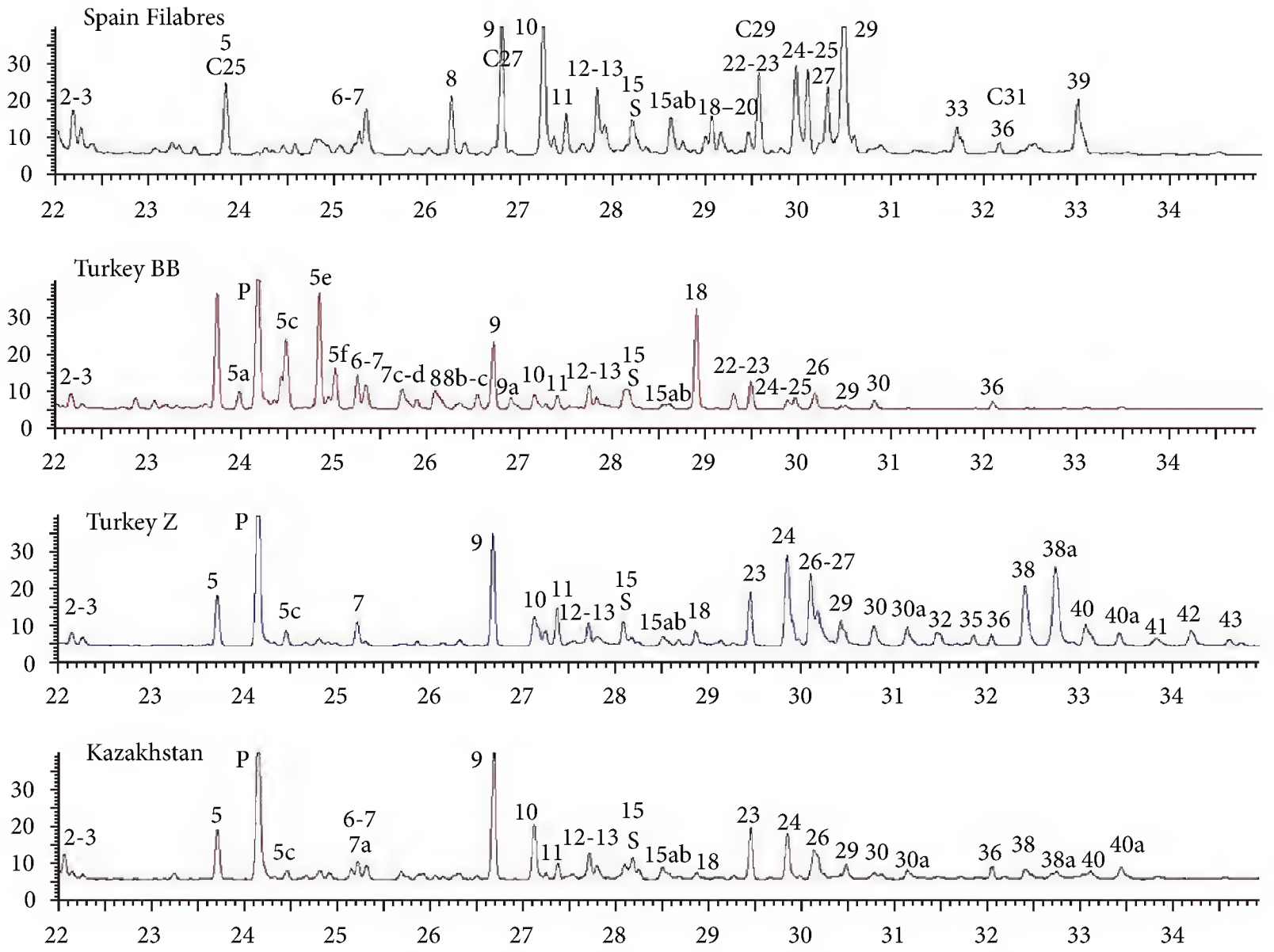


FIGURE 3: CHCs profiles for *R. minuchae* (Filabres), *R. anatolicus* (from two different populations Turkey BB = Belembaçi Beli, Turkey Z = Ziyaret Tepesi), and *R. quandratinodum* (Kazakhstan). Numbers refer to original data in [34] (P and S are pollutants).

a trait imposed by natural selection to the interaction between hosts and slave-makers (and more generally between hosts and parasites), a necessity for the system work.

5. Future Directions

A broader sampling for genetic and behavioral data, including more data on *R. quandratinodum* and *R. proformicarum-P. epinotalis*, is required to depict a more general landscape of local adaptation and coevolution in the *Proformica-Rossomyrmex* pairs.

Acknowledgments

The authors thank Patrizia D'Ettorre for review of data on *Polyergus* and *Formica sanguinea* species biology and the commentaries of two anonymous referees that improved considerably the paper.

References

[1] A. Buschinger, "Evolution of social parasitism in ants," *Trends in Ecology and Evolution*, vol. 1, no. 6, pp. 155–160, 1986.

[2] H. Topoff, "Slave-making queens," *Scientific American*, vol. 281, no. 5, pp. 84–90, 1999.

[3] P. D'Ettorre and J. Heinze, "Sociobiology of slave-making ants," *Acta Ethologica*, vol. 3, no. 2, pp. 67–82, 2001.

[4] A. Buschinger, "Social parasitism among ants: a review (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 12, pp. 219–235, 2009.

[5] E. O. Wilson, *The Insect Societies*, Belknap Press, Cambridge, Mass, USA, 1971.

[6] A. Mori and F. Le Moli, "Behavioural plasticity and domestic degeneration in facultative and obligatory slave-making ant species (Hymenoptera, Formicidae)," *Monitore Zoologico Italiano*, vol. 22, pp. 271–285.

[7] F. Ruano and A. Tinaut, "Raid process, activity pattern and influence of abiotic conditions in the slave-making ant *Rossomyrmex minuchae* (Hymenoptera, Formicidae) a slave-maker species," *Insectes Sociaux*, vol. 46, no. 4, pp. 341–347, 1999.

[8] B. H. Hölldobler and E. O. Wilson, *The Ants*, Springer, Berlin, Germany, 1990.

[9] A. Mori, D. A. Grasso, R. Visicchio, and F. Le Moli, "Comparison of reproductive strategies and raiding behaviour in facultative and obligatory slave-making ants: the case of *Formica sanguinea* and *Polyergus rufescens*," *Insectes Sociaux*, vol. 48, no. 4, pp. 302–314, 2001.

[10] M. Talbot, "Slave-raids of the ant *Polyergus lucidus* Mayr," *Psyche*, vol. 74, pp. 199–231, 1967.

[11] H. Topoff and L. Greenberg, "Mating behavior of the socially-parasitic ant, *Polyergus breviceps*: the role of the mandibular glands," *Psyche*, vol. 95, pp. 81–88, 1988.

[12] F. Le Moli, D. A. Grasso, A. Mori, and A. Ugolini, "Eco-ethological factors affecting the scouting and raiding behaviour of the slave-making ant *Polyergus rufescens* Latr. (Hymenoptera, Formicidae)," *Ethology*, vol. 96, no. 4, pp. 289–302, 1994.

[13] A. Mori, D. A. Grasso, P. D'Etorre, and F. Le Moli, "Specifity in host choice by the slave-making ant *Polyergus rufescens* Latr. (Hymenoptera, Formicidae)," *Ethology, Ecology & Evolution*, vol. 3, pp. 89–93, 1994.

[14] S. Boi, V. Capasso, and D. Morale, "Modeling the aggregative behavior of ants of the species *Polyergus rufescens*," *Nonlinear Analysis: Real World Applications*, vol. 1, no. 1, pp. 163–176, 2000.

[15] C. A. Johnson, H. Topoff, R. K. Vander Meer, and B. Lavine, "Do these eggs smell funny to you? An experimental study of egg discrimination by hosts of the social parasite *Polyergus breviceps* (Hymenoptera: Formicidae)," *Behavioral Ecology and Sociobiology*, vol. 57, no. 3, pp. 245–255, 2005.

[16] F. Ruano and A. Tinaut, "The assault process of the slave-making ant *Rossomyrmex minuchae* (Hymenoptera, Formicidae)," *Sociobiology*, vol. 43, no. 2, pp. 201–209, 2004.

[17] F. Ruano and A. Tinaut, "Mating behaviour in a slave-making ant, *Rossomyrmex minuchae* (Hymenoptera, Formicidae)," *Naturwissenschaften*, vol. 92, no. 7, pp. 328–331, 2005.

[18] K. V. Arnoldi, "Biologische beobachtungen an der neuen paläarktischen sklavenhalterameise *Rossomyrmex froformicarum* K. Arn., Nebst einigen bemerkungen über die beförderungsweise der ameisen," *Zeitschrift für Morphologie und Ökologie der Tiere*, vol. 24, no. 2, pp. 319–326, 1932.

[19] P. I. Marikovsky, "The biology of the ant *Rossomyrmex proformicarum* K. W. Arnoldi (1928)," *Insectes Sociaux*, vol. 21, no. 3, pp. 301–308, 1974.

[20] O. Sanllorente, R. L. Hammond, F. Ruano, L. Keller, and A. Tinaut, "Extreme population differentiation in a vulnerable slavemaking ant with a fragmented distribution," *Conservation Genetics*, vol. 11, no. 5, pp. 1701–1710, 2010.

[21] A. Mori, P. D. 'Ettorre, and F. Le Moli, "Mating and post-mating behaviour of the European amazon ant, *Polyergus rufescens* (Hymenoptera, Formicidae)," *Bolletino Zoologia*, vol. 61, pp. 203–206, 1994.

[22] U. Winter and A. Buschinger, "Genetically mediated queen polymorphism and caste determination in the slave-making ant *Harpagoxenus sublaevis* (Hymenoptera: Formicidae)," *Entomologia Generalis*, vol. 11, pp. 125–137, 1986.

[23] F. Ruano, A. Hefetz, A. Lenoir, W. Francke, and A. Tinaut, "Dufour's gland secretion as a repellent used during usurpation by the slave-maker ant *Rossomyrmex minuchae*," *Journal of Insect Physiology*, vol. 51, no. 10, pp. 1158–1164, 2005.

[24] C. Zamora-Muñoz, F. Ruano, C. Errard, A. Lenoir, A. Hefetz, and A. Tinaut, "Coevolution in the slave-parasite system *Proformica longiseta-Rossomyrmex minuchae* (Hymenoptera: Formicidae)," *Sociobiology*, vol. 42, no. 2, pp. 299–317, 2003.

[25] L. van Oudenhove, R. Boulay, A. Lenoir, C. Bernstein, and X. Cerdá, "Substrate temperature constrains recruitment and trail following behavior in ants," *Journal of Chemical Ecology*, vol. 38, pp. 802–809, 2012.

[26] A. Tinaut and F. Ruano, "Braquipterismo y apterismo en Formícidos. Morfología y biometría en las hembras de especies ibéricas de vida libre (Hymenoptera, Formicidae)," *Graellsia*, vol. 48, pp. 121–131, 1992.

[27] A. Tinaut, "*Rossomyrmex minuchae* nov. sp. (Hym. Formicidae) encontrada en Sierra Nevada, España," *Boletín de la Asociación española de Entomología*, vol. 4, pp. 195–203, 1981.

[28] A. Tinaut, F. Ruano, and I. Fernández-Escudero, "Descripción del macho del género *Rossomyrmex* Arnoldi, 1928 (Hymenoptera, Formicidae)," *Nouvelle Revue d'Entomologie*, vol. 4, pp. 347–351, 1994.

[29] J. Clobert, E. Danchin, A. A. Dhondt, and J. D. Nichols, *Dispersal*, Oxford University Press, New York, NY, USA, 2001.

[30] M. Brandt, J. Heinze, T. Schmitt, and S. Foitzik, "A chemical level in the coevolutionary arms race between an ant social parasite and its hosts," *Journal of Evolutionary Biology*, vol. 18, no. 3, pp. 576–586, 2005.

[31] S. Gandon, Y. Capowiez, Y. Dubois, Y. Michalakis, and I. Olivieri, "Local adaptation and gene-for-gene coevolution in a metapopulation model," *Proceedings of the Royal Society B*, vol. 263, no. 1373, pp. 1003–1009, 1996.

[32] S. Gandon, "Local adaptation and the geometry of host-parasite coevolution," *Ecology Letters*, vol. 5, no. 2, pp. 246–256, 2002.

[33] R. Frankham, J. D. Ballou, and D. A. Briscoe, *Introduction to Conservation Genetics*, Cambridge University Press, Cambridge, UK, 2002.

[34] O. Sanllorente, P. Lorite, S. Devers, F. Ruano, A. Lenoir, and A. Tinaut, "The spatial distribution does not affect host-parasite coevolution in *Rossomyrmex ants*," *Insectes Sociaux*, vol. 59, pp. 361–368, 2012.

[35] S. Gandon and Y. Michalakis, "Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time," *Journal of Evolutionary Biology*, vol. 15, no. 3, pp. 451–462, 2002.

[36] M. Brandt, B. Fischer-Blass, J. Heinze, and S. Foitzik, "Population structure and the co-evolution between social parasites and their hosts," *Molecular Ecology*, vol. 16, no. 10, pp. 2063–2078, 2007.

[37] P. S. Pennings, A. Achenbach, and S. Foitzik, "Similar evolutionary potentials in an obligate ant parasite and its two host species," *Journal of Evolutionary Biology*, vol. 24, no. 4, pp. 871–886, 2011.

[38] S. Foitzik, S. Bauer, S. Laurent, and P. S. Pennings, "Genetic diversity, population structure and sex-biased dispersal in three co-evolving species," *Journal of Evolutionary Biology*, vol. 22, no. 12, pp. 2470–2480, 2009.

[39] F. Ruano, S. Devers, O. Sanllorente, C. Errard, A. Tinaut, and A. Lenoir, "A geographical mosaic of coevolution in a slave-making host-parasite system," *Journal of Evolutionary Biology*, vol. 24, no. 5, pp. 1071–1079, 2011.

[40] P. D'Ettorre and A. Lenoir, "Nestmate recognition in ants," in *Ant Ecology*, L. Lach, C. Parr, and K. Abbott, Eds., pp. 194–209, Oxford University Press, Oxford, UK, 2010.

[41] K. A. Copren, L. J. Nelson, E. L. Vargo, and M. I. Haverty, "Phylogenetic analyses of mtDNA sequences corroborate taxonomic designations based on cuticular hydrocarbons in subterranean termites," *Molecular Phylogenetics and Evolution*, vol. 35, no. 3, pp. 689–700, 2005.

[42] S. J. Martin, H. Helanterä, and F. P. Drijfhout, "Evolution of species-specific cuticular hydrocarbon patterns in Formica ants," *Biological Journal of the Linnean Society*, vol. 95, no. 1, pp. 131–140, 2008.

[43] A. Lenoir, P. D'Ettorre, and C. Errard, "Chemical ecology and social parasitism in ants," *Annual Review of Entomology*, vol. 46, pp. 573–599, 2001.

[44] E. Nowbahari, A. Lenoir, J. L. Clément, C. Lange, A. G. Bagneres, and C. Joulie, "Individual, geographical and experimental variation of cuticular hydrocarbons of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae): their use in nest and subspecies recognition," *Biochemical Systematics and Ecology*, vol. 18, no. 1, pp. 63–74, 1990.

[45] D. R. Nash, T. D. Als, R. Maile, G. R. Jones, and J. J. Boomsma, "A mosaic of chemical coevolution in a large blue butterfly," *Science*, vol. 319, no. 5859, pp. 88–90, 2008.

[46] L. V. Ugelvig, F. P. Drijfhout, D. J. C. Kronauer, J. J. Boomsma, J. S. Pedersen, and S. Cremer, "The introduction history of invasive garden ants in Europe: integrating genetic, chemical and behavioural approaches," *BMC Biology*, vol. 6, article 11, 2008.

[47] I. Fernández-Escudero and A. Tinaut, "Factors determining nest distribution in the high-mountain ant *Proformica longiseta* (Hymenoptera Formicidae)," *Ethology Ecology and Evolution*, vol. 11, no. 4, pp. 325–338, 1999.

[48] S. Dronnet, C. Lohou, J.-P. Christides, and A.-G. Bagnères, "Cuticular hydrocarbon composition reflects genetic relationship among colonies of the introduced termite *Reticulitermes santonensis* feytaud," *Journal of Chemical Ecology*, vol. 32, no. 5, pp. 1027–1042, 2006.

[49] A. Tinaut, F. Ruano, O. Sanllorente, A. Fernández-Zambrano, C. Karaman, and Y. Kaz, "Nest composition and worker relatedness in three slave-making ants of the genus *Rossomyrmex* Arnoldi and their *Proformica* Ruzsky hosts (Hymenoptera, Formicidae)," *Insect Science*, vol. 17, no. 4, pp. 361–368, 2010.

[50] B. Fischer and S. Foitzik, "Local co-adaptation leading to a geographical mosaic of coevolution in a social parasite system," *Journal of Evolutionary Biology*, vol. 17, no. 5, pp. 1026–1034, 2004.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 201769, 3 pages
http://dx.doi.org/10.1155/2013/201769

Research Article

Defensive Glands of the Darkling Beetle *Mesomorphus villiger* Blanchard (Coleoptera: Tenebrionidae)

C. M. Seena and Sabu K. Thomas

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

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

Received 6 November 2012; Revised 23 March 2013; Accepted 13 May 2013

Academic Editor: Jan Klimaszewski

Massive home invasion by the darkling beetle *Mesomorphus villiger* Blanchard 1853 (Coleoptera: Tenebrionidae) during monsoon season make it a nuisance pest in many regions of south India. Morphology of defensive glands and mode of release and dispersal of the defensive secretion were analysed. Defensive glands were separated from the abdominal sternites by cutting along the posterior margin of the seventh sternite. Glands are evaginations of intersegmental membrane between the seventh and eighth sternites consisting of two long sac-like reservoirs, and glandular secretion is released by exudation and spread through epipleural gutter of elytra. Gradual release of the secretion is a strategy to repel the predators for a longer duration.

1. Introduction

Darkling beetle, *Mesomorphus villiger* Blanchard 1853 (Coleoptera: Tenebrionidae: Opatrini), is of cosmopolitan distribution with occurrence in Indian subcontinent, Afghanistan, Siberia, Australia, and Africa (Madagascar) [1–3]. Nibbling and gnawing at the base of the stem of newly transplanted tobacco seedlings lead to the death of the plants. Hence, they are referred as tobacco ground beetle in tobacco growing belts in India [1, 4]. However, in the Kerala state in south India, they are present in the litter of rubber (*Hevea brasiliensis* ((Willd. ex Adr. De Jus) Müll. Arg. 1865)), mango (*Mangifera indica*, Linnaeus 1753), cashew (*Anacardium occidentale,* Linnaeus 1753), and rain tree (*Samanea saman* (Jacquin) Merrill 1916) and have strong feeding preference towards fallen tender leaves (personal observation). Home invasion of huge aggregation of *M. villiger* into residential buildings with the onset of monsoon season, their nocturnal movements and release of an irritating, odoriferous quinonic secretion that causes mild skin burns, makes it a nuisance pest in many regions of the South Western Ghats.

Similarities in morphology and the aggregation pattern by *M. villiger* often lead to its misidentification as rubber litter beetle *Luprops tristis* (Fabricius, 1801). No data exists on the structure of defensive glands of the genus, and the present study analyses the structure of defensive glands and mode of release and dispersal of the defensive gland secretion in *M. villiger*.

2. Materials and Methods

Aggregated beetles were collected from a residential building at Calicut (11°15′N, 75°50′E), in south India, during the monsoon season. Adults of both sexes were killed using diethyl ether and pinned to a wax tray. Elytra and abdominal tergites were removed to expose the internal structures and observed under a stereo zoom microscope (Labomed, ASZ-99TR).

Reproductive and digestive structures and fat reserves were removed to expose defensive glands. After washing in water followed by 70% alcohol treatment, the sternites with the attached glands were separated. The defensive glands were separated from the sternites by cutting along the posterior margin of the seventh sternite. Glands were dehydrated in graded series of ethyl alcohol, brought to xylene through alcohol, and mounted on a glass slide in Canada balsam.

Live beetles were held between the left thumb and index finger and placed on the stage of a stereo zoom microscope with the ventral surface of the insect facing up and keeping

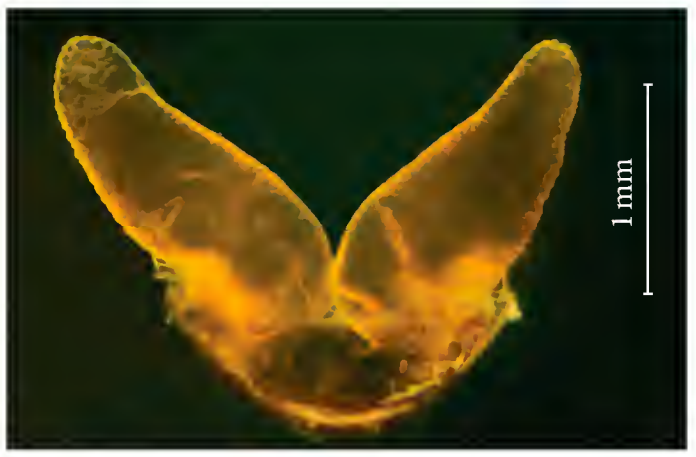


FIGURE 1: Defensive gland of *Mesomorphus villiger* cut from the remainder of the sternites.

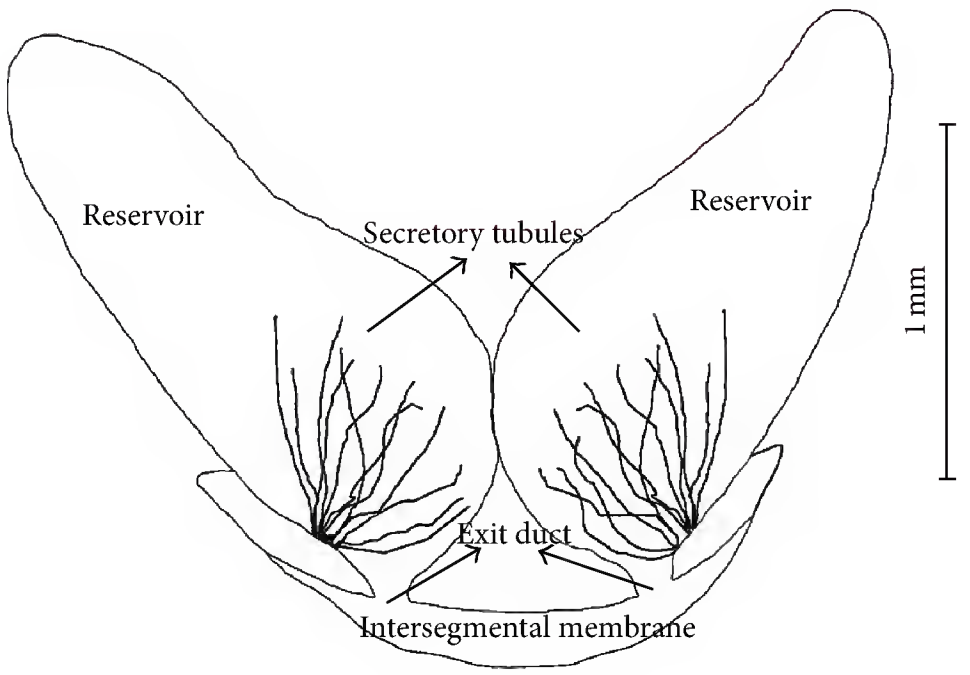


FIGURE 2: Line diagram of defensive gland of *Mesomorphus villiger.*

the posterior end away from the observer. Beetles were subjected to a graded series of stimuli, namely, by (1) pressing the abdomen; (2) tapping the body, especially the elytra, with a steel rod; and (3) pinching the legs with forceps, to observe the pattern and mode of release of gland secretion. Time taken for production of gland secretion is estimated by making the beetle to discharge the entire gland secretion and noticing the time taken to initiate the discharge again following repeated stimulation by application of successive stimuli.

3. Results and Discussion

3.1. Morphology of Defensive Glands. Defensive glands of *M. villiger* consist of a pair of non eversible reservoirs located in the abdomen between the fourth to seventh sternites irrespective of the sex. They belong to the platynotine type gland [5] with swollen base and conical tips and without prothoracic glands. Each gland opens independently on the seventh sternite beside the anus. Gland reservoirs are long and strongly annulated conical pouches (length 1.5–1.7 mm; width 0.5–0.7 mm), occur parallel to the long axis of the body, and are separated (Figure 1).

Reservoirs consist of the intersegmental membrane evaginations between the seventh and eighth sternites, occur on either side of hind gut, and are immersed in a thick matrix

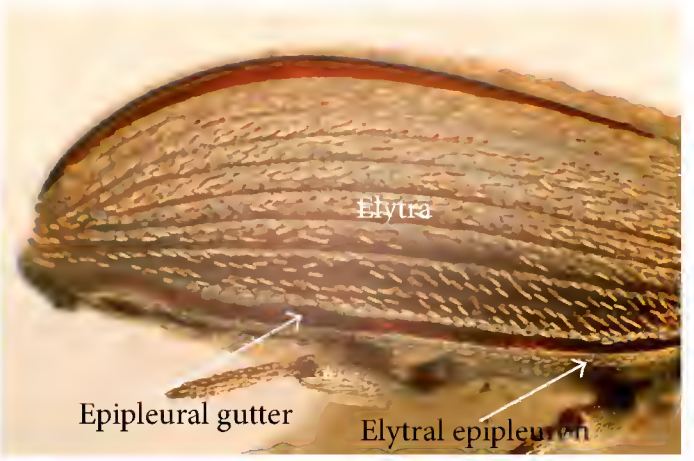


FIGURE 3: Epipleural gutter on the elytra of *Mesomorphus villiger.*

of fat reserves. No muscles were found associated with the reservoirs. Reservoirs have narrow exit ducts and backwardly directed constricted openings. Gland secretion is produced by the 10–15 secretory tubules present on the proximal dorsolateral field of the reservoirs (Figure 2).

3.2. Delivery of Secretion. On disturbance, *M. villiger* released the odoriferous secretion by exudation to the seventh abdominal sternite, the most common method of delivery among tenebrionids [5]. Exudation of defensive secretion is considered as an advanced feature compared to eversion [5, 6], a primitive character seen in *Luprops tristis* [7], where it causes rupture of the reservoirs. Entire gland secretion was not released at once, and upon maintaining the disturbance, beetles released the secretion at intervals from five to six times within a period of 10 minutes. Following the complete discharge of the secretion, beetles were defenceless for four to five days without gland secretion. Slow release of the gland secretion might be enabling the beetle to repel predators for a prolonged period of time. Beetle responded towards all the three stimuli applied, and the quickest response was towards pinching of legs. Exudation of gland secretion was side specific, as when the stimulus was applied on one side of the beetle, exudation of reservoir of that particular side alone occurred.

3.3. Dispersal of Secretion. Margins of the epipleura are formed into a gutter or channel in *M.villiger* along the lateral edge of the elytra with the posterior end opening in to the seventh abdominal sternite and the anterior end merging with the elytral humeri (Figure 3). Epipleural gutter is present in all other species of *Mesomorphus* (*M. gridelli* Kaszab 1963, *M. kulzeri* Kaszab 1963, and *M. Striolatus* Fairmaire 1896) recorded from the region. Portion of the exuded secretion was expelled as a narrow streak through the gutter as far as the elytral humeri and spread over the anterior margin of elytra as in many other tenebrionids [5]. Dispersal through the epipleural gutter has the effect of increasing the area exposed to the secretion, and the remaining secretion spreads over the posterior end of abdomen and elytra. Dispersal of secretion was very rapid, with the entire length of the elytra and posterior part of pronotum being covered within

a fraction of a second. Released secretion dried off within 10–20 seconds, and the repelling odour persisted for three to five minutes.

4. Conclusion

Defensive glands of *M. villiger* consist of a pair of abdominal glands and without prothoracic glands. Glands are evaginations of the intersegmental membrane between seventh and eighth sternites with the secretory tubules distributed at the proximal dorsolateral portion of the reservoirs. Secretion is released by exudation which constitutes a comparatively advanced mode of dispersal, and the exuded secretion spreads quickly along the epipleural gutters to reach the anterior portion.

Acknowledgments

Financial assistance received from UGC-CSIR through their Research Fellowship (JRF) to the first author is gratefully acknowledged. The authors Arunraj C, Shiju T. Raj, and Nirdev P. M. (St. Joseph's College, Devagiri, Calicut) for their assistance.

References

[1] S. Sitaramaiah, G. Rama Prasad, and U. Sreedhar, "Management of tobacco ground beetle, *Mesomorphus villiger* with insecticide baits on flue cured Virginia tobacco," *Indian Journal of Agricultural Sciences*, vol. 69, no. 9, pp. 660–663, 1999.

[2] Z. Kaszab, "Faunistik der Tenibrioniden von Sri Lanka (Coleoptera)," *Folia Entomologica Hungarica, Rovartani Kozlemenyek*, vol. 32, no. 2, pp. 43–128, 1979.

[3] W. Schawaller, "The genus Mesomorphus Seidlitz in Nepal," *Faunistische Abhandlungen Staatliches Museum Fur Tierkunde, Dresden*, vol. 22, no. 4, pp. 39–48, 2000.

[4] T. N. Ananthakrishnan and B. V. David, *General and Applied Entomology*, Tata McGraw-Hill, Noida, India, 2004.

[5] W. R. Tschinkel, "A comparative study of the chemical defensive system of tenebrionid beetles. III. Morphology of the glands," *Journal of Morphology*, vol. 145, no. 3, pp. 355–370, 1975.

[6] W. R. Tschinkel and J. T. Doyen, "Comparative anatomy of the defensive glands, ovipositors and female genital tubes of tenebrionid beetles (Coleoptera)," *International Journal of Insect Morphology and Embryology*, vol. 9, no. 5-6, pp. 321–368, 1980.

[7] P. Abhitha, K. V. Vinod, and T. K. Sabu, "Defensive glands in the adult and larval stages of *Luprops tristis* (Tenebrionidae: Lagriinae: Lupropini)," *Journal of Insect Science*, vol. 10, no. 10, 5 pages, 2010.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 539780, 10 pages
http://dx.doi.org/10.1155/2013/539780

Review Article

Leaf-Cutter Ant Parasitoids: Current Knowledge

Patricia J. Folgarait

Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Saenz Peña 352, Bernal, 1876 Buenos Aires, Argentina

Correspondence should be addressed to Patricia J. Folgarait; patricia.folgarait@gmail.com

Received 8 March 2013; Accepted 25 April 2013

Academic Editor: Alain Lenoir

This review updates and summarizes the current knowledge about the interaction of leaf-cutter ants and their parasitoids by providing comparable data for *Acromyrmex* and *Atta* ants. First, an overview of the relevant aspects of the biology and taxonomy of leaf cutters and of their parasitoids is provided. Second, I show the peculiarities of the parasitoids attacking behaviors towards their host as well as the responses or ant defenses against the phorids exhibited by their hosts. Third, I discuss relevant aspects of the interactions between hosts and parasitoids. Finally, the review ends demonstrating why these phorids could be promising biological control agents of leaf-cutter pests and suggests priority lines of research for the future.

1. Introduction

Since the Feener Jr. and Brown [1] review discussion on flies as parasitoids, there has not been a comprehensive review on Phoridae (Diptera) parasitoids specialized on attacking adult ant workers. Phorids attacking fire ants are the ones most extensively studied due to their application in biological control. The literature is vast and dispersed although there is a review about *Pseudacteon* biology and interaction with fire ants [2]. Other scarce studies were done on other ant-phorid systems such as *Pheidole* [3, 4], *Azteca* [5, 6], and *Paraponera* [7]. Until more information is gathered, generalizations will not be possible for these groups. Hsun-Yi and Perfecto [8] have done an interesting review on indirect trait mediated effects of parasitoids on ants showing general patterns such as a reduction in ant's foraging activity, body sizes as well as the amount of food retrieved by colonies.

A compilation of leaf-cutter phorid species with their known and/or potential host species has been recently made [9]. The mentioned work includes some biological data about parasitoids of *Atta*, mainly from the laboratory, but a comprehensive review about their biology and ecological interaction with their hosts, including data of *Acromyrmex,* has not been done. Furthermore, Bragança [9] has not updated the scientific names of 14 species (called as *Neodohrniphora*) according to the status change of the subgenus *Eibesfeldtphora* to genus, proposed by Disney et al. [10]. Although the great majority of data available is limited to the southern portion of South America and therefore more work is needed, it is enough to observe general patterns. This review will summarize the current information about this system and will identify key questions and gaps of knowledge where researchers should focus attention.

2. Leaf-Cutter Ants

The leaf cutters are a subgroup of the higher Attine fungus growing ants and are confined to two genera: *Acromyrmex* and *Atta*. *Acromyrmex* ants are the more diverse genus with 31 species with an additional 33 infraspecies [11]. Species that have more than 2 infraspecies, such as *Ac. coronatus, Ac. hispidus, Ac. lobicornis, Ac. lundii, Ac. octospinosus, Ac. rugosus, and Ac. subterraneous*, deserve to be studied in greater detail or using multiple techniques to avoid confusion and contradictory classification. *Atta*, on the other hand, exhibits less richness (14 spp.). *Acromyrmex* is more broadly distributed (by 10°N and S) than *Atta*, from 34°N to 41°S. Detailed maps of each species distribution can be found in Delabie et al. [11], and additional records for certain species from Argentina can be found in Elizalde and Folgarait [12].

Atta and *Acromyrmex* are larger Attines and are readily distinguishable from other ants because of their generally larger size, morphology, and behaviors. *Acromyrmex* ants are

easily recognized because all workers have at least 4 pairs of spines, 3 of which are on the thorax (promesonotum). The mesonotum spines are regular and smooth; also the frontal carinas in the head are short and never go beyond the eyes. The first abdominal tergite usually has tuberculous [13]. Their color varies from black to orange yellowish. On the other hand, *Atta* has 3 pairs of spines, 2 of which are in the promesonotum, the spines are generally curved, and the first abdominal tergite is smooth (Figure 1). Both genera are polymorphic, and although this trait is not as clear as in *Atta*, three castes of workers (tiny, small, and medium) can be differentiated in *Acromyrmex*; soldiers present in *Atta* are absent in *Acromyrmex* [14]. These ants have mass recruiting strategies, following a trail, more or less developed or clear, depending on the species, with 1 to several trails per nest, short or as long as 300 m. In *Atta* foraging trails are numerous and very conspicuous.

Acromyrmex colony nests can be completely hypogeous (underground, i.e., *A. striatus, A. aspersus*) with only small and few or variable number of entrances/exits or additionally have an epigeous mound (of variable height) such as in the case of *A. heyeri* or *A. coronatus*. Their foraging trails in general are not very conspicuous although this also depends on the taxa, the colony's age, and habitat. Although the nest's shape and appearance help render an ant's identification, more information is needed. The existent literature on the shape of *Acromyrmex* nests [15–17] is incomplete. Another complication is that certain species change greatly their type of nest in different habitats/regions (i.e., *A. lundii, A. lobicornis*) introducing confusion with others, such as *A. crassispinus, A. subterraneus*. For example, *A. lobicornis* epigeous nests are found in the southernmost part of its distribution while it barely has a mound in warmer areas (Folgarait, pers. obs.) such as in northern middle parts of Argentina. Another conspicuous feature that helps identify some species of this genus is the location of refuse dumps. Most *Acromyrmex* species have internal refuse dumps, although there are few exceptions where this characteristic is very helpful in identification (i.e., *A. lobicornis, A. crassispinus, and A. hispidus*). On the other hand, *Atta* nests are very distinctive as they create mounds of much greater size, that in general do not have vegetation on/or around them, and nests have loose soil with many holes on their surface. However, distinctions among species require an experienced eye that could also recognize key morphological characteristics of workers.

For *Acromyrmex*, climatic conditions can explain aspects of the mentioned differences regarding the presence/absence of a mound [18] and dump location either interspecifically (Farji Brener, pers. com.) as well as intraspecifically (Folgarait, pers. obs.), but other reasons such as colony sanitation and internal nest architecture may be additional factors, most likely all correlated with each other. Unfortunately, we know very little about the natural history of these species and the costs involved in dealing with trash and nest construction. For instances, is it less costly to lose additional workers by carrying the unsanitary trash outside to eliminate possible foci of infection or is it more energy efficient to close a trash filled internal chamber and not to maintain it? If the trash is internal, are these ants taking advantage of the nutrients that mineralize within those trash-decomposition hot spots? Is the heat produced by internal refuse dumps utilized by the ants for colony or fungal thermoregulation? All these questions represent interesting lines of research, and the questions can be answered using C/N tracing techniques or manipulative field experiments.

3. Leaf-Cutter Parasitoids

3.1. Richness, Distribution, and Characters Used to Distinguish among Genera. Bragança [9] cites 30 species of phorids (Diptera: Phoridae) within 8 genera associated with *Acromyrmex* ants whereas 39 species in 5 genera were recorded on *Atta*. Also, he lists 7 cases of the same phorid species seen flying or sitting beside the nests of both genera. However, if only positive-sure cases (hosts from which parasitoids emerge or phorids seen pursuing and attacking ants) are considered, these numbers decrease for *Acromyrmex* to 15 species in 4 genera, for *Atta* to 25 species, and 4 genera with only 2 observations of phorids attacking both genera (*Apocephalus setitarsus* and *Myrmosicarius crudelis*), although these could well be mistakes or trials that were seen only once. Further observations for these two species should be specifically done as one of the references for each record is very old. In fact, Elizalde and Folgarait [12, 19] argue that leaf-cutter phorid parasitoids are very specific in the sense that those attacking *Acromyrmex* ants do not attack *Atta* and vice versa. Moreover, in many instances in which one phorid species is seen "ovipositing" an ant and this ant is reared, a different phorid species is obtained [20]. Therefore, these observations could be considered mistakes or tests made by the parasitoids. What really matters is the recurrent attack of a phorid species on the same host and its possibility of emerging from that host. According to this criterion, phorids that attack *Acromyrmex* or *Atta* ants are specific to that ant genus.

Despite the fact that phorids only represent 20% of known parasitoids, flies are the insect order that has the greatest range of hosts parasitized [21], and they are the only group known to attack adult ants [22]. Recently the subgenus *Eisbesfeldtphora* was elevated to genus status [20], and a new genus with a single species has been described *Lucianophora folgaraitae* Disney [23].

So far, *Myrmosicarius* is the genus with the greatest geographical distribution ranging from 35°N to 41°S ([24]; Elizalde, Pers. Com.). However, *Eibesfeldtphora* is present in the largest number of countries [9].

Among the four most important genera attacking leaf-cutter ants, *Apocephalus* [26], *Eibesfeldtphora* [10, 27], *Myrmosicarius* [28, 29], and *Neodohrniphora* [10], it is difficult to say which one is most important. In the case of *Apocephalus*, the subgenera *Apocephalus* includes only ant-decapitating flies, and these flies are recognized for lacking tibial setae and possessing abdominal segments 7 to 10 fused to form an ovipositor, with which the eggs are inserted into the host. Segment 7 forms a rigid structure called oviscape. Another diagnostic character is the presence of a stylet comprised of segments 8 to 10 [30] (Figure 2). The mentioned subgenus has

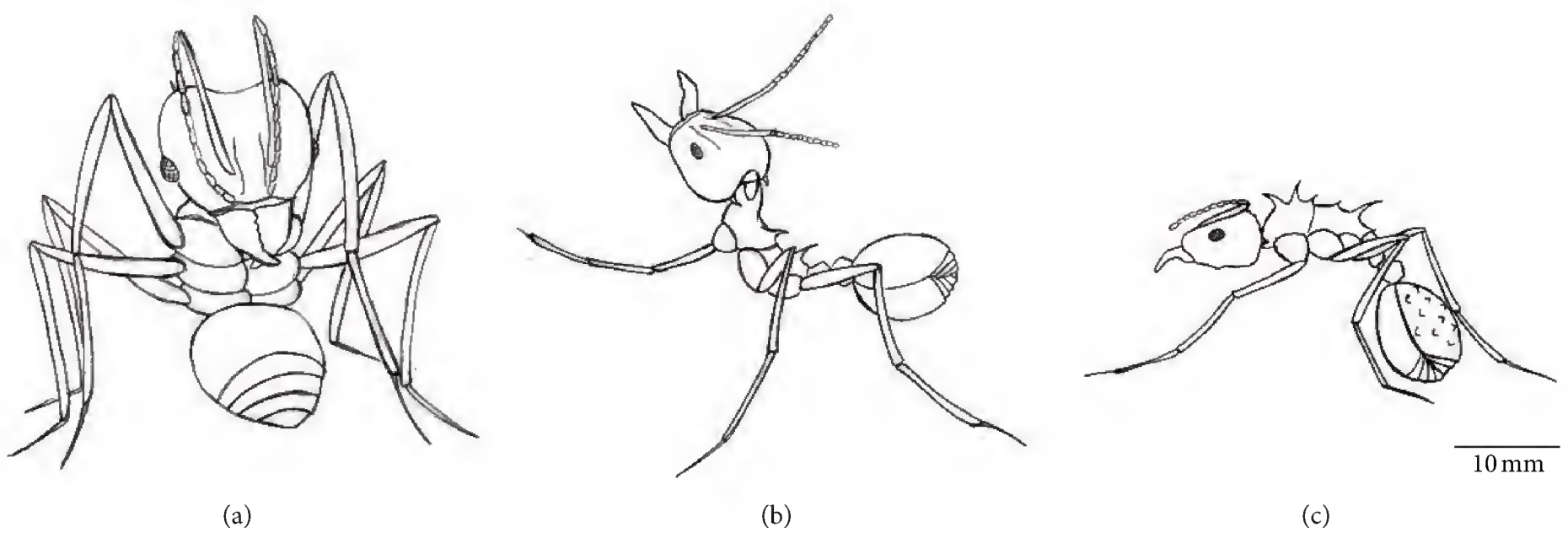


FIGURE 1: *Atta* (a, b) and *Acromyrmex* (c) morphological differences and exhibiting different body postures. (a) shows the C posture, (b) the alarm/attack phorid posture whereas (c) exhibits lowering the abdomen to avoid oviposition at the tip of the gaster.

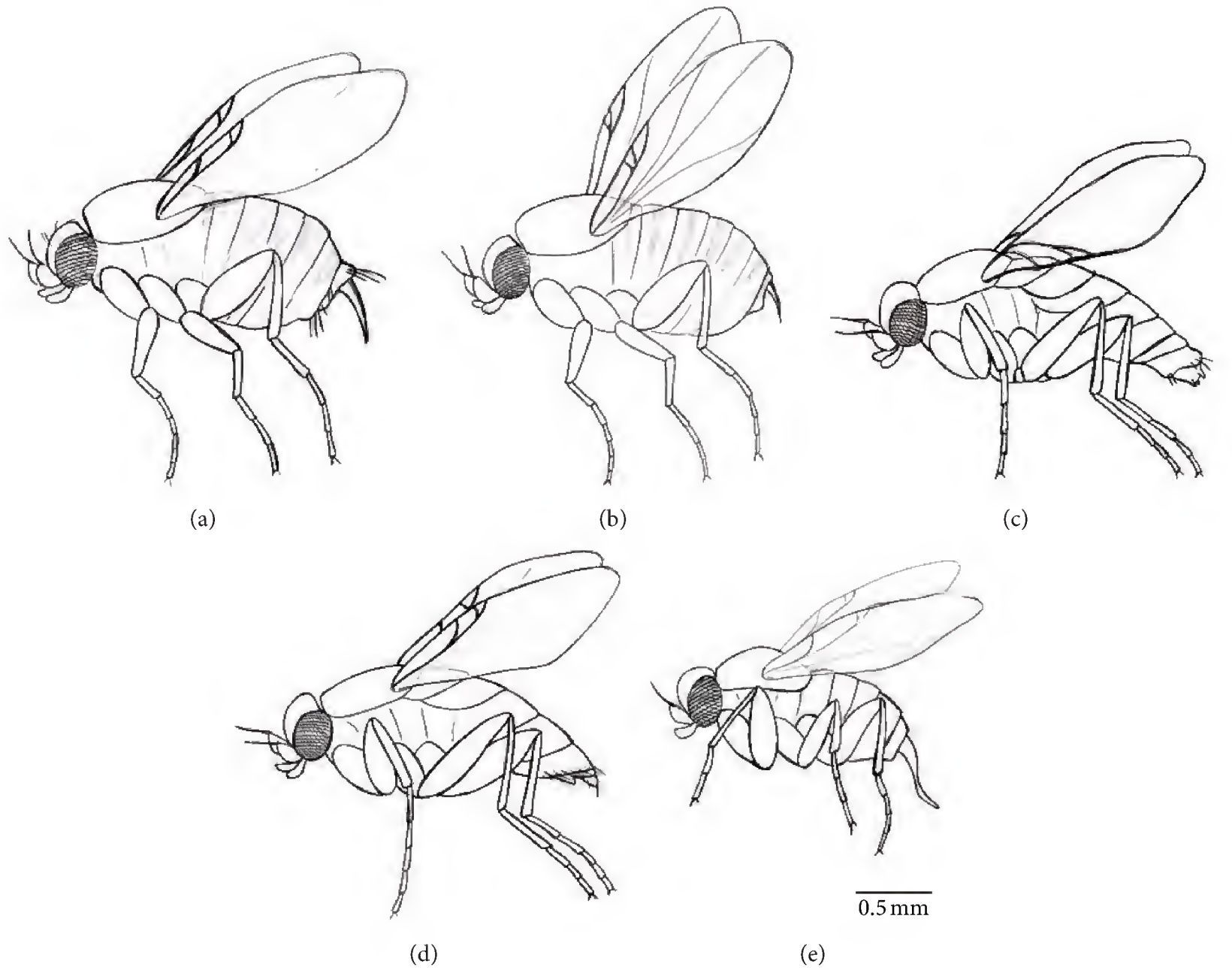


FIGURE 2: Schematic drawings of phorids showing details of the main characters that can be used to easily identify and distinguish among the main genera attacking leaf-cutter ants. Sizes represent real relative differences. (a) *Apocephalus* from the "attophilus" group and (b) from the "*grandipalpis*" group, (c) *Eibesfeldtphora,* (d) *Neodohrniphora*, and (e) *Myrmosicarius*.

subgroups specializing on different ant subfamilies. These are potentially monophyletic groups. The group "attophilus" is specialized on leaf cutters [26] and recognized because the apical sclerite is clearly separated posterior from the oviscape [30]. A few species from the "*grandipalpis*" group also attack *Acromyrmex* ants and are characterized by a short ovipositor, with a ventral sclerite wider than the dorsal one giving the very distinctive effect of a rounded and lateral concavity in dorsal view [26]. *Apocephalus* flies attack both ant genera with 8 recorded species attacking *Atta* and 6 others that use *Acromyrmex* as hosts [9]. *Neodohrniphora* at present has only two species attacking leaf cutters (*N. acromyrmecis* and *N. unichaeta*). This genus is distinctive because the front legs have 5 unusual fore-tarsal segments. Besides, abdominal

segment 6 is either reduced to hairs or has on its sides a transverse row of long hairs. Segment 7 also could be reduced to 2–4 hairy lobes or is basally articulated to form appendages. Beyond the ovipositor and below the tip of the abdomen is found a strongly sclerotized hook [31] (Figure 2). *Eibesfeldtphora* largely specializes as 9 out of 10 species are known to oviposit or develop on *Atta* hosts. It has yellow legs with dorsal enlarged hair palisade in all tibia. Fore leg with tarsomeres 4 and 5 fused, therefore with 4 distinctive tarsomeres. Abdominal segments are yellow ventrally (1–5), but segment 6 is mainly dark. Segment 7 has several lateral lobes darkly sclerotized. Segments 8–10 form at the end a pointed stylet [27] (Figure 2). There are 6 *Myrmosicarius* species that attack *Acromyrmex* whereas only 3 attack *Atta*. Females of the latter are recognized because the front tarsus is reduced to two segments; the sternite of the abdominal segment 6 is absent or vestigial and, by the characteristic oviscape tube, relatively nonornamented, that is, formed from abdominal segments 7 and 8 [29] (Figure 2).

Other features that help to identify among the mentioned genera are related to the pupae. While most *Apocephalus* species have a free pupae, the other genera have claustral pupation in the dead host head. *Apocephalus* do not decapitate their host and is unique in that more than one adult can emerge from a single host although this has not been recorded on *Acromyrmex* hosts. Also *Apocephalus vicosae* is the single exception for having a pupae coming out from the thorax. *Myrmosicarius* pupae are difficult to detect as the pupa is found deep in the head, below the tentorium arms, and the respiratory horns do not come outside of the head capsule; all these parasitoids decapitate their host. The other two genera pupae also develop in the head although they are easily seen and recognized by the exposed respiratory horns and sclerotized operculum (Figure 3); not all the species induce host decapitation [32].

3.2. Ecological Characteristics

3.2.1. Generalities. *Atta* parasitoids oviposit on workers while transporting leaves in the foraging trail or while potential hosts are cutting leaf fragments [33–36], sometimes using the load transported by the ant as a platform [37] or not [38, 39]. In the case of *Acromyrmex* parasitoids, not only these also attack ants on the foraging trail, those that are transporting a load or cutting leaves, but also while workers are repairing the nest or attending external refuse piles [19]. Both *Atta* and *Acromyrmex* parasitoids use either an ambush or an actively searching strategy and oviposit on different parts of the ant body such as through (on) the mandibles, in the head, thorax, legs, and anus [32, 38, 40]. Tables summarizing this information at the species level can be found for *Acromyrmex* [19] and for *Atta* [20].

Eibesfeldtphora females can use an ambush or active searching strategy, can land and oviposit on the head or abdomen, and always attack ants on the foraging trails while pursuing the host; in general they rest close to nest entrances. On the other hand, *Myrmosicarius* is mainly an active flyer while searching for its host. Some of them can fly onwards, backwards, or sideward. They also land and oviposit in the head (mandible, clypeus, and occiput) and abdomen (tip) and can attack while on the trails, doing nest maintenance, or at refuse dumps. *Apocephalus* females attack using an ambush strategy, landing on the leaves carried by the ants, and ovipositing close to the mandible. *Neodohrniphora* are ambush or active searching parasitoids; there are too few records so as to generalize this genus. The four genera search hosts at foraging trails [19].

3.2.2. Refuse Dumps. Phorids attacking ants at refuse dumps were observed only for *Acromyrmex* ants [19]. This behavior was recorded consistently for *M. longipalpis, M. crudelis*, and *M. gracilipes* attacking *Ac. hispidus* for the first species and *Ac. crassispinus* for the latter two. The common factor seems to be the Monte habitat and inconspicuousness of the foraging trails of the mentioned hosts (either for being subterraneus or otherwise covered with vegetation and being difficult to find). Therefore, the refuse piles could be a better place to spot the ants by these phorids in microhabitats with dense and high vegetation and low light. In fact, the mean light intensity at this habitat is 1 order of magnitude lower than for species attacking at other microhabitats [20]. Despite this capacity to oviposit at very low light levels, phorids attacking at refuse piles do not coincide with nocturnal ones (*M. brandaoi, M. gonzalezae*, *A. setitarsus,* and *A. longisetarum* for *Atta* and *M. cristobalensis, A. neivai, A. penicillatus, and A. necdivergens* for *Acromyrmex*). As nocturnal phorids are also diurnal, therefore an exact agreement between the phorid circadian rhythm and the microhabitat of attack may not be necessary. It is expected that refuse dump and nocturnal phorids rely more on close-range cues not associated with vision. This hypothesis, with the little knowledge that exists, disagrees with the data gathered for *Neodohrniphora elongata* [41]; however as it is a diurnal phorid (as far as it is known), it is reasonable that uses visual cues in motion for host location and recognition. On the other hand, another diurnal phorid, *Pseudacteon tricuspis,* uses short range chemical cues to locate their fire ant hosts [42]. This topic deserves further attention and research [43].

Phorid species that consistently attack at refuse piles such as *M. crudelis* and *M. longipalpis* seem to be very acrobatic flies, able to maneuver very rapidly, and are fast at flying forward as well as backwards, attacking the ants while being in front, back, or beside the host [32]. These abilities may be important in a small microsite, such as the refuse piles of these hosts, where many ants are together, carrying refuses and walking in a variety of directions (in comparison to the bimodal pattern on a foraging trail). Curiously, *M. crudelis* and *M. longipalpis* have the longest developmental periods recorded for leaf-cutter hosts (means of 49 and 52 days, resp.; these means are underestimated as it is not known when the oviposition occurred) [20]. Their developmental times are the longest recorded to date, even considering that developmental periods of phorids that attack *Acromyrmex* ants are longer than those coming from *Atta*. Furthermore, considering that these flies attack small ants [20], these lengthy developments are even more surprising as, in general,

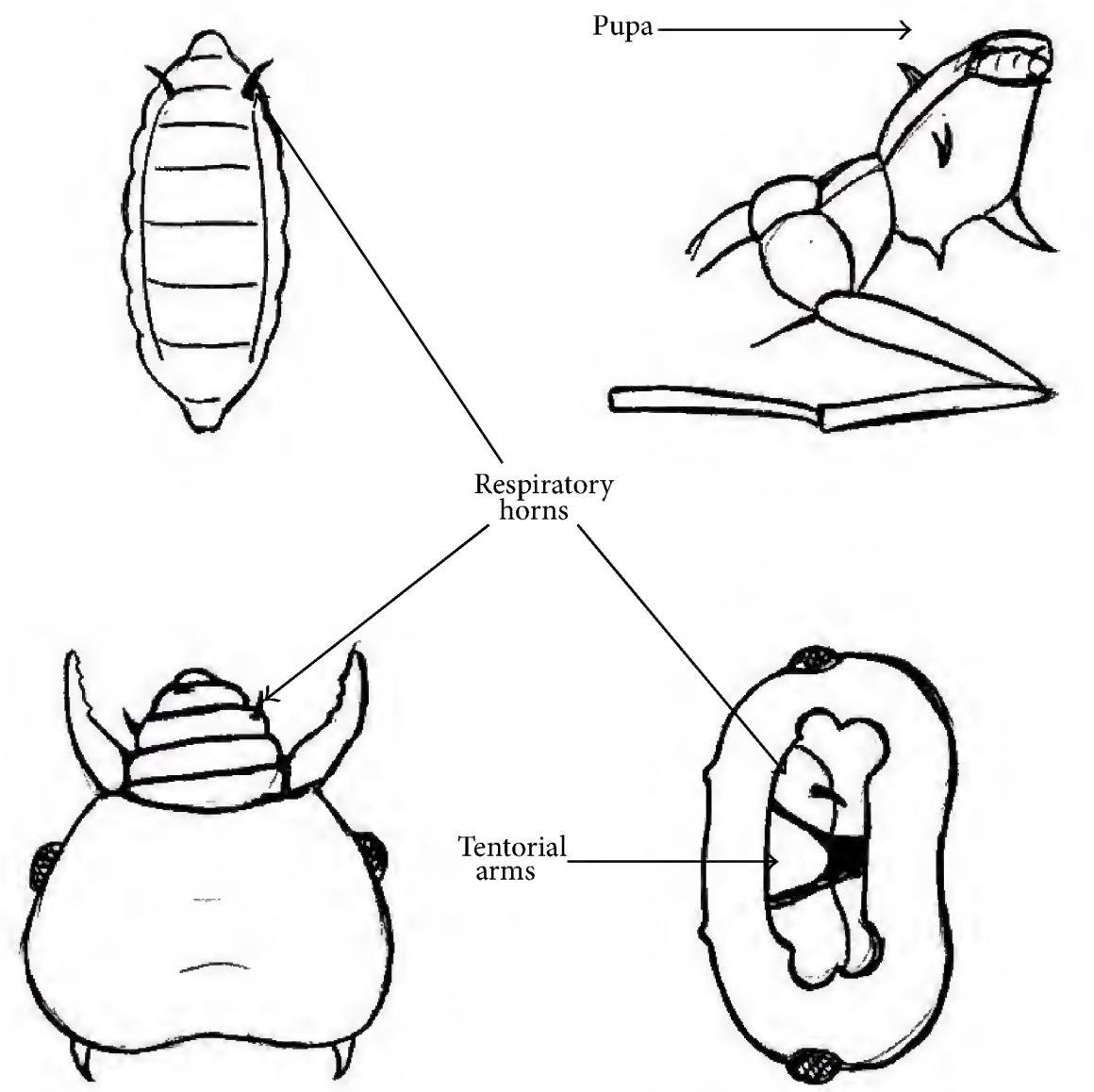


FIGURE 3: Schematic drawings showing different types of pupae according to the parasitoid genus. Top left: Dorsal view of a free pupae from *Apocephalus*, top right: claustral thoracic pupae from *Apocephalus vicosae*, viewed from ventral side, bottom left: claustral pupae from *Eibesfeldtphora* and *Neodohrniphora* coming out of the ant head between the mandibles (ventral view), and bottom right: claustral pupae from *Myrmosicarius,* viewed within ant head, under the tentorial arms (modified from [25]).

phorids attacking smaller ants develop faster than those attacking larger ones [25, 44, 45]. Probably, the ants involved in this task, such as carrying refuses plus working on them, are constantly dealing with infectious pathogens and may well be considered disposable ants from the colony point of view (either for being old or having a bad health) and, in turn, poor hosts from a phorid nutritional perspective. If this is the case, then a longer developmental time is expected.

4. Leaf-Cutters Defenses against Parasitoids

4.1. Generalities. Phorids that parasitize leaf-cutting ants affect the ant behavior which translates to a negative effect on their foraging activity. The response behaviors of *Atta* ants against phorids include dropping their load [33], retreating to the nest [46], moving legs, antennae, and mandibles [37], outrunning the phorid [40], or adopting particular body postures in order to avoid oviposition such as lowering the tip of the abdomen, having a C posture, or making a ball with their whole body (Figure 1) [33, 39]. Similar behaviors were observed in *Acromyrmex* ants [19].

The presence of phorids was a significant determinant for the display of defensive behaviors by *Acromyrmex* ants. In fact, this chance was 5 times greater in the presence of phorids than in their absence [19]. It is particularly intriguing why phorids that attack *Atta* ants are not the same as those attacking *Acromyrmex* [32] considering (1) that, in several cases, the ants are attacked by species from the same genus, (2) that hosts oviposited by different phorid species respond in such similar ways to the attacking flies, and (3) that both host genera could be present in the same habitat as well as their specific parasitoids. Besides, *Atta* parasitoids do not attack soldiers, a caste not present in *Acromyrmex* ants.

Although ant species varied in the incidence levels of defensive behaviors like the ones mentioned above, most ant species reacted against different phorids utilizing similar behaviors, as, for example, ants being attacked by an anus ovipositing fly typically lowered their abdomen, whereas ants being attacked by a head ovipositing fly adopted a C or biting posture (Figure 1). In contrast, parasitoids perform different behaviors when presented with multiple hosts [19]. Furthermore, *Acromyrmex* ants are generalist hosts in terms of being attacked by several phorid species, whereas phorids are mainly specialists (attack only one host species) [20], adding another level of asymmetry in the interaction. This pattern is not as strong for *Atta* ants [9]. As mentioned in Elizalde and Folgarait [19], parasitoids can choose their hosts whereas leaf cutters cannot easily reject or avoid a specific phorid species. Phylogenetic analyses of phorids that attack each genus may shed some light although immunological capacities could also help explain the lack of overlap. However, it will be more fruitful to first perform specificity tests offering different species of specialist parasitoids to a single

host species. Besides, it will be useful to evaluate, in long-term field studies, new communities where leaf-cutter hosts and nonhosts of several phorids species are present.

4.2. Hitchhikers. There has been a long standing controversy regarding the role(s) of hitchhikers, which are small ants riding on leaves that are transported by foraging workers. Despite the initial role proposed as defenders against parasitoids of the ants they ride [37], other functions are offered such as leaf microbes cleaners or sap ingestion from cut leaves [47–49]. Initially, it was also proposed that hitchhikers needed a flat surface where to ride [37] and were present only during the day because of the diurnal phorid activity [46]. However, in *Acromyrmex,* hitchhikers were found to ride on tips of monocots or pieces of grasses, they were present at night, and it was shown that nocturnal phorids exist [19, 35].

At present, hitchhikers are known for each of the 9 *Atta* species in which this behavior has been studied. *Acromyrmex*, however, do not have hitchhikers in about 1/3 (5 of 14 species) of the studied species; interestingly waste removers never carried hitchhikers [19]. The latter authors have shown that the chance for finding greater proportion of ants exhibiting hitchhikers was 2.5 times greater in the presence of phorids than in their absence.

5. Leaf Cutter and Their Parasitoids: Some Relevant Aspects of Their Interaction

5.1. Parasitism Rates. Natural parasitism from the same nests of *Atta* vary through time [25, 32], and these rates may reflect changes in health status of each colony or physiological tolerances of phorids to different weather conditions. For comparable data, percentages of natural parasitism in *Atta* are greater than in *Acromyrmex* in Argentina. Medians vary from 0.9–2.2% in *Acromyrmex* species to 3.8–20.2% in *Atta* [32]. However, the previous values include different species of ants and are medians. If we evaluate the parasitism rate by species and consider the maximum values, numbers are quite different. For example, a 12.5% was recorded in autumn for *A. lundi*, and a 35% maximum parasitism was found in *At. vollenweideri* in a mild winter. Evidently, parasitism rates not only change with seasons but also do across years. For example, for *At. vollenweideri* sampled at the same sampling site, maximum values range from 4% to 35% at different years [25, 32].

Rates of parasitism could also be related to the health status of the colonies, as discussed in Section 5.4.

5.2. Host Sizes. The parasitoid decision, about which host is good or not, should involve not only quality but also host size or amount of available food. In general, the larger the host selected, the bigger the resulting adult phorid [9, 25]. Host size is related to the amount of food available for the internal larvae to feed and be able to pupariate. Both, in *Atta* and *Acromyrmex*, several sizes are parasitized, but it is interesting to highlight that the ant size distribution available for parasitism does not differ statistically from that used for oviposition in *Acromyrmex*, though it does in *Atta*; for the latter the smallest, biggest, or both extremes of the ant size distribution are not used as hosts [9, 32]. It is important to know the ant distribution available and that used by the phorids for two reasons: (1) a mean will not represent the most abundant size available relative to that used by the flies if the ant size distribution is not normal (which is typically left-skewed), and (2) without the ant distribution and that used by each phorid it is not possible to make inferences about phorid competition or segregation. Furthermore, speculations of ant competition/segregation should not be done considering either only one host and several phorids or the other way around, because several species in a particular area coexist, at least, at some months per year with other competitors and hosts. Therefore, community studies are necessary to make the best inferences and understand the community assembly rules involved for the species under study.

5.3. Sex Ratios. Data recorded so far [9, 25, 32] show that there is no sexual size dimorphism in adult flies nor in the size of the heads from which females and males emerge. This pattern holds for *Acromyrmex* as well as for *Atta* phorids. Possibly as a consequence of this, the sex ratio is near 1 or does not differ statistically from one in the many instances studied for phorids attacking leaf-cutter ants. This pattern is somehow unexpected because for many fire ant parasitoids females emerge from bigger head sizes whereas smaller heads produce males within a species [44].

The host size to adult fly size pattern is also very interesting because, on the one hand, the size of phorids is very different; for example, *Eibesfeldtphora* is double the size of *Myrmosicarius*, and two species of these genera attack the same size of the same host [32]. On the other hand, because of the great intraspecific plasticity of phorids, parasitoids coming from greater head sizes produce bigger phorids in comparison to those emerging from smaller ones [25, 32]. Three lines of research are needed in order to shed light on the two mentioned patterns; it will be important (1) to evaluate the sex ratios of phorids attacking monomorphic ants, (2) to discern if monomorphic or polymorphic ants and their specific phorid genera/species are more primitive or evolved, and (3) to study genetically the mechanism of sex determination.

5.4. The Gestalt-Immunology Hypothesis. A common pattern found in parasitoids attacking soil ants is that they parasitize ants from a few nests out of the total possible ones available in the same patch. Moreover, the same nests from which phorids emerged continue as such through time. Similarly, the percentage of parasitism could vary enormously from one colony to the other close by ([20]; Guillade unpublished). The fidelity and/or the great parasitism of a particular nest(s) through time represent(s) that the nest(s) in question is (are) better to complete the parasitoid's life cycle. How do phorids assess which nest is good? If the health of a colony or its suitability as a good host is linked with a particular taste, then phorids could choose one nest but not another using sensorial cues.

It has been shown in ants the importance of a chemical signature, given by their cuticular hydrocarbons, which is used by nestmates to differentiate self from nonself [50]. This implies that the particular chemical can be sensed by other ants also. We can extend this argument involving other organisms such as phorids. In fact, there is evidence from other systems that parasitoids can cue on the volatile compounds released by the plants due to having been fed by their herbivores [51]. Also, fire ant parasitoids use long-range olfactory cues to detect their hosts [42]. Then, if the gestalt (unique chemical signature shared by all members of a nest) of a colony is somehow related/linked to the health status of that colony, the consequence is obvious. Healthy colonies with vigorous ants will better nourish the parasitoid larvae than unhealthy ones which will have an altered gestalt. As the cuticular hydrocarbons are nonvolatiles, this information should be gathered by a phorid at very close range, in fact, by touching it. Following a sequence of events involved in host location, parasitoids first may use ant's alarm and/or trail pheromones as long-range cues to locate the ants (or their nests), second they may use intermediate-distance cues, such as visual ones, to determine which is the correct host size, and finally use taste-type cues to assess the health status of the ant/colony. This hypothesis can also help explain what is normally seen in fire ants, that is, where one colony is parasitized but not another one close by and surrounded by the same vegetation. In fact, cryptic sympatric species (haplotypes) are known of *S. saevissima* based on cuticular hydrocarbons and venom alkaloids [52]. Therefore, if there is a link between the cuticular hydrocarbons and the immunological status of the colonies, then a taste mechanism can be used to explain the parasitism rates discussed.

To my knowledge nothing is known about how the gestalt and immunological status of leaf-cutter ants (or any other) relate to each other and how these parameters could affect their relationships with natural enemies. De Souza et al. [53] evaluated encapsulation rates and cuticular hydrocarbon profiles in *Acromyrmex subterraneus* but did not relate one to the other because they were interested in answering another type of question.

5.5. The Asymmetry Hypothesis. The fact that hosts respond to phorids attack with similar behaviors, whereas phorids varied substantially among species in choosing and ovipositing their host, indicates that there is a great interspecific variation found in phorid behaviors but not in their host's responses giving support to the asymmetry hypothesis [54] in which the parasitoids can evolve different behavioral strategies as they can choose their prey but the hosts cannot evolve specific responses towards each parasitoid under the uncertainty of which one they will attack [32]. In addition, the high host specificity shown for most fly species with about 3/4 of taxa utilizing one host (30 in total, with 19 attacking *Atta* and 11 on *Acromyrmex*) and 13 different phorid species (6 attacking *Atta* and 7 *Acromyrmex*) using several species [9] is a pattern that somehow favors expectations from the asymmetry hypothesis. On the other hand, these host specificity ratios reflect data obtained from several regions and seasons. It will be interesting to analyze the web of interactions at a local scale and from a richness point of view. If it holds, that is, finding more parasitoid species attacking a single species than attacking multiple hosts within each ant genus (where the immunological system might be more similar), then the asymmetry hypothesis could also help explain phorid speciation.

5.6. The Conspicuousness-Abundance-Stability Hypothesis. There might be a reason why every species of *Atta* has phorids attacking them while the same does not occur in *Acromyrmex*. One obvious hypothesis could be the conspicuousness and temporal-spatial stability of *Atta* which assures an enormous amount of resources available, relative to that for *Acromyrmex* [11]. If we define conspicuousness as any index that considers nest size, ant activity/trial, and number of trails, then a positive relationship could be expected among nests from different species that have different conspicuousness and the richness/abundance of phorids attacking them [55].

Acromyrmex species without known phorids are relatively inconspicuous with low number of individuals/colony. In fact, the species richness and abundance of hosts were the main determinants of phorid richness at the nest, hectare, and local scale, although, for the latter scale, climatic variables emerged in importance [12]. Moreover, the conspicuousness of the host was also important in explaining parasitoid richness [55]. In conjunction with the intriguing pattern that leaf-cutter phorids do not attack both genera of potential hosts, this latter result suggests that past competition could have led to segregation across different host niche axes [20, 25] whereas ecological conditions at local scales, with the availability of particular combination of hosts, may produce the final assembly that minimizes host overlap.

6. Biological Control of Leaf-Cutter Ants by Parasitoids

Leaf-cutter ant parasitoids exhibit several features that suggest they may become promising biological controls of leaf-cutter ants.

(1) They are generally species host specific, with no intergenus parasitism to the extent that *Atta* and *Acromyrmex* phorids should be considered separate guilds.

(2) They attack different sizes of hosts and in the case of *Acromyrmex* utilize most of the potential host size distribution which can assure the complete parasitism of all castes present in a colony.

(3) The percentage of parasitism is high, in comparison to other analogous parasitoids such as fire ant *Pseudacteon* spp. In addition, they have a strong negative impact on ant foraging in the field.

(4) The varied behavioral repertoire (attack strategies, presence throughout day and night and across seasons) and sites of attack (habitat and anatomical)

allow the selection of complementary species to promote broad spectrum parasitism.

(5) The 1:1 sex ratio is extremely important to warrant matings in the laboratory as well as in the field.

(6) The successful rearing of these parasitoids in the laboratory presents important baseline data that can be used to achieve mass rearing (Folgarait, unpublished).

(7) The existence of a positive relationship between host size and phorid size could allow manipulation in the laboratory to produce females of greater size that might survive longer and have greater fecundity that would lead to higher attack levels.

(8) The high resistance of some species to extreme weather and changes of climate [25] would allow for a larger area of biological control coverage.

(9) The plasticity in host size selection makes these parasitoids less dependent on the varied size of hosts available [20].

However, it should be highlighted that the single use of parasitoids may not be able to control leaf-cutter ants. The hundred to million individuals involved in the nests of this successful group of ants will certainly need the use of a combination of different strategies to control them.

7. Promising Lines of Research

Over half of the 67 known species (38) have been described since Feener Jr. and Brown [1]. In addition, a great amount of information has been gathered on the basic biology of these newly discovered species, as well as that of longer known taxa. This information is also fundamental to any applied utilization of these parasitoids for biological control, including the descriptions of life cycles of many of the extant species, their host associations, the discovery of two guilds defined by the host genus, and the oviposition behaviors and response by their hosts under different circumstances. However, much waits to be studied and discovered about the fascinating interactions within this system. To help guide us through the many possible lines of research proposed within the body of this text, I list here the lines of research that I consider to be most important.

(1) Examine how the physiological status of ant colonies, including immunological status, impacts on the performance of their parasitoids.

(2) Identify the type of cues used by parasitoids to

  (a) locate their host(s) at long and proximate distances,

  (b) asses if hosts are already parasitized,

  (c) determine if the colony is appropriate or not in order to be used as a source of ants to parasite.

(3) Understand the assembly rules involved in the leaf-cutter-parasitoid system at the community level.

(4) Determine the place where parasitoid mating, late-stage infected host ants, and pupae are located, for at least 1 species from each host genus.

(5) Develop a system by which ants can be parasitized in the laboratory without the need of the whole colony.

Acknowledgments

The author thanks all colleagues that have been contributing through time to this fascinating system of study, especially to Dr. Disney and Dr. Brown, her ex-Ph.D. student Dr. Elizalde and her current one Lic. Guillade. Dr. Patrock kindly reviewed this paper, improving it, as he always does. Drawings were made by A. Guillade. The author is also grateful to previous grants from National Geographic, National Council for Scientific and Technological Research (CONICET), National Agency for the Promotion of Science and Technology (ANPCyT), and National University of Quilmes (UNQ) that allowed her to gather data on this system. This paper was written under current Grants CONICET (PIP 11420090100195), ANPCyT (Pict Start Up 1936), and UNQ (Programa PIBMaC 1009/11).

References

[1] D. H. Feener Jr. and B. V. Brown, "Diptera as parasitoids," *Annual Review of Entomology*, vol. 42, pp. 73–97, 1997.

[2] L. W. Morrison, "Biology of *Pseudacteon* (Diptera: Phoridae) ant parasitoids and their potential to control imported *Solenopsis* fire ants (Hymenoptera: Formicidae)," *Recent Research and Development in Entomology*, vol. 3, pp. 1–13, 2000.

[3] D. H. Feener Jr., "Competition between ant species: outcome controlled by parasitic flies," *Science*, vol. 214, no. 4522, pp. 815–817, 1981.

[4] E. G. LeBrun and D. H. Feener Jr., "Linked indirect effects in ant-phorid interactions: impacts on ant assemblage structure," *Oecologia*, vol. 133, no. 4, pp. 599–607, 2002.

[5] S. M. Philpott, I. Perfecto, J. Vandermeer, and S. Uno, "Spatial scale and density dependence in a host parasitoid system: an arboreal ant, *Azteca instabilis*, and its *Pseudacteon* phorid parasitoid," *Environmental Entomology*, vol. 38, no. 3, pp. 790–796, 2009.

[6] G. L. Pardee and S. M. Philpott, "Cascading indirect effects in a coffee agroecosystem: effects of parasitic phorid flies on ants and the coffee berry borer in a high-shade and low-shade habitat," *Environmental Entomology*, vol. 40, no. 3, pp. 581–588, 2011.

[7] B. V. Brown and D. H. Feener Jr., "Life history parameters and description of the larva of *Apocephalus paraponerae* (Diptera: Phoridae), a parasitoid of the giant tropical ant *Paraponera clavata* (Hymenoptera: Formicidae)," *Journal of Natural History*, vol. 25, no. 1, pp. 221–231, 1991.

[8] H. Hsun-Yi and I. Perfecto, "Trait mediated indirect effects of phorid flies on ants," *Psyche*, vol. 2012, Article ID 380474, 11 pages, 2012.

[9] M. A. L. Bragança, "Parasitoides de formigas cortadeiras," in *Formigas-Cortadeiras: da Bioecologia ao Manejo*, T. M. Castro Della Lucia, Ed., pp. 321–343, Universidade Federal de Viçosa, Viçosa, Brasil, 2011.

[10] R. H. L. Disney, L. Elizalde, and P. J. Folgarait, "New species and new records of scuttle flies (diptera: Phoridae) that parasitize

leaf-cutter and army ants (Hymenoptera: Formicidae)," *Sociobiology*, vol. 54, no. 2, pp. 601–631, 2009.

[11] J. H. Delabie, H. S. Rodrigues Alves, G. M. Reuss-Strenzel, A. F. Ribeiro do Carmo, and I. Cardoso do Nascimento, "Distribucao das formigas-cortadeiras *Acromyrmex* e *Atta* no Novo Mundo," in *Formigas-Cortadeiras: da Bioecologia ao Manejo*, T. M. Castro Della Lucia, Ed., pp. 80–101, Universidade Federal de Viçosa, Viçosa, Brasil, 2011.

[12] L. Elizalde and P. J. Folgarait, "Host diversity and environmental variables as determinants of the species richness of the parasitoids of leaf-cutting ants," *Journal of Biogeography*, vol. 37, no. 12, pp. 2305–2316, 2010.

[13] C. R. Ferreira Brandao, A. J. Mayhe-Nunes, and C. E. Dias Sanhudo, "Taxonomia e filogenia das formigas-cortadeiras," in *Formigas-Cortadeiras: da Bioecologia ao Manejo*, T. M. Castro Della Lucia, Ed., pp. 27–48, Universidade Federal de Viçosa, Viçosa, Brasil, 2011.

[14] B. Holldobler and E. O. Wilson, *The Ants*, Cambridge University Press, New York, NY, USA, 1990.

[15] A. A. Bonetto, *Las Hormigas "Cortadoras" de la Provincia de Santa Fé (Géneros: Atta y AcromYrmex)*, Ministerio de Agricultura y Ganaderia, Provincia de Santa Fe, Argentina, 1959.

[16] L. C. Forti, M. L. de Andrade, A. P. P. Andrade, J. F. S. Lopes, and V. M. Ramos, "Bionomics and identification of *Acromyrmex* (Hymenoptera: Formicidae) through an illustrated key," *Sociobiology*, vol. 48, no. 1, pp. 135–153, 2006.

[17] L. C. Forti, A. Alves Moreira, A. P. Protti Andrade, M. A. Castellani, and N. Caldato, "Nidificaçao e arquitetura de ninhos de formigas -cortadeiras," in *Formigas-Cortadeiras: da Bioecologia ao Manejo*, T. M. Castro Della Lucia, Ed., pp. 102–125, Universidade Federal de Viçosa, Viçosa, Brasil, 2011.

[18] M. Bollazzi, J. Kronenbitter, and F. Roces, "Soil temperature, digging behaviour, and the adaptive value of nest depth in South American species of *Acromyrmex* leaf-cutting ants," *Oecologia*, vol. 158, no. 1, pp. 165–175, 2008.

[19] L. Elizalde and P. J. Folgarait, "Behavioral strategies of phorid parasitoids and responses of their hosts, the leaf-cutting ants," *Journal of Insect Science*, vol. 12, article 135, 2012.

[20] L. Elizalde, *Biogeografía y comunidades de fóridos parasitoides de hormigas cortadoras de hojas. Diversidad del sistema y partición del recurso hospedador [Ph.D. thesis]*, Universidad Nacional de Quilmes, Buenos Aires, Argentina, 2009.

[21] P. Eggleton and R. Belshaw, "Insect parasitoids: an evolutionary overview," *Philosophical Transactions of the Royal Society of London B*, vol. 337, no. 1279, pp. 1–20, 1992.

[22] R. H. L. Disney, *Scuttle Flies: The Phoridae*, Chapman and Hall, London, UK, 1994.

[23] R. H. L. Disney, L. Elizalde, and P. J. Folgarait, "New species and records of scuttle flies (Diptera: Phoridae) associated with leaf-cutter ants and army ants (Hymenoptera: Formicidae) in Argentina," *Sociobiology*, vol. 51, no. 1, pp. 95–117, 2008.

[24] L. Elizalde and P. J. Folgarait, "Primeros registros de foridos parasitoides (Phoridae: Diptera) de hormigas cortadoras de hojas (*Atta y Acromyrmex*) para Argentina y Paraguay, incluyendo su distribución en el área total donde se encuentran los hospedadores," in *VI Congreso Argentino de Entomología*, San Miguel del Tucuman, Argentina, 2005.

[25] A. C. Guillade and P. J. Folgarait, "Life-history traits and parasitism rates of four phorid species (Diptera: Phoridae), parasitoids of *Atta vollenweideri* (Hymenoptera: Formicidae) in Argentina," *Journal of Economic Entomology*, vol. 104, no. 1, pp. 32–40, 2011.

[26] B. V. Brown, "Revision of the *Apocephalus* attophilus-group of ant-decapitaiting flies (Diptera: Phoridae)," *Contribution in Science*, vol. 468, pp. 1–60, 1997.

[27] B. V. Brown, "Taxonomic revision of *Neodohrniphora*, subgenus *Eibesfeldtphora* (Diptera: Phoridae)," *Insect Systematics and Evolution*, vol. 32, no. 4, pp. 393–409, 2001.

[28] T. Borgmeier, "Investigacoes sobre Phorideos myrmecophilos (Diptera, Phoridae)," *Archivos do Instituto Biologico de Defesa Agricola e Animal São Paulo*, vol. 1, pp. 159–192, 1928.

[29] R. H. L. Disney, L. Elizalde, and P. J. Folgarait, "New species and revision of *Myrmosicarius* (Diptera: Phoridae) that parasitize leaf-cutter ants (Hymenoptera: Formicidae)," *Sociobiology*, vol. 47, no. 3, pp. 771–810, 2006.

[30] B. V. Brown, R. H. L. Disney, L. Elizalde, and P. J. Folgarait, "New species and new records of *Apocephalus* Coquillett (Diptera: Phoridae) that parasitize ants (Hymenoptera: Formicidae) in America," *Sociobiology*, vol. 55, no. 1 B, pp. 165–190, 2010.

[31] R. H. L. Disney, "A key to *Neodohrniphora* (Diptera: Phoridae), parasites of leaf-cutter ants (Hymenoptera: Formicidae)," *Journal of Natural History*, vol. 30, no. 9, pp. 1377–1389, 1996.

[32] L. Elizalde and P. J. Folgarait, "Biological attributes of Argentinian phorid parasitoids (Insecta: Diptera: Phoridae) of leaf-cutting ants, *Acromyrmex* and *Atta*," *Journal of Natural History*, vol. 45, no. 43-44, pp. 2701–2723, 2011.

[33] A. Tonhasca Jr, "Interactions between a parasitic fly, *Neodhorniphora declinata* (Diptera: Phoridae) and its host, the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae)," *Ecotropica*, vol. 2, pp. 157–164, 1996.

[34] M. Erthal Jr. and A. Tonhasca Jr., "Biology and oviposition behavior of the phorid *Apocephalus attophilus* and the response of its host, the leaf-cutting ant *Atta laevigata*," *Entomologia Experimentalis et Applicata*, vol. 95, no. 1, pp. 71–75, 2000.

[35] M. A. L. Bragança, A. Tonhasca Jr., and T. M. C. Delia Lucia, "Reduction in the foraging activity of the leaf-cutting ant *Atta sexdens* caused by the phorid *Neodohrniphora* sp," *Entomologia Experimentalis et Applicata*, vol. 89, no. 3, pp. 305–311, 1998.

[36] M. A. L. Bragança and Z. C. S. Medeiros, "Ocorrência e Características Biológicas de Forídeos Parasitóides (Diptera: Phoridae) da *Saúva Atta laevigata* (Smith) (Hymenoptera: Formicidae) em Porto Nacional, TO," *Neotropical Entomology*, vol. 35, pp. 408–411, 2006.

[37] D. H. Feener Jr. and K. A. G. Moss, "Defense against parasites by hitchhikers in leaf-cutting ants: a quantitative assessment," *Behavioral Ecology and Sociobiology*, vol. 26, no. 1, pp. 17–29, 1990.

[38] J. Tonhasca Jr., M. A. L. Bragança, and J. Erthal Jr., "Parasitism and biology of *Myrmosicarius grandicornis* (Diptera, Phoridae) in relationship to its host, the leaf-cutting ant *Atta sexdens* (Hymenoptera, Formicidae)," *Insectes Sociaux*, vol. 48, no. 2, pp. 154–158, 2001.

[39] M. A. L. Bragança, A. Tonhasca Jr., and T. M. C. Della Lucia, "Biological and behavioral characteristics of *Neodohrniphora elongata* Brown (Diptera, Phoridae), a parasitic fly of the leaf-cutting ant *Atta sexdens rubropilosa* Forel (Hymenoptera, Formicidae)," *Revista Brasileira de Entomologia*, vol. 53, no. 4, pp. 600–606, 2009.

[40] D. H. Feener Jr. and B. V. Brown, "Oviposition behavior of an ant-parasitizing fly, *Neodohrniphora curvinervis* (Diptera: Phoridae), and defense behavior by its leaf-cutting ant host *Atta cephalotes* (Hymenoptera: Formicidae)," *Journal of Insect Behavior*, vol. 6, no. 6, pp. 675–688, 1993.

[41] V. Gazal, O. Bailez, and A. M. Viana-Bailez, "Mechanism of host recognition in *Neodohrniphora elongata* (Brown) (Diptera: Phoridae)," *Animal Behaviour*, vol. 78, no. 5, pp. 1177–1182, 2009.

[42] L. W. Morrison and J. R. King, "Host location behavior in a parasitoid of imported fire ants," *Journal of Insect Behavior*, vol. 17, no. 3, pp. 367–383, 2004.

[43] K. A. Mathis and S. M. Philpott, "Current understanding and future prospects of host selection, acceptance, discrimination, and regulation of phorid fly parasitoids that attack ants," *Psyche*, vol. 2012, Article ID 895424, 2012.

[44] L. W. Morrison and L. E. Gilbert, "Parasitoid-host relationships when host size varies: the case of *Pseudacteon* flies and *Solenopsis* fire ants," *Ecological Entomology*, vol. 23, no. 4, pp. 409–416, 1998.

[45] P. J. Folgarait, O. A. Bruzzone, R. J. W. Patrock, and L. E. Gilbert, "Developmental rates and host specificity for *Pseudacteon* parasitoids (Diptera: Phoridae) of fire ants (Hymenoptera: Formicidae) in Argentina," *Journal of Economic Entomology*, vol. 95, no. 6, pp. 1151–1158, 2002.

[46] M. R. Orr, "Parasitic flies (Diptera: Phoridae) influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes* (Hymenoptera: Formicidae)," *Behavioral Ecology and Sociobiology*, vol. 30, no. 6, pp. 395–402, 1992.

[47] E. H. M. Vieira-Neto, F. M. Mundim, and H. L. Vasconcelos, "Hitchhiking behaviour in leaf-cutter ants: an experimental evaluation of three hypotheses," *Insectes Sociaux*, vol. 53, no. 3, pp. 326–332, 2006.

[48] T. A. Linksvayer, A. C. McCall, R. M. Jensen, C. M. Marshall, J. W. Miner, and M. J. McKone, "The function of hitchhiking behavior in the leaf-cutting ant *Atta* cephalotes," *Biotropica*, vol. 34, no. 1, pp. 93–100, 2002.

[49] H. M. Griffiths and W. O. H. Hughes, "Hitchhiking and the removal of microbial contaminants by the leaf-cutting ant *Atta colombica*," *Ecological Entomology*, vol. 35, no. 4, pp. 529–537, 2010.

[50] A. Lenoir, D. Fresneau, C. Errard, and A. Hefetz, "The individuality and the colonial identity in ants: the emergence of the social representation concept," in *Information Processing in Social Insects*, C. Detrain, J. L. Deneubourg, and J. Pasteels, Eds., pp. 219–237, Birkhauser, Basel, Switzerland, 1999.

[51] A. Clavijo McCormick, S. B. Unsicker, and J. Gershenzon, "The specificity of herbivore-induced plant volatiles in attracting herbivore enemies," *Trends in Plant Science*, vol. 17, pp. 303–310, 2012.

[52] E. Goncalvez Paterson Fox, A. Pianaro, D. Russ Solis et al., "Intraspecific and intracolonial variation in the profile of venom alkaloids and cuticular hydrocarbons of the fire Ant *Solenopsis saevissima* Smith (Hymenoptera: Formicidae)," *Psyche*, vol. 2012, Article ID 398061, 10 pages, 2012.

[53] D. J. de Souza, A. Lenoir, M. C. Megumi Kasuya et al., "Ectosymbionts and immunity in the leaf-cutting ant *Acromyrmex subterraneus subterraneus*," *Brain Behavior, and Immunity*, vol. 28, pp. 182–187, 2013.

[54] L. Lapchin and T. Guillemaud, "Asymmetry in host and parasitoid diffuse coevolution: when the red queen has to keep a finger in more than one pie," *Frontiers in Zoology*, vol. 2, article 4, 2005.

[55] L. Elizalde L and P. J. Folgarait, "Diversity of interactions among leaf-cutting ants and their phorid parasitoids: a community approach," in *Primer Encuentro de Mirmecologistas de las Américas y XX Simpósio de Mirmecologia*, Petropolis, Brasil, 2011.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 936341, 11 pages
http://dx.doi.org/10.1155/2013/936341

Research Article

Bacterial Infections across the Ants: Frequency and Prevalence of *Wolbachia*, *Spiroplasma*, and *Asaia*

Stefanie Kautz,[1] Benjamin E. R. Rubin,[1,2] and Corrie S. Moreau[1]

[1] *Department of Zoology, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605, USA*
[2] *Committee on Evolutionary Biology, University of Chicago, 1025 East 57th Street, Chicago, IL 60637, USA*

Correspondence should be addressed to Stefanie Kautz; skautz@fieldmuseum.org

Received 21 February 2013; Accepted 30 May 2013

Academic Editor: David P. Hughes

Bacterial endosymbionts are common across insects, but we often lack a deeper knowledge of their prevalence across most organisms. Next-generation sequencing approaches can characterize bacterial diversity associated with a host and at the same time facilitate the fast and simultaneous screening of infectious bacteria. In this study, we used 16S rRNA tag encoded amplicon pyrosequencing to survey bacterial communities of 310 samples representing 221 individuals, 176 colonies and 95 species of ants. We found three distinct endosymbiont groups—*Wolbachia* (Alphaproteobacteria: Rickettsiales), *Spiroplasma* (Firmicutes: Entomoplasmatales), and relatives of *Asaia* (Alphaproteobacteria: Rhodospirillales)—at different infection frequencies (at the ant species level: 22.1%, 28.4%, and 14.7%, resp.) and relative abundances within bacterial communities (1.0%–99.9%). *Spiroplasma* was particularly enriched in the ant genus *Polyrhachis*, while *Asaia* relatives were most prevalent in arboreal ants of the genus *Pseudomyrmex*. While *Wolbachia* and *Spiroplasma* have been surveyed in ants before, *Asaia*, an acetic acid bacterium capable of fixing atmospheric nitrogen, has received much less attention. Due to sporadic prevalence across all ant taxa investigated, we hypothesize facultative associations for all three bacterial genera. Infection patterns are discussed in relation to potential adaptation of specific bacteria in certain ant groups.

1. Introduction

Recent studies have shown that insects are associated with a broad range of unrelated microbial taxa [1, 2]. These interactions shape the ecology and evolution of hosts and bacterial symbionts and often heavily impact host biology [3, 4]. Congruent evolutionary histories between some symbiotic partners show the likely obligate nature of this relationship [5], while other associations occur sporadically and can vary both spatially and temporally [6]. Bacterial endosymbionts sometimes inhabit specialized host cells or structures [7, 8] and might even share metabolic pathways with their hosts [9], while others occur loosely in unspecific tissues or hemolymph [10].

Microbes associated with insects are extremely diverse and span-wide taxonomic groups, even within individual hosts. One of the best-characterized endosymbiont groups is comprised of insect-associated bacteria that increase the nutritive value of their hosts' diets. These bacteria are often highly specialized and coevolved associates, playing particularly important roles in insects with nutritionally limited or deficient diets. Some well-known examples of such endosymbionts include *Buchnera aphidicola* in aphids, which provide their hosts with essential amino acids lacking in the sugar-rich but nitrogen-poor phloem sap [11]. Other examples are the cospeciated and essential amino acid synthesizing *Blochmannia* endosymbionts of Camponotini ants [12, 13], nitrogen fixing taxa in the fungal gardens of the leaf-cutter ants [14], *Wigglesworthia glossinidia,* which provides vitamins that are lacking in the blood meals of its host, the tsetse fly [15], and the nitrogen-fixing microflora of termites [16, 17]. In ants, several recent studies have highlighted the importance of bacterial symbionts for nutrition, especially in ant taxa feeding low on the trophic scale [18–20].

Symbiotic bacteria can also play other beneficial roles by protecting insects from parasites and pathogens and thus

defending their hosts against natural enemies [4, 7, 21]. For example, *Spiroplasma* can convey increased resistance to nematode infections in *Drosophila* flies [22], and secondary symbionts in aphids can confer resistance to parasitic wasps [23]. Some insect-associated bacteria also contribute to nest hygiene [7]. For example, actinomycetes in the fungal gardens of leaf-cutter ants inhibit the growth of fungal pathogens, but not of mutualistic fungi [24]. Actinomycetes are also found in antennal glands of bee wolves and protect larvae in their nests against infestation by pathogens [25]. Other mutualistic bacteria can increase host tolerance to unfavorable abiotic conditions such as temperature stress [26] or facilitate the use of novel hosts [27].

While the associations described above are typically beneficial to hosts, many bacterial endosymbionts are detrimental reproductive manipulators. *Wolbachia*, for example, can cause cytoplasmic incompatibility, parthenogenesis, male killing, and male feminization [28]. There are also examples of *Wolbachia*, which protect their host against RNA viruses, thus acting as defensive mutualists [29]. An estimated 66% of insect species and about 30% of ant species have been reported to be facultatively infected with *Wolbachia* [30, 31]. Other less prevalent reproductive manipulators in insects include *Cardinium*, *Arsenophonus,* and *Spiroplasma* [32, 33]. *Spiroplasma*, although beneficial to hosts in some cases [22], can have various negative effects on their insect hosts, including manipulation of sex ratios, male killing, and entomopathenogenicity [33–35].

Despite these fascinating findings, our knowledge of bacterial symbionts is based on a relatively small number of organisms. Thus, we still know little about the identities and ecological or physiological functions of bacteria associated with most animal groups [36]. In-depth analyses and extensive surveys of the bacterial communities present in a wide range of eukaryotic taxa are required to understand the diversity and the function of microbial symbionts [37]. Here, we analyzed bacterial communities across the ants (Hymenoptera: Formicidae) using 16S rRNA tag encoded amplicon pyrosequencing (454 pyrosequencing) to survey for infection patterns with potential parasitic microbes. Due to their sporadic prevalence and unknown effects on host ant biology, we refer to these microbes as infections. In total, we screened 310 ant samples of 176 colonies from 95 ant species and encountered high prevalence of three bacterial groups: *Wolbachia*, *Spiroplasma,* and *Asaia*.

2. Materials and Methods

A total of 299 ant samples were subjected to 454 pyrosequencing and combined with data from 11 samples analyzed by Ishak et al. [38], that is for a total of 310 samples. All samples represented 176 different colonies and 95 different ant species belonging to the genera *Camponotus* (Formicinae; 1 species), *Cephalotes* (Myrmicinae; 7 species), *Crematogaster* (Myrmicinae; 6 species), *Myrmecia* (Myrmeciinae; 2 species), *Myrmecocystus* (Formicinae; 1 species), *Oecophylla* (Formicinae; 1 species), *Paraponera* (Paraponerinae; 1 species), *Polyrhachis* (Formicinae; 32 species), *Pseudomyrmex* (Pseudomyrmecinae; 36 species), *Solenopsis* (Myrmicinae; 2 species) and *Tetraponera* (Pseudomyrmecinae; 6 species). DNA extractions were either prepared from entire ants or from dissected ant parts as described in Kautz et al. [39]. A complete list of samples used for this study can be found in Supplementary Table 1 (see Supplementary material available online at http://dx.doi.org/10.1155/2013/936341).

2.1. 454 Pyrosequencing. To screen ant samples for overall bacterial diversity, bacterial tag-encoded FLX amplicon pyrosequencing was performed by the Research and Testing Laboratory (Lubbock, TX, USA) as described by Dowd et al. [40]. The 16S rRNA universal eubacterial primers 28F (5′-GAGTTTGATCITGGCTCAG) and 519R (5′-GWATTACCGCGGCKGCTG) were used to amplify approximately 500 bp of the variable regions V1–V3.

2.2. Bacterial 16S rRNA Data Processing and Analysis. All 16S rRNA pyrosequencing reads were quality controlled and denoised using the QIIME v1.5.0 implementation of AmpliconNoise v1.25 using default parameters [41]. Chimeras were removed by Perseus, a component of the AmpliconNoise pipeline [42]. All the remaining reads were then clustered into operational taxonomic units (OTUs) at 97% sequence similarity using UCLUST [43]. We used the longest sequence in a cluster as the representative sequence for that OTU. Singletons, that is, OTUs with only one read in the entire dataset, were removed. We used the QIIME implementation of the Ribosomal Database Project [44] classifier trained on the February 4, 2011 release of the greengenes database [45] to classify OTUs at the level of bacterial orders. Default settings were used, including a 0.8 confidence cutoff for classifications.

Our filtering approach recovered infections with *Wolbachia* (Alphaproteobacteria: Rickettsiales), *Spiroplasma* (Firmicutes: Entomoplasmatales), and *Asaia* (Alphaproteobacteria: Rhodospirillales). All OTUs classified as Rickettsiales, Entomoplasmatales, and Rhodospirillales that accounted for more than one percent of reads within a sample were considered as infections by the respective order and included in further analyses. This cutoff also allowed us to control the relatively high error rate of 454 pyrosequencing. We classified the sequences at the genus level using the RDP classifier (see Supplementary Table 2 for results). All OTUs used in further analyses have been deposited in GenBank (accessions KF015767-KF015856; Supplementary Table 2).

We downloaded the closest relatives of each OTU from GenBank. Additionally, we were interested in retrieving any other sequence from GenBank of those three orders that were associated with ants and insects in general. Thus, we searched for sequences using the search keywords "16S" and "symbiont" as well as the name of the respective order. GenBank sequences with 99% identity that were isolated from the same source were considered duplicates and deleted from the dataset.

2.3. Phylogenetic Tree Construction. Sequences were compiled and edited using Geneious v5.3.6 [46]. The alignment

was generated using the infernal secondary-structure-based aligner of the ribosomal database project (RDP) [44]. We inferred a maximum likelihood phylogeny of the most common OTUs and their GenBank relatives using the RAxML 7.2.8 Black Box [47] on the CIPRES web portal [48]. The model GTR+I+G was employed. We then uploaded the most likely tree to the iToL website [49] to facilitate graphical illustration of bacterial source, ant subfamily, and geographic region for each sequence. Trees with branch length and bootstrap support are provided as supplementary material (Figures S1–S3).

3. Results and Discussion

3.1. Wolbachia (Alphaproteobacteria: Rickettsiales). In our study, 21 of 95 ant species had at least one individual infected with *Wolbachia* (Table 1). Across all 304 samples from which we obtained data (Supplementary Table 1), we found 30 *Wolbachia* OTUs. Overall, with 22.1% of infected species this is a lower infection rate of *Wolbachia* across ants than has been reported before. In an extensive compilation of existing data, about 28.6% of ant species carried *Wolbachia* infections [31], while a frequency of up to 50% had been found previously [50]. This discrepancy from our study to general trends could be due to several reasons. Often a species is counted as being infected with *Wolbachia* when just one individual carries this infection. However, not all individuals of a species or individuals from the same colony need to be infected. Thus, discrepancies in infection rate across studies might merely be due to natural variation among individuals. Also, there is a strong bias in infection rate among different ant groups. Species from the genera *Acromyrmex*, *Formica*, *Solenopsis,* and *Tetraponera* are often infected with *Wolbachia,* while *Dolichoderus* and *Leptogenys* mostly lack infection [51]. For example, in a screening of 24 *Polyrhachis* species, 5 (20.8%) were infected with *Wolbachia* [31]. In the present study, we found the genera *Cephalotes* (57%) and *Solenopsis* (50%) to have particularly high infection rates, *Tetraponera* (33.3%) and *Polyrhachis* (25.0%) with intermediate rates, *Crematogaster* (16.7%) and *Pseudomyrmex* (13.9%) with rather low rates, and no infections in the samples of *Camponotus*, *Myrmecia*, *Myrmecocystus*, *Oecophylla,* and *Paraponera* included here.

Most studies that screen for *Wolbachia* use diagnostic approaches by conducting PCR with *Wolbachia*-specific primers. This is the most reliable means of *Wolbachia* detection [51]. However, even when using diagnostic PCR, negative results can occur due to variations in the primer sequence or low titers of the bacterial symbionts [52]. In our study, we found high variability in *Wolbachia* titers, ranging from 1.03% to 97.36% (Supplementary Table 1). We used a 1% relative abundance within a sample as the cutoff to control error rates of 454 pyrosequencing, which might also have led to lower detected infection rates among species.

In addition to the 30 *Wolbachia* sequences obtained in this study, we downloaded sequence data from GenBank and compiled a dataset of 111 taxa including the outgroup *Rhizobium leguminosarum* (Alpha-proteobacteria: Rhizobiales). The total alignment had a length of 1224 characters. Four ant-specific clades of *Wolbachia* were recovered in the inferred tree (Figure 1; Figure S1). Ant clade 1 comprised *Wolbachia* that was isolated from Australian *Polyrhachis* (6 sequences) as well as one sequence detected in *Cephalotes varians* from the Nearctic. Ant clade 2 included mostly Australian *Polyrhachis* (9 sequences) in addition to sequences found in Nearctic *Solenopsis* and Neotropical *Pseudomyrmex*. Ant clade 3 exclusively contained sequences from European *Formica* species, while ant clade 4 was the most diverse. This fourth clade comprised the majority of ant-associated *Wolbachia* sequences from our dataset as well as existing GenBank data and included the ant subfamilies Dolichoderinae, Ecitoninae, Formicinae, Myrmicinae, Ponerinae, and Pseudomyrmecinae from the Afrotropics, Nearctics, Neotropics, and Palearctics. Overall, 68 out of 82 (82.9%) ant-associated *Wolbachia* sequences clustered in ant-specific clades indicating a certain degree of host specialization. Even though neither ant relatedness (subfamily) nor biogeographic region (continent) was a strong determinant for infection with similar *Wolbachia* strains, related *Wolbachia* seemed to infect related hosts from the same geographic region to some extent. A rather low degree of host specificity has previously been reported for *Wolbachia* across ants and butterflies, while strict cospeciation between *Wolbachia* and its hosts has not been found [51, 53].

Wolbachia are reported to be the most prevalent bacterial symbionts across insects and ants [31], although infections with other bacterial groups were often more frequent in our present study. Despite this ubiquity, to date no studies have been able to show the functional role of *Wolbachia* in ants. This is due to the difficulty of breeding most species of ants in the laboratory, and thus, we have to restrict our knowledge to the correlations of *Wolbachia* infections with specific host traits. *Wolbachia* most commonly manipulate host reproduction, but in ants no such phenomena are known [51]. In *Formica truncorum*, *Wolbachia* infection leads to a reduced production of sexuals, although this could be due to physiological costs rather than direct manipulation [54]. However, worker production is not affected and it has been suggested that *Wolbachia* might reduce the ability of workers to provide resources to alate development [51]. Curing of *Wolbachia* infection within individuals has been observed, which seems to be unique to ants, but the mechanisms behind this phenomenon are not understood [54]. Lastly, ants often show exceptionally high levels of coinfection with multiple *Wolbachia* strains adding another layer of complexity to this poorly understood symbiosis [51]. It has been speculated that eusociality or haplodiploidy might have an impact on *Wolbachia* infection [50, 55], but such mechanisms have never been confirmed. Also, there seems to be a weak correlation of *Wolbachia* infection with colony founding mode as species that found new colonies independently are less frequently infected than species relying on dependent colony founding [50]. Speculations on effects of *Wolbachia* on colony-founding behavior and colony structure have often been made as ants can show exceptional variations in these traits ranging from a single queen that mated once to multiple queens and/or multiple matings per queen [56–58].

Table 1: *Wolbachia*, *Spiroplasma*, and *Asaia* detected by 454 amplicon pyrosequencing across 310 ant samples.

Ant genus and subfamily	Species screened	Individuals screened	Colonies screened	Number (and percent) of infected species and number of individuals/colonies		
				Wolbachia	*Spiroplasma*	*Asaia*
Camponotus (Formicinae)	1	1	1	0	1 (100%) 1/1	0
Cephalotes (Myrmicinae)	7	17	12	4 (57.1%) 4/4	2 (28.5%) 6/3	1 (14.3%) 1/1
Crematogaster (Myrmicinae)	6	6	6	1 (16.7%) 1/1	0	0
Myrmecia (Myrmeciinae)	2	3	3	0	0	0
Myrmecocystus (Formicinae)	1	1	1	0	0	0
Oecophylla (Formicinae)	1	1	1	0	0	0
Paraponera (Paraponerinae)	1	23	9	0	1 (100%) 2/2	1 (100%) 1/1
Polyrhachis (Formicinae)	32	64	60	8 (25.0%) 10/10	15 (46.9%) 15/15	0
Pseudomyrmex (Pseudomyrmecinae)	36	88	72	5 (13.9%) 5/5	5 (13.9%) 5/5	12 (33.3%) 15/15
Solenopsis (Myrmicinae)	2	11	5	1 (50%) 1/1	2 (100%) 2/1	0
Tetraponera (Pseudomyrmecinae)	6	6	6	2 (33.3%) 2/2	1 (16.7%) 1/1	0
Total	95	221	176	21 (22.1%)	27 (28.4%)	14 (14.7%)

3.2. Spiroplasma (Tenericutes: Entomoplasmatales). A total of 27 (28.4%) ant species were infected with *Spiroplasma* relatives (Mollicutes: Entomoplasmatales) leading to one of the highest frequency estimates of this bacterial group across the ants to date (Table 1). Previously, an infection rate of 6.2% across ant species had been reported, and the infection rates of approximately 6% were documented for Coleoptera, Diptera, Hymenoptera, and Lepidoptera in general, while 23.1% of spiders (Araneae) carried *Spiroplasma* symbionts [31]. There appears to be a strong bias towards certain groups of ants that are more often associated with this group of bacteria [31, 59]. The ant genus *Polyrhachis* showed a high infection rate of 46.9% (15 of 32 species were infected). The phenomenon of enriched *Spiroplasma* symbionts in this ant genus is in line with a study by Russell et al. [31] and is particularly interesting as ants of the tribe Camponotini, to which *Polyrhachis* belong, carry obligate *Blochmannia* endosymbionts, which are housed in specific bacteriocytes and provide essential amino acids to the ant host [12, 13]. Studying the prevalence of spiroplasmas in more genera of the Camponotini, particularly the hyperdiverse genus *Camponotus*, would reveal whether these bacteria are likely to interact within their hosts. Infections per species were high in *Camponotus* (1/1), *Paraponera* (1/1), and *Solenopsis* (2/2). However, these values are not representative due to the low number of species included. Outside of *Polyrhachis,* infection rates were moderate in the better sampled genera *Cephalotes* (2/7), *Pseudomyrmex* (5/36), and *Tetraponera* (1/6). No infection was detected in *Crematogaster*, *Myrmecia*, *Myrmecocystus*, and *Oecophylla* (Table 1). Again, sampled species numbers were low for these ant genera so infection frequency can only be regarded as preliminary.

An alignment of 175 taxa and 1311 characters was generated including *Selenomonas ruminantium* (Firmicutes: Selenomonadales) as an outgroup. In this molecular phylogeny, three large ant-specific clades of spiroplasmas were identified: ant clade 1 that includes endosymbionts of *Cephalotes*, *Solenopsis*, *Tetraponera*, *Pseudomyrmex* and *Neivamyrmex*; ant clade 2 that comprises spiroplasma-associates of the ant genera *Polyrhachis*, *Camponotus*, *Pseudomyrmex,* and *Cephalotes;* and ant clade 3 which was dominated by army ants (subfamilies Aenictinae, Dorylinae, and Ecitoninae) (Figure 2). Additionally, several small clades containing only ant-associated spiroplasmas were scattered throughout the phylogeny as well as several individual ant-associated OTUs. Overall, bioregion did not seem to be a strong predictor for relatedness among *Spiroplasma* symbionts (Figure 2; Figure S2).

Clade 3, which is dominated by army ants from the New and Old World, has been identified before [60]. In our analysis, GenBank-derived *Spiroplasma* sequences that were isolated from the ant genera *Odontomachus* and *Pachycondyla* also fell into this clade (Figure 2). Army ants are characterized by the "army ant syndrome" of nomadism and group predation [61]. Due to their specialized diet and a weak correlation of Entomoplasmatales infection with trophic position, a nutritive symbiosis between army ants and Entomoplasmatales has been suggested [60]. Even though this clade of Entomoplasmatales is highly dominated by army ants, the association is not obligate as infection rates vary with respect to species and individuals, and the symbionts are not necessary for host development and reproduction [60]. As Entomoplasmatales are generally absent in eggs and larvae, horizontal transmission is assumed.

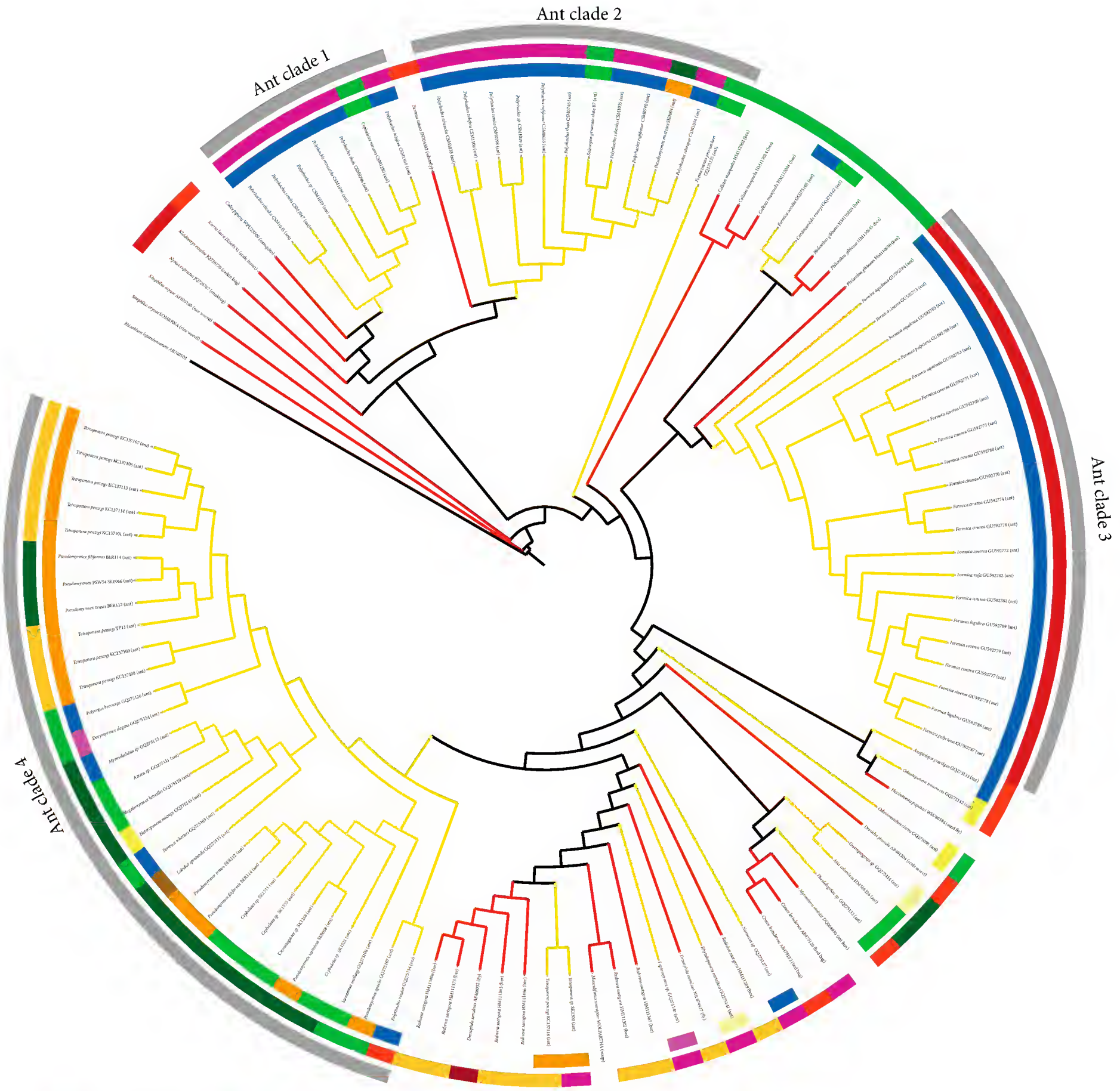


FIGURE 1: Phylogenetic tree of *Wolbachia* symbionts associated with ants and their closest relatives with sequence data available in GenBank. A maximum likelihood phylogeny of the 16S rRNA region of bacterial symbionts is shown. The host name is given together with the GenBank accession number (GenBank sequences) or collection code (sequences generated in the present study). Yellow and red branches represent bacteria isolated from ant hosts and other insect hosts, respectively. The inner circle shows ant subfamily, and the outer circle refers to the continent from which host organisms were collected. Four ant-specific clades of *Wolbachia* symbionts are highlighted (Ant clades 1–4). *Rhizobium leguminosarum* was used as an outgroup.

Even outside the army ants, a certain degree of host specificity of Entomoplasmatales bacteria is evident from our phylogeny and has been described for ants, *Drosophila,* and other arthropod-associated spiroplasmas [60]. In our molecular phylogeny, clades 1 and 2 exclusively contained ant-associated Entomoplasmatales (Figure 2). However, both clades contained symbionts from different ant subfamilies and biogeographic regions indicating that neither phylogeny nor geographic range drives the association with these symbionts, and repeated environmental acquisition is common.

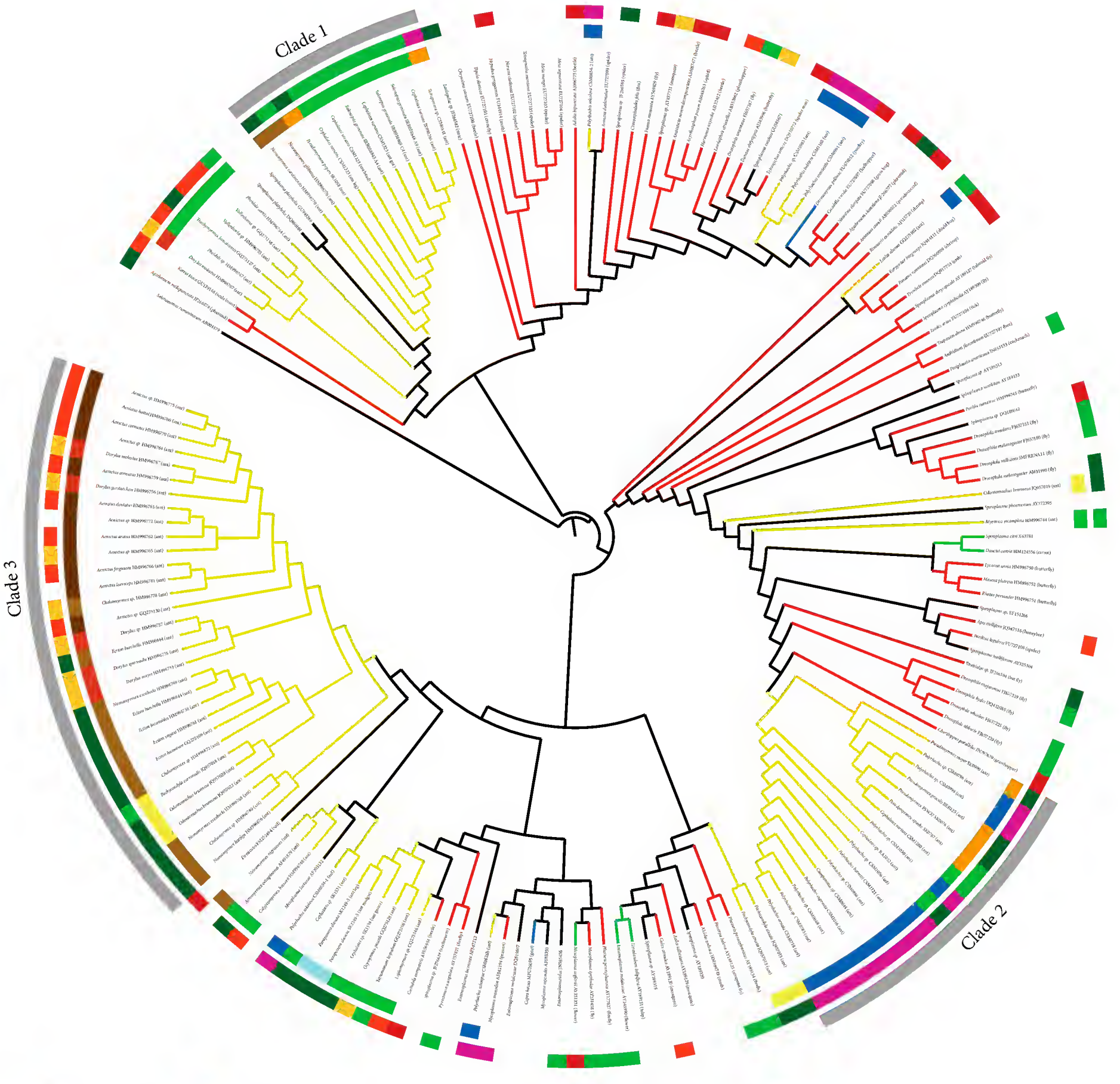


FIGURE 2: Phylogenetic tree of *Spiroplasma*-related ant symbionts and their closest relatives with sequence data available in GenBank. A maximum likelihood phylogeny of the 16S rRNA region of bacterial symbionts is shown. The host name is given together with the GenBank accession number (GenBank sequences) or collection code (sequences generated in the present study). The branch color refers to the source from which the bacteria were isolated with yellow representing ant hosts, red other insect hosts, blue vertebrates, and green plants. The inner circle refers to the ant subfamily, and the outer circle refers to the continent from which samples were collected. The three largest ant-specific clades of *Spiroplasma* symbionts are indicated (Clades 1–3). *Selenomonas ruminantium* was used as an outgroup.

The infection with *Spiroplasma* seems to be systemic, as we found high titers of this bacterium in association with ant guts, heads, and legs (Supplementary Table 1).

Entomoplasmatales can be pathogenic to plants and vertebrates [59, 62] and have been isolated from various insect taxa including aphids, ants, bees, beetles, butterflies, fruit flies, and horse flies [63–68]. Mutualistic spiroplasmas can grant insects resistance to parasitic nematodes [22] and an increased ability to overwinter [69]. Pathogenic phenotypes usually lead to insect death [34] and reproductive manipulation includes altered sex ratios [33] and male killing [35, 70, 71]. In ants, spiroplasmas have been surveyed, and

biocontrol potential has been hypothesized, but their role remains elusive to date [31, 38, 60]. Functional studies that compare the performance of infected and uninfected individuals would improve our understanding of the role of these facultative symbionts.

3.3. Asaia (Alphaproteobacteria: Rhodospirillales). Of 95 ant species, 14 hosted bacteria related to *Asaia* (Alphaproteobacteria: Rhodospirillales) (Table 1). For these bacteria, no previous surveys on their prevalence across the ants have been conducted. We found a particularly high infection rate of 33.3% (12/36 species) in *Pseudomyrmex*. In contrast, *Asaia*-related symbionts were lacking in *Camponotus*, *Crematogaster*, *Myrmecia*, *Myrmecocystus*, *Polyrhachis*, *Oecophylla,* and *Solenopsis*. Low infection frequency was present in *Cephalotes* (1/7) and *Paraponera* (1/1 species) (Table 1). The enrichment of *Asaia* symbionts in *Pseudomyrmex* is particularly interesting as this ant genus is arboreal and contains several obligate plant ants, which exclusively feed on plant-derived food sources [58, 72]. However, this bacterial group occurred facultatively in arboreal generalists and plant mutualists alike indicating that even if these symbionts are more frequent in arboreal or mutualistic *Pseudomyrmex* ants, the association is not obligate.

In total, we obtained 25 *Asaia*-related OTUs in our dataset. Of these OTUs, 21 were associated with *Pseudomyrmex*, 3 with *Paraponera,* and 1 with *Cephalotes*. We inferred a maximum likelihood phylogeny of these OTUs, their closest GenBank relatives, and other endosymbiotic Rhodospirillales bacteria from GenBank. The total alignment consisted of 91 taxa and had a length of 1313 characters. We used *Wolbachia pipientis* (Alphaproteobacteria: Rickettsiales) as an outgroup. The phylogenetic tree shows three clades in which ant-associated *Asaia* OTUs cluster together (Figure 3): (1) a small clade with two *Pseudomyrmex*-associated OTUs and one *Paraponera*-associated OTU, (2) a clade that appears to be Hymenoptera specific containing the bulk of *Pseudomyrmex*-associated OTUs, a *Formica*-associated sequence from GenBank, and bacteria isolated from several bee species, and (3) a clade comprised of many insect-associated *Asaia* bacteria and five of our OTUs. This last clade is particularly interesting as it comprised several strains that were isolated from different mosquito species as well as three ant-associated *Asaia* sequences from GenBank. One sequence (JF514556), was isolated and cultivated from *Tetraponera rufonigra* in India [73]. The *nifH* gene, a gene associated with the fixation of atmospheric nitrogen, has also been found in this bacterium (GenBank accession JF736510) and it has been experimentally shown that this strain is capable of fixing nitrogen *in vitro* suggesting possible nitrogen fixing attributes in its natural environment, the ant body cavity [73]. The two other sequences are cultivated bacteria from *Cephalotes varians* and were generated in the framework of a previous study from our lab (GenBank accessions JX445137 and JX445138) [39].

Bacteria from the family Acetobacteraceae are commonly known as "acetic acid bacteria" and have the metabolic capacity to oxidize ethanol to acetic acid [74]. *Asaia*, also a member of the Acetobacteraceae, however, only weakly oxidizes ethanol and shows higher rates of sugar oxidation [74]. These bacteria are environmentally ubiquitous, but have also been found in association with insects, such as bees [75, 76], mosquitoes [77], *Drosophila melanogaster* [78], leafhoppers [79], and mealybugs [80]. All these insects rely on sugar-rich and often nitrogen-limited diets, and it has been suggested that the bacteria function as nutritional symbionts. Some acetic acid bacteria have the capacity to fix atmospheric nitrogen [73]. However, it remains entirely speculative whether this function can be retained in the insect gut environment and whether these bacteria actually contribute to insect nitrogen metabolism or recycling [81]. Interestingly, neither acetic acid bacteria nor lactic acid bacteria are commonly found in the core gut microbiota of arboreal Cephalotini ants, an ant group with one of the most thoroughly studied microbiomes [18, 19, 39]. The metabolic capacities of the core gut microbiota of the Cephalotini consisting of Burkholderiales, Opitutales, Pseudomonadales, Rhizobiales, and Xanthomonadales might be redundant with the role that acetic acid bacteria play in other insects.

In *Drosophila,* acetic acid bacteria are part of the normal commensal bacterial gut community and can be involved in the regulation of the innate immune system. In healthy flies, a stable equilibrium of different gut microbes is maintained. Perturbation of the normal gut community, which can be caused by a defective regulation of antimicrobial peptide, leads to the dominance of the pathogenic commensal *Gluconobacter morbifer* and ultimately to gut apoptosis [82]. Potential other mechanisms by which acetic acid bacteria benefit insect immunity are by decreasing the gut pH making it an unfavorable environment for pathogenic microorganisms or by competitive exclusion [81]. However, these acetic acid bacteria are not essential for the fitness and reproduction of most insects as even in the well-studied *Asaia*-mosquito interaction, experimental removal of bacteria had no detectable negative impact on the host [81].

Several studies have been conducted to analyze the microbial diversity associated with ants [18, 19, 39, 60, 83]. However, the symbiotic relationships of ants with Rhodospirillales have rarely been observed. In fact, only two ant-associated Rhodospirillales sequences had been deposited in GenBank (GQ275104 from *Formica occulta* and JF514556 from *Tetraponera rufonigra*) prior to work from our group [39]. Clone libraries generated for the Cephalotini ants [18, 19] as well as tag-encoded amplicon data sets [38, 39] are among the most extensive microbial data collections available for ants to date, and acetic acid bacteria were only sporadically associated with the ant taxa that were investigated. Thus, the interaction of *Asaia* relatives with ants is generally poorly understood, but due to the metabolic capacities of these bacteria to utilize sugar-rich substrates and fix nitrogen, they might play an important nutritional role. Particularly, they might be functionally important in the ant subfamily Pseudomyrmecinae, in which they seem to be enriched as indicated by our present study.

The phylogenetic history of ant-associated Rhodospirillales does not show host specificity and suggests likely acquisition from the environment (Figure 3). These observations

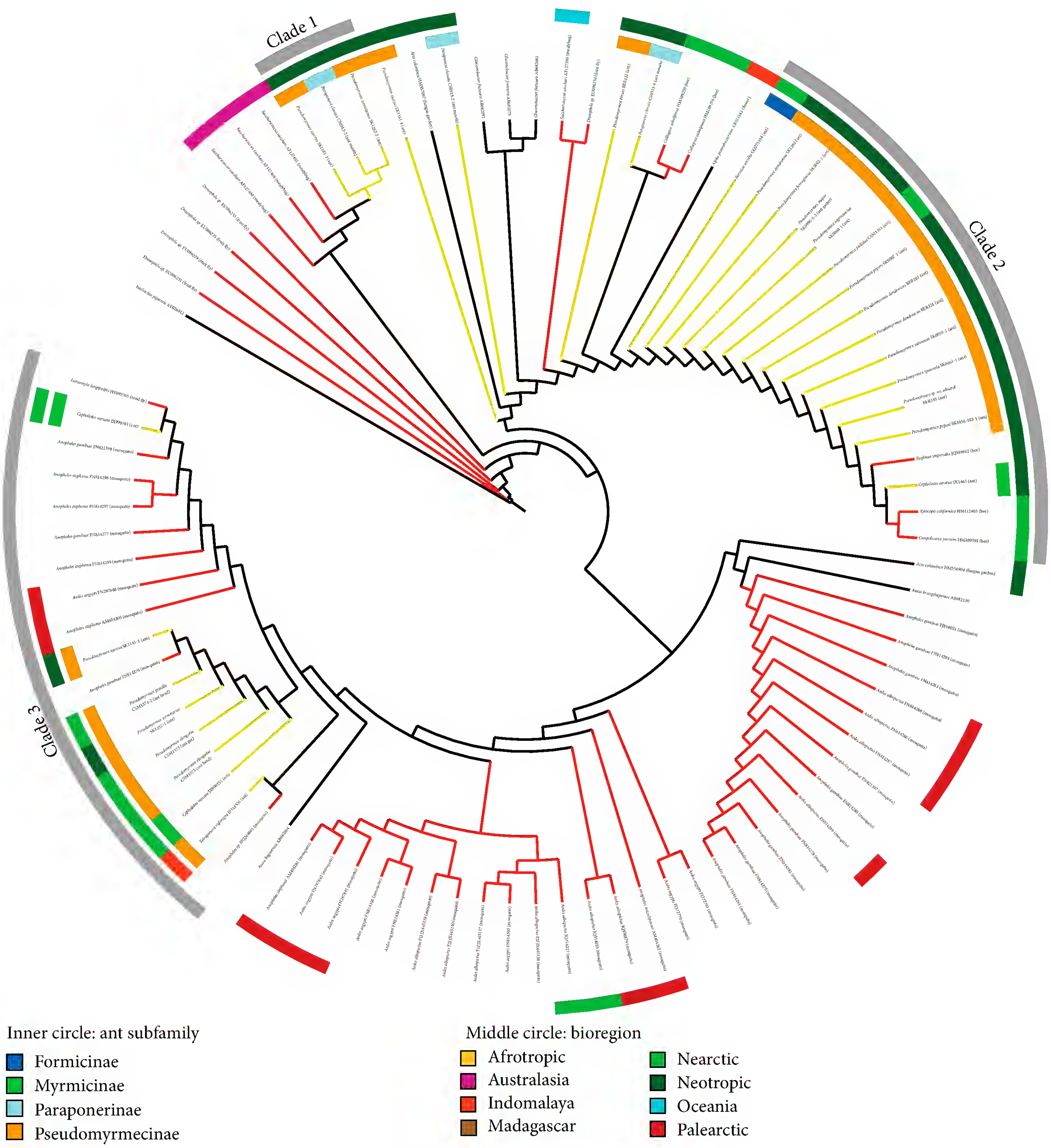


Figure 3: Phylogenetic tree of *Asaia*-related symbionts associated with ants and closest relatives with sequence data available in GenBank. A maximum likelihood phylogeny of the 16S rRNA region of bacterial symbionts is shown. The host name is given together with the GenBank accession number (GenBank sequences) or collection code (sequences generated in the present study). The branch color refers to the source from which the bacteria were isolated with yellow representing ant hosts and red other insect hosts. The inner circle refers to the ant subfamily, and the middle circle refers to the bioregion from which samples were collected. The outer circle indicates three clades (Clades 1–3), which contained several ant-associated symbionts. *Wolbachia pipientis* was used as an outgroup.

indicate that Rhodospirillales are most likely environmentally transmitted and support the hypothesis that they are only facultative associates of ants. One clade of ant-associated Rhodospirillales was closely related to endosymbionts isolated from mosquitos (Figure 3). It has been experimentally shown that mosquito-associated *Asaia* can successfully colonize leafhoppers further emphasizing the low-host specificity of this bacterial group [77].

4. Conclusion

Our broad bacterial screening approach has contributed to our understanding of the prevalence of ant-associated microbes, particularly with regard to their *Wolbachia* and *Spiroplasma* symbionts. Furthermore, we provide the first extensive survey for ant-associated *Asaia*-related symbionts. While these symbionts of the order Rhodospirillales infect

ants only sporadically, some strains are capable of fixing atmospheric nitrogen and might retain this function in ants. Alternatively, these bacteria might have an important functional role for upgrading nitrogen-poor diets of some herbivorous ants, which comprise the majority of all ant taxa [20]. Even though we do not have experimental evidence of the role of most bacterial symbionts in ants, previous studies illustrate a broad variety of effects of these bacteria on insect hosts [4, 7, 9]. Even a single group of microbes can have very different effects on different hosts. Our study shows that despite several extensive bacterial surveys across the ants, the diversity and functional role of ant-associated microbes is far from being fully understood, and broad next generation sequencing approaches will provide a fast and cost-effective tool to deepen our knowledge of the rare (and not so rare) biosphere.

Acknowledgments

The authors thank Daniel Ballhorn and Tina Schroyer for helpful comments and discussions. Phil Ward kindly identified *Pseudomyrmex* specimens. This study was supported in part by National Science Foundation DEB Grant no. 1050243 to Corrie S. Moreau, a Grainger Foundation grant to Corrie S. Moreau, a Negaunee Foundation grant to Corrie S. Moreau, and the Pritzker Laboratory for Molecular Evolution and Systematics. Stefanie Kautz acknowledges a postdoctoral fellowship from the German Academy of Sciences Leopoldina (Grant no. LPDS 2009-29). Benjamin E. R. Rubin was supported in part by an NSF Graduate Research Fellowship.

References

[1] H. Koch, D. P. Abrol, J. Li, and P. Schmid-Hempel, "Diversity of evolutionary patterns of bacterial gut associates of corbiculate bees," *Molecular Ecology*, vol. 22, no. 7, pp. 2028–2044, 2013.

[2] R. J. Dillon and V. M. Dillon, "The Gut bacteria of insects: nonpathogenic interactions," *Annual Review of Entomology*, vol. 49, pp. 71–92, 2004.

[3] S. Fraune and T. C. G. Bosch, "Why bacteria matter in animal development and evolution," *BioEssays*, vol. 32, no. 7, pp. 571–580, 2010.

[4] H. Feldhaar, "Bacterial symbionts as mediators of ecologically important traits of insect hosts," *Ecological Entomology*, vol. 36, no. 5, pp. 533–543, 2011.

[5] J. M. Urban and J. R. Cryan, "Two ancient bacterial endosymbionts have coevolved with the planthoppers (Insecta: Hemiptera: Fulgoroidea)," *BMC Evolutionary Biology*, vol. 12, article 87, 2012.

[6] H. Toju and T. Fukatsu, "Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: relevance of local climate and host plants," *Molecular Ecology*, vol. 20, no. 4, pp. 853–868, 2011.

[7] M. Kaltenpoth, "Actinobacteria as mutualists: general healthcare for insects?" *Trends in Microbiology*, vol. 17, no. 12, pp. 529–535, 2009.

[8] N. A. Moran, J. P. McCutcheon, and A. Nakabachi, "Genomics and evolution of heritable bacterial symbionts," *Annual Review of Genetics*, vol. 42, pp. 165–190, 2008.

[9] J. P. McCutcheon and C. D. Von Dohlen, "An interdependent metabolic patchwork in the nested symbiosis of mealybugs," *Current Biology*, vol. 21, no. 16, pp. 1366–1372, 2011.

[10] C. B. Montllor, A. Maxmen, and A. H. Purcell, "Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress," *Ecological Entomology*, vol. 27, no. 2, pp. 189–195, 2002.

[11] A. E. Douglas, "The nutritional physiology of aphids," *Advances in Insect Physiology*, vol. 31, pp. 73–140, 2003.

[12] J. J. Wernegreen, S. N. Kauppinen, S. G. Brady, and P. S. Ward, "One nutritional symbiosis begat another: phylogenetic evidence that the ant tribe Camponotini acquired *Blochmannia* by tending sap-feeding insects," *BMC Evolutionary Biology*, vol. 9, no. 1, article 292, 2009.

[13] H. Feldhaar, J. Straka, M. Krischke et al., "Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*," *BMC Biology*, vol. 5, article 48, 2007.

[14] A. A. Pinto-Tomás, M. A. Anderson, G. Suen et al., "Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants," *Science*, vol. 326, no. 5956, pp. 1120–1123, 2009.

[15] R. Pais, C. Lohs, Y. Wu, J. Wang, and S. Aksoy, "The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the tsetse fly," *Applied and Environmental Microbiology*, vol. 74, no. 19, pp. 5965–5974, 2008.

[16] J. A. Breznak, "Intestinal microbiota of termites and other xylophagous insects," *Annual Review of Microbiology*, vol. 36, pp. 323–343, 1982.

[17] J. A. Breznak, W. J. Brill, J. W. Mertins, and H. C. Coppel, "Nitrogen fixation in termites," *Nature*, vol. 244, no. 5418, pp. 577–579, 1973.

[18] J. A. Russell, C. S. Moreau, B. Goldman-Huertas, M. Fujiwara, D. J. Lohman, and N. E. Pierce, "Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 50, pp. 21236–21241, 2009.

[19] K. E. Anderson, J. A. Russell, C. S. Moreau et al., "Highly similar microbial communities are shared among related and trophically similar ant species," *Molecular Ecology*, vol. 21, pp. 2282–2296, 2012.

[20] D. W. Davidson, S. C. Cook, R. R. Snelling, and T. H. Chua, "Explaining the abundance of ants in lowland tropical rainforest canopies," *Science*, vol. 300, no. 5621, pp. 969–972, 2003.

[21] J. C. Brownlie and K. N. Johnson, "Symbiont-mediated protection in insect hosts," *Trends in Microbiology*, vol. 17, no. 8, pp. 348–354, 2009.

[22] J. Jaenike, R. Unckless, S. N. Cockburn, L. M. Boelio, and S. J. Perlman, "Adaptation via symbiosis: recent spread of a *Drosophila* defensive symbiont," *Science*, vol. 329, no. 5988, pp. 212–215, 2012.

[23] K. M. Oliver, J. A. Russell, N. A. Morant, and M. S. Hunter, "Facultative bacterial symbionts in aphids confer resistance to parasitic wasps," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 4, pp. 1803–1807, 2003.

[24] C. R. Currie, J. A. Scott, R. C. Summerbell, and D. Malloch, "Fungus-growing ants use antibiotic-producing bacteria to control garden parasites," *Nature*, vol. 398, pp. 701–704, 1999.

[25] M. Kaltenpoth, W. Göttler, G. Herzner, and E. Strohm, "Symbiotic bacteria protect wasp larvae from fungal infestation," *Current Biology*, vol. 15, no. 5, pp. 475–479, 2005.

[26] H. E. Dunbar, A. C. C. Wilson, N. R. Ferguson, and N. A. Moran, "Aphid thermal tolerance is governed by a point mutation in bacterial symbionts," *PLoS Biology*, vol. 5, no. 5, pp. 1006–1015, 2007.

[27] T. Tsuchida, R. Koga, S. Matsumoto, and T. Fukatsu, "Interspecific symbiont transfection confers a novel ecological trait to the recipient insect," *Biology Letters*, vol. 7, no. 2, pp. 245–248, 2011.

[28] R. Stouthamer, J. A. J. Breeuwer, and G. D. D. Hurst, "*Wolbachia* pipientis: microbial manipulator of arthropod reproduction," *Annual Review of Microbiology*, vol. 53, pp. 71–102, 1999.

[29] L. M. Hedges, J. C. Brownlie, S. L. O'Neill, and K. N. Johnson, "*Wolbachia* and virus protection in insects," *Science*, vol. 322, no. 5902, p. 702, 2008.

[30] K. Hilgenboecker, P. Hammerstein, P. Schlattmann, A. Telschow, and J. H. Werren, "How many species are infected with *Wolbachia*?—a statistical analysis of current data," *FEMS Microbiology Letters*, vol. 281, no. 2, pp. 215–220, 2008.

[31] J. A. Russell, C. F. Funaro, Y. M. Giraldo et al., "A veritable menagerie of heritable bacteria from ants, butterflies, and beyond: broad molecular surveys and a systematic review," *PLoS ONE*, vol. 7, no. 12, Article ID e51027, 2012.

[32] O. Duron, D. Bouchon, S. Boutin et al., "The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone," *BMC Biology*, vol. 6, article 27, 2008.

[33] D. L. Williamson and D. F. Poulson, "Sex ratio organisms (Spiroplasmas) of *Drosophila*," in *The Mycoplasmas III: Plant and Insect Mycoplasmas*, R. F. Whitcomb and J. G. Tully, Eds., pp. 175–208, Academic Press, New York, NY, USA, 1979.

[34] J. M. Bové, "Spiroplasmas: infectious agents of plants, arthropods and vertebrates," *Wiener Klinische Wochenschrift*, vol. 109, no. 14-15, pp. 604–612, 1997.

[35] M. C. Tinsley and M. E. N. Majerus, "A new male-killing parasitism: *Spiroplasma* bacteria infect the ladybird beetle *Anisosticta novemdecimpunctata* (Coleoptera: Coccinellidae)," *Parasitology*, vol. 132, no. 6, pp. 757–765, 2006.

[36] A. Dobson, K. D. Lafferty, A. M. Kuris, R. F. Hechinger, and W. Jetz, "Homage to Linnaeus: how many parasites? How many hosts?" *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 1, pp. 11482–11489, 2008.

[37] S. T. Kelley and S. Dobler, "Comparative analysis of microbial diversity in *Longitarsus flea* beetles (Coleoptera: Chrysomelidae)," *Genetica*, vol. 139, no. 5, pp. 541–550, 2011.

[38] H. D. Ishak, R. Plowes, R. Sen et al., "Bacterial diversity in *Solenopsis invicta* and *Solenopsis geminata* ant colonies characterized by 16S amplicon 454 pyrosequencing," *Microbial Ecology*, vol. 61, no. 4, pp. 821–831, 2011.

[39] S. Kautz, B. E. R. Rubin, J. A. Russell, and C. S. Moreau, "Surveying the microbiome of ants: comparing 454 pyrosequencing with traditional methods to uncover bacterial diversity," *Applied and Environmental Microbiology*, vol. 79, no. 2, pp. 525–534, 2013.

[40] S. E. Dowd, T. R. Callaway, R. D. Wolcott et al., "Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP)," *BMC Microbiology*, vol. 8, article 125, 2008.

[41] J. G. Caporaso, J. Kuczynski, J. Stombaugh et al., "QIIME allows analysis of high-throughput community sequencing data," *Nature Methods*, vol. 7, no. 5, pp. 335–336, 2010.

[42] C. Quince, A. Lanzen, R. J. Davenport, and P. J. Turnbaugh, "Removing noise from pyrosequenced amplicons," *BMC Bioinformatics*, vol. 12, article 38, 2011.

[43] R. C. Edgar, "Search and clustering orders of magnitude faster than BLAST," *Bioinformatics*, vol. 26, no. 19, pp. 2460–2461, 2010.

[44] J. R. Cole, Q. Wang, E. Cardenas et al., "The Ribosomal database project: improved alignments and new tools for rRNA analysis," *Nucleic Acids Research*, vol. 37, no. 1, pp. D141–D145, 2009.

[45] T. Z. DeSantis, P. Hugenholtz, N. Larsen et al., "Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB," *Applied and Environmental Microbiology*, vol. 72, no. 7, pp. 5069–5072, 2006.

[46] A. J. Drummond, B. Ashton, S. Buxton et al., "Geneious v5. 4," 2011, http://www.geneious.com/ .

[47] A. Stamatakis, P. Hoover, and J. Rougemont, "A rapid bootstrap algorithm for the RAxML web servers," *Systematic Biology*, vol. 57, no. 5, pp. 758–771, 2008.

[48] M. A. Miller, M. T. Holder, R. Vos et al., "The CIPRES portals," http://www.phylo.org/sub_sections/portal .

[49] I. Letunic and P. Bork, "Interactive Tree of Life v2: online annotation and display of phylogenetic trees made easy," *Nucleic Acids Research*, vol. 39, no. 2, pp. W475–W478, 2011.

[50] T. Wenseleers, F. Ito, S. van Borm, R. Huybrechts, F. Volckaert, and J. Billen, "Widespread occurrence of the micro-organism *Wolbachia* in ants," *Proceedings of the Royal Society B*, vol. 265, no. 1404, pp. 1447–1452, 1998.

[51] J. A. Russell, "The ants (Hymenoptera: Formicidae) are unique and enigmatic hosts of prevalent *Wolbachia* (Alphaproteobacteria) symbionts," *Myrmecological News*, vol. 16, pp. 7–23, 2012.

[52] W. Arthofer, M. Riegler, D. Schneider, M. Krammer, W. J. Miller, and C. Stauffer, "Hidden *Wolbachia* diversity in field populations of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae)," *Molecular Ecology*, vol. 18, no. 18, pp. 3816–3830, 2009.

[53] J. A. Russell, B. Goldman-Huertas, C. S. Moreau et al., "Specialization and geographic isolation among *Wolbachia* symbionts from ants and lycaenid butterflies," *Evolution*, vol. 63, no. 3, pp. 624–640, 2009.

[54] T. Wenseleers, L. Sundström, and J. Billen, "Deleterious *Wolbachia* in the ant *Formica truncorum*," *Proceedings of the Royal Society B*, vol. 269, no. 1491, pp. 623–629, 2002.

[55] F. Vavre, F. Fleury, J. Varaldi, P. Fouillet, and M. Boulétreau, "Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic and evolutionary consequences," *Evolution*, vol. 54, no. 1, pp. 191–200, 2000.

[56] J. Heinze, "The demise of the standard ant (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 11, pp. 11–20, 2008.

[57] S. Kautz, S. U. Pauls, D. J. Ballhorn, H. T. Lumbsch, and M. Heil, "Polygynous supercolonies of the acacia-ant *Pseudomyrmex peperi*, an inferior colony founder," *Molecular Ecology*, vol. 18, no. 24, pp. 5180–5194, 2009.

[58] S. Kautz, D. J. Ballhorn, J. Kroiss et al., "Host plant use by competing acacia-ants: mutualists monopolize while parasites share hosts," *PLoS ONE*, vol. 7, no. 5, Article ID e37691, 2012.

[59] J. Fletcher, G. A. Schultz, R. E. Davis, C. E. Eastman, and R. M. Goodman, "Brittle root disease of horseradish-evidence for an etiological role of *Spiroplasma citri*," *Phytopathology*, vol. 71, pp. 1073–1080, 1981.

[60] C. F. Funaro, D. J. C. Kronauer, C. S. Moreau, B. Goldman-Huertas, N. E. Pierce, and J. A. Russell, "Army ants harbor a host-specific clade of *Entomoplasmatales* bacteria," *Applied and Environmental Microbiology*, vol. 77, no. 1, pp. 346–350, 2011.

[61] D. J. C. Kronauer, "Recent advances in army ant biology (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 12, pp. 51–65, 2009.

[62] F. Thiaucourt and G. Bölske, "Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats," *OIE Revue Scientifique et Technique*, vol. 15, no. 4, pp. 1397–1414, 1996.

[63] T. Fukatsu, T. Tsuchida, N. Nikoh, and R. Koga, "*Spiroplasma* Symbiont of the pea aphid, *Acyrthosiphon pisum* (Insecta: Homoptera)," *Applied and Environmental Microbiology*, vol. 67, no. 3, pp. 1284–1291, 2001.

[64] T. S. Haselkorn, T. A. Markow, and N. A. Moran, "Multiple introductions of the *Spiroplasma* bacterial endosymbiont into *Drosophila*," *Molecular Ecology*, vol. 18, no. 6, pp. 1294–1305, 2009.

[65] J. G. Lundgren, R. M. Lehman, and J. Chee-Sanford, "Physiology, biochemistry, and topology: bacterial communities within digestive tracts of ground beetles (Coleoptera: Carabidae)," *Annals of the Entomological Society of America*, vol. 100, no. 2, pp. 275–282, 2007.

[66] I. Meeus, V. Vercruysse, and G. Smagghe, "Molecular detection of *Spiroplasma apis* and *Spiroplasma melliferum* in bees," *Journal of Invertebrate Pathology*, vol. 109, no. 1, pp. 172–174, 2012.

[67] S. van Borm, J. Billen, and J. J. Boomsma, "The diversity of microorganisms associated with *Acromyrmex* leafcutter ants," *BMC Evolutionary Biology*, vol. 2, article 9, 2002.

[68] J. Wedincamp, F. E. French, R. F. Whitcomb, and R. B. Heneger, "Laboratory infection and release of *Spiroplasma* (Entomoplasmatales: Spiroplasmataceae) from horse flies (Diptera: Tabanidae)," *Journal of Entomological Science*, vol. 32, no. 4, pp. 398–402, 1997.

[69] M. A. Ebbert and L. R. Nault, "Improved overwintering ability in *Dalbulus maidis* (Homoptera: Cicadellidae) vectors infected with *Spiroplasma kunkelii* (Mycoplasmatales: Spiroplasmataceae)," *Environmental Entomology*, vol. 23, no. 3, pp. 634–644, 1994.

[70] F. M. Jiggins, G. D. D. Hurst, C. D. Jiggins, J. H. G. V. D. Schulenburg, and M. E. N. Majerus, "The butterfly *Danaus chrysippus* is infected by a male-killing *Spiroplasma* bacterium," *Parasitology*, vol. 120, no. 5, pp. 439–446, 2000.

[71] J. H. G. V. D. Schulenburg, G. D. D. Hurst, D. Tetzlaff, G. E. Booth, I. A. Zakharov, and M. E. N. Majerus, "History of infection with different male-killing bacteria in the two-spot ladybird beetle *Adalia bipunctata* revealed through mitochondrial DNA sequence analysis," *Genetics*, vol. 160, no. 3, pp. 1075–1086, 2002.

[72] S. Kautz, H. T. Lumbsch, P. S. Ward, and M. Heil, "How to prevent cheating: a digestive specialization ties mutualistic plant-ants to their ant-plant partners," *Evolution*, vol. 63, no. 4, pp. 839–853, 2009.

[73] N. Samaddar, A. Paul, S. Chakravorty et al., "Nitrogen fixation in *Asaia* sp. (Family Acetobacteraceae)," *Current Microbiology*, vol. 63, no. 2, pp. 226–231, 2011.

[74] Y. Ano, H. Toyama, O. Adachi, and K. Matsushita, "Energy metabolism of a unique acetic acid bacterium, *Asaia bogorensis*, that lacks ethanol oxidation activity," *Bioscience, Biotechnology and Biochemistry*, vol. 72, no. 4, pp. 989–997, 2008.

[75] V. G. Martinson, B. N. Danforth, R. L. Minckley, O. Rueppell, S. Tingek, and N. A. Moran, "A simple and distinctive microbiota associated with honey bees and bumble bees," *Molecular Ecology*, vol. 20, no. 3, pp. 619–628, 2011.

[76] N. A. Moran, A. K. Hansen, J. E. Powell, and Z. L. Sabree, "Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees," *PLoS ONE*, vol. 7, no. 4, Article ID e36393, 2012.

[77] E. Crotti, C. Damiani, M. Pajoro et al., "*Asaia*, a versatile acetic acid bacterial symbiont, capable of cross-colonizing insects of phylogenetically distant genera and orders," *Environmental Microbiology*, vol. 11, no. 12, pp. 3252–3264, 2009.

[78] C. R. Cox and M. S. Gilmore, "Native microbial colonization of *Drosophila* melanogaster and its use as a model of *Enterococcus faecalis* pathogenesis," *Infection and Immunity*, vol. 75, no. 4, pp. 1565–1576, 2007.

[79] M. Marzorati, A. Alma, L. Sacchi et al., "A novel Bacteroidetes symbiont is localized in *Scaphoideus titanus*, the insect vector of Flavescence Dorée in *Vitis vinifera*," *Applied and Environmental Microbiology*, vol. 72, no. 2, pp. 1467–1475, 2006.

[80] N. J. Ashbolt and P. A. Inkerman, "Acetic acid bacterial biota of the pink sugar cane mealybug, *Saccharococcus sacchari*, and its environs," *Applied and Environmental Microbiology*, vol. 56, no. 3, pp. 707–712, 1990.

[81] E. Crotti, A. Rizzi, B. Chouaia et al., "Acetic acid bacteria, newly emerging symbionts of insects," *Applied and Environmental Microbiology*, vol. 76, no. 21, pp. 6963–6970, 2010.

[82] J. Ryu, S. Kim, H. Lee et al., "Innate immune homeostasis by the homeobox gene *Caudal* and commensal-gut mutualism in *Drosophila*," *Science*, vol. 319, no. 5864, pp. 777–782, 2008.

[83] S. Stoll, J. Gadau, R. Gross, and H. Feldhaar, "Bacterial microbiota associated with ants of the genus *Tetraponera*," *Biological Journal of the Linnean Society*, vol. 90, no. 3, pp. 399–412, 2007.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 264279, 5 pages
http://dx.doi.org/10.1155/2013/264279

Editorial

Ants and Their Parasites 2013

Jean-Paul Lachaud,[1,2] Alain Lenoir,[3] and David P. Hughes[4]

[1] *El Colegio de la Frontera Sur, Departamento de Conservación de la Biodiversidad, Avenida Centenario Km. 5.5, AP 424, 77014 Chetumal, Quintana Roo, Mexico*
[2] *Centre de Recherches sur la Cognition Animale, CNRS-UMR 5169, Université de Toulouse UPS, 118 route de Narbonne, 31062 Toulouse Cedex 09, France*
[3] *IRBI, CNRS-UMR 7261, Faculté des Sciences, Université François Rabelais, Parc de Grandmont, 37200 Tours, France*
[4] *Department of Entomology and Biology, Center for Infectious Disease Dynamics, Penn State University, University Park, PA 16802, USA*

Correspondence should be addressed to Jean-Paul Lachaud; jlachaud@ecosur.mx

Received 12 June 2013; Accepted 12 June 2013

Ants, like any animal, are subject to parasitism. However, as they are also superorganisms living in common nests, their parasites experience environments wholly different from those of parasites affecting solitary organisms [1]. The nests of most ant species are relatively stable microhabitats prone to provide both readily available resources and some degree of protection against predators to many organisms. Consequently, ant-parasite (or ant-myrmecophile) associations gather a great deal of diversity ranging from the casual, opportunistic, unspecialized interaction—through temporary protection or sharing of some resources or even predation—, to obligate, specific mutualism that may involve coevolution of both the host and the parasite [2–5].

The first issue of this series examined a wide range of species: viruses, bacteria, fungi, nematodes, silverfishes, flies, butterflies, beetles, spiders, wasps, and ants themselves. However, it could not cover all possible ant parasites. More studies examining their complex interactions from every possible angle, attempting to bring a more global vision of the functioning of such an evolutionary important relationship, are a challenging and fascinating goal. In this second volume, we continued giving specific attention to both the mechanisms used by ant parasites to integrate into their host colony and the way parasite pressure could affect patterns of reproduction and life history in ant hosts. Moreover, considering the increasing pace of losses in biodiversity due to habitat destruction and climate change, we also wanted to reflect the effort towards accurate faunistic surveys of the diversity of the associations involving ants as hosts and the exact nature of these associations.

This volume is divided into two main sections: (1) ant-parasite interactions and the mechanisms of integration into the host colony, in which both already known and new associations between ants and a diverse fauna including numerous beetle families, phorid and syrphid flies, diapriid, eucharitid and eurytomid wasps, myrmecophilid crickets, spiders, and bacteria are reviewed and/or discussed considering behavioral, taxonomical, phylogenetical, and even conceptual aspects; (2) social parasitism involving ant-ant interactions, in which different interspecific associations between ant species are reviewed, from the most basic forms illustrated by independent plesiobiotic associations to sophisticated, permanent ones found between slave-making ants or inquiline species and their single specialized hosts.

Ant-Parasite Interactions and the Mechanisms of Integration into the Host Colony. Even if we tried to give more importance to the diversity of ant social parasites and the other kinds of myrmecophiles not tackled in the first issue, Coleoptera remains the most documented group among the myrmecophiles and various contributions still deal with beetles in this second issue. Though the first pioneering lists of ant-associated beetles by Märkel [6, 7] dealt with European fauna, few faunistic works have focussed on this part of

the world in the last decades. For such a reason, the sound up-to-date compilation and review of literature—along with some few new data—provided by A. Lapeva-Gjonova on myrmecophilous beetles of Bulgaria, their host specificity, and the nature of their relations with their hosts, is particularly welcome. Apart from resulting in a comprehensive list of 121 myrmecophilous beetle species from 14 families, associated to 22 out of the 170 ant species of Bulgaria, this review brings an opportunity to our community to access some poorly known or difficult to obtain literatures. Due to their specialized behavioral and morphological adaptations, some groups of myrmecophilous beetles are particularly well documented in different regions. However, determining how complete and accurate their taxonomic status is remains an open question. S. Fattorini and colleagues, through a synthesis of the present knowledge of the alpha and beta taxonomy levels of the Paussini group and a modelling of synonym accumulation curves using logistic functions, show that this tribe is taxonomically stable. Relatively few species are expected to be described in the future on morphological basis (but the existence of cryptic species is still possible) and few currently accepted taxa will be recognized to be synonymous. It appears that morphological characters are not fully adequate to resolve infrageneric relationships and that future works using molecular approach are needed along with more accurate survey in poorly studied zones such as Australian and Oriental regions. Since the first attempts by Wasmann [8, 9], various classifications have tried to organize into a hierarchy the diverse myrmecophile habits of Coleoptera. However, the lack of knowledge on the biology of the myrmecophiles is one of the main problems of such classification and has resulted, in many instances, in discrete groups but with overlapping behavioral categories, confusing our knowledge of the real interactions with the host. Moreover, the fact that scientists attribute the same kind of behavior to an insect solely based on morphological similarities is highly problematic. G. Mynhardt discusses the effectivity of such classification systems, and her main goal merely focuses on a declassification and on the fact that we urgently need more in-depth studies in order to know what is really happening biologically before attempting to place beetles or other myrmecophiles into discrete classification schemes.

The lack of knowledge for numerous associations with ants, which can have high implications in their social structure or may be of potential economic interest, is a general problem and numerous studies have tried to fill this gap. Recent discoveries on bacteria [10] show that they are more and more involved in the evolution of their hosts and raise the question of how much do microbes shape animal development? The maternally transmitted bacteria from the genus *Wolbachia*, for example, represent a widespread, active component in the conflict of interests within ant colonies [11]. Furthermore, phylogenetic analyses have demonstrated that related *Wolbachia* commonly infect related hosts and that their host associations show a strong pattern of specialization [12]. In the aim of broadly sampling and searching for those groups of potential interest before performing more targeted studies, Kautz and colleagues show how deep sequencing can be used for a broad screening of infectious bacteria. Using both already available data and new data from a large 16S amplicon 454 pyrosequencing to survey ant associated bacteria, they investigate associations of ants with three genera of bacteria (*Wolbachia*, *Spiroplasma*, and *Asaia*). On the base of available data they conclude that phylogeny and geography are not strong determinants of infection rate. In the past decades, a growing set of literatures has focused on other groups of organisms associated with ants and on their possible use as biological control agents against invasive or economically important species (see [13–15]). This is particularly the case for numerous dipteran and hymenopteran parasitoids, most often closely restricted to specific hosts. An overview of taxonomical, biological, and behavioral aspects of the interaction between leaf-cutting ants of the genera *Atta* and *Acromyrmex* and the main four genera of phorid flies attacking them is given by P. J. Folgarait. Focussing on the peculiarities of the parasitoids attacking behaviors towards their host and the defensive responses of the ants against the parasitoids, she both suggests some predictive hypothesis related to phorid-ant interactions and proposes priority lines of research to enhance the use of parasitoids in leaf-cutting ant control. Concerning the hymenopteran parasitoids, J. Torréns offers an up-to-date, well-illustrated review of what is known, for Argentina, about the obligatory ant-associated family Eucharitidae, along with valuable new information on ant-host and/or plant-host associations for various of these species. In particular, he reports an interesting example of concurrent parasitism for the ectatommine ant *Ectatomma brunneum*, which is parasitized by three eucharitid species from three different genera, a case known previously for only one other species of the same ant genus, *E. tuberculatum* [15, 16]. Various other groups of dipteran and hymenopteran parasites are associated with ants, but the biology of only a very small fraction is known and, for most species, the real nature of their interactions with ant-hosts remains uncertain. This is typically the case of diapriid-ant relationships for which there has been a lot of speculation. True associations with ants occur only for a fraction of the diapriid species. The paper by M. S. Loiácono and colleagues gives both useful information on type material recently curated in the Museum of La Plata, in Buenos Aires, and an overview of the presence of the ant-associated species in Argentina. It summarizes a lot of the authors past work on diapriid-ant relationships and more specifically some of the very few cases of true ant parasitoidism in this family. Amongst the dipteran, the hoverflies of the syrphid subfamily Microdontinae illustrate another group for which the relationships with ants need more detailed studies. Whereas all of the species of the genus *Microdon* for which the natural history is known have been found within ant nests or in their immediate vicinity, with their immature stages developing as predators of the ant brood, such relationships are poorly known for the majority of microdontine taxa. Through a review of the 109 published and unpublished records of associations between microdontine flies and ants, M. Reemer provides a phylogenetic evaluation showing that the microdontine taxa found in association with ants occur scattered throughout their phylogenetic tree, suggesting that myrmecophily would

be a dominant feature of larval biology for all microdontine flies.

As for all the parasites associated with ants, microdontine species need some mechanism preventing aggressiveness from the ants to allow their integration into the host nest. For some species of *Microdon*, it has been established that the larvae manage to integrate the host colony using chemical mimicry [17] and, in some cases, biosynthesizing cuticular hydrocarbons similar to those of their host [18], a very uncommon mechanism recently demonstrated to occur also in an histerid beetle [19]. However, even when their integration in the ant nest can be secure, the integration process is not necessarily complete and they do not always lure natural enemies like parasitoid wasps which can locate and parasitize their primary host within the ant nest. This is what occurs for the myrmecophilous wasp, *Camponotophilus delvarei*, as reported by G. Pérez-Lachaud and colleagues who describe, in various nests of the neotropical weaver ant *Camponotus* sp. aff. *textor*, the first case of parasitism of a species of microdontine fly by an eurytomid wasp. Due to the very specific habitat where this association was found, the authors stressed the urgent need to improve our understanding of the biology of both microdontine flies and their natural enemies before their natural habitat is lost. T. Komatsu and colleagues report on another case of apparent incomplete integration, showing an unexpected absence of behavioral integration of the specialist myrmecophilous cricket, *Myrmecophilus tetramorii*, within the colony of their host, *Tetramorium tsushimae*. As such integration does exist for other specialized congeneric species like *M. kubotai*, also found in the colonies of *T. tsushimae*, this suggests that specialization in the genus *Myrmecophilus* does not necessarily correlate with intimate behavior of the ant-host and that some species can reach high degree of adaptation to a specific host without sophisticated integration cues. In that particular case, the authors conclude that *M. tetramorii* could be specialized to exploit the host by means other than chemical integration. Nevertheless, as noted previously for *Microdon* larvae, numerous myrmecophiles do mimic the cuticular hydrocarbon pattern of their host to be accepted or use some chemical mechanism to achieve it. The paper by M. Stoeffler and colleagues deals with the exceptional release of monoterpenes by the tergal gland of two extremely rare Lomechusini species of the rove beetle genus *Zyras* from Germany, for which both the ant host and the nature of the myrmecophilic relationships were not known with certainty. The similarity between these monoterpenes and those present in some ant-attended aphids and aphid honeydew suggests that *Z. collaris* and *Z. haworthi* could achieve acceptance by their putative host, *Lasius fuliginosus*, mimicking aphid compounds to stimulate more antennation by the ants and no aggression. Moreover, this finding supports recent data on the molecular phylogeny of Lomechusini indicating that the genus *Zyras* is much more distant from the genus *Pella* than previously assumed. Apart from chemical mimicry, ant-mimicking through morphological and/or behavioral mechanisms is largely used by numerous arthropods, and in spiders in particular, to deceive their ant associates, a topic already reviewed in the previous volume [20], but still as fascinating as ever. F. S. Ceccarelli tackles it in a complementary way, focussing on the behavioral aspect of ant-associating spiders (in particular for myrmecomorph species which apparently do not use chemical mimicry) that allow them to live close to the ants and to minimize the costs of this potentially lethal association. The central idea is that the existence of such a diversity of species involved in myrmecomorphy inevitably implies that the benefits (essentially the protection against natural enemies, not against the ants themselves) must overweight the costs.

Social Parasitism Involving Ant-Ant Interactions. The amazing diversity of the forms that can take the dependence of an ant species on one or more other free-living ant species is a fascinating topic that has been recently and excellently reviewed by Buschinger [21]. However, reviewing more basic associations without interdependence, like the plesiobiosis, has barely been tackled. O. Kanizsai and colleagues fill this gap through a preliminary review of our current understanding of ant-ant nesting associations consisting in the casual or regular nesting in close vicinity of two ant species. They establish a list of 48 different plesiobiotic species pairs that have been recorded from various habitat types of the Holarctic region and provide a good discussion of the possible reasons for the associations that have been recorded and of their possible role in the formation of other types of interspecific associations like cleptobiosis or lestobiosis. Pointing out the lack of reliable data, this review raises numerous questions that, hopefully, will promote collecting more and better defined data and extend our knowledge to arboreal species and to Tropical and Neotropical regions. More intricately specialized ant-ant relationships, involving permanently parasitizing species depending upon their hosts throughout their lives, have attracted more attention from numerous scientists. For slave-making ants and their hosts, most of the work has been made on *Harpagoxenus* and *Polyergus* [21–23], but some groups of species are less well known. This is the case for the four species of the obligate slave-maker genus *Rossomyrmex*, each one specializing in raiding a specific species of the genus *Proformica* in a large geographical area. In their review, F. Ruano and colleagues compile all the available data from the *Rossomyrmex-Proformica* associations and contrast them with observations on other slave-makers, providing a useful comparative overview. In particular, they emphasize the distinctive biological traits of these associations, namely, concerning their reproductive strategy, some characteristics of their raids, and their dispersal abilities. Addressing the problem of the evolutionary potential for host and parasite in two pairs of *Rossomyrmex-Proformica* associations presenting contrasting ecological characteristics, they interestingly hypothesize that parasite migration would be counter-selected in fragmented habitats because distant dispersal could lead to get away from the distribution area of the potential host colonies. Among the numerous examples of social parasitism, one of the highest degree of biological interdependence between two species of ants is inquilinism where one species acts as a permanent parasite, but without enslaving the host species. In most cases, the parasite queens do not produce a worker caste and coexist with the host

queens in the host colony [21]. Until now, only one case of inquilinism has been reported within the poneromorph ants [24], involving a facultative polygynous population of the common Neotropical ectatommine ant *Ectatomma tuberculatum* and miniature queens of the sibling species *E. parasiticum*. R. Fénéron and colleagues provide an up-to-date survey of the biological, genetical, and behavioral data accumulated since the first discovery of *E. parasiticum*, fourteen years ago [25], and try to shed light on the evolutionary history of the parasitic relationships between both species. The phylogenetical proximity between both species, along with the fact that the parasite queens are clearly discriminated from conspecifics by the host workers and, apparently, are not well integrated into the host colony, suggest a recent sympatric speciation from the host. The authors also emphasize the endangered status of this inquiline species known but from a single, extremely restricted location in Mexico.

Both this special issue and the one before have demonstrated that a great deal of interest still surrounds parasites that live in ant societies. The intersection between collective groups that have long inspired biologists with studies of the organisms that have evolved to break into the fortress of the nest is an exciting field. Because all fields require a solid, but expanding, foundation of detailed biology from which to progress, we rather feel that the contributions gathered here signal a very bright future for studies into ants and their parasites.

Acknowledgments

The guest editors would like to thank all of the authors who accepted to participate in this second volume of the annual issue "Ants and Their Parasites". Particular thanks are due to the numerous referees who generously helped improving the quality of the papers, making this special issue possible.

Jean-Paul Lachaud
Alain Lenoir
David P. Hughes

References

[1] D. P. Hughes, "Parasites and the superorganism," in *Host Manipulation by Parasites*, D. P. Hughes, J. Brodeur, and F. Thomas, Eds., pp. 140–154, Oxford University Press, Oxford, UK, 2012.

[2] W. M. Wheeler, *Ants, Their Structure, Development and Behavior*, The Columbia University Press, New York, NY, USA, 1910.

[3] D. H. Kistner, "The social insects' bestiary," in *Social Insects*, H. R. Hermann, Ed., vol. 3, pp. 1–244, Academic Press, New York, NY, USA, 1982.

[4] B. Hölldobler and E. O. Wilson, *The Ants*, Springer, Berlin, Germany, 1990.

[5] P. Schmid-Hempel, "Parasites in social insects," in *Monographs in Behavior and Ecology*, J. R. Krebs and T. Clutton-Brock, Eds., Princeton University Press, Princeton, NJ, USA, 1998.

[6] F. Märkel, "Beiträge zur Kenntniss der unter Ameisen lebenden, Insekten, Erstes Stück," *Germar's Zeitschrift für die Entomologie*, vol. 3, pp. 203–225, 1841.

[7] F. Märkel, "Beiträge zur Kenntniss der unter Ameisen lebenden, Insekten, Zweites Stück," *Germar's Zeitschrift für die Entomologie*, vol. 5, pp. 193–271, 1844.

[8] E. Wasmann, "Vergleichende studien über ameisengäste und termitengäste," *Tijdschrift voor Entomologie*, vol. 33, pp. 27–96, 1890.

[9] E. Wasmann, *Kritisches Verzeichniss der Myrmekophilen und Termitophilen Arthropoden mit Angabe der Lebensweise und mit Beschreibung Neuer Arten*, F. L. Dames, Berlin, Germany, 1894.

[10] E. Pennisi, "How do microbes shape animal development?" *Science*, vol. 340, no. 6137, pp. 1159–1160, 2013.

[11] T. Wenseleers, F. Ito, S. Van Borm, R. Huybrechts, F. Volckaert, and J. Billen, "Widespread occurrence of the micro-organism *Wolbachia* in ants," *Proceedings of the Royal Society B*, vol. 265, no. 1404, pp. 1447–1452, 1998.

[12] J. A. Russell, B. Goldman-Huertas, C. S. Moreau et al., "Specialization and geographic isolation among *Wolbachia* symbionts from ants and lycaenid butterflies," *Evolution*, vol. 63, no. 3, pp. 624–640, 2009.

[13] D. H. Feener Jr. and B. V. Brown, "Diptera as parasitoids," *Annual Review of Entomology*, vol. 42, pp. 73–97, 1997.

[14] L. W. Morrison, "Biological control of *Solenopsis* fire ants by *Pseudacteon* parasitoids: theory and practice," *Psyche*, vol. 2012, Article ID 424817, 11 pages, 2012.

[15] J.-P. Lachaud and G. Pérez-Lachaud, "Diversity of species and behavior of hymenopteran parasitoids of ants: a review," *Psyche*, vol. 2012, Article ID 134746, 24 pages, 2012.

[16] G. Pérez-Lachaud, J. A. López-Méndez, and J.-P. Lachaud, "Eucharitid parasitism of the Neotropical ant *Ectatomma tuberculatum*: parasitoid co-occurrence, seasonal variation, and multiparasitism," *Biotropica*, vol. 38, no. 4, pp. 574–576, 2006.

[17] R. W. Howard, R. D. Akre, and W. B. Garnett, "Chemical mimicry in an obligate predator of carpenter ants (Hymenoptera: Formicidae)," *Annals of the Entomological Society of America*, vol. 83, no. 3, pp. 607–616, 1990.

[18] R. W. Howard, D. W. Stanley-Samuelson, and R. D. Akre, "Biosynthesis and chemical mimicry of cuticular hydrocarbons from the obligate predator, *Microdon albicomatus* Novak (Diptera: Syrphidae) and its ant prey, *Myrmica incompleta* Provancher (Hymenoptera: Formicidae)," *Journal of the Kansas Entomological Society*, vol. 63, no. 3, pp. 437–443, 1990.

[19] A. Lenoir, Q. Chalon, A. Carvajal et al., "Chemical integration of myrmecophilous guests in *Aphaenogaster* ant nests," *Psyche*, vol. 2012, Article ID 840860, 12 pages, 2012.

[20] P. E. Cushing, "Spider-ant associations: an updated review of myrmecomorphy, myrmecophily, and myrmecophagy in spiders," *Psyche*, vol. 2012, Article ID 151989, 23 pages, 2012.

[21] A. Buschinger, "Social parasitism among ants: a review (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 12, pp. 219–235, 2009.

[22] P. D'Ettorre and J. Heinze, "Sociobiology of slave-making ants," *Acta Ethologica*, vol. 3, no. 2, pp. 67–82, 2001.

[23] A. Lenoir, P. D'Ettorre, C. Errard, and A. Hefetz, "Chemical ecology and social parasitism in ants," *Annual Review of Entomology*, vol. 46, pp. 573–599, 2001.

[24] R. M. Feitosa, R. R. Hora, J. H. C. Delabie, J. Valenzuela, and D. Fresneau, "A new social parasite in the ant genus *Ectatomma* F. Smith (Hymenoptera, Formicidae, Ectatomminae)," *Zootaxa*, no. 1713, pp. 47–52, 2008.

[25] R. R. da Hora, R. Fénéron, J. Valenzuela, M. E. Favila, and D. Fresneau, "Queen-size dimorphism in the ant *Ectatomma tuberculatum* (Hymenoptera: Formicidae: Ponerinae)," *Sociobiology*, vol. 38, no. 3A, pp. 407–420, 2001.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 917847, 8 pages
http://dx.doi.org/10.1155/2013/917847

Research Article

A New Species of Afrotropical Ants in the Genus *Bothroponera* (Hymenoptera: Formicidae: Ponerinae)

Abdulmeneem M. A. Joma[1,2] and William P. Mackay[2]

[1] *Department of Zoology, Faculty of Sciences, The University of Sebha, P.O. Box 18758, Sebha, Libya*
[2] *Department of Biological Sciences, Ecology and Evolutionary Biology Program, The University of Texas at El Paso, 500 West University Avenue, El Paso, TX 79968, USA*

Correspondence should be addressed to Abdulmeneem M. A. Joma; alnoure69@yahoo.com

Received 3 April 2013; Accepted 24 June 2013

Academic Editor: Abraham Hefetz

We describe a new species of Afrotropical *Bothroponera* from Whittlesea City, Eastern Cape Province, South Africa. This species is unique among the African *Bothroponera* as it is the only species with a horizontal propodeal spiracle. It is also the largest species of African *Bothroponera* (total length 14.80–15.65). The clypeus lacks a medial longitudinal carina, the head is subquadrate, the sculpture is mostly foveolate, and the second gastral segment nearly lacks sculpturing. We compare the new species to the similar *B. cavernosa* and *B. cavernosa* var. *montivaga*. We also compare the new species to all of the other 10 taxa that belong to the *cavernosa* complex. A key to the *cavernosa* complex species of the Afrotropical *Bothroponera* is provided along with diagnosis, comparison, distribution, habitat, biology, and etymology for the new species.

1. Introduction

1.1. Ants of Africa and Their Importance. Ants are generally considered a remarkable model for the study of population dynamics and ecosystem structure and function, especially in tropical, subtropical, and biodiversity hotspot areas, because of the ecological roles of these organisms in ecosystems. Ant species are considered keystone species in several terrestrial ecosystems and are unique in that they can interact biologically and ecologically with other organisms and display huge positive and negative effects on ecosystem [1–6]. They play almost all of the roles of symbiotic relationships with other organisms. Biologists study ant species for several reasons: they are easy to handle, able to survive in various habitats, adapt to extreme environments, and are small in size. Afrotropical regions are especially rich with ant species, where they disperse seeds in the Fynbos biome in South Africa [3, 7, 8]. Ant species play a central role in maintaining the vegetation at the appropriate density. Afrotropical ants are very important in optimal ecosystem management, such as *Oecophylla longinoda* in South Africa [9].

1.2. Current Estimations of African Ant Diversity. Studies are still insufficient to estimate the actual number of species of African ants, which includes about 16 subfamilies and 83 to 154 genera [2, 10]. The largest subfamilies are Myrmicinae with about 6983 species, Formicinae with about 3709 species, and Ponerinae with about 1250 species. Studies on the biodiversity of African ants demonstrate that these important organisms have a high species richness and great biodiversity in African ecosystems. Afrotropical ant genera have been sampled in several projects. Belshaw and Bolton [11] collected 47 genera in Ghana; Lindsey and Skinner [12] recorded 17 genera in Tussen die Riviere Game Reserve, Free State in South Africa; Parr and Chown [13] collected 16 genera from the central Satara area of the Kruger National Park, South Africa; Fisher [14] collected 56 genera on Monts Doudou in Southwestern Gabon; Yanoviak et al. [15] collected 14 genera in Gamba, Gabon; Braschler et al. [16] collected 35 genera in South Africa; Schoeman and Foord [17] collected 29 genera at the Marakele National Park, Limpopo province, South Africa; Hita Garcia et al. [18] recorded 52 genera from Kakamega Forest, Western Kenya. Robertson [2] listed 83 genera of

Afrotropical ants. Some genera (44) have received modern taxonomic revision, while 39 genera including *Pachycondyla* have not been revised. By simple calculations, based on the previous studies and [10, 19], the approximate number of African ant genera (excluding Malagasy genera) is about 126–154. The number will increase soon as a result of the current active work on revisions of Afrotropical ants.

1.3. Subfamily Ponerinae and Tribe Ponerini Characters. *Bothroponera* belongs to the subfamily Ponerinae, which includes three tribes, Platythyreini, Ponerini, and Thaumatomyrmecini [20]. The worker of the subfamily Ponerinae can be recognized by several characters [20], including a well-developed sting, the toruli are completely fused to the frontal lobes, which are greatly narrowed posteriorly, and the palpal segments are reduced in length. The promesonotal suture is present and flexible with the pronotum and mesonotum capable of movement relative to each other. The metapleural gland orifice is simple. The clypeus is well developed. The petiole is present, the postpetiole is not separated from the gaster [21]. The petiole represents the second abdominal segment, the postpetiole is the third abdominal segment (first gastral segment), and the rest of abdominal segments are gastral segments 4–7. The Ponerinae can often be recognized by a stridulatory organ on the dorsal surface of the second acrotergite [20, 21]. The petiole is without tergosternal fusion [20]. The Ponerinae is considered to be a heterogeneous assemblage of ants [6]. The worker of the tribe Ponerini also has special characteristics such as the petiole has a slender articulation on the ventral side of the first gastral segment, palpal joints are reduced in number, and the frontal carinae converge posteriorly [21]. The clypeus has an anterior medial raised area and is narrowed from the sides of the head. The frontal lobes cover the base of the insertion of the scapes. The helcium projects from very low down on the anterior face of third abdominal segment, the latter with a high vertical anterior face above the helcium. This tribe is considered as a paraphyletic group based on apomorphic characteristics [20].

1.4. Genus Pachycondyla. Since 1858, when Smith described the genus *Pachycondyla* [22], myrmecologists have continued to add new subgenera, and recently this genus and the proposed generic synonyms have reached a state of unclear taxonomic identity. This genus includes 18 subgenera, which in the future will probably be separated into more than 18 genera, *Bothroponera* being one of them. The taxonomic level of *Pachycondyla* is still under extensive study in that it could be a paraphyletic or even a polyphyletic assemblage. The morphological characters of these ants are heterogeneous; there is no apomorphic character that defines the genus [23], which suggests several separate genera within *Pachycondyla* [23, 24]. Genetic studies [25] revealed that the karyotypes of *Pachycondyla* species are extremely variable; most karyotypes are with large chromosome numbers, (more than 11 chromosomes), while others have smaller chromosome numbers (fewer than 11 chromosomes), and the morphological characters of the chromosomes are variable. Since there are independent patterns of karyotype evolution in the *Pachycondyla* genome, there are several genera that should be distinct from *Pachycondyla* [25].

1.5. Genus Bothroponera. The genus *Bothroponera* was described by Mayr in 1862 [26]. The majority of myrmecologists, such as von Dalla Torre, Bingham, Ashmead, W. M. Wheeler, Bernard, Taylor et al., G. C. Wheeler and J. Wheeler, Taylor, and Dlussky and Fedoseeva [27–35], agreed that *Bothroponera* is a separate genus; however, *Bothroponera* and *Pachycondyla* are closely related to each other. The characteristics that are used to distinguish the genus *Bothroponera* in this paper are based on descriptions of the entire group of African *Bothroponera* species (~40). The genus is characterized by the narrowed, convex, and medially raised clypeus. The mandibles are triangular or narrowed with 6–9 teeth. The frontal lobes are rounded or semioval, divided by a well-developed frontal furrow. The pronotum of the worker lacks any evidence of a carina or shelf. The mesonotum is completely fused with the propodeum, and the notopropodeal suture is completely absent. The petiole is thick with a developed ventral process. The mesopleuron is not divided by an anapleural suture and is well separated from the metapleuron by the mesometapleural suture.

1.6. Global Distribution of the Genus Bothroponera. The genus *Bothroponera* in Africa occurs mainly in tropical and subtropical areas. The biogeographical information shows that the genus *Bothroponera* is present only in the Afrotropical, Oriental, and Australian Regions. There are about 92 species of *Bothroponera* distributed worldwide, but *Bothroponera* is absent from the Palearctic, Nearctic, and Neotropical Regions. The *Bothroponera* species are distributed as the following: Madagascar 9 taxa (*Bothroponera cambouei*, *B. comorensis*, *B. masoala*, *B. perroti*, *B. perroti admista*, *B. planicornis*, *B. tavaratra*, *B. vazimba*, and *B. wasmannii* revised as *Pachycondyla* [36]; Australia 16 taxa (*Bothroponera excavata* var. *acuticostata*, *B. astuta*, *B. barbata*, *B. denticulata*, *B. dubitata*, *B. excavata*, *B. piliventris* var. *intermedia*, *B. sublevis* subsp. *kurandensis*, *B. mayri*, *B. sublevis* var. *murina*, *B. piliventris*, *B. porcata*, *B. piliventris* subsp. *regularis*, *B. sublaevis* r. *reticulata*, *B. sublaevis* var. *rubicunda*, and *B. sublaevis*) [33]; New Guinea has five species (*Bothroponera incisa*, *B. obesa*, *B. simillima*, *B. striata*, and *B. verecunda*); and India has eight taxa (*Bothroponera sulcata* var. *fossulata*, *B. bispinosa*, *B. henryi*, *B. leeuwenhoeki*, *B. rufipes*, *B. sulcata*, *B. sulcata* var. *sulcatotesserinoda*, and *B. tesseronoda*). In Asia there are about 15 taxa distributed as the following: Philippines two species (*Bothroponera glabripes* and *B. williamsi*); Indonesia four species (*B. insularis*, *B. solitaria*, *B. unicolor*, and *B. vermiculata*); Borneo four taxa (*B. sandakana*, *B. insularis* var. *brevior*, *B. tridentata*, and *B. tridentata* r. *debilior*); Myanmar one species (*B. rubiginosa*); Sri Lanka one taxon (*B. rufipes* subsp. *ceylonensis*); West Malaysia one taxon (*Bothroponera tridentata* var. *exasperans*); Singapore one species (*B. havilandi*); and Vietnam one species (*Bothroponera annamita*) [10, 37].

In Africa there are about 40 species of *Bothroponera* distributed as the following: Guinea one species, Sierra

Leone one species, Ghana one species, Cameroun four species, Democratic Republic of Congo four species, Congo Brazzaville four species, Angola one species, Ethiopia four species, Kenya three species, Tanzania one species, Malawi one species, Mozambique one species, Zimbabwe two species, and South Africa ten species. They are clustered in three major areas in African continent according to their complexes: eastern countries are the favorite habitat for the *crassa* complex, western countries are the habitat for the *talpa* complex, and southern countries are the main ecosystems for the *cavernosa* complex. The African taxa in the *cavernosa* complex include *Bothroponera berthoudi* Forel, 1901, *B. cariosa* Emery, 1895, *B. cavernosa* (Roger, 1860), *B. cavernosa* var. *montivaga* Arnold, 1947, *B. granosa* (Roger, 1860), *B. laevissima* Arnold, 1915, *B. laevissima* var. *aspera* Arnold, 1962, *B. pumicosa* (Roger, 1860), *B. strigulosa* Emery, 1895, and *B. variolosa* Arnold, 1947.

In this paper, the morphological description of a new species of African *Bothroponera* is provided. The illustrations of the head (full face) and the lateral view of the body are included. The lateral view shows the horizontal propodeal spiracle on the lateropropodeum. A taxonomic key to the species complexes of the Afrotropical *Bothroponera* as well as taxonomic key of the *cavernosa* complex species is provided along with diagnosis, comparison, distributional information, habitat, biology, and etymology of the new species.

2. Material and Methods

2.1. Specimens Collections. The two worker specimens of the new species were obtained from the Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA (MCZC). The male and queen are unknown. The other museums that provided us with the African *Bothroponera* specimens are the following:

Naturhistorisches Museum (NHMB), Basel, Switzerland.

Muséum d'Histoire Naturelle (MHNG), Geneva, Switzerland.

Iziko South African Museum (SAM).

Dr. William Mackay's collection (CWEM) the University of Texas at El Paso.

British Natural History Museum (BMNH), London.

Museum für Naturkunde (ZMHU), Berlin, Germany.

Museum Nationale d'Histoire Naturelle (MNHN), Paris, France.

Museo Civico di Storia Naturale (MCSN), Genova, Italy.

American Museum of Natural History (AMNH), New York.

Los Angeles County Museum of Natural History (LACM), California.

2.2. Measurement Abbreviations. The specimens were examined with a Zeiss binocular microscope with an ocular micrometer. All measurements are in millimeters.

Head length (HL), in full face view, the maximum length of the head excluding the mandibles, from the midpoint of the anterior clypeal margin to the midpoint of the posterior margin of the head.

Head width (HW), in full face view, the maximum width of the head from the extreme side of head to the other extreme side excluding the eyes.

Mandible length (ML), the distance from the mandible's base to the apex of the apical tooth.

Eye length (EL), the maximum diameter of the eye as seen from the side.

Eye Width (EW), the maximum distance of the eye from the anterior edge to the posterior edge as seen from the side.

Scape length (SL), the maximum length of the scape from the proximal to the distal extremes, excluding the basal constriction.

Funiculus length (FL), the measurement of the distal 11 segments of the antenna including the club and all of the funicular segments.

Weber length (WL), the length in lateral view, from the anterior edge of the pronotum to the end of posterior margin of the propodeal lobes.

Petiole length (PL), in lateral view, the maximum distance of the petiole from the anterior face to the posterior side excluding the helcium.

Petiole width (PW), in dorsal view, the maximum side to side thickness of the petiole, generally at the posterior edge since it has the largest width.

Petiole height (PH), in lateral view, the maximum length from the lower point of the sternopetiolar process, excluding the petiolar teeth, to the highest point at the apex of the petiolar node.

The following indices are used:

cephalic index (CI), $HW/HL \times 100$,

ocular index (OI), $EL/HW \times 100$,

mandible index (MandI), $ML/HL \times 100$,

scape index (SI), $SL/HW \times 100$,

petiole index (PetI), $PW/PL \times 100$.

2.3. Further Measurements and Descriptions. In each specimen, we measured the hair length, the total body length, the malar space length (from lower edge of the eye to the base of the mandible), and the length of the side of the head from the upper margin of the eye to the highest point of the posterior lateral corner of the head (side view). There are other characters that are taken into account including the shape of the head, eyes (large or small), pronotum, mesopleuron, propodeum, petiole, and postpetiole. The shape of the pronotal shoulder, lower margin of the pronotum, basalar sclerite, and propodeal spiracle are important. The entire body color including the antennae, clypeus, mandibles, and legs was described as well.

2.4. Nomenclatural Acts. The new name contained in this paper is available under the International Code of Zoological Nomenclature (ICZN). This work and the nomenclatural acts it contains have been registered in ZooBank. Zoobank life science identifier (LSID) for this publication is urn:lsid:zoobank.org:pub:29432EB5-CF41-4FF6-BFC9-F63DD64C502E. The LSID registration and any associated information can be viewed in a web browser by adding the LSID to the prefix "http://zoobank.org/."

3. Results and Discussion

The following are the diagnosis of the worker, description, and measurements of the new species, *Bothroponera umgodikulula n* sp. (Figures 1 and 2).

3.1. Diagnosis of the Worker. The main distinguishable characters of *B. umgodikulula* are the lack of sculpture on the tergum of fourth abdominal segment (second gastral segment), which is mostly smooth and glossy, and the horizontal propodeal spiracle. The worker is also characterized by the large total length, which is 14.80–15.65 mm. The head is subquadrate (CI 95.00–95.16). The clypeus is convex, "v" shaped, and covered with striae, except for the medial area. The anterior medial area is raised and coarsely punctate on the sides, and smooth and glossy in the middle, but an actual carina is absent. The mandibles are triangular, shorter than the head length (MandI 50.00–54.83), smooth and glossy with scattered elongated coarse punctures and about 7 teeth. The scape reaches or extends slightly past the posterior border of the head (SI 81.35–82.45). The compound eyes are relatively large (OI 15.25–15.78). The lower margins of the frontal lobes are smooth; the upper part is punctate. The maximal frontal lobe width is 1.10–1.20 mm. The head is subquadrate and coarsely foveolate. The length of the malar space on the side of the head is (0.65–0.70 mm); the length from the upper edge of the eye to the edge of posterior lobe is 1.35–1.50 mm.

The pronotum, dorsum of the mesonotum, and dorsum of the propodeum are coarsely foveolate and rough. The dorsum of the petiole and postpetiole are coarsely foveolae and punctate. The mesopleuron and lateropropodeum are coarsely grooved and covered with striae, foveolae, and punctures. The propodeal spiracle is unusual in being nearly horizontal. The pronotal shoulder is rounded. The antennae, legs, and posterior edge of each gastral tergite are shiny. The petiole is rounded and slightly narrowed anteriorly, while it is slightly concave posteriorly (PetI 115.38–125.92).

The entire head, pronotum, mesonotum, propodeum, petiole, and postpetiole are covered with short (0.03–0.10 mm) fine golden hairs. The ventral side of the postpetiole and fourth-seventh gastral segments are covered with relatively long (0.20–0.25 mm) golden suberect hairs. The hairs on underside of the head range from 0.25 to 0.50 mm in length.

The head, pronotum, mesonotum, mesopleuron, propodeum, petiole, postpetiole, and entire gaster are black. The legs, antennae, mandibles are red. The clypeus is dark brown.

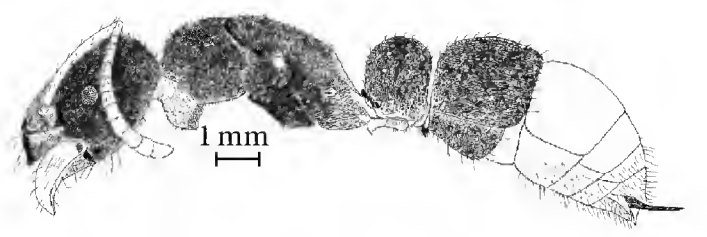


FIGURE 1: Side view of the holotype worker of *B. umgodikulula*.

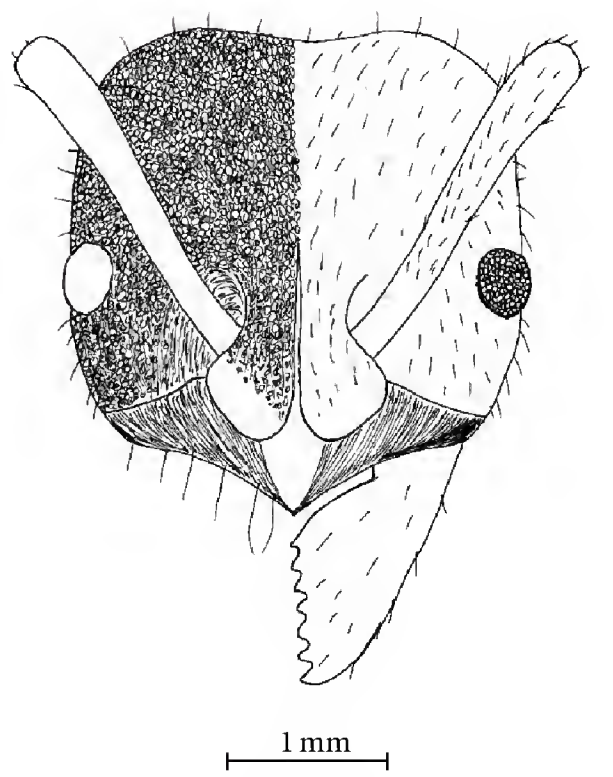


FIGURE 2: Full face view of the holotype worker of *B. umgodikulula*.

3.2. Description

3.2.1. Worker Measurements and Indices (n = 2). HL 3.00–3.10, HW 2.85–2.95, ML 1.50–1.70, EW 0.40–0.45, EL 0.45, SL 2.35–2.40, FL 3.65–3.75, WL 4.20, WPL 5.00–5.50, PL 1.30–1.35, PW 1.50–1.70, PH 1.75–1.80, CI 95.00–95.16, OI 15.78–15.25, MandI 50.00–54.83, SI 82.45–81.35, and PetI 115.38–125.92.

3.2.2. Worker Characteristics. Head excluding mandibles subquadrate with rounded sides; mandibles smooth, glossy with scattered elongated coarse punctures and fine longitudinal striolae, with about 7 teeth, mandible shorter than head length; clypeus covered with longitudinal striae (except medial area), medial area raised, without forming carina, disc smooth and glossy, sides coarsely punctate; eyes relatively large; scape reaches or extends slightly past posterior border of head; lower lateral margins of frontal lobes smooth, upper part punctate; dorsum of head mostly foveolate; pronotum, mesonotum, propodeum rough, and coarsely foveolate; mesopleuron, lateropropodeum covered with foveolae, with punctures, striae, and coarse grooves; pronotal shoulder rounded; basalar sclerite nearly oval; propodeal spiracle nearly horizontal; antennae, legs shiny; petiole rounded, slightly narrowed anteriorly, slightly concave posteriorly; dorsum and sides of petiole, postpetiole (first gastral segment) coarsely foveolate, punctate; second gastral segment to tip of gaster mostly smooth, punctate, with weak markings of foveolae; entire head, pronotum, mesonotum, propodeum,

petiole, and postpetiole covered with short golden erect hairs; hairs on underside of head long; head, pronotum, mesonotum, mesopleuron, propodeum, petiole, postpetiole, entire gaster black; legs, antennae, and mandibles red; clypeus reddish brown.

3.3. Comparison. The new species is a member of the African *cavernosa* species complex of the genus *Bothroponera*, in which the metatibial gland on the ventral tibial hind leg is absent and the clypeus is convex with "v" shaped anterior margin. *Bothroponera umgodikulula* is easily recognized by the horizontal propodeal spiracle (parallel to the postero-propodeum in the remainder of the *Bothroponera* species). In addition, the smooth and glossy 4th abdominal segment (second gastral segment) of *B. umgodikulula* is different from the sculptured segments of the similar *B. cavernosa* and *B. cavernosa* var. *montivaga*. The 4th abdominal segment of *B. cavernosa* is rough, moderately shiny with few scattered hairs and fine poorly defined striae, while that of *B. cavernosa* var. *montivaga* is somewhat smooth and moderately shiny (less so than *B. umgodikulula*) with a few scattered punctures. The other taxa that can be confused with *B. umgodikulula* are *B. laevissima* and *B. laevissima* var. *aspera* because they both have smooth and glossy 4th abdominal segments, but it is easy to recognize them by the body surface. Most surfaces of *B. umgodikulula* are coarsely foveolate whereas they are smooth and shiny in *B. laevissima* and with a few punctures in *B. laevissima* var. *aspera*. The new species *B. umgodikulula* is also characterized by the larger body size (total length 14.80–15.65 mm) compared to *B. cavernosa* (11.90 mm) and *B. cavernosa* var. *montivaga* (12.20–12.65 mm). In fact, the new species is the largest species among the other *cavernosa* complex species (*B. granosa* 13.75–14.50 mm, *B. variolosa* 12.15–12.75 mm, *B. strigulosa* 12.20 mm, *B. laevissima* 11.80–13.00 mm, *B. laevissima* var. *aspera* 11.70–12. 70 mm, *B. pumicosa* 11.00–11.65 mm, *B. cariosa* 11.50 mm, and *B. berthoudi* 9.60 mm). *Bothroponera cavernosa* var. *montivaga* and *B. cavernosa* share most of the characteristics with the new species except the propodeal spiracle, which is obliquely vertical in *B. cavernosa* var. *montivaga* and *B. cavernosa*, while it is horizontal in *B. umgodikulula*. The new species *B. umgodikulula* is the only species among the *cavernosa* complex species that has a horizontal propodeal spiracle. The anterior medial margin of the clypeus is "v" shaped in *B. umgodikulula* similarly to that of *B. granosa*, *B. cavernosa*, *B. cavernosa* var. *montivaga*, and *B. laevissima* var. *aspera*. Conversely, the anterior medial margin of the clypeus is "u" shaped in *B. cariosa*, *B. strigulosa*, *B. pumicosa*, *B. laevissima*, *B. berthoudi*, and *B. variolosa*. The clypeus has a sharp carina on the raised area in *B. granosa*; it is partially carinated in *B. cavernosa* and *B. cavernosa var. montivaga*, and the lower parts are smooth. The anterior medial raised area of *B. umgodikulula* is completely smooth (lacking the carina), and shiny, but sculptured and punctate on sides of the medial raised area. The scape of *B. umgodikulula* reaches or slightly exceeds the posterior lateral corner of the head, while it is slightly shorter in *B. cavernosa* var. *montivaga* and slightly longer in *B. cavernosa*.

3.4. Material Examined. Type series, South Africa: Eastern Cape, Bulhoek, Klaver-Clanw., Bulhoek at 32°10′0″ S; 26°49′0″E, Mus. Expd. October 1950, (2 w, holotype (MCZC) and paratype (SAM) no. C005835) *Bothroponera cavernosa* Roger, 1860, F. W. G., SAM-HYM SAM.

3.5. Distribution. The species is known only from Bulhoek (Whittlesea), Eastern Cape, South Africa.

3.6. Biology and Habitat. The new species (*Bothroponera umgodikulula*) is collected from the Bulhoek area in South Africa. Bulhoek is the former name of Whittlesea in the Eastern Province of South Africa. The average elevation of the area is about 1,060 m. The main vegetation in Whittlesea is grassland [38, 39]. The Eastern Cape Province includes about six different types of biomes: fynbos, savanna, thicket, grassland, nama karoo, and forest biomes. The area is characterized by different habitats that results in high biodiversity. In fact, most of the *cavernosa* complex species were found in South Africa especially in the Cape Provinces. There are at least 24 taxa that belong to *Pachycondyla* and *Bothroponera* collected from South Africa, including *Bothroponera berthoudi*; *B. cavernosa*; *B. cavernosa* var. *montivaga*; *B. granosa*; *B. kruegeri*; *B. laevissima* var. *aspera*; *B. pumicosa*; *B. strigulosa*; *B. variolosa*; *Pachycondyla aenigmatica*; *P. caffraria*; *P. elisae rotundata*; *P. fossigera*; *P. hartwigi*; *P. havilandi*; *P. havilandi fochi*; *P. havilandi godfreyi*; *P. havilandi marleyi*; *P. hottentota*; *P. peringueyi*; *P. peringueyi saldanhae*; *P. tarsata*; *P. wroughtonii* and *P. wroughtonii crudelis*. This high diversity is not only because of the extensive studies that have been conducted in South Africa, but it is also because of the numerous habitats and ecosystems that characterize the country.

3.7. Etymology. The name of the new species of African *Bothroponera* "*umgodikulula*" comes from isiZulu, one of the major South African languages. The word "umgodi" means hole, and "kulula" means level, to express that this species has a hole for respiration that is situated horizontally on the lateropropodeum.

3.8. Key to the Afrotropical Bothroponera Complexes

(1) Metatibial gland present; scape extends at least first funicular segment past posterior lateral corner of head; lower margin of anterior medial area of clypeus convex, straight, or slightly concave; eyes range from small to large (EW 0.05–0.45, EL 0.05–0.70) . . . *crassa* complex.

– Metatibial gland absent; scape shorter, barely reaches posterior lateral corner of head, or extends past less than length of first funicular segment . . . 2.

(2) Anterior margin of anterior medial area of clypeus convex, "u" or "v" shaped; eyes relatively large (EW and EL 0.30–0.45 mm) . . . *cavernosa* complex.

– Anterior margin of clypeus straight, or slightly concave, or slightly convex but not "v" or "u" shaped; eyes

relatively small (EW and EL 0.15–0.40 mm) . . . *talpa* complex.

3.9. Preliminary Key to the Afrotropical Cavernosa Complex Species of the Genus Bothroponera

(1) Scapes relatively short, barely reaching, or slightly exceeding posterior lateral corner of head, (SI 78.00–87.17); head subquadrate or suborbicular (CI 78.18–100); clypeus convex, anterior border "v" or "u" shaped, and sharp medial longitudinal carina present on clypeus, or partially carinated, or lacking carina; mandibles with 7-8 teeth without striae (MI 48.27–59.61); body sculptured with foveolae or punctae; fourth abdominal segment (second gastral segment) smooth, slightly rough, or sculptured; hairs length ranges from 0.03 to 0.55 mm . . . 2.

– Scapes slightly longer, extending past posterior lateral corner of head (SI 77.77); head suborbicular, cephalic index 90.00; clypeus convex, anterior border "v" shaped with partial longitudinal carina on the upper part; mandibles with 7 teeth, covered with fine striae (MI 48.33); body strongly sculptured by foveolae; fourth abdominal segment slightly rough with fine hairs scattered on dorsum; hairs lengths ranges from 0.03–0.20 mm . . . *cavernosa.*

(2) Hairs on scape short, 1/2-1/3 greatest diameter of scape (0.07–0.17 mm); hairs on posterior tibia 0.15–0.30 mm, with few to many suberect to erect hairs throughout length; few hairs on mandibles (fewer than 0.20 mm) with about 7 teeth, at least in part glossy, shiny, and possibly with coarse punctures or with few fine striae, mandibular index (48.27–59.61); anterior border of clypeus "v" or "u" shaped, lacking carina, or partially carinated; fourth abdominal segment smooth, or slightly rough, or sculptured . . . 3.

– Hairs on scape long, at least as long as the greatest diameter of scape (0.50–0.55 mm); hairs on posterior tibia about 0.30–0.35 mm; mandibles hairy (~0.20 mm) with about 8 teeth, mandibular index 50.90–52.00; anterior border of clypeus "u" shaped, anterior medial area raised to form partial carina on upper part; fourth abdominal segment sculptured, covered with shallow foveolae . . . *pumicosa.*

(3) Entire body smooth, shiny; ocular index 14.00–15.09 . . . *laevissima.*

– Entire body coarsely sculptured with foveolae or punctae, ocular index 15.25–21.42 . . . 4.

(4) Propodeal spiracle nearly horizontal; anterior border of clypeus "v" shaped with longitudinal raised medial area, but lacking carina; head subquadrate (CI 95.00–95.16); fourth abdominal segment smooth, glossy . . . *umgodikulula.*

– Propodeal spiracle nearly vertical; anterior border of clypeus "v" or "u" shaped, sharp carina present on clypeus, partially carinated, or lacking carina; head subquadrate or suborbicular (CI 82.75–100); fourth abdominal segment slightly rough or sculptured . . . 5.

(5) Body with sparse punctures, moderately shiny, black; anterior border of clypeus "v" shaped, lacking carina; head suborbicular (CI 90.19–90.90); fourth abdominal segment smooth shiny . . . *laevissima* var. *aspera.*

– Body sculptured foveolae or punctate, black, brownish, or brownish dark, brown or reddish brown appendages; anterior border of clypeus "u" or "v" shaped, carina present, lacking carina, or partially carinated; head suborbicular or subquadrate; fourth abdominal segment slightly roughened or sculptured . . . 6.

(6) Anterior border of clypeus "u" shaped with or without carina; scape reaching or slightly surpassing posterior lateral corner of head . . . 7.

– Anterior border of clypeus "v" shaped with at least partial carina; scape not reaching posterior lateral corner of head . . . 8.

(7) Mandible with scattered coarse punctures that unite to some degree, forming poorly defined rugulae, mandible shorter than head length (MI 59.61); fine carina present on anterior medial raised area of clypeus; posterior border of petiolar node (seen from above) with depression medially; fourth abdominal segment sculptured, covered with shallow foveolae . . . *cariosa.*

– Mandible with scattered isolated punctures that do not unite, completely smooth and glossy between punctures with little evidence of striae or rugulae, mandible shorter than head length (MI 48.27–56.45); anterior medial raised area of clypeus with or without sharp carina or lacking carina; posterior border of petiolar node with little evidence of depression; fourth abdominal segment (second gastral segment) slightly roughened or sculptured . . . 9.

(8) Eyes relatively smaller (OI 16.66–18.18); clypeus with sharp longitudinal carina on anterior border; petiolar index (116.66–123.07); fourth abdominal segment (second gastral segment) sculptured, covered with shallow foveolae, striae . . . *granosa.*

– Eyes relatively larger (OI 18.36–18.75); partial carina present on anterior border of clypeus; petiolar index (130.00); fourth abdominal segment smooth with few scattered punctures . . . *cavernosa* var. *montivaga.*

(9) Erect hairs on scape short (most less than 0.10 mm in length, few up to 0.20 mm), scape not reaching posterior lateral corner of head (SI 78.57); erect golden hairs on entire dorsum of body (0.07–0.13 mm, a few up to 0.16 mm), hairs on petiole (0.15–0.18 mm); clypeus forming sharp medial carina on the medial raised area; mandibular index (54.00) . . . *strigulosa.*

– Erect hairs on scape short (0.07–0.10 mm), scape reaching or nearly reaching posterior lateral corner of head (SI 82.22–87.17); moderately short erect golden

hairs on mesosoma (most over 0.06 mm, few over 0.25 mm in length), but longer (most over 0.25 mm) suberect hairs on petiole and postpetiole (0.25 mm); clypeal medial area not forming carina (smooth and rounded); mandibular index (55.10–55.55) ... *variolosa* and *berthoudi*.

Acknowledgments

The authors would like to thank the curators and museums who provided Afrotropical ant specimens including Dr. Claire Villemant (MNHN), Dr. Bernhard Merz (MHNG), Drs. Stefan Cover, Philip Perkins, Jack Boyle, and Charles Whittemore Farnum (MCZC), Drs. Frank Koch and Viola Richter (ZMHU), Drs. Roberto Poggi, Giuliano Doria, and Maria Tavano (MCSN), Drs. Isabelle Zürcher-Pfander, and Daniel Burckhardt (NHMB), Drs. Gavin Broad and Suzanne Ryder (BMNH), Drs. James Carpenter and Christine LeBeau (AMNH), Drs. Weiping Xie and Laura McGover (LACM), and Dr. Dawn Larsen (SAM). Special thanks are due to Dr. Gary Alpert who offered to take photos of the *Bothroponera* species and allowed the authors lodging during the study period at the Museum of Comparative Zoology. Dr. Alain Dejean provided several publications on African ants; Dr. Hamish Robertson provided his ant publication website; Dr. Brigitte Braschler offered useful information about African ant richness and biodiversity, and Dr. Nigel Worden, from the University of Cape Town, offered historical information about South Africa. Thanks are also due to Dr. Barry Bolton, who suggested useful comments for ant character descriptions and identifications. An anonymous reviewer made important suggestions to improve the paper, for which the authors are grateful. The authors also thank Dr. Robert Kirken, Chairman of the Department of Biological Sciences of the University of Texas at El Paso, who facilitated procedures to borrow specimens from SAM, Dr. Elizabeth Walsh, Ph.D. advisor and the doctoral committee members Dr. Carl Lieb, Dr. Eli Greenbaum, Dr. Michael Moody, and Dr. Mahesh Narayan, as well as Dr. Shawn Dash and Dr. Jerry Johnson, for assistance and useful suggestions. This research was supported by Grant no. G12MD007592 from the National Institutes on Minority Health and Health Disparities (NIMHD), a component of the National Institutes of Health (NIH), and also supported by the Libyan Ministry of Higher Education, Libyan-North American Scholarship Program, and Canadian Bureau for International Education. This paper fulfills one of the requirements for the Doctor of Philosophy degree in Ecology and Evolutionary Biology at the University of Texas at El Paso.

References

[1] B. Hölldobler and E. O. Wilson, *The Ants*, The Belknap Press of Harvard University Press, Cambridge, Mass, USA, 1990.

[2] H. G. Robertson, "Afrotropical ants (Hymenoptera: Formicidae): taxonomic progress and estimation of species richness," *Journal of Hymenoptera Research*, vol. 9, no. 1, pp. 71–84, 2000.

[3] K. French and R. E. Major, "Effect of an exotic *Acacia* (Fabaceae) on ant assemblages in South African fynbos," *Austral Ecology*, vol. 26, no. 4, pp. 303–310, 2001.

[4] D. Grimaldi and M. S. Engel, *Evolution of the Insects*, Cambridge University Press, Cambridge, Mass, USA, 2005.

[5] R. M. Pringle, D. F. Doak, A. K. Brody, R. Jocqué, and T. M. Palmer, "Spatial pattern enhances ecosystem functioning in an African savanna," *PLoS Biology*, vol. 8, no. 5, Article ID e1000377, 2010.

[6] P. S. Ward, "Taxonomy, phylogenetics, and evolution," in *Ant Ecology*, L. Lori, C. L. Parr, and K. L. Abbott, Eds., chapter 1, Oxford University Press, New York, NY, USA, 2010.

[7] N. Farwig and D. G. Berens, "Imagine a world without seed dispersers: a review of threats, consequences and future directions," *Basic and Applied Ecology*, vol. 13, no. 2, pp. 109–115, 2012.

[8] M. A. Rodriguez-Cabal, K. L. Stuble, B. Guénard, R. R. Dunn, and N. J. Sanders, "Disruption of ant-seed dispersal mutualisms by the invasive Asian needle ant (*Pachycondyla chinensis*)," *Biological Invasions*, vol. 14, no. 3, pp. 557–565, 2012.

[9] M. J. Way and K. C. Khoo, "Role of ants in pest management," *Annual Review of Entomology*, vol. 37, pp. 479–503, 1992.

[10] http://www.antweb.org/.

[11] R. Belshaw and B. Bolton, "A survey of the leaf litter ant fauna of Ghana, West Africa (Hymenoptera: Formicidae)," *Journal of Hymenoptera Research*, vol. 3, pp. 5–16, 1994.

[12] P. A. Lindsey and J. D. Skinner, "Ant composition and activity patterns as determined by pitfall trapping and other methods in three habitats in the semi-arid Karoo," *Journal of Arid Environments*, vol. 48, no. 4, pp. 551–568, 2001.

[13] C. T. Parr and S. L. Chown, "Inventory and bioindicator sampling: testing pitfall and winkler methods with ants in a South African savanna," *Journal of Insect Conservation*, vol. 5, no. 1, pp. 27–36, 2001.

[14] B. L. Fisher, "Diversity patterns of ants (Hymenoptera: Formicidae) along an elevational gradient on Monts Doudou in southwestern Gabon. In: Monts Doudou, Gabon: A Floral and Faunal Inventory with Reference to Elevational Variation," *California Academy of Sciences*, vol. 28, pp. 269–286, 2004.

[15] S. P. Yanoviak, B. L. Fisher, and A. Alonso, "Arboreal ant diversity (Hymenoptera: Formicidae) in a central African forest," *African Journal of Ecology*, vol. 46, no. 1, pp. 60–66, 2008.

[16] B. Braschler, S. L. Chown, and K. J. Gaston, "The Fynbos and Succulent Karoo biomes do not have exceptional local ant richness," *PLoS ONE*, vol. 7, no. 3, Article ID e31463, 2012.

[17] C. S. Schoeman and S. H. Foord, "A checklist of epigaeic ants (Hymenoptera: Formicidae) from the Marakele National Park, Limpopo, South Africa," *Koedoe*, vol. 54, no. 1, pp. 1–7, 2012.

[18] F. Hita Garcia, G. Fischer, M. K. Peters, R. R. Snelling, and J. W. Wägele, "A preliminary checklist of the ants (Hymenoptera: Formicidae) of Kakamega Forest (Kenya)," *Journal of East African Natural History*, vol. 98, pp. 147–165, 2009.

[19] http://www.webarchive.org.uk/wayback/archive/20100617220041/http://antbase.org/ants/africa/contents.htm.

[20] B. Bolton, "Synopsis and classification of Formicidae," *Memoirs of the American Entomological Institute*, vol. 71, pp. 1–370, 2003.

[21] W. M. Wheeler, *Ants: Their Structure, Development and Behavior*, Columbia University Press, New York, NY, USA, 1910.

[22] F. Smith, *Catalogue of Hymenopterous Insects in the Collection of the British Museum*, Part VI. Formicidae, British Museum, London, UK, 1858.

[23] W. P. Mackay and E. E. Mackay, *The Systematics and Biology of the New World Ants of the Genus Pachycondyla (Hymenoptera: Formicidae)*, The Edwin Mellen Press, Lewiston, NY, USA, 2010.

[24] W. P. Mackay and E. E. Mackay, "A new species of the ant genus *Pachycondyla* F. Smith, 1858 from Ecuador (Hymenoptera: Formicidae)," *Myrmecologische Nachrichten*, vol. 8, pp. 49–51, 2006.

[25] C. D. S. F. Mariano, S. D. G. Pompolo, J. G. Silva, and J. H. C. Delabie, "Contribution of cytogenetics to the debate on the paraphyly of Pachycondyla spp. (Hymenoptera, Formicidae, Ponerinae)," *Psyche*, vol. 2012, Article ID 973897, 9 pages, 2012.

[26] G. Mayr, "Myrmecologische Studien," *Verhandlungen der Kaiserlich-Koniglichen Zoologisch-Botanischen Gesellschaft in Wien*, vol. 12, pp. 649–776, 1862.

[27] K. W. von Dalla Torre, *Catalogus Hymenopterorum hucusque descriptorum systematicus et synonymicus, Formicidae (Heterogyna)*, vol. 7, W. Engelmann, Leipzig, Germany, 1893.

[28] C. T. Bingham, *The Fauna of British India, Including Ceylon and Burma. Hymenoptera, Ants and Cuckoo-Wasps*, vol. 2, Taylor and Francis, London, UK, 1903.

[29] W. H. Ashmead, "A skeleton of a new arrangement of the families, subfamilies, tribes and genera of the ants, or the superfamily Formicoidea," *The Canadian Entomologist*, vol. 37, pp. 381–384, 1905.

[30] W. M. Wheeler, "Ants of the American Museum Congo expedition, A contribution to the myrmecology of Africa, IX. A synonymic list of the ants of the Malagasy region," *Bulletin of the American Museum of Natural History*, vol. 45, pp. 1005–1055, 1922.

[31] F. Bernard, "Les fourmis du Tassili des Ajjer. Pp. 121–250 in: Bernard, F., (ed.) Mission scientifique au Tassili des Ajjer (1949), Recherches zoologiques et médicales, Paris," *P. Lechevalier*, vol. I, p. 302, 1953.

[32] R. W. Taylor, D. R. Brown, and J. C. Cardale, *Zoological Catalogue of Australia*, vol. 2 of *Hymenoptera: Formicoidea, Vespoidea and Sphecoidea*, Canberra, Australia, 1985.

[33] G. C. Wheeler and J. Wheeler, "A simplified conspectus of the Formicidae," *Transactions of the American Entomological Society*, vol. 111, pp. 255–264, 1985.

[34] R. W. Taylor, "A checklist of the ants of Australia, New Caledonia and New Zealand," *CSIRO Division of Entomology Report*, vol. 41, pp. 1–92, 1987.

[35] G. M. Dlussky and E. B. Fedoseeva, "Origin and early stages of evolution in ants," in *Cretaceous Biocenotic Crisis and Insect Evolution*, A. G. Ponomarenko, Ed., pp. 70–144, Nauka, Moscow, Russia, 1988.

[36] J. C. Rakotonirina and B. L. Fisher, "Revision of the *Pachycondyla wasmannii*-group (Hymenoptera: Formicidae) from the Malagasy region," *Zootaxa*, vol. 3609, no. 2, pp. 101–141, 2013.

[37] B. Bolton, G. Alpert, P. S. Ward, and P. Naskrecki, *Bolton's Catalogue of Ants of the World 1758–2005*, Harvard University Press, 2007.

[38] http://www.mbendi.com/a_sndmsg/place_view.asp?pid=9678.

[39] http://en.wikipedia.org/wiki/Whittlesea.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 693856, 8 pages
http://dx.doi.org/10.1155/2013/693856

Research Article

Hive Relocation Does Not Adversely Affect Honey Bee (Hymenoptera: Apidae) Foraging

Fiona C. Riddell Pearce, Margaret J. Couvillon, and Francis L. W. Ratnieks

Laboratory of Apiculture & Social Insects, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK

Correspondence should be addressed to Francis L. W. Ratnieks; f.ratnieks@sussex.ac.uk

Received 6 June 2013; Revised 29 July 2013; Accepted 29 July 2013

Academic Editor: Felipe Andrés León Contrera

Honey bees, *Apis mellifera*, face major challenges including diseases and reduced food availability due to agricultural intensification. Additionally, migratory beekeeping may subject colonies to a moving stress, both during the move itself and after the move, from the bees having to forage in a novel environment where they have no knowledge of flower locations. This study investigated the latter. We moved three colonies housed in observation hives onto the campus from a site 26 km away and compared their foraging performance to three similarly sized colonies at the same location that had not been moved. We obtained data on (1) foraging performance by calculating distance by decoding waggle dances, (2) hive foraging rate by counting forager departure rate, (3) forage quality by assessing sugar content of nectar from returning foragers, and (4) forager success by calculating the proportion of bees returning to the nest entrance with nectar in their crop. We repeated this 3 times (August 2010, October 2010, and June 2011) to encompass any seasonal effects. The data show no consistent difference in foraging performance of moved versus resident hives. Overall the results suggest that moving to a new location does not adversely affect the foraging success of honey bees.

1. Introduction

Beekeepers routinely move colonies of honey bees *Apis mellifera* L. to pollinate crops and to take advantage of asynchrony in nectar flows. For example, in the United Kingdom some hives are moved into apple (*Malus domestica* Borkh.) farms for pollination in early April and onto heather (*Calluna vulgaris*) moors in late July for obtaining heather honey. In the USA, hives are moved from as far away as Florida to pollinate California almonds in February, a distance of 3000 miles that will take a minimum of 2 to 3 days by truck. Bees are also moved extensively in many other countries (e.g., Turkey [1]). Moving hives has been suggested as an aggravating factor in the decline in colony numbers in the United States [2–4].

Honey bees are an important commercial crop pollinator [4, 5]. Although the number of managed hives has increased by about 45% globally since 1961, the rate of expansion of pollinator-dependant crops is greater than the increase in the number of managed hives, creating increased demand for pollination ([6], although see [7]). In addition, the number of managed hives has declined in Europe and North America, at an average of 1.79% annually [6]. This potential pollinator shortage is most serious in the USA, where insect pollinated crops are widely grown. For example, the California almond crop, which currently uses over 1 million bee hives for pollination, is worth over $1.6 billion annually [4].

Honey bee diseases [8] and the reduction of available bee forage due to agricultural intensification [9–11] are two important challenges facing honey bees. Additionally, beekeeping practices such as hive relocation may also cause undesirable consequences. Close contact of colonies during transport can increase the likelihood of horizontal transfer of pathogens and pests among colonies, and hive movement can spread any newly introduced pathogen in a new geographic area [12]. The process of transport may directly cause stress, leading to brood mortality [13]. Additionally, being moved to a novel environment requires the bees to discover new foraging locations. Previously it was shown that relocation of hives to a new apiary site can cause significant increase in the disorientation and loss of bees via drifting, particularly on the first day after the move [14]. Moving a colony of bees from a familiar landscape into one they have never experienced may hamper the bees' ability to rapidly locate food.

The aim of this study was to investigate the effect of relocation on colony foraging. To do this, we compared relocated and control colonies for four indicators of foraging success: number of bees leaving the hive, proportion of returning bees with nectar, duration of the straight run of the waggle dance which encodes foraging distance [15], and nectar concentration. The results show no consistent effect of relocating hives on the foraging performance of moved colonies versus resident control colonies.

2. Materials and Methods

2.1. Study Colonies and Experimental Setup. Each trial used six *A. mellifera* colonies, each housed in a glass-walled observation hive with three deep Langstroth frames. Each hive was connected to the outside via a tube through the laboratory wall. Colonies were set up in the laboratory several weeks or more before a trial. Each had a laying queen, two frames of brood each with patches covering approximately half the frame, and sufficient worker bees to cover the frames. Each hive had half a frame of capped honey but also had space for further food storage.

Three "resident" colonies were located in the Laboratory of Apiculture and Social Insects (LASI) at the University of Sussex campus in East Sussex. Three weeks or more before a trial, three "moved" colonies of similar size were established at the Royal Botanic Gardens Wakehurst Place, near Ardingly, West Sussex, and allowed to forage naturally. The Wakehurst Place "moved" colonies were therefore 26 km from LASI, which makes the foraging range of the two locations nonoverlapping [16]. This is important because the "moved" colonies, once they are relocated to LASI, must have no experience of the area.

Data were gathered from the 6 colonies (3 moved and 3 resident) for 2 days (b1 and b2) prior to the relocation of the moved hives to LASI to establish baseline data on foraging performance. Moved hives were then carefully loaded and transported (<1 h) by car in the evening, to avoid losing foragers, and set up at LASI. Observation hive entrances were 0.9 m or more apart, each with a distinctly coloured and patterned surround (50 × 50 cm) to aid learning of nest location and reduce drifting. Data collection resumed the next day (foraging day 1) on all 6 colonies, now all located at LASI.

2.2. Choice of Foraging Performance Indicators and Trial Seasons. We chose four indicators of foraging performance: waggle run duration, which encodes distance [15, 17]; crop nectar concentration in nectar foragers, which is a measure of forage quality [18]; returning bee forage success (whether or not their crops are empty); and departing bees per minute.

For bee departures per minute, one possible outcome could be that colonies with no information on local foraging locations (moved colonies) would send a greater proportion of bees into the field to locate resources. Alternatively it may be that moved hives would show lower departure rates, as they do not know where flowers are. We expect the resident colonies with clear, filtered, public, and private information on available forage should be exploiting the landscape efficiently [19, 20].

Waggle run duration encodes foraging distance [15, 17] and is useful in measuring efficiency, as flying is costly in terms of energy expenditure and increased predation risk [18]. Honey bees only forage at greater distances when food is in short supply [11, 18]. We anticipated that moved colonies would spread their foraging efforts over a wider range in an attempt to locate the resources, especially immediately after the move, because they would not yet have the benefit of local knowledge of where to find the best resources. In other words, we expect the move to compromise the optimality of foraging efficiency, which would be reflected in greater communicated distance. In contrast, the resident colonies would already have such information and be foraging over shorter distances. Alternatively, perhaps resident colonies, with knowledge of the most profitable resources, go further, but they bring back better quality forage.

Nectar concentration is a measure of forage quality, as sugar is the main energy source for a honey bee colony, and honey bees are very sensitive to this in their foraging [15, 18, 19]. A crop full of sugar-rich nectar is worth more to the colony than the same volume of low-sugar nectar. Honey bee colonies should therefore aim to collect nectar with high sugar content. We predicted that moved colonies immediately after relocation would initially bring back lower quality nectar than moved colonies until they discover the better quality nectar sources.

For our location and for many other temperate areas, the high summer (early July–late August) flowers are in less abundance compared to spring [11, 16, 21]. Therefore, we predicted that during the August trial, foraging-moved bees would take longer to adjust to the new foraging site than in June, when weather is normally conducive to foraging, and flowers are more abundant. In contrast, during October, flowering ivy is the major source of forage in the study area [22] and is locally abundant, so it should be that moved bees are able to forage comparably to resident colonies quickly, although weather conditions may have more of an impact.

2.3. Collection of Performance Indicator Data. Bees leaving the entrance were counted for 30 minutes per day per hive. 30-minute counting periods were initiated at 0900, 1200, and 1500 h, 10 minutes per hive with 2 observers working simultaneously on different hives.

Nectar quality was determined by using a refractometer (Kruss HR25/800, 21°C) to measure the percentage of sugar in the crop contents of bees returning to each hive. Hive entrances were blocked, and ten returning bees (without visible pollen) from each hive were captured three times daily at 0900, 1200, and 1500 h. Nectar was expelled from the bees' crop by applying gentle pressure to the abdomen of a chilled (immobile) bee. The droplet emerging from the mouth was analysed. Bees were unharmed and were released to resume foraging. Success rate was defined as the percentage of these returning bees with a measurable amount of nectar in their crop.

We decoded waggle dances to determine foraging distances from the waggle run, which is the information-rich

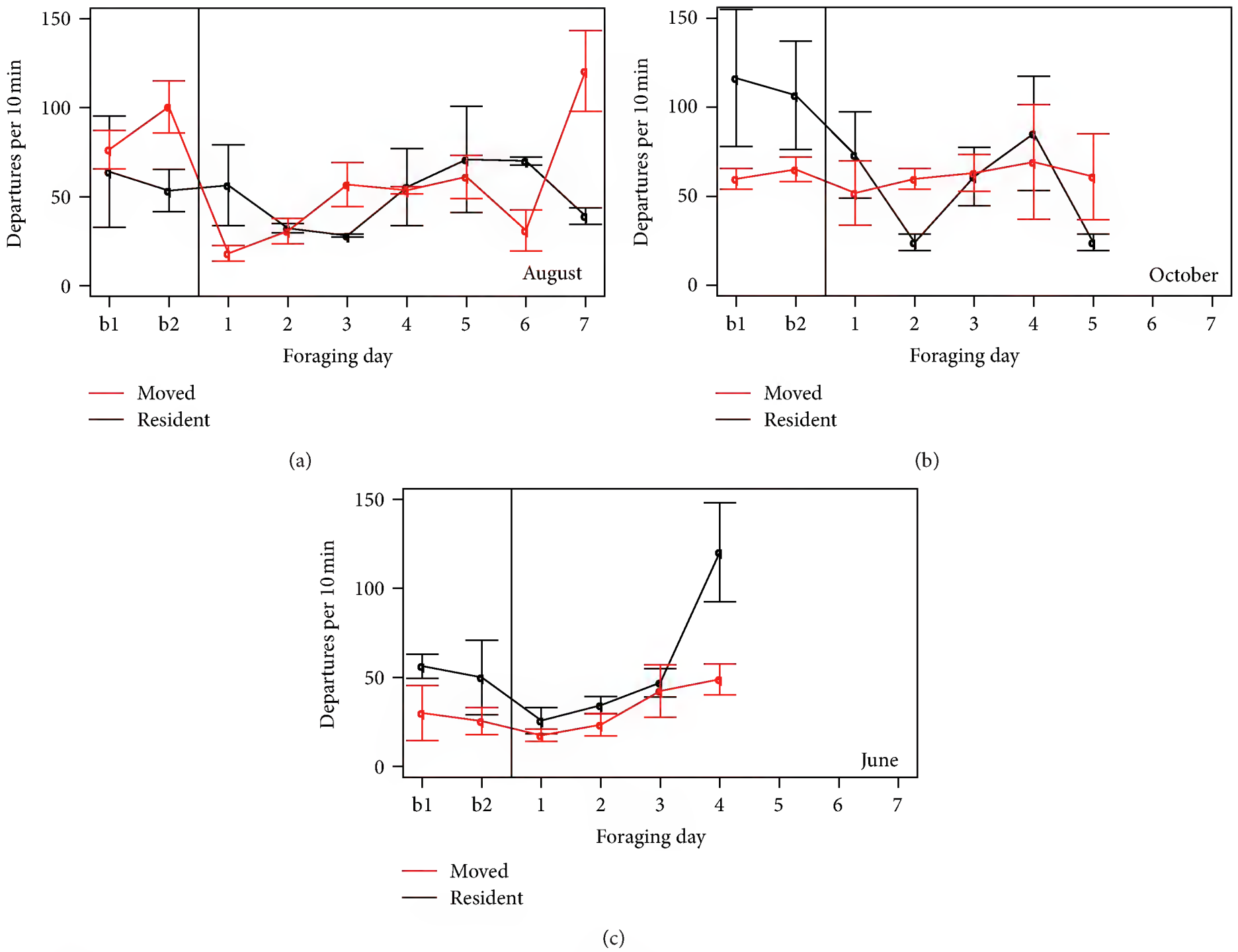


FIGURE 1: (a) Mean number of worker bees departing per minute during the three trials of August, October, and June. Days b1 and b2 are the two days immediately before the "moved" hives were relocated to the same location as the resident hives. Error bars are s.e.m. between hives (3 resident (black) and 3 moved (red)). Vertical line represents the day the hives were moved.

portion of the dance [17, 23]. Hives were filmed during three periods each day (0900–1000, 1200–1300, and 1500–1600) using CCTV cameras (Sony Super HAD 27X VHR30) to record the waggle dances made by returning foragers. Footage was then uploaded to an iMac computer. On playback, the duration of the waggle phase was determined to the nearest frame (1/25 second) using the timestamp in the video analysis software (MPEG streamclip v1.9.2) [24]. Videos were made August 23,– September 3, 2010, October 7–13, 2010, and June 7–14, 2011. Days where bees did not forage due to bad weather were excluded from the analysis.

We decoded the duration of the waggle run, as this is more accurate than using the whole dance circuit, given that the return phase of the dance circuit can vary in length due to factors such as resource quality [17, 23, 24]. Dances were decoded according to previously published protocols [24], with only the cameras and video playback software differing.

3. Results

3.1. Departing Bees per Minute. Figure 1(a) shows the mean number of bees departing the hive entrances for the 6 study colonies in the 3 trial periods before and after moving. For moved colonies, in all three trials the relative departure rates dropped from before (b1 and b2) to first day after relocation (foraging day 1; 80.1, 32.3, and 37.1% decreases in August, October, and June, resp.). However, the rates for resident colonies also decreased (3.95, 16.96, and 51.5%) over the same time period (Figure 1). There was no significant difference between the moved and resident colonies' departing worker rates when we look at differences in b1 (day immediately before the move/before vertical line in graph) from day 1 (day immediately after the move/after vertical line in graph; Mann-Whitney, $W = 7.0$, $P = 0.19$; Figure 1). In fact, there is no significant difference between the moved and resident colonies' departing worker rates on foraging day 1 (first day after the move) in August (Kruskal-Wallis, $H = 3.61$, $P = 0.57$), October (Kruskal-Wallis, $H = 0.44$, $P = 0.507$), or June (Kruskal-Wallis, $H = 0.38$, $P = 0.535$). There was also no significant difference in the number of bees departing per minute on any day after the move other than day 6 of the August trial (Kruskal-Wallis, $H = 4.26$, $P = 0.039$) and day 4 for the June trial (Kruskal-Wallis, $H = 10.39$, $P = 0.001$).

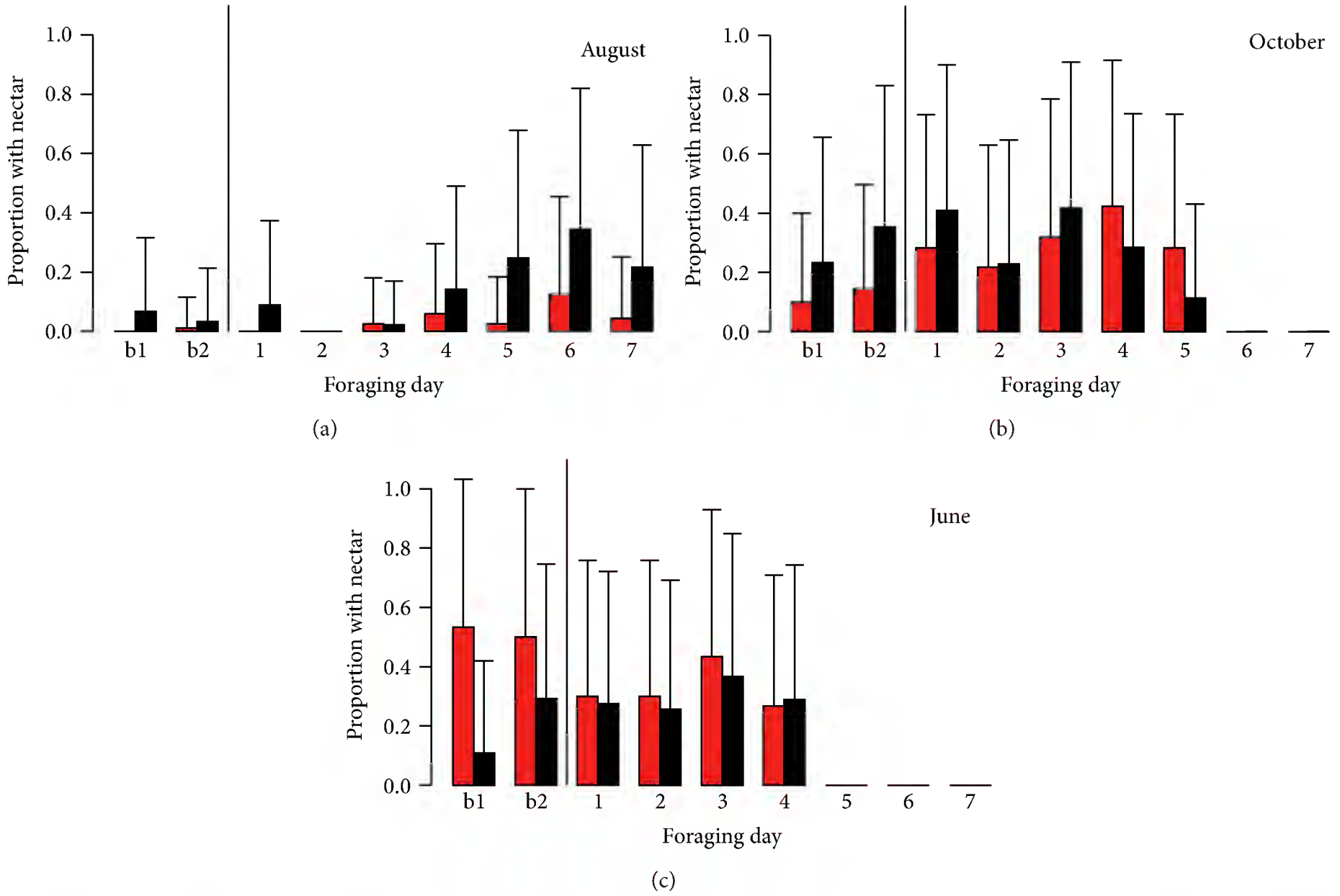


FIGURE 2: Proportion of returning worker bees with detectable nectar amounts in their crop in the August, October, and June trials before (b1 and b2) and after (days 1–7) the move for both resident (black) and moved (red) hives. Vertical line represents the day the hives were moved.

3.2. Proportion of Returning Workers with Nectar Loads. Overall, low proportions (<0.5) of returning workers carried nectar, and this metric showed large fluctuations (Figure 2). Overall success decreased after the move for both resident and moved colonies in August (mean of days b1 and b2 = 5%, mean of days 1 and 2 = 4.45% for resident; mean of b1 and b2 = 0.55%, mean of days 1 and 2 = 0% for moved colonies), increased for both resident and moved in October trial (resident, 29.3% to 31.9%; moved, 12.2% to 39.1%), and increased for resident colonies and decreased for moved colonies in June trials (resident, 10.6% to 31.1%; moved, 35.6% to 22.2%).

Specifically, we compared moved and resident hives immediately before the move (foraging day b2) and immediately after the move (foraging day 1). Despite these fluctuations in foraging success, the resident colonies were not consistently more successful than moved colonies (Figure 2). In August on foraging day 1, moved colonies did possess significantly more successfully returning nectar foragers (two-way contingency, $\chi^2 = 12.8$, df = 1, and $P = 0.003$); however, in October and June, this relationship was highly nonsignificant (October: $\chi^2 = 0.06$, df = 1, and $P = 0.8$; June: $\chi^2 = 0.317$, df = 1, and $P = 0.57$), with in June, the trend being for a higher proportion of moved returning foragers to have nectar compared to resident. When we looked at specifically at foraging day b2 versus 1, in August, both the resident and moved colonies performed significantly worse on the day after the move (resident: $\chi^2 = 12$, df = 1, and $P = 0.0005$; moved: $\chi^2 = 6.61$, df = 1, and $P = 0.01$). In October and June, the resident colonies performed better on the day after the move, but not significantly so (October: $\chi^2 = 0.87$, df = 1, $P = 0.35$; June: $\chi^2 = 0.11$, df = 1, $P = 0.74$). The moved hives performed significantly better on the day after the move in October (October: $\chi^2 = 745$, df = 1, and $P = 0.006$), and in June, the moved hive performance on the day after the move was slightly worse, but not significantly so (June: $\chi^2 = 0.54$, df = 1, and $P = 0.46$).

3.3. Waggle Run Duration. On the first foraging day after the move, moved and resident hives foraged at similar distances, as indicated by similar waggle run durations, in the August and June trials (one-way ANOVA: August, $F = 1.54$, $P = 0.22$; June $F = 0.24$, $P = 0.631$; Figure 3). However, in the October trial, the resident hives performed no dances on day 1, while the moved hives did dance. In August, the resident and moved colonies foraged over similar distances on all days other than days 5 and 6, when the moved colonies foraged at greater distances than the resident colonies (One-way ANOVA: $F = 4.55$, $P = 0.037$; $F = 12.38$, $P = 0.001$ for days 5 and 6, resp.) and over a greater range of distances (mean waggle phase duration, resident = 1.76 s, moved = 2.27 s; range, resident = 3.75 s, moved = 7.86 s).

During October, the foraging distances of resident and moved colonies were different before the move but not

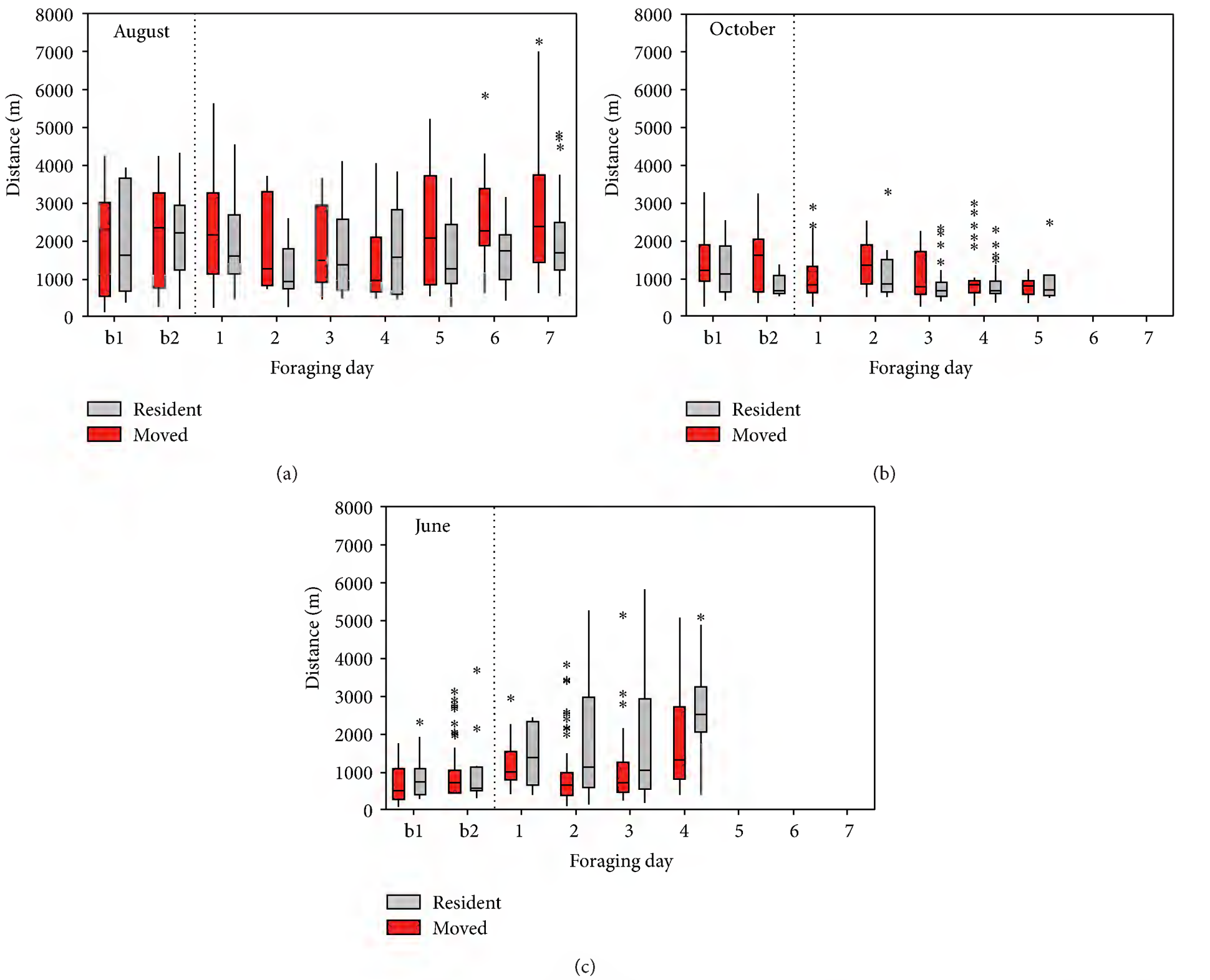


FIGURE 3: Durations of waggle runs of dancing bees in August, October, and June trials. Longer waggle runs indicate greater foraging distance. Days b1 and b2 are the two days immediately before the "moved" hives were relocated, in the evening after foraging had ended, to the same location as the resident hives. Dashed grey line indicates relocation, and stars represent a single datum point that does not fall within the whisker range.

significantly different on any day after the move ($F = 0.21$, $P = 0.646$) (other than foraging day 1, see above). In June, resident colonies foraged at greater distances on days 2, 3, and 4 (mean, resident, 1.9 s; moved, 1.3 s) than moved hives (one-way ANOVA: $F = 18.56$, $P < 0.005$; $F = 6.73$, $P = 0.011$; $F = 8.12$, $P = 0.005$ on days 2, 3, and 4, resp.).

3.4. Nectar Concentration. Moved hives failed to return with measurable nectar until day 3 after the relocation in August. The resident hives collected nectar with a mean concentration of 18.2% and 18.6% on days b1 and 1 but failed to collect nectar on days b2 and 2 (Figure 4). There were fluctuations in the concentration of nectar collected between hives and trial periods, but moved and resident hives brought back similar concentrations of nectar after the move with the exception of day 4 in August where moved hives found better quality nectar (56.8% versus 45.1%; one-way ANOVA: $F = 15.29$, $P = 0.001$) and day 3 in October where the resident hives brought back higher quality nectar (32.2 versus 28.0%; one-way ANOVA: $F = 4.45$, $P = 0.037$). In October, moved hives found poorer quality nectar before the move than resident colonies (one-way ANOVA: b1, $F = 31.18$, $P < 0.000$; b2, $F = 21.66$, $P < 0.001$); however, on the first day after the move, there was no significant difference between the nectar concentration found by moved and resident colonies (one-way ANOVA: $F = 0.13$, $P = 0.721$).

4. Discussion

Our results show no consistent differences in the four measures of foraging performance for resident colonies versus colonies relocated into the same location. There were differences in foraging performance before the move, such as lower mean nectar concentration for moved hives in October (14.5% versus 35%) and lower mean departures per minute for the moved hives than the resident hives in June (12.4

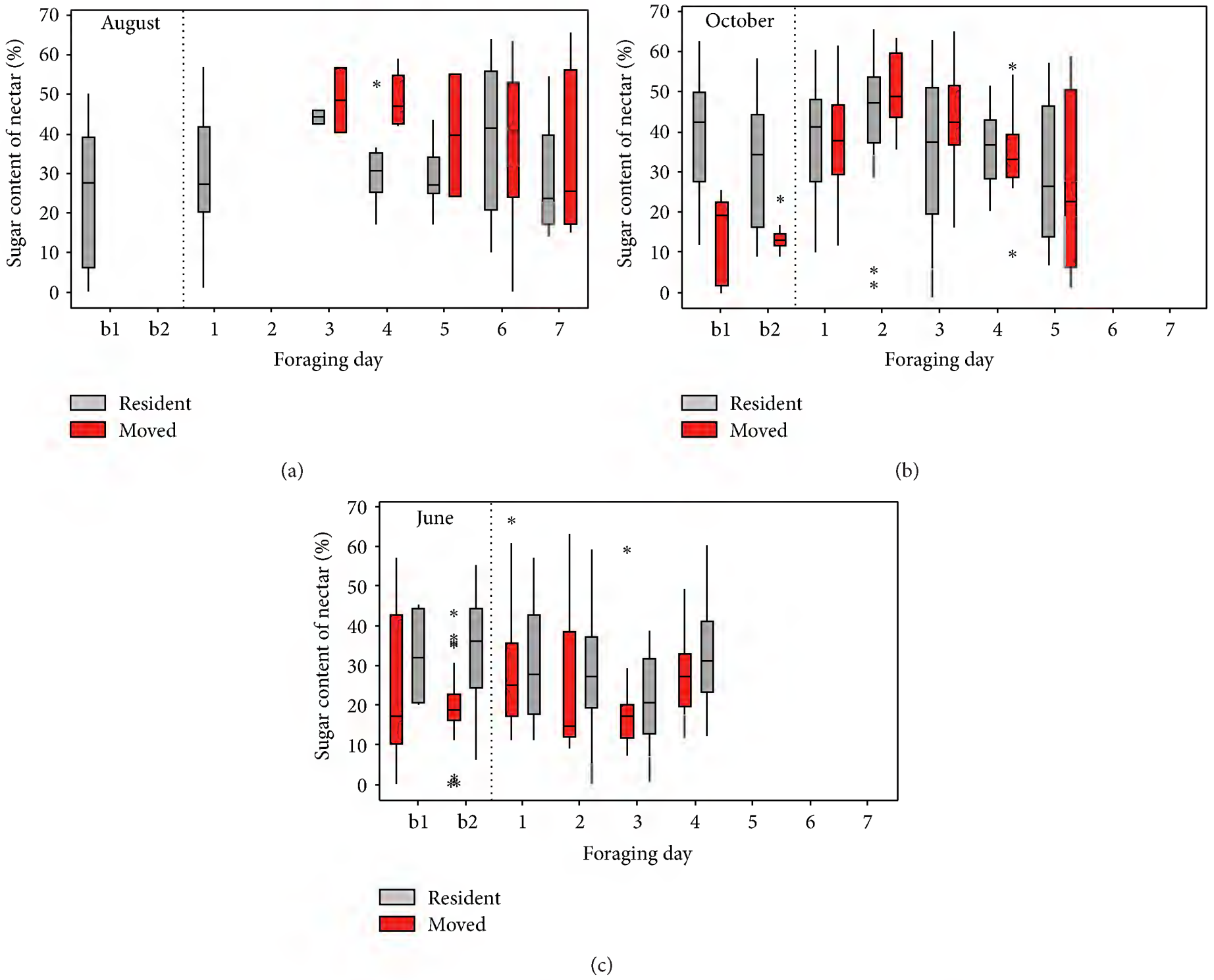


FIGURE 4: Sugar concentration of nectar in returning worker bees in the three study periods, August, October, and June. Days b1 and b2 are the two days immediately before the "moved" hives were moved, in the evening after foraging had ended, to the same location as the resident hives. Dashed grey line indicates relocation, and stars represent a single datum point that does not fall within the whisker range.

versus 6.5), presumably due to the difference in location and food availability. Overall, climatic conditions and seasonal resource availability were more likely an influence on the foraging performance of both moved and resident colonies than the relocation to a novel environment. Bees were rapidly able to find new and high quality sources of food after the move, with moved hives bringing back similar quality of nectar compared to resident colonies on the first day after the move in October and June (for August, see below). If foraging performance was poor, it was poor for both resident and moved colonies.

In the case of departure rates, there was no consistent trend for the moved hives to have lower rates with the exception of day 1 in the August trial (Figure 1(a)). Although departure rates of moved colonies dropped immediately after the move, they also dropped for the resident colonies, suggesting that weather conditions were more likely to be the cause with 0.2 mm and 0.6 mm of rain on b1 and b2 in August and 0 mm on days 1–7. This would be because, after rainfall, nectaries are often washed out, and pollen is too soggy to collect. We found a great deal of overlap in the changes in departure rates of the colonies in all three trials, indicating that moved and resident colonies were changing their departure rates by similar proportions (Figure 1(b)).

There was no clear or consistent trend in the success of returning workers other than the fact that the success was surprisingly low, with less than 50% of returning workers having nectar in their crop. In August workers experienced the lowest success, and less than 10% of both resident and moved returning workers had measurable nectar, with both groups having 0% success on day 3. Cool, damp weather reduces nectar availability to insects (Peat and Goulson, 2005) and was almost certainly an important factor in poor foraging performance in the August trial in addition to August being the most challenging month of the year for bees to find food, with the bees needing to travel at greater distances in our region [11], which is temperate.

In the October and June trials, during which the weather was drier than in the August trial (1 mm of rain fell on day 1 and 0.2 mm on day 2 in June, but this was overnight and did not interrupt foraging), success was greater, but never more than 50%. The nectar collected on successful

foraging days after the moves was of similar concentrations for resident and moved hives on all days other than day 6 in August and day 4 in June. The nectar collected by the moved hives before the move was of lower concentration than that collected after the move by both groups and before the move by resident colonies. However, on the first day after the move, both groups had located similar quality nectar. Variation in plant availability at the two sites can account for this difference. Greater coverage of ivy (*Hedera helix*), which is a major food source for honeybees from early September to early November [22], was observed at the laboratory than at Wakehurst Place.

It is interesting to examine departure rate data in light of the proportion returning with nectar. If departing bees also included large numbers of scouts, we would expect the moved hives to experience higher departure rates than before the move compared to the resident hives; additionally, we would expect noticeably fewer returning bees to contain nectar in crops. However, the success of returning foragers did not follow a clear trend, with resident colonies always returning a higher proportion of bees with full crops; additionally, there were no clear increases in the number of departing foragers from moved hives.

In the case of foraging distance, as shown by dance decoding, foragers from both moved, and resident colonies foraged at similar distances during most foraging days. On the days where the resident and moved hives did forage at different distances, the moved hives foraged at greater distances than resident hives in August (days 5 and 6), but the reverse was the case in June (days 2, 3, and 4). In October, the resident hives performed no dances on day 1, while the moved hives danced normally. One possible explanation is that the resident hives did not dance, as they knew where flowers were, but they were not sufficiently exciting to elicit dancing, whereas the threshold for the moved hives was lower, so they did dance.

Sherman and Visscher found that the waggle dance was more important to colony fitness in southern California under winter foraging conditions, with colonies prevented from performing oriented waggle dances losing more weight [25]. We found that colonies performed more dances per day in June, and there was no significant difference in the mean number of dances per day for either resident or moved hives before or after the move in August or October. A possible explanation for this is that there was more dancing in June because there was more available food, whereas although dancing may be more important in August, there were fewer sources available worth advertising.

Why might it be that we did not see a significant effect of relocation on the foraging performance of the moved bees versus the resident bees? A colony of bees has many foragers in the field at once, up to 25% of the colony's workers [19]. These foragers collect information on food availability over an area surrounding the hive of up to 100 km^2 [16] and share this information with their nestmates via the waggle dance. Seeley [26] showed that if a food patch (100 m^2) is within 1000 m of the hive, there is a 70% chance of the colony locating it. This chance drops to 50% for a patch located 2000 m from the hive entrance. Once a resource has been located by a scout, the number of visiting foragers increases rapidly as recruits are informed via the waggle dance [15]. Seeley and Visscher also showed that the waggle dance allows colonies to locate better quality food and that they can do so quickly. Scout bees are able to discover a flower patch 610 m away within 200 minutes of the resources being placed. this 200 minutes is lessened for closer resources [27]. Large numbers of recruit bees, presumably directed by the scout's waggle dances, then arrived within 50 minutes of its discovery by the scout. This shows that honey bee colonies have considerable ability rapidly to track both spatial and temporal changes in food availability. If food is available in the landscape, it is likely that a honey bee colony will locate it. The location of floral resources varies with season, but also from day to day, and even at different times of day, as some plants only produce nectar at certain times of day [28]. The fact that honey bee colonies have evolved mechanisms to track these changes may mean that a colony moved to a novel environment is not at a great disadvantage. In addition, honey bee colonies naturally change their location when a swarm establishes a new nest. In European *A. mellifera*, the new nest is within a few kilometres of the natal nest [29] However, swarms of African *A. mellifera* [30] and Asian *Apis dorsata* [31] migrate longer distances.

This study involved moving bees to a novel location, which was similar in terms of climate and available forage. It is possible that moving bees over much larger distances into different climatic conditions and resource availability may have a much greater impact on foraging efficiency. It is also possible that it may take longer to adjust to a new foraging location if the plant species are different from the known location, as odour memory plays an important role in foraging and location of food sources [23, 32]. It was shown that after a move, foragers tended to forage on species they had previously visited if they were available [33].

Our study has produced some encouraging results. With many studies focusing on factors that can potentially harm bees, such as pesticides, pest and pathogens, and lack of forage [8, 11, 34] and much of the media focused on the decline in honey bees and other pollinators, it is reassuring to find a factor which seems not be detrimental to honey bee colonies.

Acknowledgments

Fiona C. Riddell Pearce was funded by Waitrose, and Margaret J. Couvillon was funded by the Nineveh Charitable Trust. Thanks are due to Ashleigh Bates and Aurelie Croizille for their help in decoding dances.

References

[1] I. Kandemir, M. Kence, and A. Kence, "Genetic and morphometric variation in honeybee (*Apis mellifera* L.) populations of Turkey," *Apidologie*, vol. 31, no. 3, pp. 343–356, 2000.

[2] B. P. Oldroyd, "What's killing American honey bees?" *PLoS Biology*, vol. 5, no. 6, article e168, 2007.

[3] F. L. W. Ratnieks and N. L. Carreck, "Clarity on honey bee collapse?" *Science*, vol. 327, no. 5962, pp. 152–153, 2010.

[4] M. Berenbaum, Committee on the Status of Pollinators in North America, and National Research Council, *Status of Pollinators in North America*, The National Academies Press, Washington, DC, USA, 2007.

[5] K. S. Delaplane and D. F. Mayer, *Crop Pollination by Bees*, CABI, New York, NY, USA, 2000.

[6] M. A. Aizen and L. D. Harder, "The global stock of domesticated honey bees is growing slower than agricultural demand for pollination," *Current Biology*, vol. 19, no. 11, pp. 915–918, 2009.

[7] L. G. Carvalheiro, W. E. Kunin, P. Keil et al., "Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants," *Ecology Letters*, vol. 16, no. 7, pp. 870–878, 2013.

[8] D. L. Cox-Foster, S. Conlan, E. C. Holmes et al., "A metagenomic survey of microbes in honey bee colony collapse disorder," *Science*, vol. 318, no. 5848, pp. 283–287, 2007.

[9] S. Kluser and United Nations Environment Programme, *Global Honey Bee Colony Disorders and Other Threats to Insect Pollinators*, UNEP Emerging Issues, United Nations Environment Programme, Geneva, Switzerland, 2011.

[10] P. A. Matson, W. J. Parton, A. G. Power, and M. J. Swift, "Agricultural intensification and ecosystem properties," *Science*, vol. 277, no. 5325, pp. 504–509, 1997.

[11] M. J. Couvillon, R. Schürch, and F. L. W. Ratnieks, "Dancing bees provide an integrative picture of seasonal foraging challenges," submitted.

[12] E. Crane, *Bees and Beekeeping: Science, Practice and World Resources*, Heinemann Newnes, 1990.

[13] K. Hackett and United States Department of Agriculture, *Colony Collapse Disorder Progress Report*, United States Department of Agiculture, Beltsville, Md, USA, 2010.

[14] D. Nelson and S. Jay, "The effect of colony relocation on loss and disorientation of honeybees," *Apidologie*, vol. 20, no. 3, pp. 245–250, 1989.

[15] K. von Frisch, *The Dance Language and Orientation of Bees*, Harvard University Press, Cambridge, Mass, USA, 1967.

[16] M. Beekman and F. L. W. Ratnieks, "Long-range foraging by the honey-bee, *Apis mellifera* L," *Functional Ecology*, vol. 14, no. 4, pp. 490–496, 2000.

[17] T. D. Seeley, A. S. Mikheyev, and G. J. Pagano, "Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability," *Journal of Comparative Physiology A*, vol. 186, no. 9, pp. 813–819, 2000.

[18] T. D. Seeley, "Honey bee foragers as sensory units of their colonies," *Behavioral Ecology and Sociobiology*, vol. 34, no. 1, pp. 51–62, 1994.

[19] T. D. Seeley, *The Wisdom of the Hive*, Harvard University Press, Cambridge, Mass, USA, 1995.

[20] C. Grüter, E. Leadbeater, and F. L. W. Ratnieks, "Social learning: the importance of copying others," *Current Biology*, vol. 20, no. 16, pp. R683–R685, 2010.

[21] B. Heinrich and P. H. Raven, "Energetics and pollination ecology," *Science*, pp. 597–602, 1972.

[22] M. Garbuzov and F. L. W. Ratnieks, "Ivy: an underappreciated key resource to flower visiting insects in autumn," *Insect Conservation and Diversity*, 2013.

[23] M. J. Couvillon, "The dance legacy of Karl von Frisch," *Insectes Sociaux*, vol. 59, no. 3, pp. 297–306, 2012.

[24] M. J. Couvillon, F. C. Riddell Pearce, E. L. Harris-Jones et al., "Intra-dance variation among waggle runs and the design of efficient protocols for honey bee dance decoding," *Biology Open*, vol. 1, no. 5, pp. 467–472, 2012.

[25] G. Sherman and P. K. Visscher, "Honeybee colonies achieve fitness through dancing," *Nature*, vol. 419, no. 6910, pp. 920–922, 2002.

[26] T. D. Seeley, "The effectiveness of information collection about food sources by honey bee colonies," *Animal Behaviour*, vol. 35, no. 5, pp. 1572–1575, 1987.

[27] T. D. Seeley and P. K. Visscher, "Assessing the benefits of cooperation in honeybee foraging: search costs, forage quality, and competitive ability," *Behavioral Ecology and Sociobiology*, vol. 22, no. 4, pp. 229–237, 1988.

[28] R. E. Irwin, L. S. Adler, and A. K. Brody, "The dual role of floral traits: pollinator attraction and plant defense," *Ecology*, vol. 85, no. 6, pp. 1503–1511, 2004.

[29] T. D. Seeley, *Honeybee Democracy*, Princeton University Press, Princeton, NJ, USA, 2010.

[30] F. L. W. Ratnieks, "Africanized bees: natural selection for colonizing ability," in *The "African" Honey Bee*, pp. 213–234, Westview Press, Boulder, Colo, USA, 1991.

[31] J. Paar, B. P. Oldroyd, and G. Kastberger, "Giant honeybees return to their nest sites," *Nature*, vol. 406, no. 6795, p. 475, 2000.

[32] M. Hammer and R. Menzel, "Learning and memory in the honeybee," *Journal of Neuroscience*, vol. 15, no. 3 I, pp. 1617–1630, 1995.

[33] J. B. Free, "The effect of moving colonies of honeybees to new sites on their subsequent foraging behaviour," *Journal of Agricultural Science*, vol. 53, no. 1, pp. 1–9, 1959.

[34] M. Henry, M. Béguin, F. Requier et al., "A common pesticide decreases foraging success and survival in honey bees," *Science*, vol. 336, no. 6079, pp. 348–350, 2012.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 768108, 8 pages
http://dx.doi.org/10.1155/2013/768108

Research Article

Social Learning in Bumblebees (*Bombus impatiens*): Worker Bumblebees Learn to Manipulate and Forage at Artificial Flowers by Observation and Communication within the Colony

Hamida B. Mirwan and Peter G. Kevan

Canadian Pollination Initiative (CANPOLIN), School of Environmental Sciences, University of Guelph, Guelph, ON, Canada N1G 2W1

Correspondence should be addressed to Hamida B. Mirwan; hmirwan@uoguelph.ca

Received 17 May 2013; Revised 7 September 2013; Accepted 8 September 2013

Academic Editor: James C. Nieh

Social learning occurs when one individual learns from another, mainly conspecific, often by observation, imitation, or communication. Using artificial flowers, we studied social learning by allowing test bumblebees to (a) see dead bumblebees arranged in foraging positions or (b) watch live bumblebees actually foraging or (c) communicate with nestmates within their colony without having seen foraging. Artificial flowers made from 1.5 mL microcentrifuge tubes with closed caps were inserted through the centres of blue 7 cm plastic discs as optical signals through which the bees could not forage. The reinforcer reward syrup was accessible only through holes in the sides of the tubes beneath the blue discs. Two colonies (A and B) were used in tandem along with control (C and D) colonies. No bee that was not exposed (i.e., from the control colonies (C and D)) to social learning discovered the access holes. Inside colony B, we imprisoned a group of bees that were prevented from seeing or watching. Bees that saw dead bumblebees in foraging positions, those that watched nest-mates foraging, and those that had only in-hive communication with successful foragers all foraged successfully. The means of in-hive communication are not understood and warrant intense investigation.

1. Introduction

Social learning is defined by ethologists as any learning from conspecifics [1] (but we note that social learning between species is known) and mostly involves observation, imitation by observing and replicating another's behavior, and modeling to transmit the learned behaviour from one individual to others [2]. Social learning through individuals' interactions with other animals or their products encompasses attention, memory, and motivation; social theory calls social learning a bridge between behaviourism (i.e., learning based upon behaviour that is acquired through conditioning which occurs through interaction with the environment) and cognitive learning (i.e., learning by using reason, intuition, and perception) [3–6]. Research on social learning has focused largely on vertebrates [7, 8]. However, a growing number of researchers have shown recently that bees and other small brained animals can also learn through acquisition of information by social transmission [9–12]. Nonetheless, the possibility that social learning might extend to practical knowledge (skills), in addition to simple declarative knowledge (facts), remains mostly untested in invertebrates [9].

Insects, especially eusocial bees, show remarkably complex learning abilities [11, 13–15], and social information often leads to the relatively long-term changes in behaviours that constitute social learning. The dance communication of honeybees (*Apis* spp.) [16, 17], sounds in *Melipona costaricensis* [18], and other means of communication in other bees [19], ants [20], wasps [21, 22], and Octopuses [23] serve as examples. As Giurfa's short but informative review notes simple mechanisms based on elemental associations, either Pavlovian or operant conditions may account for social learning in animals with miniature brains, so social learning should not be considered surprising or a highly cognitive ability [11].

To assess the potential for social learning in bumblebees (*Bombus impatiens*), we investigated the spread of foraging techniques from experienced bees to inexperienced bees in the same and different colonies. We explored the following three different paradigms: (a) using a model (positioned dead bees), (b) observation with imitation (of foraging live bees), and (c) intracolony communication within the domicile.

2. Material and Methods

2.1. General Methods. Experiments were made in indoor screened flight cages (2.15 m long × 1.20 m wide × 1.80 m tall) with grey floors. The bees used were foragers of *Bombus impatiens* (Cresson, 1863) (Hymenoptera: Apoidea) from queen-right colonies of 30–40 workers/colony (supplied by BioBest Biological Systems, Canada (Leamington, Ontario)). Moveable screens on one side of the cages allowed experimenter access. Four colonies were used in this experiment, colony A was placed in cage I, colony B was placed in cage II, and colonies C and D (control) were placed in cages III and IV. Each was connected to a small, outer cage (30 × 23 × 20 cm) (holding area) attached to the main flight cage (testing arena) by gated, wire-mesh tunnels that allowed experimental control of the bees' entry to and egress from the flight cage. Colonies, when not being tested, had constant pollen supplies and their diets were supplemented with sugar syrup. Individually, foragers were marked on the thoracic dorsal surface with uniquely numbered and coloured tags (Opalith Plättchen, Christian Graze KG, Germany).

The experimental arena of artificial flowers (Figure 1) was placed in the flight cage 165 cm from where the bees entered and exited. It comprised a green Styrofoam base 45 × 35 × 5 cm with 8 artificial flowers. The first step was to allow naive bees to encounter simple centrifuge tubes which were mounted in a green Styrofoam base (the tubes were hidden and the forager could access the syrup only through the opening of the tube). Once they were accustomed to foraging at those tubes for a week to ten days, they were marked individually and then challenged with learning tasks as described for each experiment (below).

2.1.1. Artificial Flowers. Artificial flowers were made of 1.5 mL centrifuge tubes inserted into the centres of blue plastic discs, 7 cm in diameter. The centrifuge tube was capped so that the bees could not obtain the contained syrup (50% sucrose w:w as the reinforcer reward; the amount of syrup was not controlled but was replenished as soon as it was exhausted) from the surface of the plastic disc. Instead, a small hole (0.5 cm in diameters) had been drilled into one side of each centrifuge tube just below the lip; see Figure 2. The artificial flower was then attached to a yellow pipette tube mounted on 35 × 52 cm Styrofoam base. Eight flowers in two rows of four flowers were each arranged with the bored holes facing the central aisle between the rows of flowers and so presented to the bees in each experiment.

Thus, the bees could orientate to the blue disc of the artificial flower but could not obtain syrup except by going under the disc to the hole in the tube's wall. Test bees were assessed based on their abilities to learn and replicate foraging behaviours without actually having performed them.

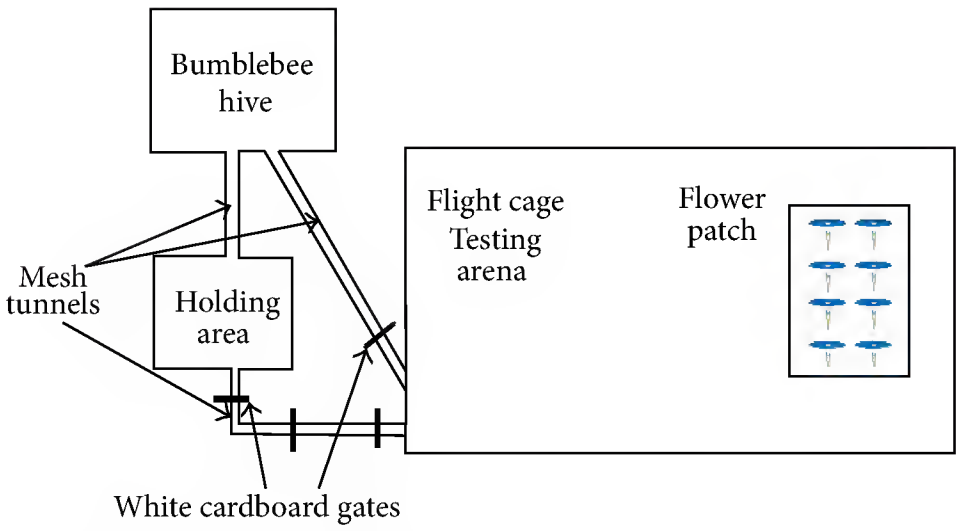


Figure 1: Experiment setup with hive, holding area, flight cage, testing arena, patch of artificial flowers, and mesh tube routes with gates by which the bees were allowed to enter and exit the flight cage. The bees, in training or trained, exited from the hive and could take only one route through the holding area to the testing arena in the main flight cage. The exiting bees were not allowed to use the diagonal route because the gate in it was kept closed. The gates after the holding area were opened and closed to allow single bees to enter the testing arena during testing. The bees returned to their hive from the testing area via the diagonal mesh tube route, the gate of which was opened as necessary. Note that the main flight cage's end wall, through which the mesh tunnels ran, was a wooden panel so that the bees in the tunnels or in the holding area could not see the flower patch.

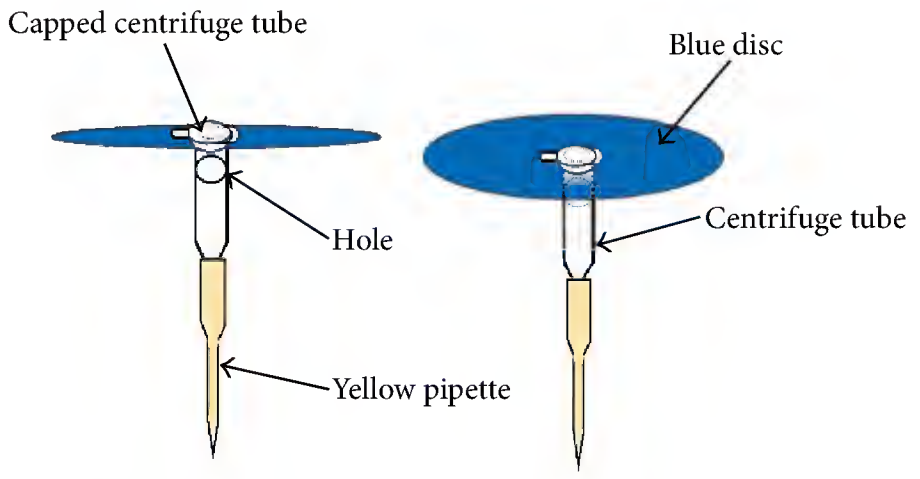


Figure 2: Artificial flowers were made of 1.5 mL centrifuge tubes inserted into the centres of blue plastic discs, 7 cm in diameter. The centrifuge tube was capped so that the bees could not obtain the contained syrup from the surface of the plastic disc. Instead, a small hole (0.5 cm in diameters) had been drilled into one side of each centrifuge tube just below the lip.

The Experimental Groups Were as Follows

(1) A group of foragers from colony A (A2) were used from which bees were tested without a model for 2 trials with 30 minutes of giving-up time; then, the model (dead bee) was introduced and the group was allowed to forage alongside models pinned in the robbing position.

(2) A group of foragers from colony A (A1) were provided with enough food (syrup and pollen) while imprisoned so that they could observe group A2 foragers for 10 hours. They were then released and allowed to forage alone.

(3) A group of foragers from colony B (B1) were provided with enough food (syrup and pollen), while imprisoned and treated as foragers in group A1, except that they were able to watch foragers from a different colony (colony A) rather than from their own colony.

(4) A group of foragers from colony B (B2) were kept contained inside the colony and not allowed to forage from the artificial flowers. They had the opportunity to interact (inside the nest) with group B1 for 24 hours and then were allowed to forage alone.

(5) Foragers from control colonies (colony C with 15 subject bees and colony D with 10 subject bees) were challenged to forage through "the access holes" of the artificial flowers without models nor opportunity to watch other foragers on the artificial flowers nor opportunity to communicate with foragers that had successfully foraged at the artificial flowers. They were allowed 30 minutes to succeed, but none did.

2.1.2. Experimental Procedures. Over a period of several days, individually marked bees were trained to forage from simple centrifuge tubes (described above).

Following the initial training, the bees from the control colonies, colonies C and D, were tested by challenging them with the 8 artificial flowers described and arranged above with 30 minutes of giving-up time. There was no need to replace the flowers in this experiment because no bee foraged successfully at them.

From test colony A, which was placed in cage I, individually marked worker bees were segregated into two groups of bees (A1 with 12 bees and A2 with 14 bees). One group (A1) was removed from the colony and imprisoned in a mesh tube (20 cm long and 3 cm diameter with 0.4 × 0.4 mm mesh) kept out of sight of the experimental cage; these bees were provided with enough food (syrup and pollen). These bees were to be placed later in the aisle between the two rows of artificial flowers. Group A2 (14 individuals) were prevented from leaving the colony and foraging until testing could be started the next day. Once the A1 bees had been sequestered, bees in group A2 were used for testing one by one. Each bee from group A2 was released and allowed to forage at the artificial flowers without dead bees in place for two trials with 30 minutes of giving-up time; none of them were successful to forage. After two trials of giving up, a model (dead bee) was introduced. At this point, newly killed bees were placed on the artificial flowers with their heads at the access hole. The dead bees came from the same colony (A) and had been killed by freezing at −18°C one day before the experiment and allowed to thaw and warm to ambient air temperature for 3 hours before the experiment started. Each bee from group A2 was released and allowed to forage at the artificial flowers with dead bees in place. After each bee from group A2 had made three successful foraging visits to any one of the artificial flowers, dead bees in place, we replaced the used artificial flowers with cleaned ones that did not have dead bees in place. This avoided the possibility that pheromone signals could influence the results. The visits of each of the A2 bees to the artificial flowers with (3 trials for each of 10 bees) and without dead bees (7 trials for each of the same 10 bees) were observed and timed for a total of 10 foraging bouts; access time was measured by using a stop watch, and the time started when the subject bee entered the testing arena and stopped when the subject bee started to probe for the syrup.

In the follow-up experiment, the cohort of 10 bees from the same colony (group A1) that had been imprisoned was placed in a mesh tube size (as described above) between the array of artificial flowers so that they could watch the successful experienced foragers (A2 bees) noted above. The A1 bees had the opportunity to watch the A2 bees at work for 10 daylight hours and were not allowed to return home until the next morning (at 8 am.), so preventing them from having communication with their nestmates (except for watching during the day), for 20 hours. The A1 bees, upon release in the morning, voluntarily and immediately returned to their hive but within 5 minutes started to reemerge from the domicile. They were then allowed to forage singly at the experimental array of new and clean artificial flowers without the experienced A2 bees present. The visits of each of these A1 bees to the artificial flowers were observed and timed for a total of 10 foraging bouts.

In a tandem experiment to test if the bees could communicate within the hive how to forage on the artificial flowers, we used a completely different colony (B) which was placed in cage II. In colony B, we segregated two cohorts of 12 sister or half-sister worker bees each of individually marked bees (as above). The workers in colony B (cage II) were allowed to forage freely from 8 microcentrifuge tubes not provided with artificial floral discs or holes in the walls. One cohort (B1) was later imprisoned in a mesh tube; these bees were provided with enough food (syrup and pollen) (as described above) and transported to cage I, where they were placed in the array of artificial flowers (as described above for A1 bees) and allowed to watch foragers from colony A forage for 10 hours. The same protocol for A1 bees was used to treat the imprisoned workers from colony B, except that the mesh tube prison and its inmates were removed from cage I for the night to the bench supporting the cage. In the morning the prison and its inmates of B1 bees were returned to cage II, where the inmates were released. As with the A1 bees as described above, the B1 bees voluntarily and immediately returned to their hive but within 5 minutes started to reemerge from the domicile. At the same time, the second cohort (B2) was allowed to forage freely at plain microcentrifuge tubes. Thus, the B2 bees had no opportunity to come into contact with, or to see, the artificial flowers with the holes in the microcentrifuge walls; group B2 was prevented from leaving the colony and foraging until they were tested after their nestmate B1 finished testing.

After the B1 bees had been returned to their home cage in the morning, after being imprisoned in the mesh tube overnight, and had reentered their home domicile, bees from both cohorts started to exit from their domicile but were denied access to the main cage. At this time, an array of

8 artificial flowers (with discs and holes in the walls) was placed into cage II. Then, only B1 bees were allowed to forage individually at that array and the B2 bees were denied entry into the main part of the cage. The B1 bees were each allowed to forage from the artificial flowers (newly cleaned for each trial and each bee) three times. After that, they were allowed to forage at the flowers 7 more times. Thus, the B2 bees still had no opportunity to come into contact with, or to see, the artificial flowers with the holes in the microcentrifuge walls, but they had contact with experienced nestmates, the B1 bees. The next day, B2 bees were allowed to forage at newly cleaned artificial flowers in the standard array. These bees were observed for 3 trials, followed by another 7 (as described above), and the durations of the foraging bouts were recorded. At this time, all bees from the first cohort (B1) were prevented from entering the main cage. At no time during the experiment were bees of both cohorts allowed to forage at the artificial flowers at the same time.

2.2. Statistical Analyses. To compare between groups and trials, we used one-way repeated measurement (using sigma plot statistic v12.0), and to isolate the group or groups that differed from the others we used a multiple comparison procedure. The duration for the manipulation of the artificial flowers on the first visit by foragers was used for interexperimental comparisons both within and between colonies (groups). Power of performed test with alpha = 0.050 : 1.000. We used Multiple Comparison Procedures (Holm-Sidak method): All pairwise and overall significance level = 0.05.

For comparison between the two groups of learning through observation, we used *t*-test.

3. Results

Bees from the control colonies (C and D) in cages III and IV had no opportunity for social learning, and all subject bees, 15 bees from colony C and 10 bees from colony D, which were observed proved incapable of foraging successfully at the artificial flowers, with 30 minutes of giving-up time.

None of the 14 tested bees colony A (in cage I) group (A2) when challenged by presenting the artificial flowers without dead bees in place for two trials with 30 minutes of giving-up time were successful to forage. 10 out of 14 (the rest gave up and did not show up for more testing) of the group (A2) were able to see dead bees at all of the 8 flowers as they foraged freely from their colony. When they foraged, they did so by climbing the artificial stem (pipette tube) of the flower, positioning themselves beside the dead bee under the disc, and taking syrup. These bees were not at first fully adept at foraging beside the dead bees, but after about 3 trials they became adept at the task (Figure 3). After having had that experience and when the dead bee was absent, those same experienced bees foraged successfully from new and cleaned artificial flowers. However, they did not require familiarization with the dead bee-less artificial flowers and were fully adept on their first visit (Figure 3).

In the follow-up experiment, a cohort of 10 different bees from the same colony (A1) that had been imprisoned in the

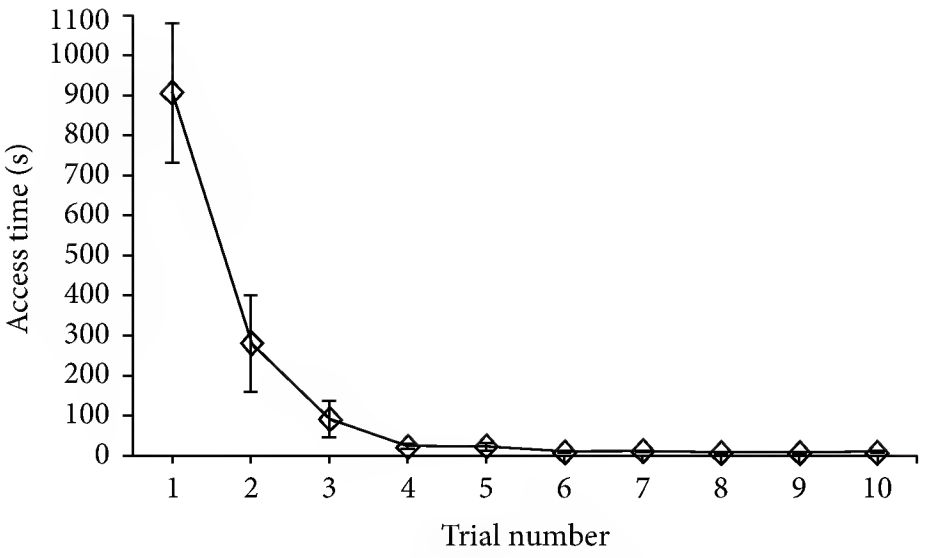


Figure 3: The learning curve (time/sec) (±SE) taken to access and forage on syrup for 10 initially naive workers of *Bombus impatiens* which were allowed to forage freely, but only one at a time, at artificial flowers with and without dead bees present. After the bees had demonstrated their ability to forage at the flowers with dead bees present (i.e., after 3 trails), those flowers were replaced with cleaned ones without dead bees present. The activities of the foragers were recorded for a further 7 visits. H_0 showing that the durations for successful foraging are independent of experience is rejected ($F_{9,9} = 19.7$; $P < 0.001$).

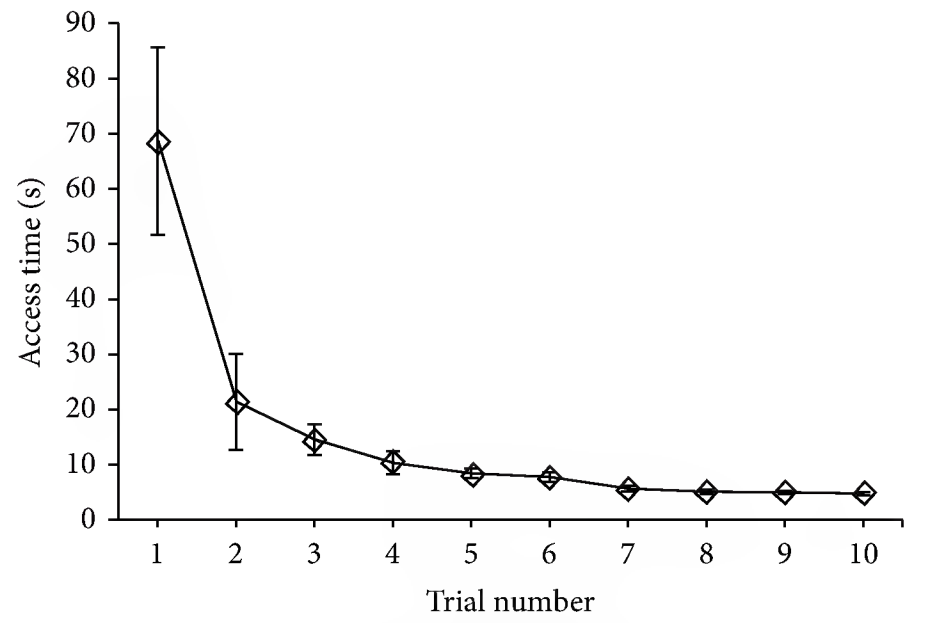


Figure 4: The learning curve (time/sec) (±SE) taken to access and forage on syrup for 9 workers of *Bombus impatiens* which were allowed to watch experienced foragers at artificial flowers for 10 hours and held incommunicado overnight. In the morning these bees demonstrated their ability to forage at the flowers after 3 trails. The flowers were replaced with cleaned ones after each of the first three trials and for each individual bee tested. The activities of the foragers were recorded for a further 7 visits. H_0 showing that the durations for successful foraging are independent of experience of having watched nestmates forage is rejected ($F_{8,9} = 10.7$; $P < 0.001$).

mesh tube was placed between the arrays of artificial flowers, so that they could watch successful experienced foragers for a day (the A2 bees) at first, typically land on the upper surface of the coloured disc of the artificial flower, and then crawl under and down to access the reinforcer syrup through the holes in the sides of the microcentrifuge tubes. After about 3 visits, these A1 bees flew directly to the openings on the sides of the microcentrifuge tubes to forage (Figure 4).

To assess the importance of watching active foragers versus the presence of the dead-bee model, we compared

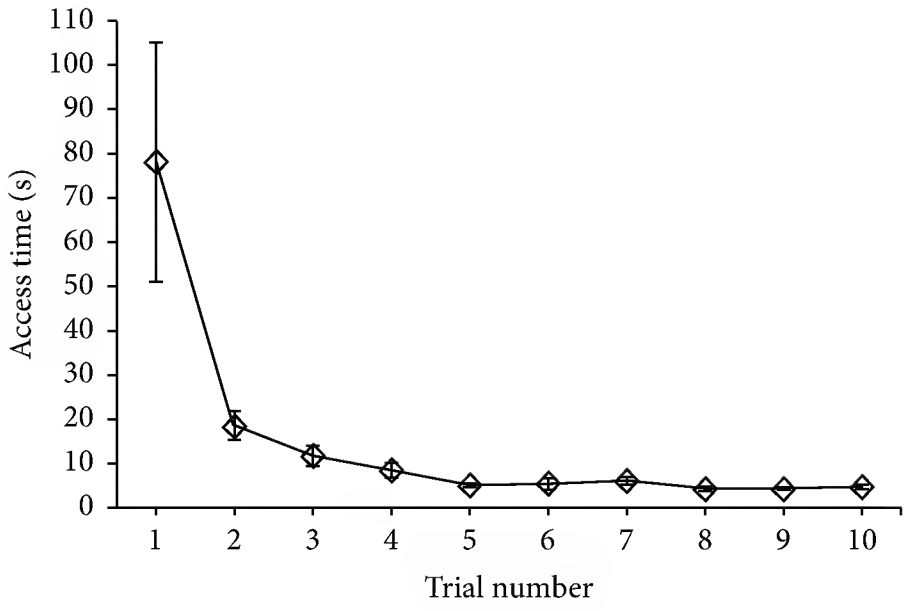


Figure 5: The learning curve (time/sec) (±SE) taken to access and forage on syrup for 9 workers of *Bombus impatiens* which were allowed to watch experienced foragers (from different colony (colony A)) at artificial flowers for 10 hours and held incommunicado overnight. In the morning these bees demonstrated their ability to forage at the flowers after 3 trails. The flowers were replaced with cleaned ones after each of the first three trials and for each individual bee tested. The activities of the foragers were recorded for a further 7 visits. H_0 showing that the durations for successful foraging are independent of experience of having watched other, non-nestmate, bees forage is rejected ($F_{8,9} = 7.7$; $P < 0.001$).

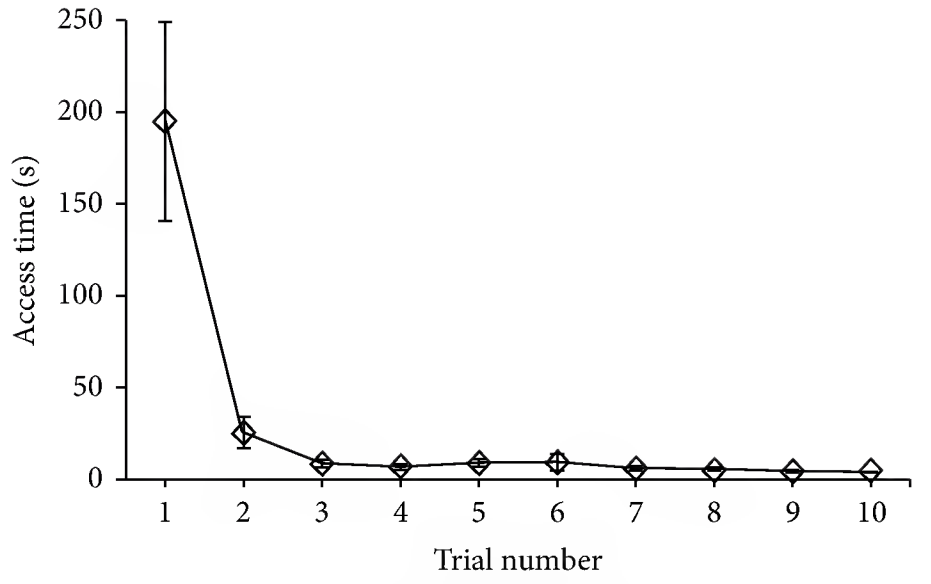


Figure 6: The learning curve (time/sec) (±SE) taken to access and forage on syrup for 9 workers of *Bombus impatiens* which were allowed to contact with their nestmates B1 (i.e., they were watching the experienced bees from colony A). The B2 bees were kept inside the hive and then, after release to forage, had apparently learned to manipulate the artificial flowers through communication with their nestmates. H_0 showing that the durations for successful foraging are independent of experience of having communicated with their nestmates is rejected ($F_{8,9} = 21.4$; $P < 0.001$).

the time it took for the bees to manipulate (i.e., to land on the flowers, orient to their correct positions to forage, and then to imbibe syrup) the flowers on their first visit (cf. Figures 4 and 3). The difference in time is huge. In the model with dead bees, the initial visit to succeed at obtaining the reinforcer syrup was 900 ± 174.8 secs (mean ± SE; $n = 10$ bees), whereas after watching, the bees took only 69 ± 17.4 secs ($n = 9$ bees) to forage successfully (Student's $t = 4.53$; df = 17; $P = 0.0003$).

Following experiments on the first colonies (colonies A, C, and D), we introduced to the experimental set-up, another colony (colony B) in another cage (II).

The watcher worker bees from colony B, cohort 1 (B1 bees), showed the same behaviour as A1 bees (from colony A) when challenged with the artificial flowers (see Figures 4 and 5).

To assess the importance of watching active nestmate foragers versus non-nestmate foragers, we compared the time it took for the bees to manipulate the flowers on their first visit (cf. Figures 4 and 5). There is no statistical difference in time for either group to succeed at obtaining the reinforcer syrup which was 69 ± 17.42 secs (mean ± SE; $n = 9$ bees) after watching nestmates versus 78 ± 27.14 secs ($n = 8$ bees) after watching non-nestmates (Student's $t = 0.3$; df = 15; $P = 0.77$).

The bees in cohort B2 had no chance to see artificial flowers with or without dead bees in foraging positions nor to observe their nestmates or non-nestmates foraging at the artificial flowers. Cohort B2 bees had only the opportunity to communicate with their nestmates, while their nestmates were foraging, with exclusive access, to the artificial flowers. Figure 6 presents the surprising results that B2 bees had somehow learned how to forage from the artificial flowers.

To assess the importance of communicating with active nestmate foragers versus no communication and versus learning by observing a model (dead bees) or active foragers (nestmates or not), we compared the time it took for the bees to manipulate the flowers on their first visit (cf. Figures 6, 3, 4, and 5). The bees that had opportunity for in-nest communication only before foraging took longer time than the bees that had watched either nestmates (Figure 5) or non-nestmates (Figure 4) forage. However, they were quicker than the bees that had learned by having only the dead-bee models in place (Figure 3). Statistical analysis by ANOVA supports those observations ($F_{3,9} = 2.3$; $P = 0.046$); durations to successfully obtaining the reinforcer syrup on the first experimental encounter rank in the following order: watcher of nestmates (69 secs; Figure 4) = watcher of non-nestmates (78 secs; Figure 5) < communicators (195 secs; Figure 6) < observers of dead bees (906 secs; Figure 3) < no clues provided (all 15 bees unsuccessful; ∞ secs).

We provide the detailed statistical tables for the results of our one-way repeated measures for ANOVA (Table 1).

4. Discussion

When naive bumblebee workers first encounter a flower from which they can obtain a reward (e.g., nectar or pollen), they must learn how to manipulate it. Laverty [24] has shown that bumblebee workers (*Bombus impatiens*, *B. fervidus*, *B. vagans*, *B. rufocinctus*, and *B. consobrinus*) become increasingly adept (i.e., by speed and accuracy of manipulation) with increasing experience. Moreover, Dornhaus and Chittka [25, 26] noted that returning foragers stimulated colony-level foraging activity. Baude et al. [12] described the intercolony facilitation in foraging by *B. terrestris* as the use of inadvertent social information (ISI), whereby foragers watched each other's activities and learned from that. Leadbeater and Chittka [27] showed that worker bumblebees (*B. terrestris*) learned to discriminate between two kinds of flowers, depending on

Table 1: Statistical values from repeated one-way Analysis of Variance of the findings from experiments in which dead bees were used as models to aid in the learning process for foraging by living bees, in which living bees were able to watch other living bees (nestmates and nonnestmates) forage to aid in the learning process and in which living bees which had no opportunity to observe models or other living bees foraging learned to forage by within-colony communication.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	Probability
Using dead bees in the foraging position on the artificial flowers (Figure 3)					
Between bees	9	992368	110263		
Between trials	9	7234272	803808	19.68	<0.001
Residual	81	3306794	40824		
Total	**99**	**11533434**			
Watching nestmates (Figure 4)					
Between bees	8	5425	678.14		
Between trials	9	30850	3427.83	10.74	<0.001
Residual	72	22961	318.91		
Total	**89**	**59237**			
Watching nonnestmates (Figure 5)					
Between bees	8	3948	493.55		
Between trials	9	38014	4223.78	7.69	<0.001
Residual	72	39528	549		
Total	**89**	**81490**			
Communication within the domicile (Figure 6)					
Between bees	8	20619	2577.37		
Between trials	9	286156	31795.11	21.37	<0.001
Residual	72	107107	1487.61		
Total	**89**	**413882**			
The difference between four groups of tested bees					
Between learning type	3	105010	35003.61		
Between trial	9	331231	36803.44	2.30	0.046
Residual	27	431755	15990.95		
Total	**39**	**867997**			

whether or not they contained nectar, faster if conspecific foragers were present and foraging at the same time than if they were alone. They also noted that if dead bumblebee models were present in posed foraging positions, the effect was the same; the experimental bees learned faster than if no dead bee was present. Their results indicate that social learning at flowers can be a component of foraging efficiency. More recently they have shown that nectar robbing can spread socially among bumblebees foraging at horizontally oriented tubular artificial flowers [9, 28] (probably by watching other bees and encountering holes already made in the flowers). They state that social learning within the nest is unlikely, but our results indicate otherwise. It is possible that measures of rates of learning (e.g., Figures 3–6 in our study) also indicate effects of stimulation by experience or the presence of other foragers. Even though our results indicate that those bees that watched living foragers (i.e., nestmate or none nestmate) learned faster than those which could see dead bees (cf. Figures 3, 4, and 5). We raise the idea that the difference could reflect stimulation's accelerates social learning. We also noted that Worden and Papaj [29] used stationary and moving model bees and found quicker responses of trained forager bees to the latter. It is also known that bumblebees, as other bees, communicate socially through pheromones [30] and can discriminate between recently visited flowers and flowers which have not been visited for some time [31–33]. Renner and Nieh [34] showed that foragers of *B. impatiens* can associate scentedness of rewarding food sources (flowers) and share this ability with their nestmates. The same phenomenon has also been shown for other species, for example, *B. terrestris* [35–38]. Physical contact, especially antennal and body contact, may be important in the transmission of information on the location, quality, quantity, and nature of floral resources in honeybees [39] and stingless bees [40, 41]. However, little is known about the role of physical contact in the lives of bumblebees. Food exchange (trophallaxis) may be the most primeval form of social communication in eusocial bees, but not bumblebees [42, 43], and may provide information about food quality and odour for some species. Bumblebees may be able to gain such information by sampling resources (nectar and pollen) once deposited in the colony. Observation and social learning strengthen a colony's

foraging efficiency both by intake of more resources by the same colony and by promoting a competitive stratagem by learning from rival colonies [44].

Our experiments were designed to extend our understanding of the potential for social learning in bumblebees following from the work of Worden and Papaj [29], Kawaguchi et al. [45], and Leadbeater and Chittka [9, 46].

We controlled for external cues, such as scentedness of or pheromone residues on the artificial flowers (cleaned as used) and reinforcer syrup (sucrose in water has no vapour pressure and was always made fresh for each experiment). The domiciles used were always in the same locations relative to the arrays of artificial flowers. The visual signals were highly controlled such as the colour of the artificial flowers, the dead bees were posed on them and the active foragers that imprisoned bees could watch.

5. Conclusion

Our results indicate that workers of *B. impatiens* are highly observant and learn through social communication. Although they were relatively slow to learn to forage from artificial flowers with dead conspecifics posed as foragers, they were much faster if they had the opportunity to observe, but not join, active foragers from either their own colony or from another. Surprisingly, when we allowed workers that had never had a chance to visit nor see an artificial flower, but had had contact with nestmates that were successful foragers, the experimental (naive) workers were adept at handling the artificial flowers. All workers that were confronted with the artificial flowers but no opportunity to see posed dead bees, active foragers, or communicate within the colony failed to forage successfully. It would be useful for other researchers to repeat our experiment, with appropriate modifications, to test if our results can be repeated or explained.

It is often assumed that observational learning and imitation (or copying) lie at the heart of social transmission of information and learning [47, 48]; there are other ways novel behaviour can be transmitted socially (e.g., through tactile, vibratory, and olfactory senses), especially in bees. We are not able to explain how workers that had never had a chance to visit nor even see an artificial flower, but only had had contact with nestmates that were successful foragers and only in the nest, became so quickly adept at handling the artificial flowers.

Evidence, including that which we present herein, continues to mount that there is no strict dichotomy between vertebrate and invertebrate cognition [23, 49, 50]. Our work adds to the growing body of research in social Hymenoptera that demonstrates that brain size does not necessarily limit an animal's cognitive abilities. More imaginative experiments are needed to determine the role of social learning, the amount and type of information that need to be transmitted, and how that body of information contributes to Darwinian fitness.

Acknowledgments

The authors thank the Ministry of Higher Education, Libya, Canadian Bureau for International Education, Ottawa, Canada, and the Canadian Pollination Initiative (NSERC-CANPOLIN for which this is contribution no. 99) for funding. BioBest Canada, Leamington, Ontario, kindly provided the colonies of *Bombus impatiens* for this study.

References

[1] S. J. Shettleworth, *Cognition, Evolution, and Behavior*, Oxford University Press, Oxford, UK, 1998.

[2] A. Whiten and R. Ham, "On the nature and evolution of imitation in the animal kingdom: reappraisal of a century of research," *Advances in the Study of Behavior*, vol. 21, no. C, pp. 239–283, 1992.

[3] A. Bandura, *Social Learning Theory*, General Learning Press, New York, NY, USA, 1977.

[4] A. Bandura, *Social Foundations of Thought and Action*, Prentice-Hall, Englewood Cliffs, NJ, USA, 1986.

[5] L. A. Giraldeau and T. Caraco, *Social Foraging Theory*, Princeton University Press, Englewood Cliffs, NJ, USA, 2000.

[6] L. A. Giraldeau, T. Caraco, and T. J. Valone, "Social foraging—individual learning and cultural transmission of innovations," *Behavioral Ecology*, vol. 5, no. 1, pp. 35–43, 1994.

[7] L. Lefebvre, "The opening of milk bottles by birds: evidence for accelerating learning rates, but against the wave-of-advance model of cultural transmission," *Behavioural Processes*, vol. 34, no. 1, pp. 43–53, 1995.

[8] C. Heyes and B. J. Galef, *Social Learning and the Roots of Culture*, Academic Press, San Diego, Calif, USA, 1996.

[9] E. Leadbeater and L. Chittka, "Social transmission of nectar-robbing behaviour in bumble-bees," *Proceedings of the Royal Society*, vol. 275, no. 1643, pp. 1669–1674, 2008.

[10] E. Leadbeater and L. Chittka, "Do inexperienced bumblebee foragers use scent marks as social information?" *Animal Cognition*, vol. 14, no. 6, pp. 915–919, 2011.

[11] M. Giurfa, "Social learning in insects: a higher-order capacity," *Frontiers in Behavioral Neuroscience*, vol. 6, article 57, 2012.

[12] M. Baude, I. Dajoz, and É. Danchin, "Inadvertent social information in foraging bumblebees: effects of flower distribution and implications for pollination," *Animal Behaviour*, vol. 76, no. 6, pp. 1863–1873, 2008.

[13] M. Giurfa, "Cognitive neuroethology: dissecting non-elemental learning in a honeybee brain," *Current Opinion in Neurobiology*, vol. 13, no. 6, pp. 726–735, 2003.

[14] R. Menzel, G. Leboulle, and D. Eisenhardt, "Small brains, bright minds," *Cell*, vol. 124, no. 2, pp. 237–239, 2006.

[15] P. G. Kevan and R. Menzel, "The plight of pollination and the interface of neurobiology, ecology and food security," *Environmentalist*, vol. 32, no. 3, pp. 300–310, 2012.

[16] K. von Frisch, *The Dance Language and Orientation of Bees*, Belknap Press, Cambridge, Mass, USA, 1993.

[17] R. W. K. Punchihewa, N. Koeniger, P. G. Kevan, and R. M. Gadawski, "Observations on the dance communication and natural foraging ranges of the three honeybee species in Sri Lanka," *Journal of Apicultural Research*, vol. 24, no. 2, pp. 168–175, 1985.

[18] I. Aguilar and D. Briceño, "Sounds in *Melipona costaricensis* (Apidae: Meliponini): effect of sugar concentration and nectar source distance," *Apidologie*, vol. 33, no. 4, pp. 375–388, 2002.

[19] J. C. Nieh, "Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini)," *Apidologie*, vol. 35, no. 2, pp. 159–182, 2004.

[20] D. E. Jackson and F. L. W. Ratnieks, "Communication in ants," *Current Biology*, vol. 16, no. 15, pp. R570–R574, 2006.

[21] R. L. Jeanne, "Chemical communication during swarm emigration in the social wasp *Polybia sericea* (Olivier)," *Animal Behaviour*, vol. 29, no. 1, pp. 102–113, 1981.

[22] H. Schöne and J. Tengo, "Competition of males, courtship behaviour and chemical communication in the digger wasp *Bembix rostrata* (Hymenoptera, Sphecidae)," *Behaviour*, vol. 77, pp. 44–66, 1981.

[23] G. Fiorito and P. Scotto, "Observational learning in *Octopus vulgaris*," *Science*, vol. 256, no. 5056, pp. 545–547, 1992.

[24] T. M. Laverty, "Bumble bee learning and flower morphology," *Animal Behaviour*, vol. 47, no. 3, pp. 531–545, 1994.

[25] A. Dornhaus and L. Chittka, "Information flow and regulation of foraging activity in bumble bees (*Bombus* spp.)," *Apidologie*, vol. 35, no. 2, pp. 183–192, 2004.

[26] A. Dornhaus and L. Chittka, "Bumble bees (*Bombus terrestris*) store both food and information in honeypots," *Behavioral Ecology*, vol. 16, no. 3, pp. 661–666, 2005.

[27] E. Leadbeater and L. Chittka, "Social information use in foraging insects," in *Food Exploitation by Social Insects: Ecological, Behavioral, and Theoretical Approaches*, S. Jarau and M. Hrncir, Eds., CRC Press, Boca Raton, Fla, USA, 2009.

[28] D. F. Sherry, "Social learning: nectar robbing spreads socially in bumble bees," *Current Biology*, vol. 18, no. 14, pp. R608–R610, 2008.

[29] B. D. Worden and D. R. Papaj, "Flower choice copying in bumblebees," *Biology Letters*, vol. 1, no. 4, pp. 504–507, 2005.

[30] T. D. Wyatt, *Pheromones and Animal Behaviour: Communication by Smell and Taste*, Cambridge University Press, Cambridge, UK, 2003.

[31] S. A. Cameron, "Chemical signals in bumble bee foraging," *Behavioral Ecology and Sociobiology*, vol. 9, no. 4, pp. 257–260, 1981.

[32] D. Goulson, S. A. Hawson, and J. C. Stout, "Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species," *Animal Behaviour*, vol. 55, no. 1, pp. 199–206, 1998.

[33] J. Wilms and T. Eltz, "Foraging scent marks of bumblebees: footprint cues rather than pheromone signals," *Naturwissenschaften*, vol. 95, no. 2, pp. 149–153, 2008.

[34] M. A. Renner and J. C. Nieh, "Bumble bee olfactory information flow and contact-based foraging activation," *Insectes Sociaux*, vol. 55, no. 4, pp. 417–424, 2008.

[35] U. Schmitt and A. Bertsch, "Do foraging bumblebees scent-mark food sources and does it matter?" *Oecologia*, vol. 82, no. 1, pp. 137–144, 1990.

[36] U. Schmitt, G. Lübke, and W. Francke, "Tarsal secretion marks food sources in bumblebees (Hymenoptera: Apidae)," *Chemoecology*, vol. 2, no. 1, pp. 35–40, 1991.

[37] D. Goulson, J. C. Stout, J. Langley, and W. O. H. Hughes, "Identity and function of scent marks deposited by foraging bumblebees," *Journal of Chemical Ecology*, vol. 26, no. 12, pp. 2897–2911, 2000.

[38] S. Witjes and T. Eltz, "Influence of scent deposits on flower choice: experiments in an artificial flower array with bumblebees," *Apidologie*, vol. 38, no. 1, pp. 12–18, 2007.

[39] K. Rohrseitz and J. Tautz, "Honey bee dance communication: waggle run direction coded in antennal contacts?" *Journal of Comparative Physiology*, vol. 184, no. 4, pp. 463–470, 1999.

[40] M. Hrncir, S. Jarau, R. Zucchi, and F. G. Barth, "Recruitment behavior in stingless bees, *Melipona scutellaris* and *M. quadrifasciata* II. Possible mechanisms of communication," *Apidologie*, vol. 31, no. 1, pp. 93–113, 2000.

[41] V. M. Schmidt, R. Zucchi, and F. G. Barth, "Recruitment in a scent trail laying stingless bee (*Scaptotrigona* aff. *depilis*): Changes with reduction but not with increase of the energy gain," *Apidologie*, vol. 37, no. 4, pp. 487–500, 2006.

[42] R. J. De Marco and W. M. Farina, "Trophallaxis in forager honeybees (*Apis mellifera*): resource uncertainty enhances begging contacts?" *Journal of Comparative Physiology*, vol. 189, no. 2, pp. 125–134, 2003.

[43] A. G. Hart and F. L. W. Ratnieks, "Task-partitioned nectar transfer in stingless bees: work organisation in a phylogenetic context," *Ecological Entomology*, vol. 27, no. 2, pp. 163–168, 2002.

[44] M. Baude, É. Danchin, M. Mugabo, and I. Dajoz, "Conspecifics as informers and competitors: an experimental study in foraging bumble-bees," *Proceedings of the Royal Society B*, vol. 278, no. 1719, pp. 2806–2813, 2011.

[45] L. G. Kawaguchi, K. Ohashi, and Y. Toquenaga, "Do bumble bees save time when choosing novel flowers by following conspecifics?" *Functional Ecology*, vol. 20, no. 2, pp. 239–244, 2006.

[46] E. Leadbeater and L. Chittka, "The dynamics of social learning in an insect model, the bumblebee (*Bombus terrestris*)," *Behavioral Ecology and Sociobiology*, vol. 61, no. 11, pp. 1789–1796, 2007.

[47] A. D. Brian, "Differences in the flowers visited by four species of bumble-bees and their causes," *Journal of Animal Ecology*, vol. 26, pp. 71–98, 1957.

[48] E. Leadbeater and L. Chittka, "A new mode of information transfer in foraging bumblebees," *Current Biology*, vol. 15, no. 12, pp. R447–R448, 2005.

[49] M. Giurfa, S. Zhang, A. Jenett, R. Menzel, and M. V. Srinivasan, "The concepts of "sameness" and "difference" in an insect," *Nature*, vol. 410, no. 6831, pp. 930–933, 2001.

[50] M. Srinivasan and S. Zhang, "Small brains, smart minds: vision, perception and "cognition" in honeybees," *IETE Journal of Research*, vol. 49, no. 2-3, pp. 127–134, 2003.

Hindawi Publishing Corporation
Psyche
Volume 2013, Article ID 859835, 5 pages
http://dx.doi.org/10.1155/2013/859835

Research Article

Does Experience Affect the Outcome of Male-Male Contests in the Burying Beetle, *Nicrophorus quadripunctatus*?

Seizi Suzuki

Ecology & Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Correspondence should be addressed to Seizi Suzuki; seizis@res.agr.hokudai.ac.jp

Received 20 May 2013; Accepted 13 November 2013

Academic Editor: Donald M. Windsor

The bigger individual in a fight usually wins unless the smaller individual is a resident or has recently won a fight. I conducted three experiments on the effects of body size, residency, and fight history on the outcome of male-male fights in a burying beetle. Fights were staged between an intruding male and the male of a male-female pair. When males differed in size, the larger male usually won regardless of residency or individual fight histories. Residents and winners of previous fights won only when competing males were similar in size. Hence, male body size largely determines the outcomes of fights in this beetle.

1. Introduction

Fighting between males over mating opportunities is a widespread phenomenon in the animal kingdom and has received much empirical attention [1]. Fighting ability is often correlated with morphological and physiological attributes such as body size, weaponry, and ornaments [1], but these are not the only attributes that may determine fighting ability. It has been hypothesized that the "prior residence effect" can also affect the outcome of fighting contests in accordance with the convention "resident wins, intruder retreats" [2]. A third effect is the "winner-loser effect," in which winners are more likely to win again and losers are more likely to lose again [3]. These two effects sometimes counteract morphological and physiological attributes (e.g., [4]).

The complex parental behaviour of burying beetles (*Nicrophorus*: Silphidae) has been well-studied (reviewed in [5, 6]). *Nicrophorus* exploits small vertebrate carrions as food for its young. A male-female pair prepares a carcass by burying it, removing its hair, and rounding it into a ball. Eggs are then laid in the soil adjacent to the carrion ball. After hatching, the larvae crawl to the carrion ball, where they are fed by parental regurgitations. *Nicrophorus* is generally monogamous [7–9], and both sexes display intense intrasexual competition [10, 11]. Two or more individuals of both sexes often locate the same carcass, but usually only a single dominant pair eventually occupies the carcass. Resident males are more likely to be injured than resident females [12], and males have a greater tendency to guard [13, 14]. Contests between males are expected to be more intense than those between females.

Larger individuals of *Nicrophorus* usually win contests among conspecifics in *N. humator* [10] or in *N. quadripunctatus* [11]. However, the presence of the winner effect is supported by a previous study of *N. humator* [15], and it is possible that other attributes affect the outcomes of such contests. In this study, I investigated whether the outcomes of male-male contests in *Nicrophorus quadripunctatus* differ in accordance with the prior residence effect and/or winner-loser effect.

2. Materials and Methods

All beetles were caught in the field in Nagaoka City, Niigata Prefecture, Japan using hanging traps baited with rotten meat. Injured individuals were excluded from the experiments. The beetles were maintained individually for more than 7 days so that they would lose the memories of past fighting. They were fed small pieces of chicken *ad libitum*. The pronotal width of each beetle was measured before the experiment. Medium-sized beetles (pronotal width 4.5–5.0 mm) were used to make pairs. A male and female pair was placed along with a small piece of chicken meat (approx. 15 g) in a plastic

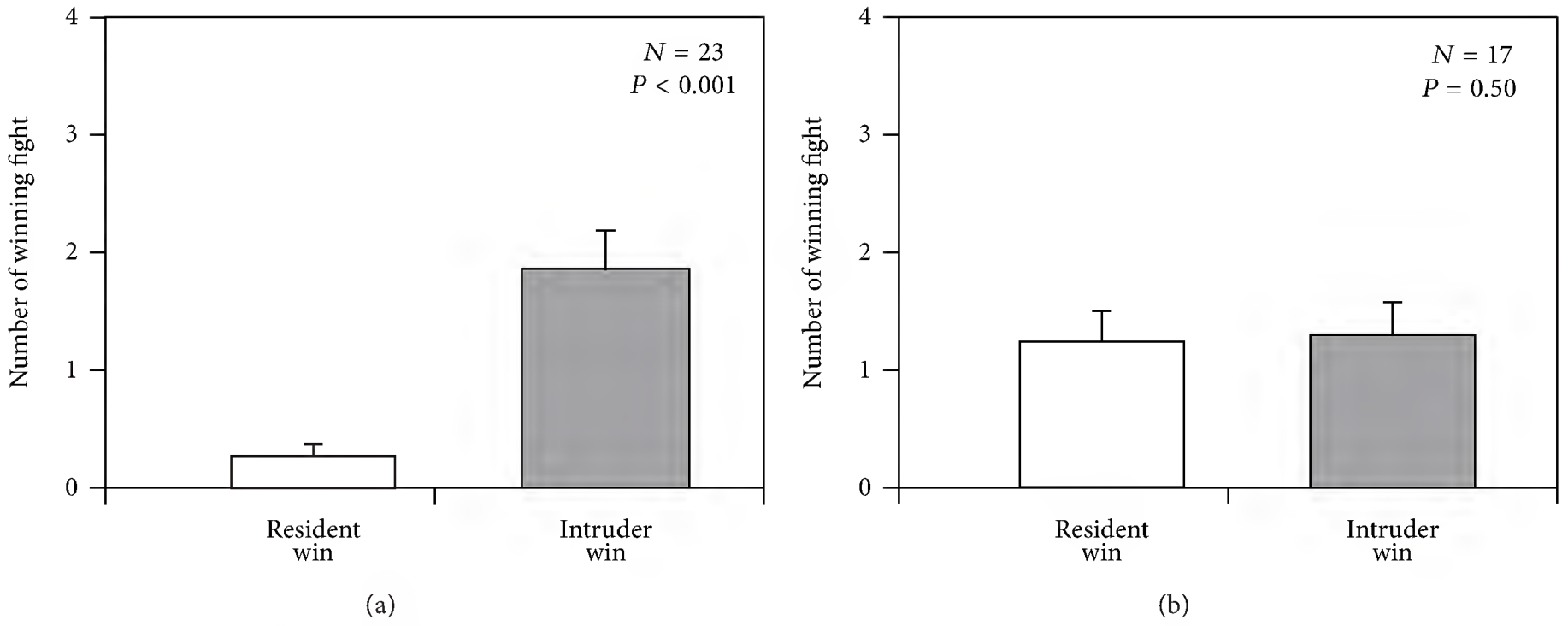


Figure 1: The times individuals took in winning fights in the control contest (experiment 1). (a) A larger intruder was introduced. (b) A similar-sized intruder was introduced. The data are means ± standard error (SE).

arena (150 × 150 × 50 mm). The arenas were maintained under standard laboratory conditions of light and ambient temperature. Beetles for intruder males were sorted into large (pronotal width 5.5–6.5 mm) and same-sized males (pronotal width less than 0.2 mm different from that of the resident male).

Experiment 1 (control contest:): the male/female pair remained in an arena for 1 h. A large male ($N = 23$) or a same-sized male ($N = 17$) was introduced as the intruder.

Experiment 2 (effect of residence): a beetle pair was maintained in an arena for 6 h. A large ($N = 20$) or same-sized male ($N = 22$) was introduced in the arena at 6 h as an intruder. Because many of the carcasses had been buried within 8 hours [16], I regarded 6 hours as enough time for pair formation.

Experiment 3 (effect of prior experience): a pair was maintained in an arena for 1 h. A larger or a smaller-size male (pronotal width difference more than 10% of the resident male) was introduced and observed until the first contest. The beetle that escaped the place first was regarded as the loser, and the beetle that stayed as the winner if body contact between males had occurred. All beetles that were placed with larger male had lost and all beetles that were placed with smaller male had won the first contest. After the fate of the contest between first introduced beetle was confirmed, the introduced male was removed and another new large male ($N = 16$), or same-sized male (winner: $N = 20$, loser: $N = 20$), was immediately introduced as an intruder. If the resident male injured its antenna or a leg in the first contest, it was excluded from the experiment.

After a male was introduced as an intruder, the behavioural interactions of all of the beetles were recorded for 1 h. When an aggressive interaction [11] occurred between the males, the number of contest and the fate of the contest were recorded. The beetle that escaped the place first was regarded as the loser, and the beetle that stayed was regarded as the winner if body contact between males had occurred. Because the contest was repeated during observation time, the fate of all contests was recorded. The number of contests that residents or intruders won was regarded as the indicator of the fate of contests.

Generalized linear models (GLM) with binomial distributions were used to examine differences in the fate of contests between resident and intruder male. Significance was accepted at $P < 0.05$.

3. Results

Experiment 1 (control contest): when the intruder was larger than the resident male, the resident male usually lost the contest ($t = 7.32$, $P < 0.001$). In contrast, when the intruder was same-sized, about half of the residents lost the contest ($t = 0.42$, $P = 0.50$, Figure 1).

Experiment 2 (effect of resident): when the intruder was larger than the resident male, the resident usually lost the contest ($t = 5.21$, $P < 0.001$). In contrast, when the intruder was same-sized, most of the residents won the contest ($t = 4.08$, $P < 0.001$, Figure 2).

Experiment 3 (effect of prior experience): whether the resident males had winning or losing contest experiences, most of them lost to the next large intruder (Figure 3). When the intruder was same-sized, the residents that had had a winning experience usually won the contest ($t = 2.52$, $P = 0.008$, Figure 3). In contrast, most of the residents that had had a losing experience did not win these contests ($t = 1.29$, $P = 0.21$, Figure 3). In addition, fewer fights were started by the males that had had a losing experience compared to the males that had had a winning experience (t-test, $t = 2.04$, $P = 0.04$).

In all experiments, no intersexual contests were observed.

4. Discussion

In *Nicrophorus* spp., even after oviposition and hatching, infanticidal takeovers by intruding individuals occur regularly in the field [17]. Intruders kill the eggs and larvae of

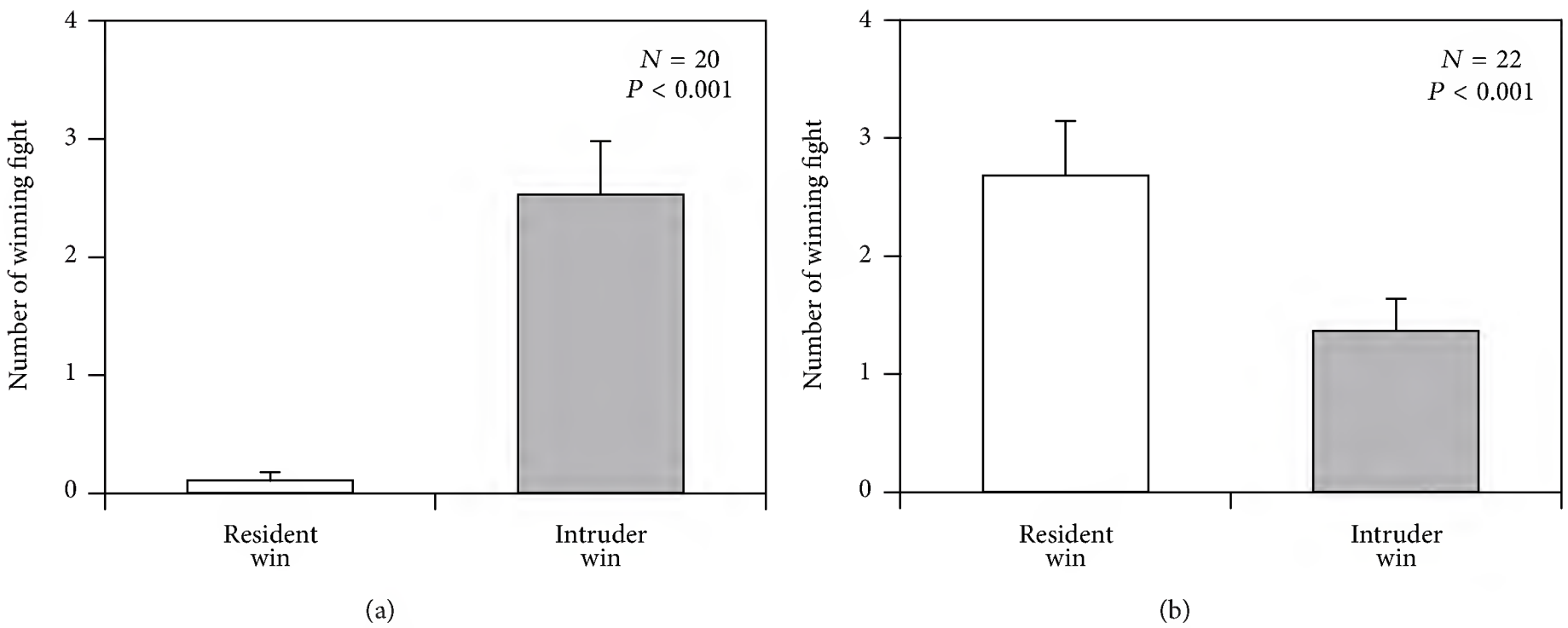


FIGURE 2: The times individuals took in winning fights in the prior experienced contest (experiment 2). (a) A larger intruder was introduced. (b) A similar-sized intruder was introduced. The data are means ± SE.

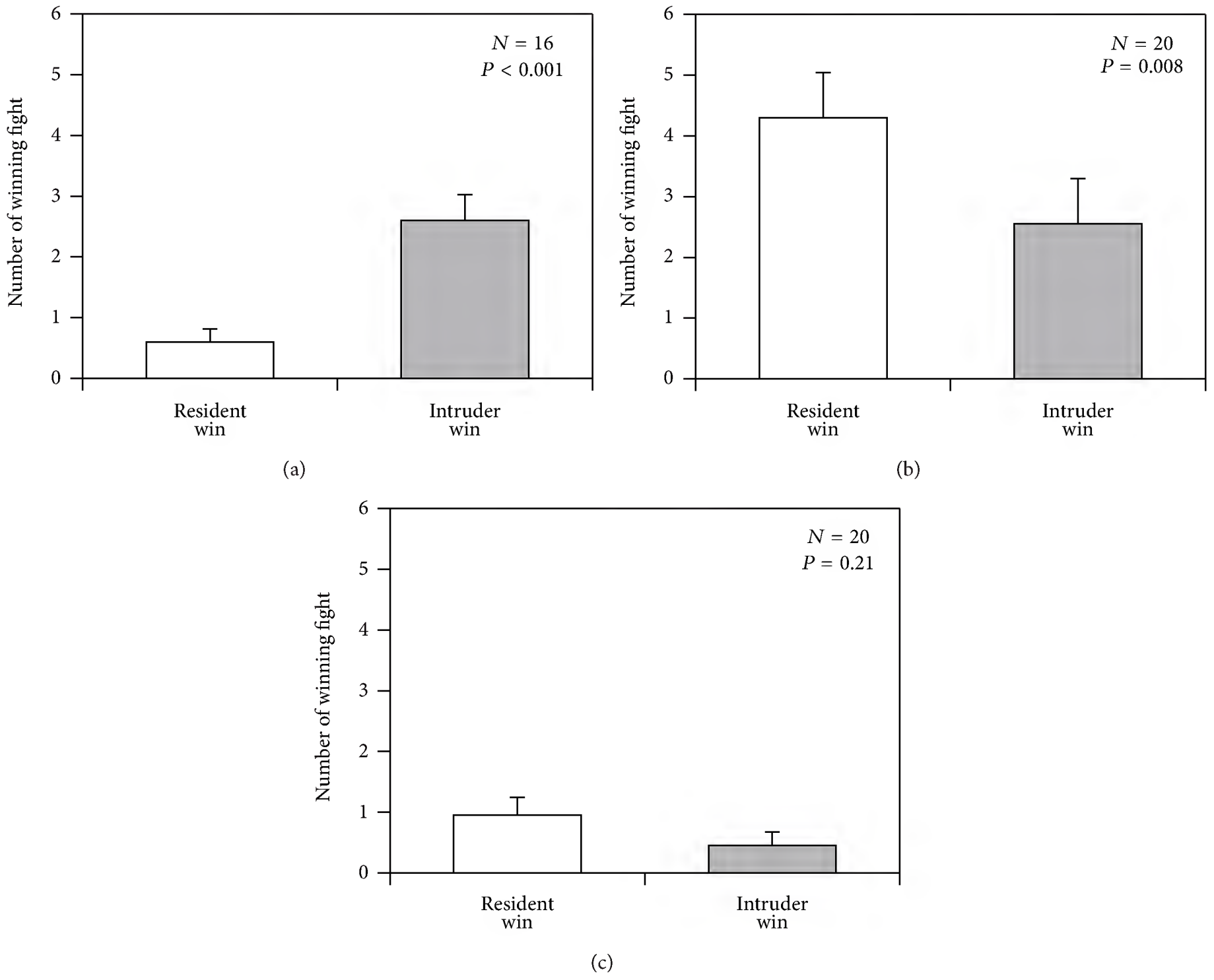


FIGURE 3: The times individuals took in winning fights in the winner-loser contest (experiment 3). (a) A larger intruder was introduced to the arena where the male had just had a winning experience. (b) A similar-sized intruder was introduced to the arena where the male had just had a winning experience. (c) A similar-sized intruder was introduced to the arena where the male had just had a losing experience. The data are means ± SE.

the residents and reproduce on the carcass [18]. Since infanticide reduces the reproductive success of the resident parents, it is advantageous for both parents to reduce the possibility of infanticide. Many studies have shown that size is an important asymmetry in *Nicrophorus* competition [10, 11, 19]. Larger beetles have a better chance of possessing a carcass and of displacing a resident and taking over a buried carcass [12, 17]. The results of the present study also indicated that larger males are more likely to win a male-male contest. However, it has been reported that smaller *Nicrophorus* individuals can repel a larger intruder [20, 21]. Biparental cooperation can also affect the fate of contests; a male-female pair can usually repel a larger male intruder [20]. However, because most contests involve resident males and because repelling larger intruders occurs after the burial of carcasses [21], the possibility of the effect of male prior residence or other effects remains.

In the present study, when the body size of the intruder male was similar to that of the resident male, the effect of prior residence seems to be paramount, but the effect of prior residence did not override the difference in body size (Figure 2).

The experience of winning had an effect on the contests between same-sized males; however, the winner effect also did not override the difference in body size (Figure 3). It has been said that the loser effect often has more effect than the winner effect [22]. According to the self-assessment hypothesis, prior fighting experience could be used to assess one's own fighting ability relative to that of others in the population [23]. The present study's results showed that the males that had losing experiences had not only lower rates of winning but also fewer contests with same-sized males. When a burying beetle loses a fight, body damage is often incurred [21].

Because the fighting ability of burying beetles depends in part on their body size, a male that has already lost a fight may be reluctant to fight even a similar-sized intruder. Males that have had a losing experience may avoid fighting altogether. Males that have experience winning will defend resources more aggressively, and losing males will defend them less aggressively when the body size of the intruder is not larger.

Biparental cooperation can repel intruder beetles [20]. In the present experiments, no females attacked the intruder males, and thus, biparental cooperative defence did not occur. Intersexual contests in *N. quadripunctatus* before carrion burial have not been reported [11], and biparental cooperation is restricted after parental care begins [20]. Thus, the outcome of fights will be determined by the resource-holding power of resident males. Since the effect of prior residence seems not to override the difference of body size, the fate of male-male contests will be body size-dependent. Thus, if a male is challenged by a larger male before the completion of a carrion burial, they may lose the carcass.

The results of the present study suggest the presence of an effect of prior residence and the winner-loser effect in *Nicrophorus* males, but larger males still usually win the male-male contests. Only biparental cooperation has been reported to override body-size differences in male-male competitions among *Nicrophorus* burying beetles.

Acknowledgment

This study was supported in part by a Grant-in-Aid for Scientific Research (no. 24570013) from the Japan Society for the Promotion of Science.

References

[1] F. A. Huntingford and A. K. Turner, *Animal Conflict*, Chapman & Hall, New York, NY, USA, 1987.

[2] J. Maynard Smith and G. A. Parker, "The logic of asymmetric contests," *Animal Behaviour*, vol. 24, no. 1, pp. 159–175, 1976.

[3] Y. Hsu and L. L. Wolf, "The winner and loser effect: integrating multiple experiences," *Animal Behaviour*, vol. 57, no. 4, pp. 903–910, 1999.

[4] K. Okada and T. Miyatake, "Effect of losing on male fights of broad-horned flour beetle, *Gnatocerus cornutus*," *Behavioral Ecology and Sociobiology*, vol. 64, no. 3, pp. 361–369, 2010.

[5] A. K. Eggert and J. K. Müller, "Biparental care and social evolution in burying beetles: lessons from the larder," in *The Evolution of Social Behavior in Insects and Arachnids*, J. C. Choe and B. J. Crespi, Eds., pp. 216–236, Cambridge University Press, Cambridge, UK, 1997.

[6] M. P. Scott, "The ecology and behavior of burying beetles," *Annual Review of Entomology*, vol. 43, pp. 595–618, 1998.

[7] A.-K. Eggert and S. K. Sakaluk, "Benefits of communal breeding in burying beetles: a field experiment," *Ecological Entomology*, vol. 25, no. 3, pp. 262–266, 2000.

[8] S. T. Trumbo, "Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*)," *Ecological Entomology*, vol. 17, no. 3, pp. 289–298, 1992.

[9] S. T. Trumbo and A. K. Eggert, "Beyond monogamy: territory quality influences sexual advertisement in male burying beetles," *Animal Behaviour*, vol. 48, no. 5, pp. 1043–1047, 1994.

[10] M. Otronen, "The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*)," *Annales Zoologici Fennici*, vol. 25, no. 2, pp. 191–201, 1988.

[11] S. Suzuki, M. Nagano, and S. T. Trumbo, "Intrasexual competition and mating behavior in *Ptomascopus morio* (Coleoptera: Silphidae Nicrophorinae)," *Journal of Insect Behavior*, vol. 18, no. 2, pp. 233–242, 2005.

[12] S. T. Trumbo, "Defending young biparentally: female risk-taking with and without a male in the burying beetle, *Nicrophorus pustulatus*," *Behavioral Ecology and Sociobiology*, vol. 61, no. 11, pp. 1717–1723, 2007.

[13] I. A. Fetherston, M. P. Scott, and J. F. A. Traniello, "Parental care in burying beetles: the organization of male and female brood-care behavior," *Ethology*, vol. 85, pp. 177–190, 1990.

[14] I. A. Fetherston, M. P. Scott, and J. F. A. Traniello, "Behavioural compensation for mate loss in the burying beetle *Nicrophorus orbicollis*," *Animal Behaviour*, vol. 47, no. 4, pp. 777–785, 1994.

[15] M. Otronen, "The effect of prior experience on the outcome of fights in the burying beetle, *Nicrophorus humator*," *Animal Behaviour*, vol. 40, no. 5, pp. 980–982, 1990.

[16] S. Suzuki, "Biparental burying beetles, *Nicrophorus quadripunctatus* (Coleoptera, Silphidae), do not show a division of labor in their carrion-burial behavior," *Elytra New Series*, vol. 1, pp. 41–45, 2011.

[17] M. P. Scott, "Brood guarding and the evolution of male parental care in burying beetles," *Behavioral Ecology and Sociobiology*, vol. 26, no. 1, pp. 31–39, 1990.

[18] S. T. Trumbo, "Reproductive benefits of infanticide in a biparental burying beetle *Nicrophorus orbicollis*," *Behavioral Ecology and Sociobiology*, vol. 27, no. 4, pp. 269–273, 1990.

[19] S. A. Safryn and M. P. Scott, "Sizing up the competition: do burying beetles weigh or measure their opponents?" *Journal of Insect Behavior*, vol. 13, no. 2, pp. 291–297, 2000.

[20] S. Suzuki, "Cooperative defence of brood in *Nicrophorus quadripunctatus* (Coleoptera: Silphidae)," *European Journal of Entomology*, vol. 108, no. 4, pp. 561–563, 2011.

[21] S. T. Trumbo, "Infanticide, sexual selection and task specialization in a biparental burying beetle," *Animal Behaviour*, vol. 72, no. 5, pp. 1159–1167, 2006.

[22] Y. Hsu, R. L. Earley, and L. L. Wolf, "Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes," *Biological Reviews of the Cambridge Philosophical Society*, vol. 81, no. 1, pp. 33–74, 2006.

[23] C. Rutte, M. Taborsky, and M. W. G. Brinkhof, "What sets the odds of winning and losing?" *Trends in Ecology and Evolution*, vol. 21, no. 1, pp. 16–21, 2006.


Hindawi