

Psyche

Ants and Their Parasites 2013

Guest Editors: Jean-Paul Lachaud, Alain Lenoir, and David P. Hughes





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Psyche: A Journal of Entomology

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Editorial

Ants and Their Parasites 2013

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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, diapiid, 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 plesio-biotic 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. Torr ns 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 diapiid-ant relationships for which there has been a lot of speculation. True associations with ants occur only for a fraction of the diapiid species. The paper by M. S. Loi cono 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 diapiid-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 microdentine flies.

As for all the parasites associated with ants, microdentine 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 microdentine 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 microdentine 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 outweigh 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. Féneron 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.

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Research Article

Bacterial Infections across the Ants: Frequency and Prevalence of *Wolbachia*, *Spiroplasma*, and *Asaia*

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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 sugary but nitrogen-poor phloem sap [11]. Other examples are the cospeciating 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 entomopathogenicity [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 | | |
|--|------------------|----------------------|-------------------|---|---------------------|---------------------|
| | | | | <i>Wolbachia</i> | <i>Spiroplasma</i> | <i>Asaia</i> |
| <i>Camponotus</i> (Formicinae) | 1 | 1 | 1 | 0 | 1 (100%) 1/1 | 0 |
| <i>Cephalotes</i> (Myrmicinae) | 7 | 17 | 12 | 4 (57.1%) 4/4 | 2 (28.5%) 6/3 | 1 (14.3%) 1/1 |
| <i>Crematogaster</i> (Myrmicinae) | 6 | 6 | 6 | 1 (16.7%) 1/1 | 0 | 0 |
| <i>Myrmecia</i> (Myrmeciinae) | 2 | 3 | 3 | 0 | 0 | 0 |
| <i>Myrmecocystus</i> (Formicinae) | 1 | 1 | 1 | 0 | 0 | 0 |
| <i>Oecophylla</i> (Formicinae) | 1 | 1 | 1 | 0 | 0 | 0 |
| <i>Paraponera</i> (Paraponerinae) | 1 | 23 | 9 | 0 | 1 (100%) 2/2 | 1 (100%) 1/1 |
| <i>Polyrhachis</i> (Formicinae) | 32 | 64 | 60 | 8 (25.0%) 10/10 | 15 (46.9%) 15/15 | 0 |
| <i>Pseudomyrmex</i> (Pseudomyrmecinae) | 36 | 88 | 72 | 5 (13.9%) 5/5 | 5 (13.9%) 5/5 | 12 (33.3%) 15/15 |
| <i>Solenopsis</i> (Myrmicinae) | 2 | 11 | 5 | 1 (50%) 1/1 | 2 (100%) 2/1 | 0 |
| <i>Tetraponera</i> (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.

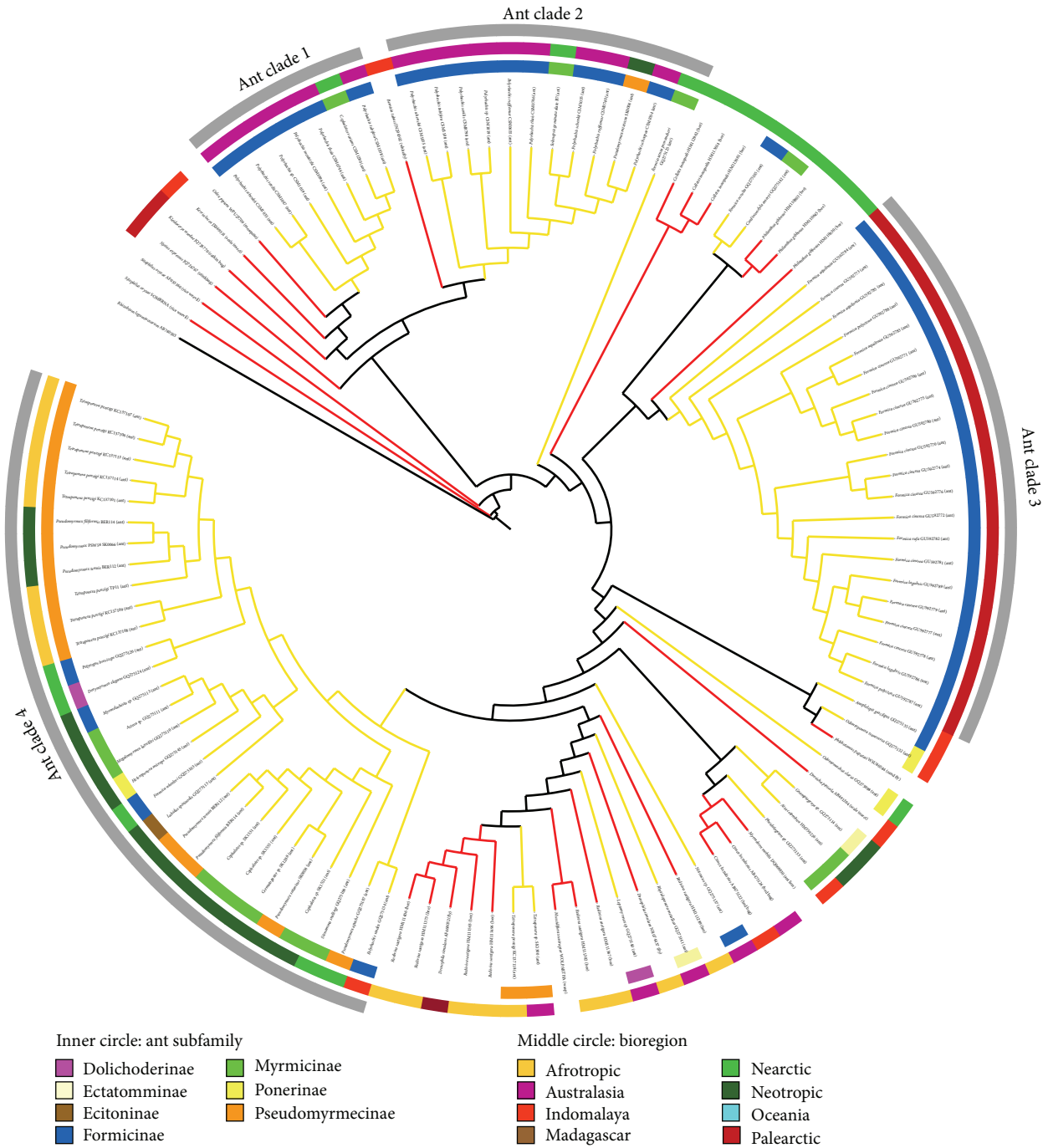


FIGURE 1: Phylogenetic tree of *Wolbachia* symbionts associated with ants and their closest relatives with sequence data available in GenBank. A maximum likelihood phylogeny of the 16S rRNA region of bacterial symbionts is shown. The host name is given together with the GenBank accession number (GenBank 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.

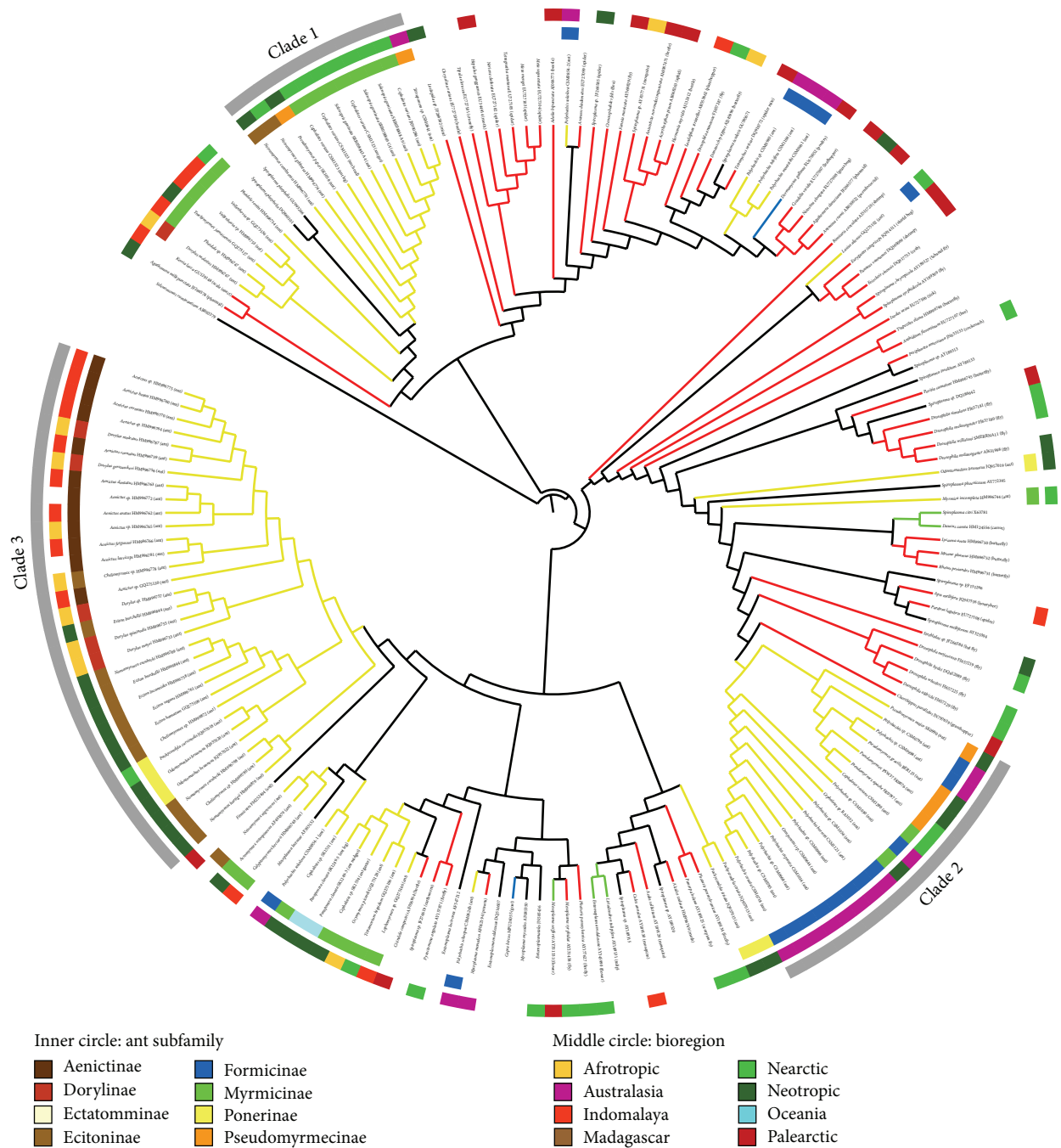


FIGURE 2: Phylogenetic tree of *Spiroplasma*-related ant symbionts and their closest relatives with sequence data available in GenBank. A maximum likelihood phylogeny of the 16S rRNA region of bacterial symbionts is shown. The host name is given together with the GenBank accession number (GenBank sequences) 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

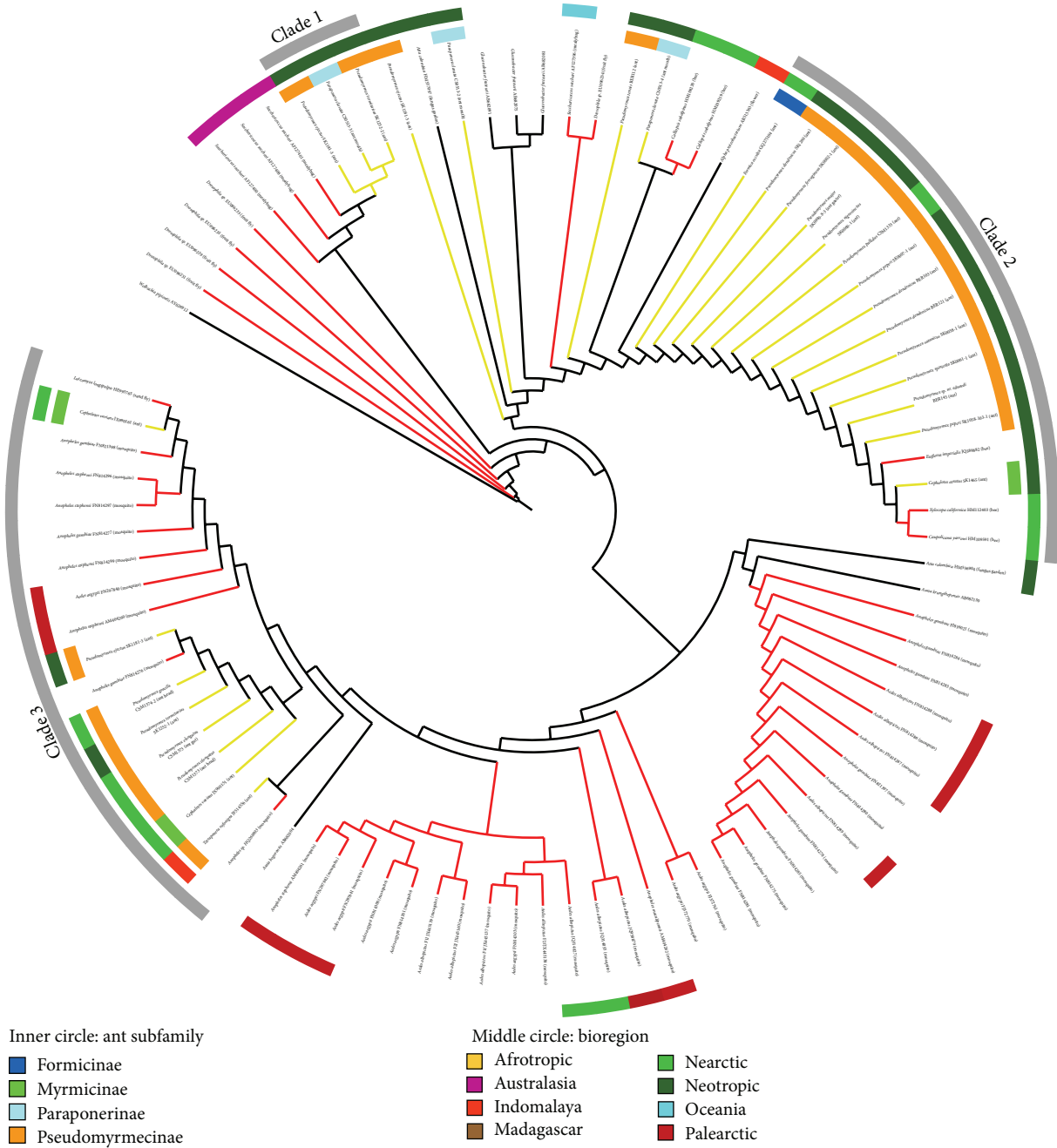


FIGURE 3: Phylogenetic tree of *Asaia*-related symbionts associated with ants and closest relatives with sequence data available in GenBank. A maximum likelihood phylogeny of the 16S rRNA region of bacterial symbionts is shown. The host name is given together with the GenBank accession number (GenBank 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 pipitensis* 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.

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Review Article

Leaf-Cutter Ant Parasitoids: Current Knowledge

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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. subterraneus*, 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, *Eisbesfeldtphora* is present in the largest number of countries [9].

Among the four most important genera attacking leaf-cutter ants, *Apocephalus* [26], *Eisbesfeldtphora* [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

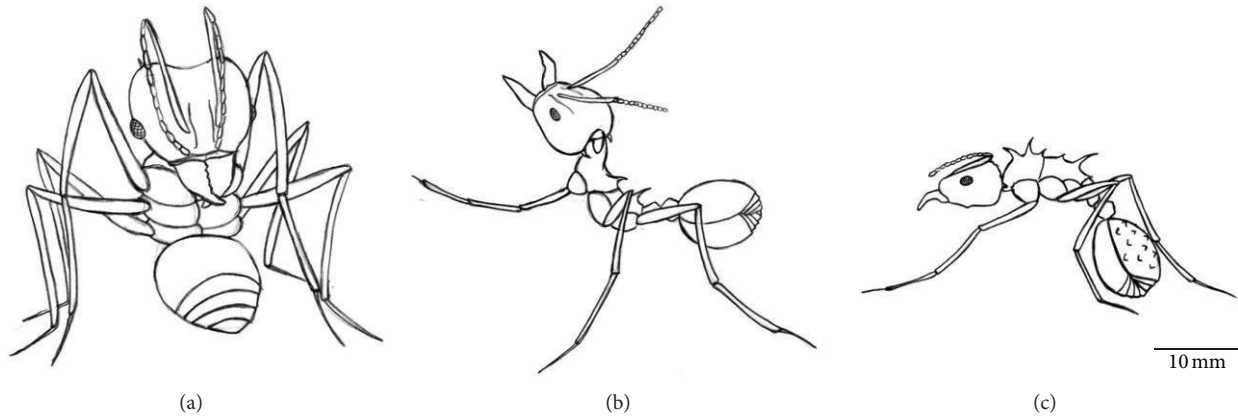


FIGURE 1: *Atta* (a, b) and *Acromyrmex* (c) morphological differences and exhibiting different body postures. (a) shows the C posture, (b) the alarm/attack phorid posture whereas (c) exhibits lowering the abdomen to avoid oviposition at the tip of the gaster.

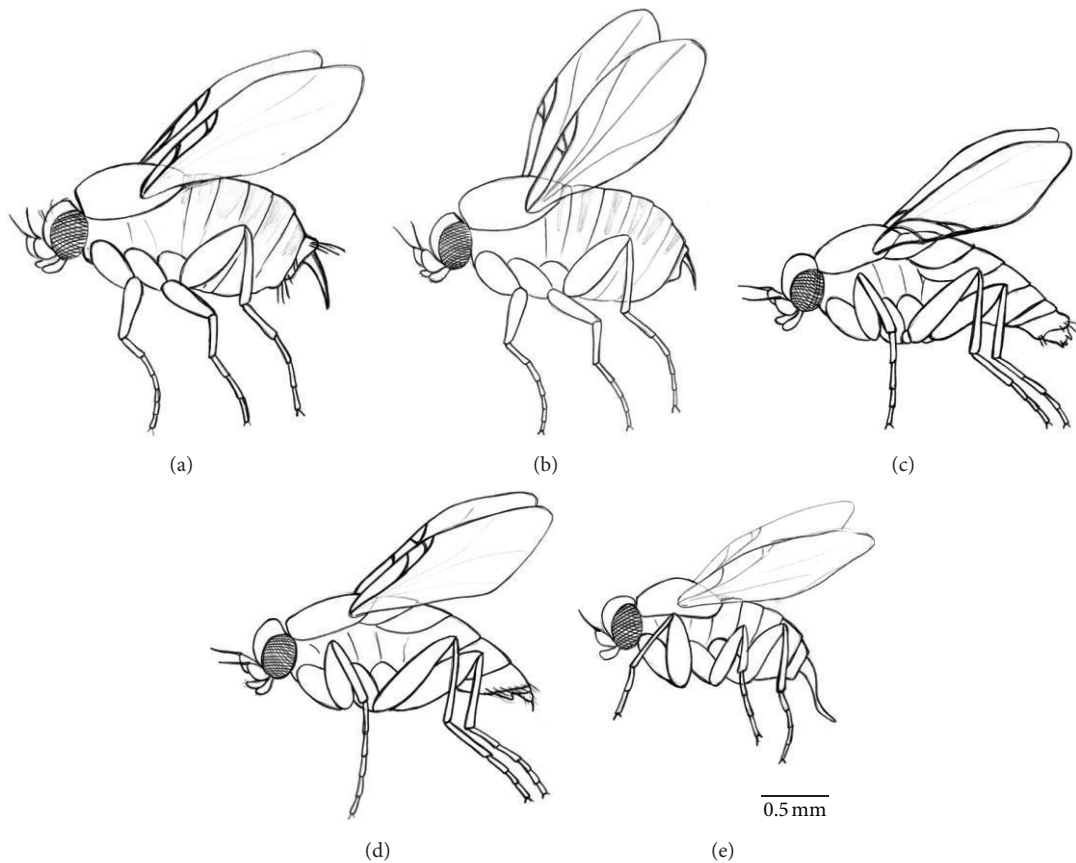


FIGURE 2: Schematic drawings of phorids showing details of the main characters that can be used to easily identify and distinguish among the main genera attacking leaf-cutter ants. Sizes represent real relative differences. (a) *Apocephalus* from the “attophilus” group and (b) from the “grandipalpis” group. (c) *Eibesfeldtphora*, (d) *Neodohniphora*, and (e) *Myrmosciarius*.

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 oviscap [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]. *Neodohniphora* 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 *Myrmosciarius* 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. *Myrmosciarius* 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, *Myrmosciarius* 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. *Neodohniphora* 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 subterranean 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 *Neodohniphora 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,

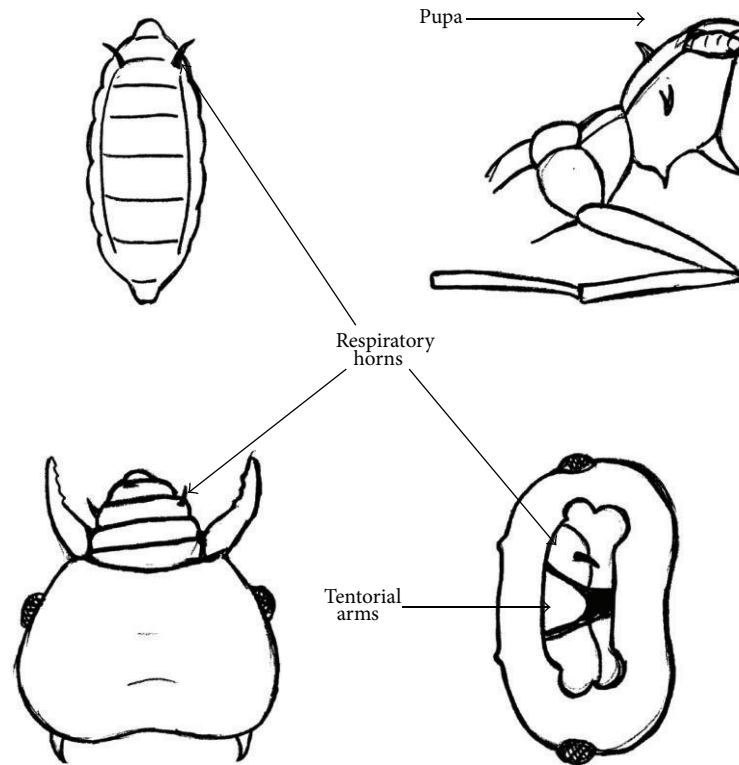


FIGURE 3: Schematic drawings showing different types of pupae according to the parasitoid genus. Top left: Dorsal view of a free pupae from *Apocephalus*, top right: claustral thoracic pupae from *Apocephalus vicosae*, viewed from ventral side, bottom left: claustral pupae from *Eibesfeldtphora* and *Neodohniphora* 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. lundii*, 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]; Guillaude 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/trail, 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) assess 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 parasitize.
- (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.

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Review Article

Rossomyrmex, the Slave-Maker Ants from the Arid Steppe Environments

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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. quadratinodum* 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.

| | <i>Rossomyrmex</i> | <i>Polyergus</i> | <i>F. sanguinea</i> 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. quadratinodum* population: CC = Charyn Canyon) (from [20]).

parasite-host pairs live mostly in extended plains whereas the Spanish pair *R. minuchae*-*P. longiseta* 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. quadratinodum*-*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.

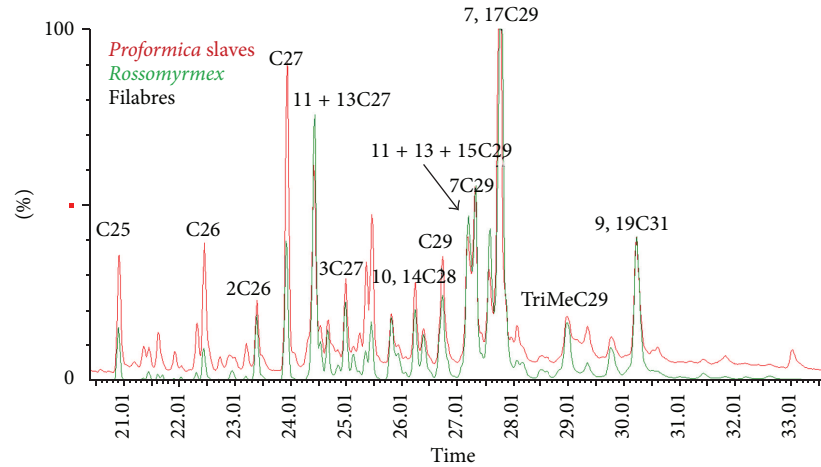


FIGURE 2: CHCs profiles of *R. minuchae* and *P. longiseta* (Sierra de Filabres population). The profiles are superposed to show the similarity between the host and parasite with some differences, for example, in alkanes C25, C26, and C27.

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. quadratinodum* 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. quadratinodum* 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

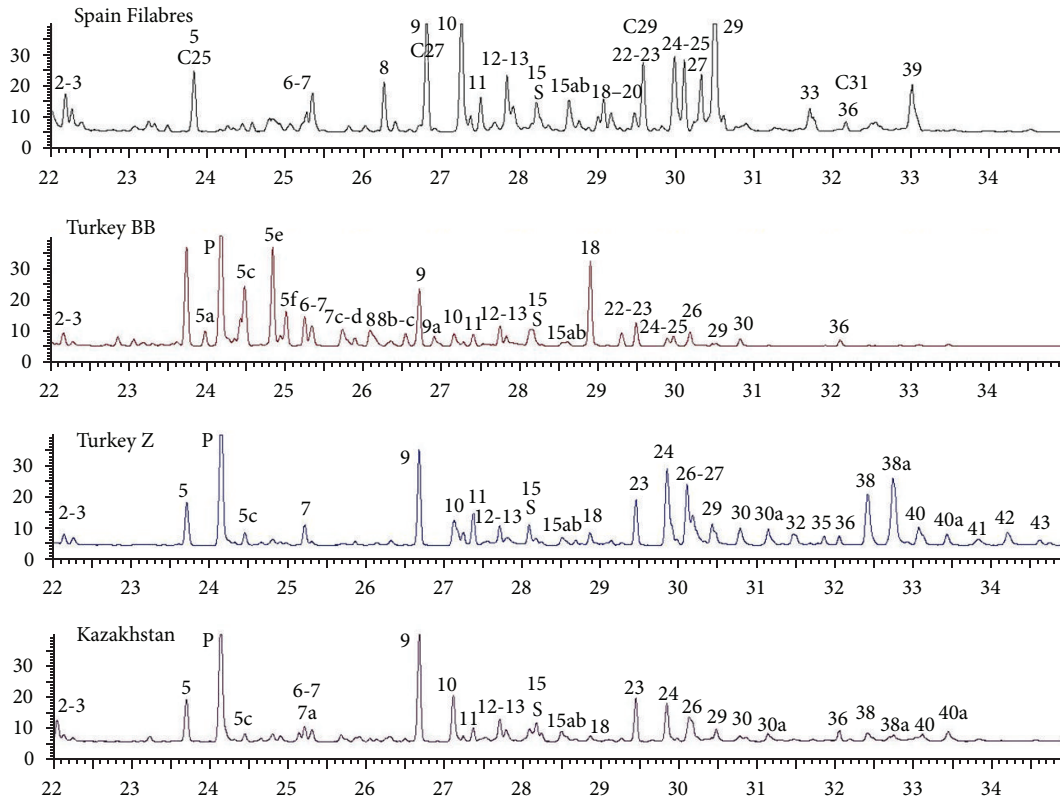


FIGURE 3: CHCs profiles for *R. minuchae* (Filabres), *R. anatolicus* (from two different populations Turkey BB = Belebachi Beli, Turkey Z = Ziyaret Tepesi), and *R. quadratinodum* (Kazakhstan). Numbers refer to original data in [34] (P and S are pollutants).

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. quadratinodum* and *R. proformicarum*-*P. epinotalis*, is required to depict a more general landscape of local adaptation and coevolution in the *Proformica*-*Rossomyrmex* pairs.

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Review Article

A Review of the Biology of Eucharitidae (Hymenoptera: Chalcidoidea) from Argentina

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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, *Pseudabutylon 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 mm² 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, *Isomeralla* 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 | | | |
| <i>Psilocharis</i> Heraty | <i>Psilocharis</i> sp.* | ? | — |
| Eucharitini | | | |
| <i>Colocharis</i> Heraty | <i>Colocharis hungi</i> Torrén | ? | — |
| <i>Dicoelothorax</i> Ashmead | <i>Dicoelothorax parviceps</i> Cameron | ? | — |
| | <i>Dicoelothorax platycerus</i> Ashmead | HP, HA, IS | [22] |
| <i>Dilocantha</i> Shipp | <i>Dilocantha bennetti</i> Heraty | ? | — |
| | <i>Dilocantha flavicornis</i> (Walker) | ? | — |
| <i>Galearia</i> Brullé | <i>Galearia latreillei</i> (Guérin-Méneville) | HP, HA, IS | *** |
| | <i>Kapala argentina</i> Gemignani | ? | — |
| | <i>Kapala chacoensis</i> Gemignani | ? | — |
| <i>Kapala</i> Cameron | <i>Kapala furcata</i> (Fabricius) | HP | [2, 3] |
| | <i>Kapala splendens</i> Ashmead | ? | — |
| | <i>Kapala sulcifacies</i> (Cameron)** | HP, IS | [23, 24] |
| | <i>Latina bonariensis</i> (Gemignani) | ? | — |
| <i>Latina</i> Koçak & Kemal | <i>Latina rugosa</i> (Torrén, Heraty & Fidalgo) | HP, HA, IS | [25], HA*** |
| | <i>Latina vianai</i> (Gemignani) | ? | — |
| <i>Lophyrocera</i> Cameron | <i>Lophyrocera daguerrei</i> (Gemignani) | ? | — |
| | <i>Lophyrocera plagiata</i> (Walker)** | ? | — |
| | <i>Lophyrocera variabilis</i> Torrén, Heraty & Fidalgo | HP, HA, IS | [26] |
| <i>Neolirata</i> Torrén & Heraty | <i>Neolirata alta</i> (Walker) | HP, IS | [15] |
| | <i>Neolirata daguerrei</i> (Gemignani) | HP, IS | [15] |
| | <i>Neolirata furcula</i> Torrén & Heraty | ? | — |
| <i>Obeza</i> Heraty | <i>Obeza maculata</i> (Westwood) | ? | — |
| | <i>Obeza nigriceps</i> (Ashmead) | ? | — |
| | <i>Obeza semifumipennis</i> (Girault)** | ? | — |
| <i>Parakapala</i> Gemignani | <i>Parakapala decarloi</i> Gemignani | ? | — |
| | <i>Pseudochalcura alba</i> Heraty & Heraty | ? | — |
| | <i>Pseudochalcura americana</i> (Howard) | ? | — |
| <i>Pseudochalcura</i> Ashmead | <i>Pseudochalcura frustrata</i> Heraty | ? | — |
| | <i>Pseudochalcura pauca</i> Heraty | ? | — |
| | <i>Pseudochalcura prolata</i> Heraty | ? | — |
| | <i>Thoracantha spegazzinii</i> (Gemignani) | HP | [17] |
| <i>Thoracantha</i> Latreille | <i>Thoracantha striata</i> Perty | HP, IS | [8] |
| Oraseminae | | | |
| <i>Orasema</i> Cameron | <i>Orasema aenea</i> Gahan | HP, HA, IS | *** |
| | <i>Orasema argentina</i> Gemignani | HA | [6, 17] |
| | <i>Orasema deltae</i> Gemignani | ? | — |
| | <i>Orasema freychei</i> (Gemignani) | ? | — |
| | <i>Orasema gemignanii</i> De Santis | ? | — |
| | <i>Orasema salebrosa</i> Heraty | HA | [11, 27] |
| | <i>Orasema simplex</i> Heraty | HA, HP | [11, 28, 29] |
| | <i>Orasema susanae</i> Gemignani | HA | [6] |
| | <i>Orasema vianai</i> Gemignani | ? | — |
| | <i>Orasema worcesteri</i> (Girault) | HA | [17] |
| <i>Orasema xanthopus</i> (Cameron) | HP, HA, IS | [6, 11, 27, 28, 30–39] | |

Abbreviations: * doubtful record [5]; ** new record of presence in Argentina; *** new biological record.

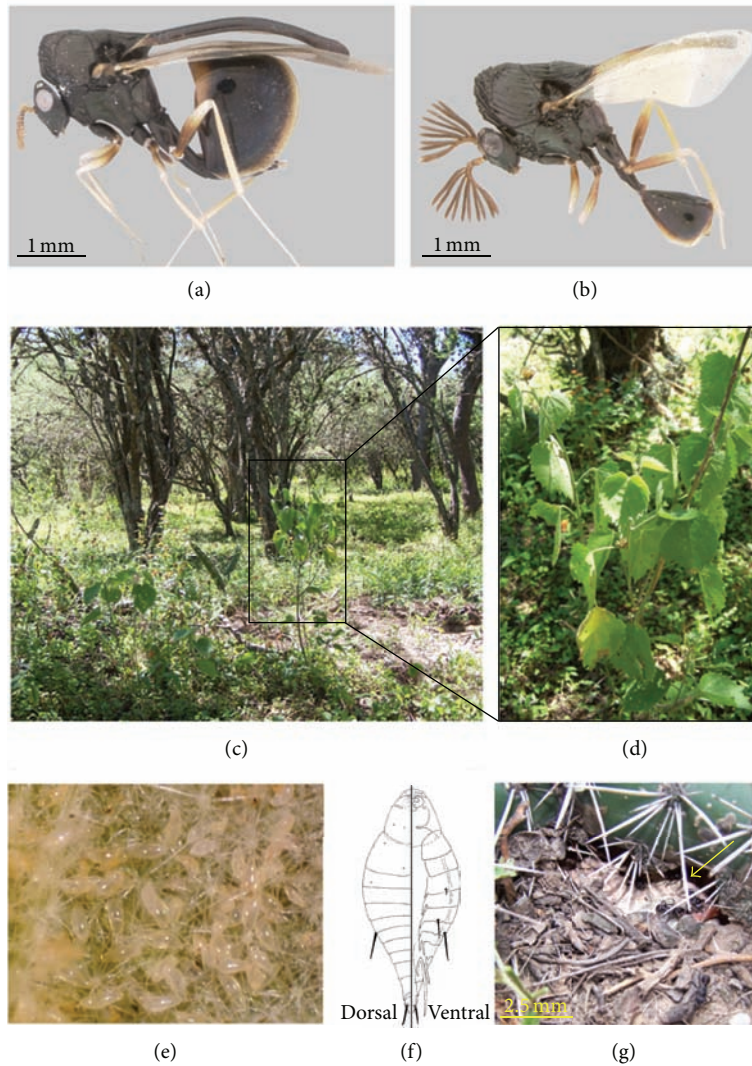


FIGURE 1: *Dicoelothorax platycerus*: (a) habitus (female); (b) habitus (male). Biology and immature stage of *D. platycerus*: (c) habitat; (d) *Pseudabutilon virgatum*; (e) underside of leaf of *P. virgatum* with eggs; (f) planidium (dorsal and ventral); (g) nest entrance of *Ectatomma brunneum* (opening 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 curasavica* (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

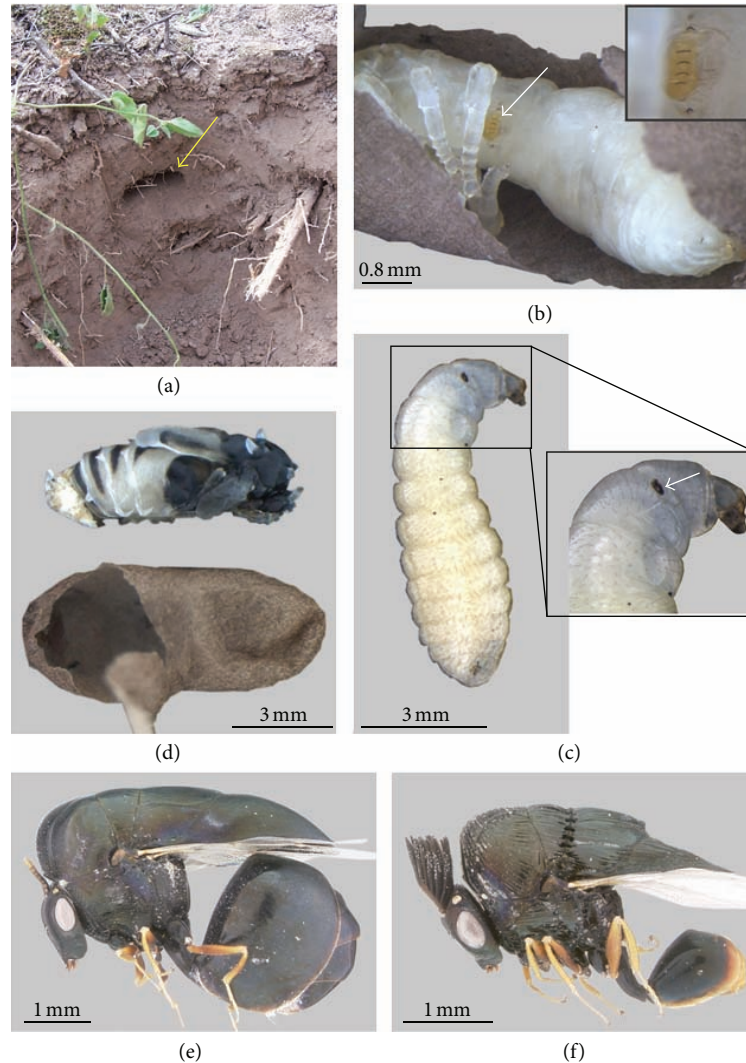


FIGURE 2: Biology and immature stage of *D. platycerus*: (a) brood chamber (indicated); (b) prepupa parasitized (2nd instar larva indicated and magnified); (c) ant larva parasitized (attached planidium magnified and indicated); (d) pupa extracted with ant cocoon (female, lateral). *Galearia latreillei*: (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. guri-ana* (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 Torrén, Heraty, and Fidalgo [25].

3.4.1. *Latina rugosa* (Torrén, 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 mm² 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

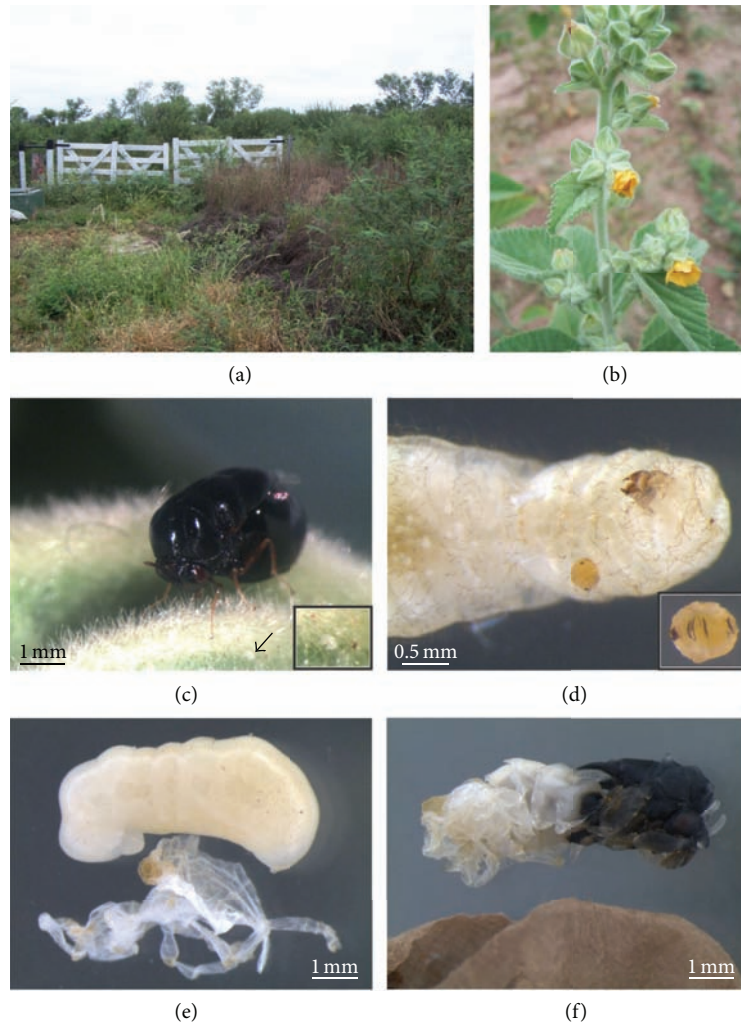


FIGURE 3: Biology and immature stage of *Galearia latreillei*: (a) habitat; (b) *Sida cordifolia*; (c) female of *G. latreillei* ovipositing on leaf of *S. cordifolia* (eggs indicated and magnified); (d) prepupa parasitized (1st instar larva indicated and magnified); (e) third instar (with remains of 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* Torrén 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* Torrén, 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].

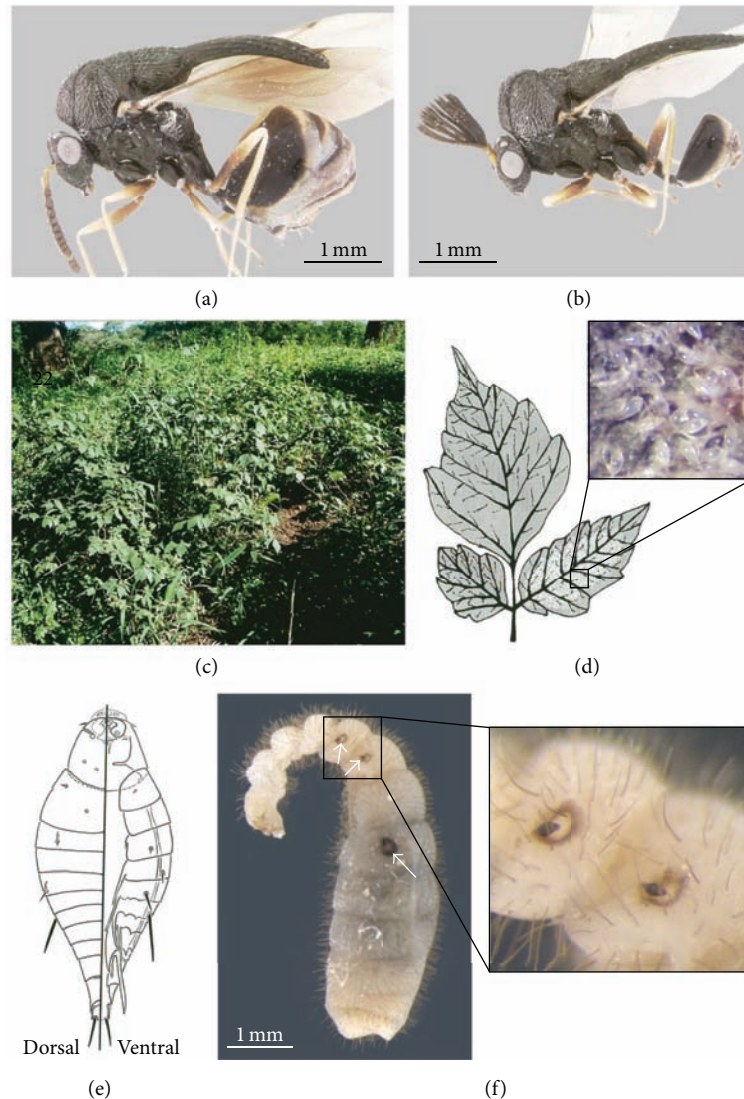


FIGURE 4: *Latina rugosa*: (a) habitus (female); (b) habitus (male). Biology and immature stage of *L. rugosa*: (c) habitat; (d) underside of leaf of *Serjania glabrata* with eggs (eggs represented in white area and magnified); (e) planidium (dorsal and ventral view); (f) ant larva parasitized (attached 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* Torr ns 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* Torr ns and Heraty (Brazil) [15].

Neolirata alta and *N. daguerrei* were collected in north-western Argentina, and their taxonomic and biological information is given in Torr ns and Heraty [15].

3.6.1. *Neolirata alta* (Walker). Habitat and location are as follows. Specimens were collected in Los Ba os and Rosario de la Frontera (Salta) and Tapia and San Vicente (Tucum n). In Los Ba os, the vegetation corresponds to the transition

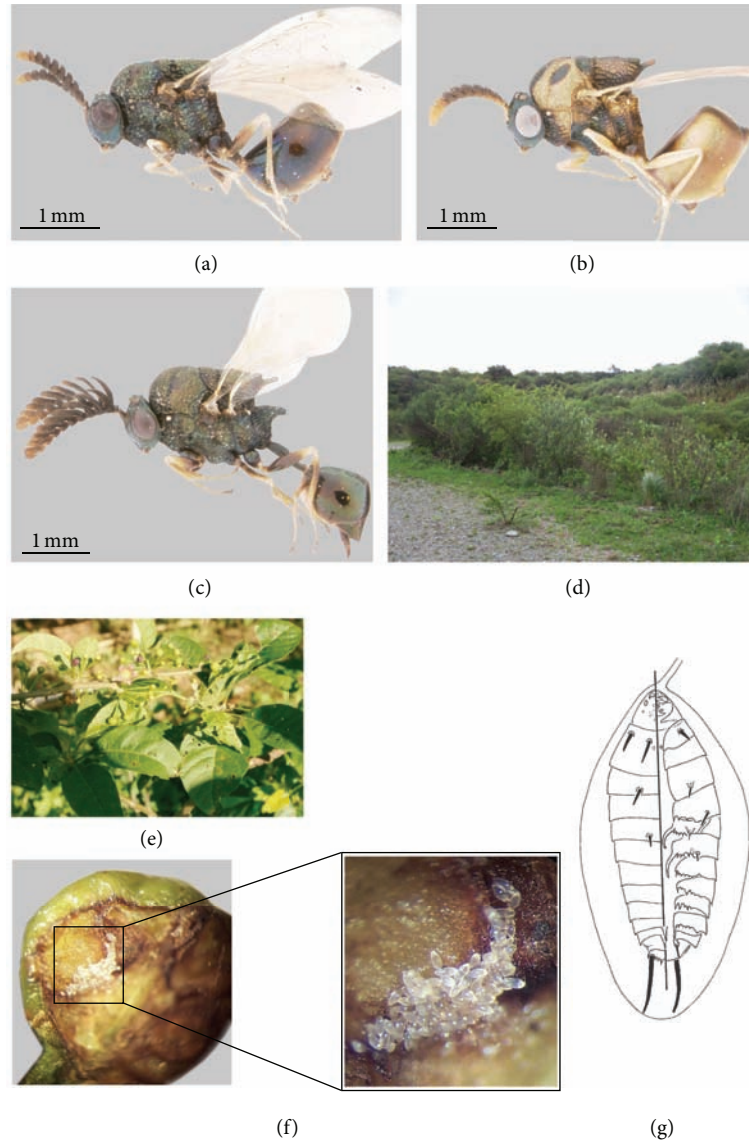


FIGURE 5: *Lophyrocera variabilis*: (a) and (b) habitus (female); (c) habitus (male). Biology and immature stages of *L. variabilis*: (d) habitat; (e) *Vasobia breviflora*; (f) saggittal section of unripe fruits of *V. breviflora* with eggs (egg mass magnified); (g) planidium (before hatching; dorsal 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, *Pseud-abutilon 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:

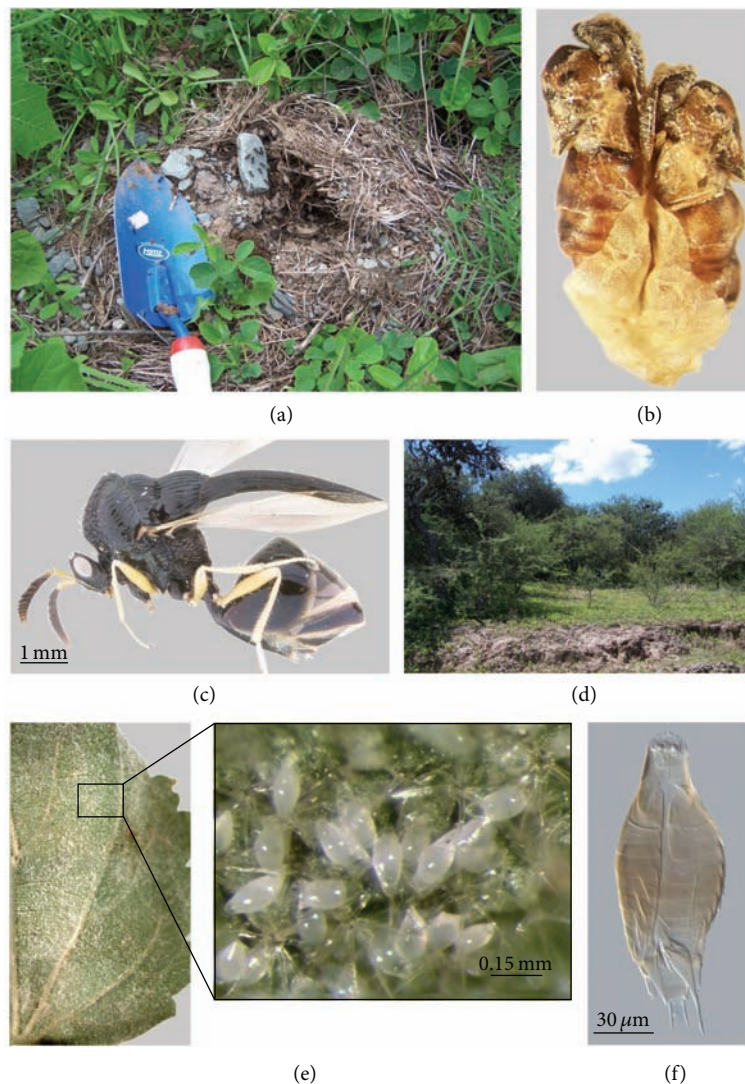


FIGURE 6: Biology and immature stage of *Lophyrocera variabilis*: (a) ant nest of *Camponotus* sp.; (b) two *Lophyrocera* pupae extracted from same ant cocoon (females). *Neolirata alta*: (c) habitus (female). Biology and immature stage of *N. alta*: (d) habitat; (e) underside of leaf of *P. virgatum* 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.

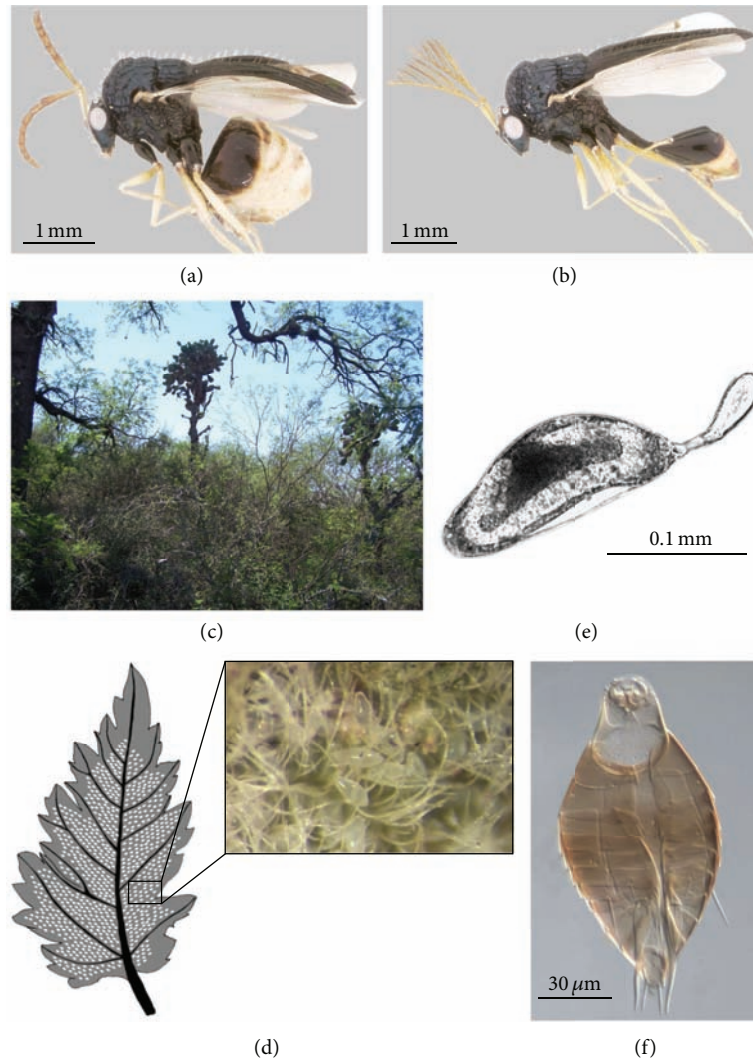


FIGURE 7: *Neolirata daguerrei*: (a) habitus (female); (b) habitus (male). Biology and immature stage of *N. daguerrei*: (c) habitat; (d) underside of leaf of *Urvillea chacoensis* with eggs (eggs represented in white area and magnified); (e) egg; (f) planidia. Figures extracted from [15].

Although host ants were not located in the area, the host has been reported as *Solenopsis quinquecupis* 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. quinquecupis* (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].

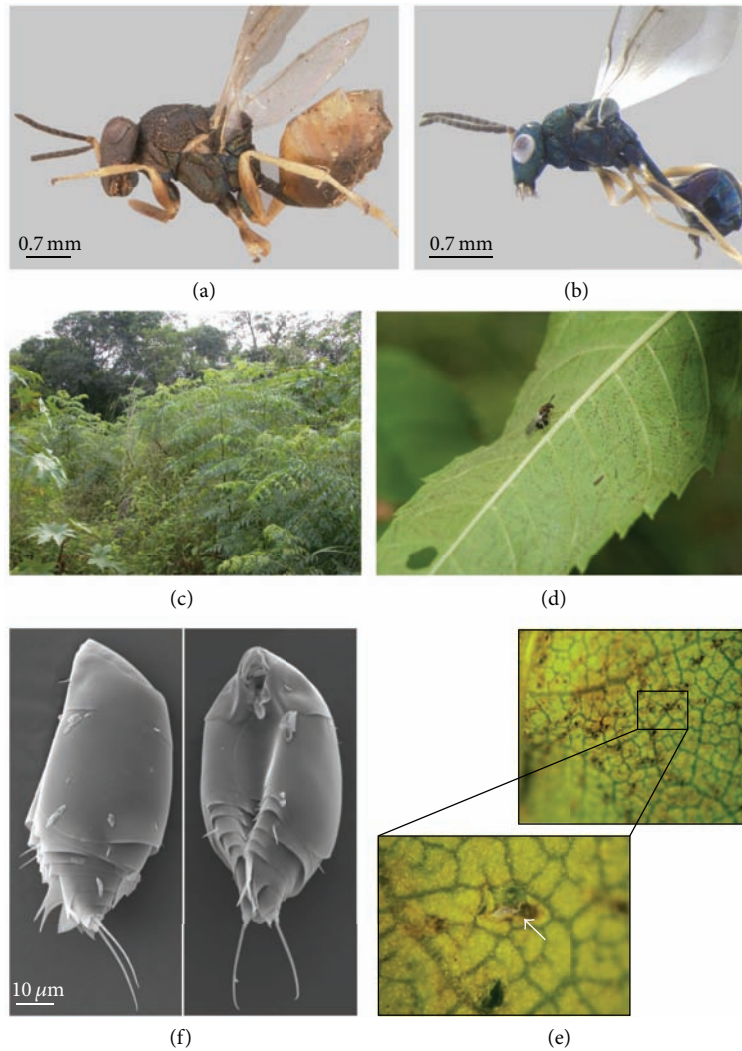


FIGURE 8: *Orasema aenea*: (a) habitus (female); (b) habitus (male). Biology and immature stage of *O. aenea*: (c) habitat; (d) female of *O. aenea* ovipositing on leaf of *Tecoma stans*; (e) underside of leaf of *T. stans* with incisions (magnified area with egg extracted from incision); (f) planidia (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

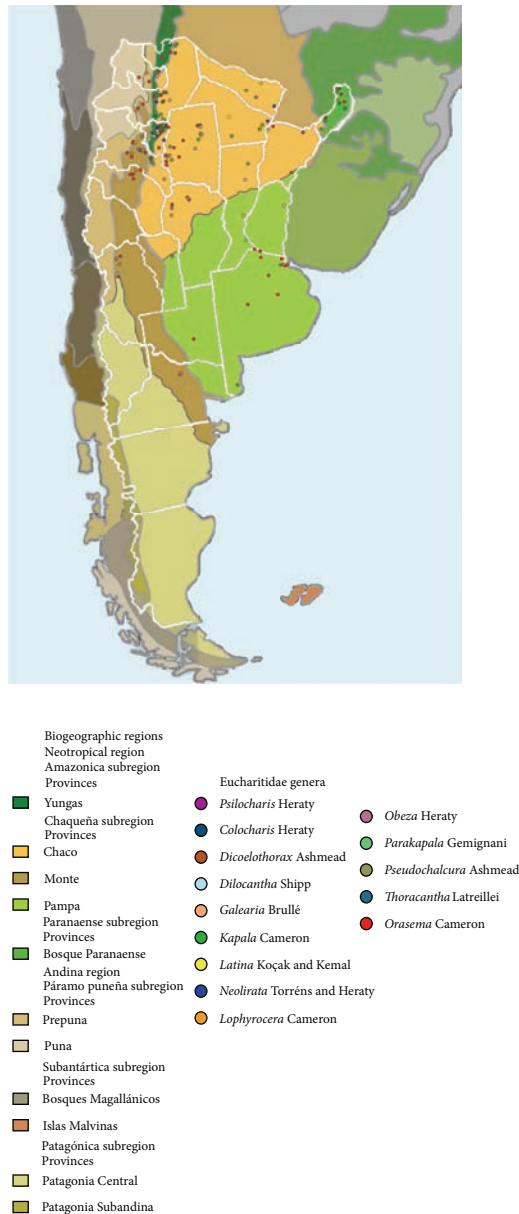


FIGURE 9: Distribution of genera of Eucharitidae in Argentina.

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 Torrén 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 scarring 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.

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Research Article

Discrimination of the Social Parasite *Ectatomma parasiticum* by Its Host Sibling Species (*E. tuberculatum*)

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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 ($T = 28 \pm 2^\circ\text{C}$, 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

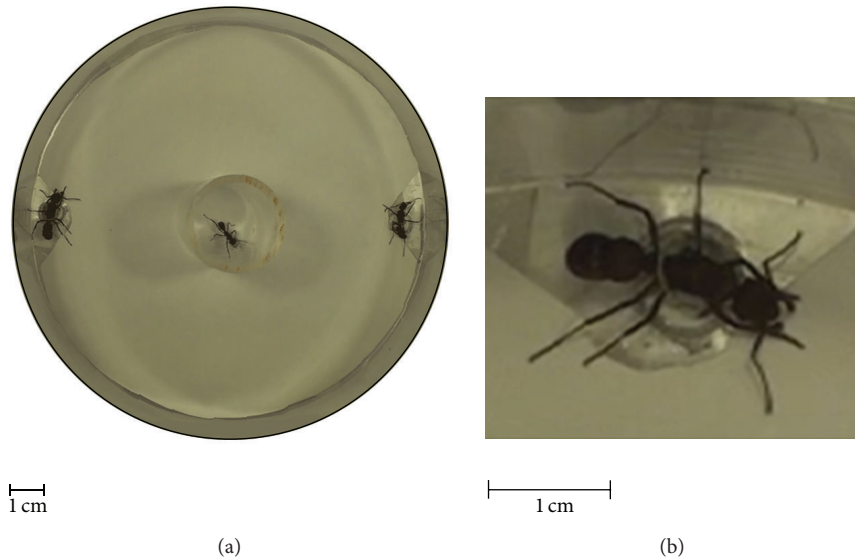


FIGURE 1: The experimental device used for discrimination tests. (a) Overview of the device composed of a round plastic box (11.8 cm diameter) and two fixation systems. The test-worker faced two immobilized stimuli-ants, here a parasite and a conspecific queen. (b) Detailed view of the fixation system 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

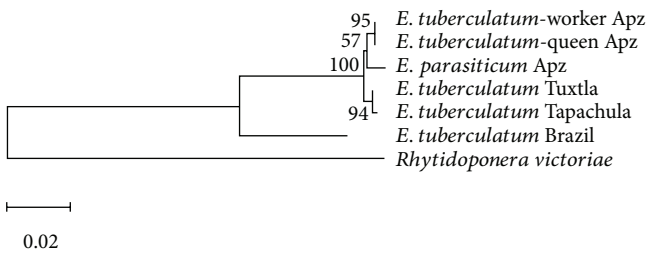


FIGURE 2: Neighbor-joining tree for the different populations. Bootstrap values (1000 replicates) are shown for each node.

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. Intracolony 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

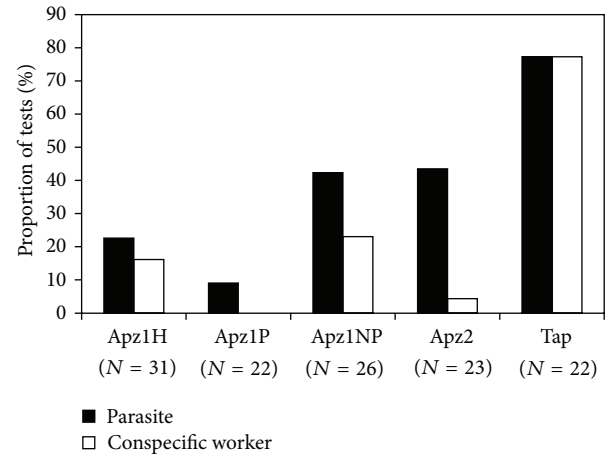


FIGURE 3: Proportions of tests including aggression towards the social parasite or the conspecific worker according to the type of tests. Apz1H = homocolonial tests, Apz1P = tests between nonnestmates from parasitized colonies, Apz1NP = tests between nonnestmates from parasitized and nonparasitized colonies of the same site, Apz2 = tests between sites, Tap = tests between populations, and N = number of tests.

1 transversion, 0.81%) whereas the parasite diverged from Tapachula colonies by 9 variable sites (8 transitions and 1 transversion, 1.08%).

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.

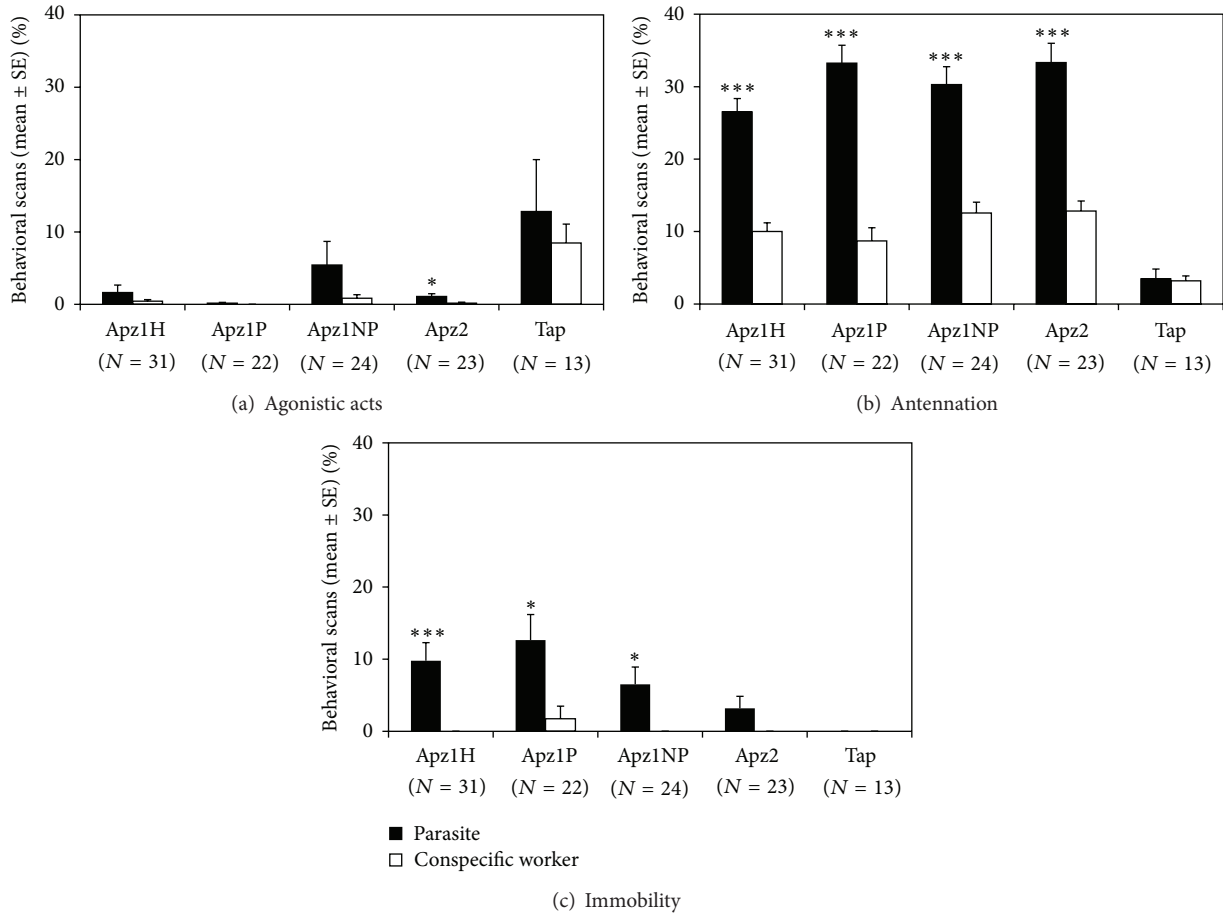


FIGURE 4: Comparison of the behavioral reactions towards the social parasite (black bars) and the conspecific worker (white bars) in the different types of tests (see Figure 3 for the abbreviations). Pairwise comparisons were made with Permutation tests: * $P < 0.05$, *** $P < 0.001$. N = number of tests.

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 | | |
|------------------|-----------|---------------|---------------|-----------|---------------|----------------|-----------|---------------|--------------|
| | Collected | N of colonies | N of workers | Collected | N of colonies | N of workers | Collected | N of colonies | N of workers |
| September 1999 | 3 | 3 | 51 (12–120) | — | — | — | — | — | — |
| June–July 2000 | 21 | 12 | 118 (15–428) | — | — | — | — | — | — |
| March–April 2002 | 19 | 0 | 178 (64–358) | 7 | 0 | 146 (55–243) | 6 | 0 | 139 (51–225) |
| January 2009 | 8 | 0 (3*) | 263 (163–341) | 15 | 0 | 198 (46–287) | — | — | — |
| September 2010** | — | — | — | 8 | 0 | 68 (47–150) | — | — | — |
| November 2011 | 4 | 0 | 162 (95–383) | 4 | 0 | 242 (74–254) | 3 | 0 | 94 (57–148) |
| Total | 55 | 18 | 157 (12–428) | 34 | 0 | 146.5 (46–287) | 9 | 0 | 99 (51–225) |

—: no sampled data; * emergence of parasites from larvae collected in field; ** data from Pérez-Lachaud et al. [38].

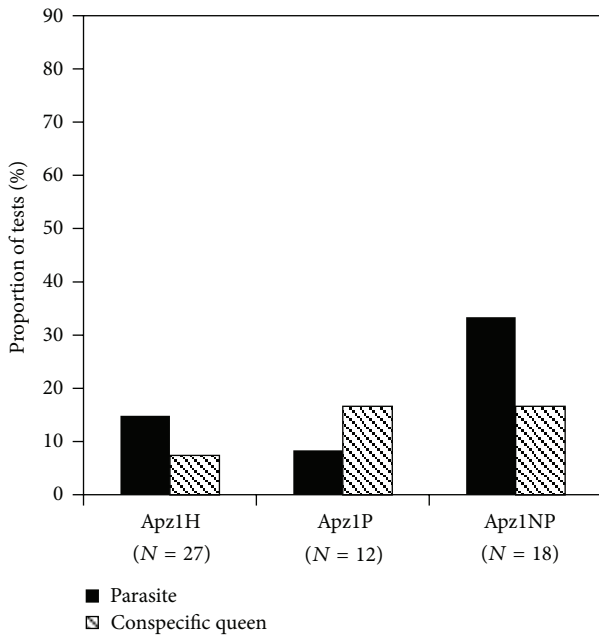


FIGURE 5: Proportions of tests including aggression towards the social parasite or the conspecific queen according to the type of tests (see Figure 3 for the abbreviations). N = number of tests.

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 acceptance 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.

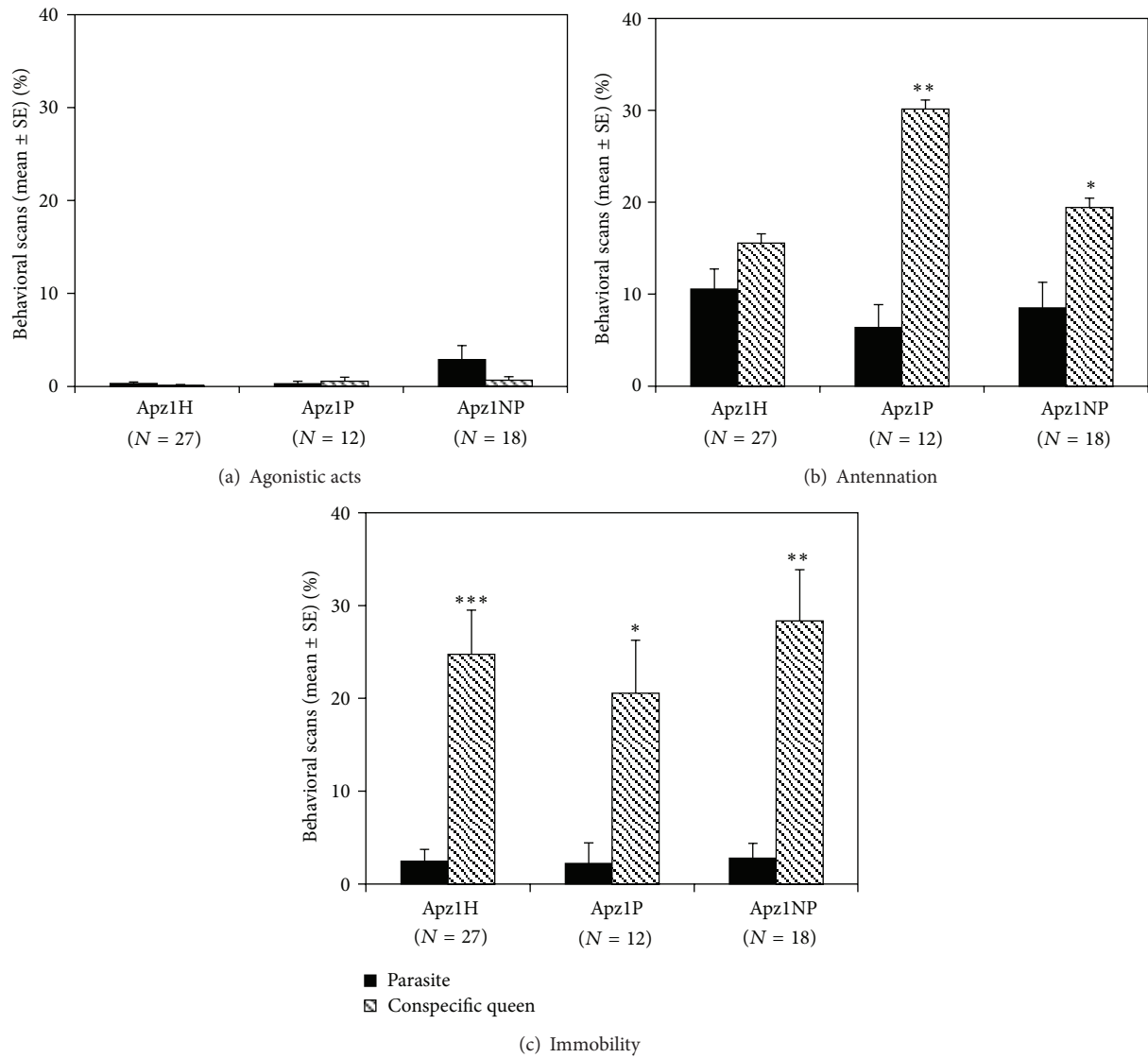


FIGURE 6: Comparison of the behavioral reactions towards the social parasite (black bars) and the conspecific queen (dashed bars) in the different types of tests (see Figure 3 for the abbreviations). Pairwise comparisons were made with Permutation tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. 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, intracolony 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.

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Review Article

Nesting Associations without Interdependence: A Preliminary Review on Plesiobiosis in Ants

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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 plesio-biotic 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 plesio-biosis [5, 6, 22]. According to the classical definition, plesio-biotic 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 outweigh 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. Plesio-biotic Association

Regarding the lack of biotic interdependence between the associated colonies [1, 5, 6, 12, 30], plesio-biosis 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 plesio-biotic colonies occupy the same nesting shelter (e.g., in or under logs, stumps, rocks, etc.). On the basis of the currently available data on plesio-biotic associations, this close nesting can occur occasionally or regularly, depending on the species and/or habitat type (as discussed below). Although plesio-biotic 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]. Plesio-bionts 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, plesio-biotic 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 plesio-biotic relationship in order to avoid intraspecific competition.

4. A Synthesis of the Recorded Cases of Plesio-biosis

4.1. Plesio-bionts and Plesio-biotic Partners. In Table 1, we list 49 species that have been observed so far in plesio-biotic 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 plesio-biotic 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 plesio-biosis revealed that two species, *Formica fusca* (Linnaeus, 1758) and *Lasius flavus* (Fabricius, 1782), can be considered as two of the most frequent plesio-bionts, on the basis of the total number of their so far known plesio-biotic partner species (Table 1).

Up to the present, at least 48 different plesio-biotic 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 plesio-biotic associations—where the exact number of the observed cases was given—was 69, from which the two most frequent plesio-bionts participated in 46 associations, *F. fusca* in 28 cases (60.9%) and *L. flavus* in 18 cases (39.1%) (Table 1). *F. fusca* established plesio-biotic associations with species belonging to 6 different genera of two subfamilies (Myrmicinae and Formicinae). Its typical plesio-biotic 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*). Plesio-biotic 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 | <i>Formica fusca</i> - <i>Myrmica rubra</i> | 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 | <i>Formica fusca</i> - <i>Myrmica ruginodis</i> | Poland | Clearcut of managed forest | In tree stumps | 1 | Włodarczyk et al. 2009 [36] |
| 3 | <i>Formica fusca</i> - <i>Tetramorium caespitum</i> | Poland | Clearcut of managed forest | In tree stumps | 3 | Włodarczyk et al. 2009 [36] |
| 4 | <i>Formica fusca</i> - <i>Leptothorax acervorum</i> | Finland | Different successional series of rocky habitats | Mound of <i>F. lugubris</i> | 1 | Czechowski 2004 [34] |
| 5 | <i>Formica fusca</i> - <i>Lasius flavus</i> | Finland | Different successional series of rocky habitats | Under wood; in rock crevice; under stone | 4 | Czechowski 2004 [34] |
| 6 | <i>Formica fusca</i> - <i>Lasius platythorax</i> | Finland; Poland | Forest on rocks; clearcut of managed forest | In decaying wood; in tree stump | 3 | Włodarczyk et al. 2009 [36] |
| 7 | <i>Formica fusca</i> - <i>Lasius niger</i> | UK | Foreshore | Under stone | ? | Morley 1945 [25] |
| 8 | <i>Formica fusca</i> - <i>Camponotus herculeanus</i> | Poland | Forest edge | Under wood | 1 | Czechowski 2005 [35] |
| 9 | <i>Formica fusca</i> - <i>Camponotus vagus</i> | Hungary | Pine and poplar forest patches | In/under wood | 10 | Kanizsai (unpubl.) |
| 10 | <i>Formica fusca</i> - <i>Formica lugubris</i> | Finland | Different successional series of rocky habitats | Mound of <i>F. lugubris</i> | 1 | Czechowski 2004 [34] |
| 11 | <i>Formica fusca</i> - <i>Formica aquilonia</i> | Finland | Forest on rocks | Mound of <i>F. lugubris</i> | 1 | Czechowski and Vepsäläinen 1999 [29] |
| 12 | <i>Formica fusca</i> - <i>Formica truncorum</i> | Finland | Different successional series of rocky habitats | In rock crevice | 1 | Czechowski 2004 [34] |
| 13 | <i>Lasius flavus</i> - <i>Formica cunicularia</i> | UK | Foreshore | Under stone | ? | Morley 1945 [25] |
| 14 | <i>Lasius flavus</i> - <i>Formica aquilonia</i> | Finland | Different successional series of rocky habitats | Mound of <i>F. aquilonia</i> | 1 | Czechowski 2004 [34] |
| 15 | <i>Lasius flavus</i> - <i>Formica fuscocinerea</i> | Poland | Grassy mountain slope | Under stone | 1 | Czechowski & Czechowska 2000 [30] |
| 16 | <i>Lasius flavus</i> - <i>Tetramorium caespitum</i> | Finland | Different successional series of rocky habitats | Under stone | 1 | Czechowski 2004 [34] |
| 17 | <i>Lasius flavus</i> - <i>Myrmica scabrinodis</i> | UK | Foreshore | Under stone | ? | Morley 1945 [25] |
| 18 | <i>Lasius flavus</i> - <i>Lasius niger</i> | Finland; UK | Rocky outcrop; shore meadow, foreshore | In rock crevice/under stone | 12; ? | Czechowski 2004 [34], Morley 1945 [25] |
| 19 | <i>Lasius flavus</i> - <i>Lasius platythorax</i> | Finland | Different successional series of rocky habitats | In rock crevice/under stone/overgrown soil | 3 | Czechowski 2004 [34] |
| 20 | <i>Monomorium minimum</i> - <i>Pachycondyla harpax</i> | USA | ? | ? | ? | Wheeler 1901 [5] |
| 21 | <i>Monomorium minimum</i> - <i>Pogonomyrmex barbatus</i> | USA | ? | ? | ? | Wheeler 1901 [5] |
| 22 | <i>Monomorium minimum</i> - <i>Camponotus festinatus</i> | USA | ? | ? | ? | Wheeler 1901 [5] |
| 23 | <i>Monomorium minimum</i> - <i>Camponotus sansabeanus</i> | USA | ? | ? | ? | Wheeler 1901 [5] |
| 24 | <i>Monomorium minimum</i> - <i>Formica gnava</i> | 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 | <i>Myrmecina americana-Myrmica pinetorum</i> | USA | ? | In the sand | 1 | Wheeler 1905 [21] |
| 26 | <i>Myrmecina americana-Pheidole picea</i> | USA | ? | ? | ? | Wheeler 1901 [5] |
| 27 | <i>Myrmecina americana-Ponera pennsylvanica</i> | USA | ? | ? | ? | Wheeler 1901 [5] |
| 28 | <i>Myrmecina americana-Formica gnava</i> | USA | ? | Under stone | 1 | Wheeler 1901 [5] |
| 29 | <i>Myrmica rubra-Lasius niger</i> | Finland | Shore meadow; at road | Under stone; between asphalt edge and grass | 2 | Czechowski 2004 [34] |
| 30 | <i>Myrmica rubra-Lasius platythorax</i> | Finland | Forest | In decaying wood | 1 | Czechowski 2004 [34] |
| 31 | <i>Myrmica rubra-Leptothorax muscorum</i> | Finland | Shore meadow | Under stone | 1 | Czechowski 2004 [34] |
| 32 | <i>Lasius umbratus-Formica sanguinea</i> | Poland | Clearings in a pine forest | In the sandy soil | 1 | Czechowski & Rotkiewicz 1997 [27] |
| 33 | <i>Lasius umbratus-Polyergus rufescens</i> | Poland | Clearings in a pine forest | In the sandy soil | 1 | Czechowski & Rotkiewicz 1997 [27] |
| 34 | <i>Lasius umbratus-Lasius sabularum</i> | Poland | Stand of oak trees | Under stone | 1 | Borowiec 2011 [40] |
| 35 | <i>Pheidole picea-Lasius minutus</i> | USA | Hardwood forest | In a stump | 1 | Gaige 1914 [23] |
| 36 | <i>Pheidole picea-Lasius nearcticus</i> | USA | Hardwood forest | Under rock | 1 | Gaige 1914 [23] |
| 37 | <i>Camponotus fallax-Lasius brunneus</i> | Poland | Urban park | In decaying wood | 1 | Czechowski 2004 [33] |
| 38 | <i>Camponotus herculeanus-Lasius platythorax</i> | Finland | ? | In decaying wood | 1 | Czechowski 2004 [33] |
| 39 | <i>Camponotus yogi-Temnothorax andrei</i> | USA | Chaparral | In living stems of <i>Haploppappus pinifolius</i> | 1 | Creighton & Snelling 1966 [26] |
| 40 | <i>Camponotus modoc-Leptothorax calderoni</i> | USA | Pine forest | In log/in stump | ? | Wheeler 1917 [24] |
| 41 | <i>Camponotus pennsylvanicus-Formica subaenescens</i> | USA | Hardwood forest | Under log | 1 | Gaige 1914 [23] |
| 42 | <i>Camponotus festinatus-Pachycondyla harpax</i> | USA | At road | Under stone | 2 | Wheeler 1901 [5] |
| 43 | <i>Camponotus sansabeanus-Pachycondyla harpax</i> | USA | ? | ? | ? | Wheeler 1901 [5] |
| 44 | <i>Camponotus ligniperdus-Aphaenogaster subterranea</i> | Hungary | Pine forest | Under stone | 3 | Lőrinczi (unpubl.) |
| 45 | <i>Formica japonica-Tetramorium tsushimae</i> | Japan | Urban area | In the soil | 1 | Czechowski & Yamauchi 1998 [28] |
| 46 | <i>Formica rufa-Leptothorax muscorum</i> | Sweden | ? | ? | ? | Wheeler 1901 [5] |
| 47 | <i>Myrmecina graminicola-Ponera coarctata</i> | Hungary | Pine forest | Under stone | 2 | Lőrinczi (unpubl.) |
| 48 | <i>Strumigenys pergandei-Formica</i> 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 plesio-biotic colonies and force the relocation of one of the associated plesio-bionts.

(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 plesio-biotic 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 plesio-biotic 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,” plesio-biotic 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 plesio-biosis emphasise the importance of the close proximity of plesio-biotic 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 plesio-biotic 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 plesio-biotic relationship.

(4) *Plesio-biotic associations of arboreal species.* Most of the recorded cases of plesio-biotic 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 plesio-biotic 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 plesio-biosis.

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Review Article

Ant-Mimicking Spiders: Strategies for Living with Social Insects

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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 reinforce 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*

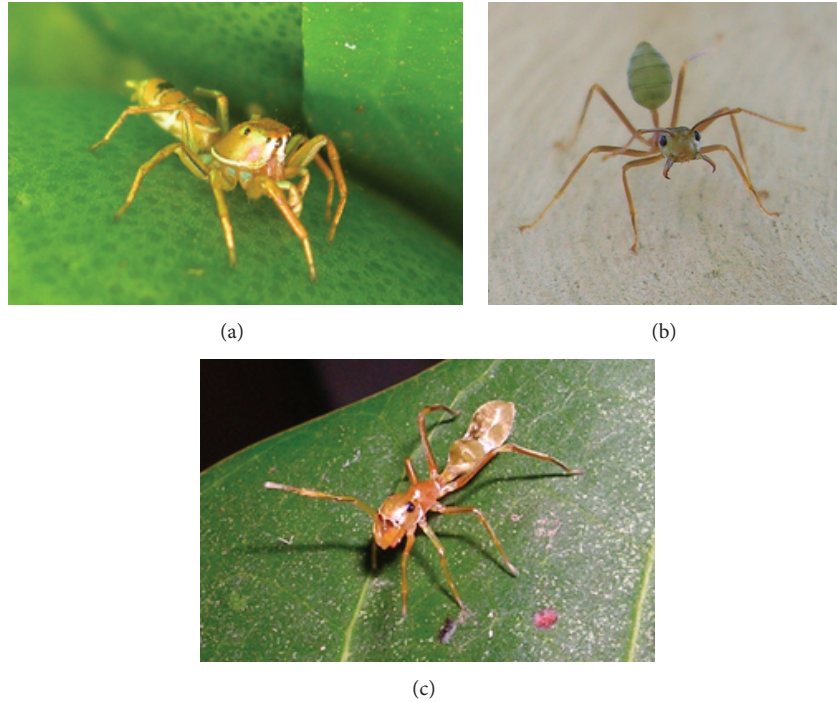


FIGURE 1: Ant-associating salticids (a) *Cosmophasis bitaeniata*, chemical, aggressive myrmecophile and (c) *Myrmarachne smaragdina*, myrmecomorphic Batesian mimic, and their common model ant species (b) *Oecophylla smaragdina* in an aggressive display posture.

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.

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Research Article

New Host Record for *Camponotophilus delvarei* (Hymenoptera: Eurytomidae), a Parasitoid of Microdentine Larvae (Diptera: Syrphidae), Associated with the Ant *Camponotus* sp. aff. *textor*

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Microdentine 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 microdentine 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 microdentine 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 Microdentinae, 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 Microdentinae 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: Microdentinae, Eristalinae, and Syrphinae [11, 12], with Microdentinae 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 microdentine 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 microdentinae associated with ants include taxa known only from the immature stages. Presently, only 126 of 454 valid species of Microdentinae 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 microdentine species.”

Members of the Microdentinae 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 μ 1020) 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).

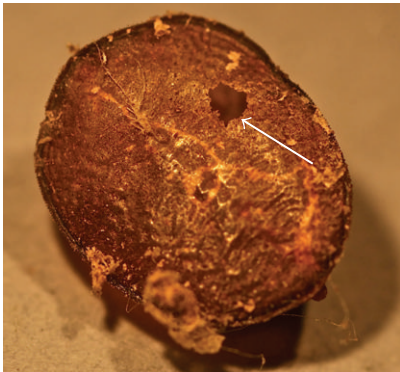
Of the parasitized puparia, two presented an exit hole on their dorsal surface (Figure 2(a)), from which wasp parasitoids had already emerged. Another puparium contained 31 *C. delvarei* pupae (29 females; 2 males). These pupae filled the entire space inside the host puparium (Figure 3). Another parasitized 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 microdentine 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 Microdentinae in Uganda [31]. The associated ant for *E. ugandensis* is unknown, but both eulophids are gregarious endoparasitoids of larvae of Microdentinae 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 microdentine 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].

Our record is thus the fourth reliable report of a parasitoid attacking Microdentinae. From our observations, it could be concluded that *Camponotophilus delvarei* is a gregarious, primary ectoparasitoid of larvae and pupae of microdentine 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 microdentinae [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. Microdentine 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



FIGURE 4: The silk nest of the weaver ant host *Camponotus* sp. aff. *textor*. Photo: G. Pérez-Lachaud.

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 microdentine 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 aztecica* (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 microdentine 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 microdentine 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.

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Review Article

Evaluating Alpha and Beta Taxonomy in Ant-Nest Beetles (Coleoptera, Carabidae, Paussini)

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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 uncertainty about their relationships with other Paussinae lineages [6, 7]. These studies have prompted our knowledge of Paussini biology,

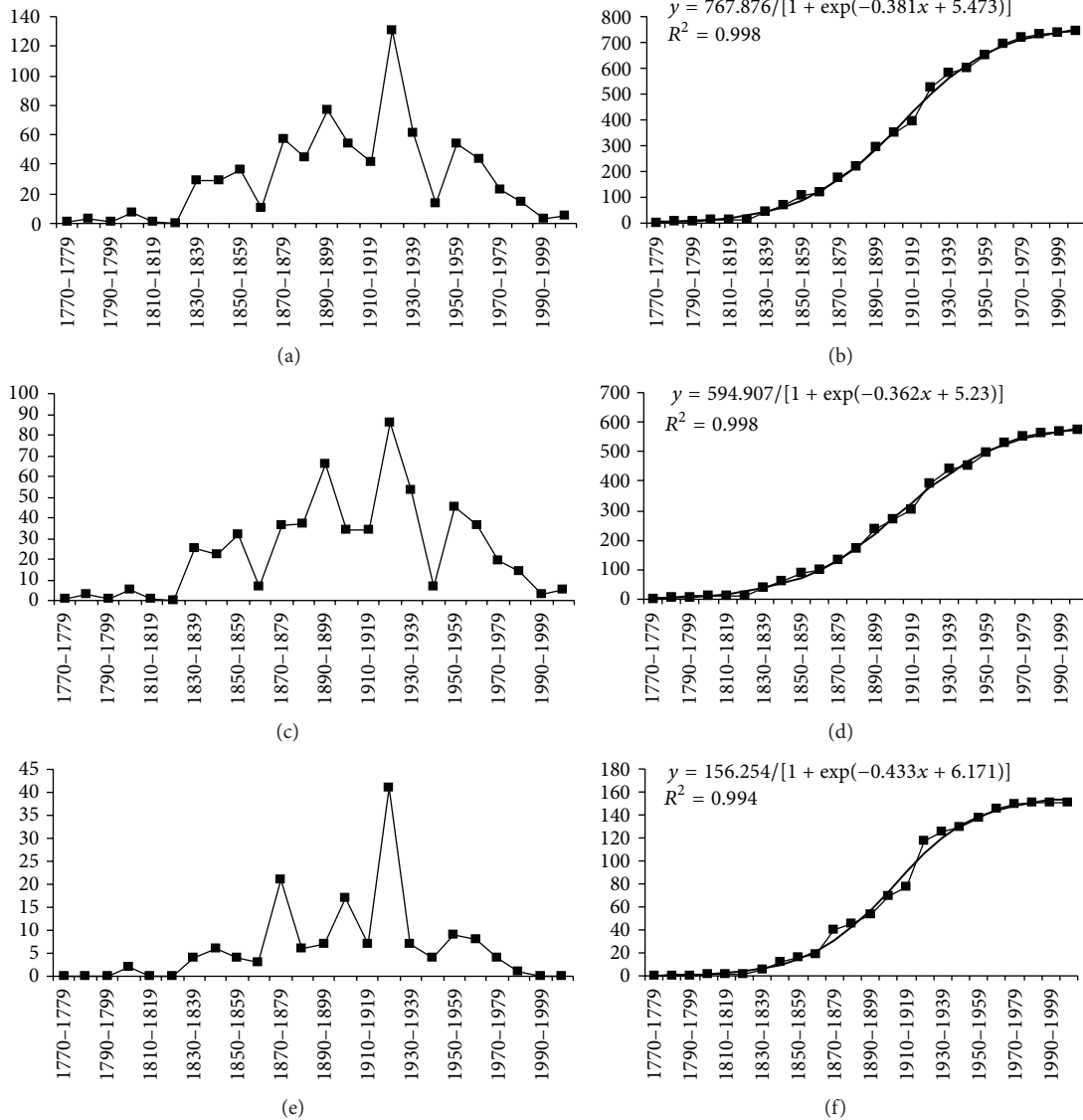


FIGURE 1: Numbers of total described taxa (a, b), valid species (c, d), and synonyms (e, f) of Paussini by decade. Figures (a), (c), and (e) report the absolute numbers, and Figures (b), (d), and (f) the cumulative numbers along with the equations of the fitted curves.

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

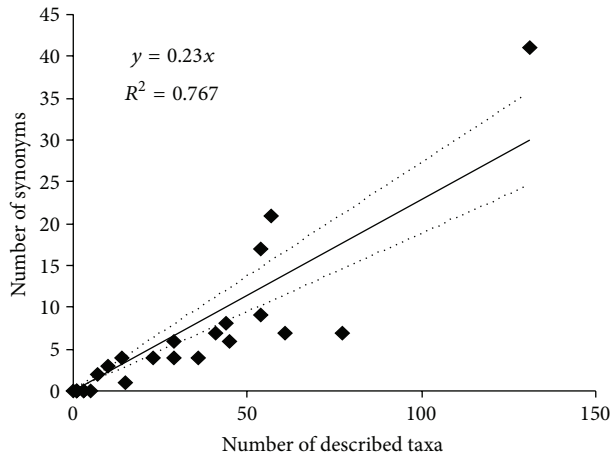


FIGURE 2: Relationship between number of synonyms and total number of described taxa per decade. Ordinary least square (OLS) regression forced to pass through the origin.

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

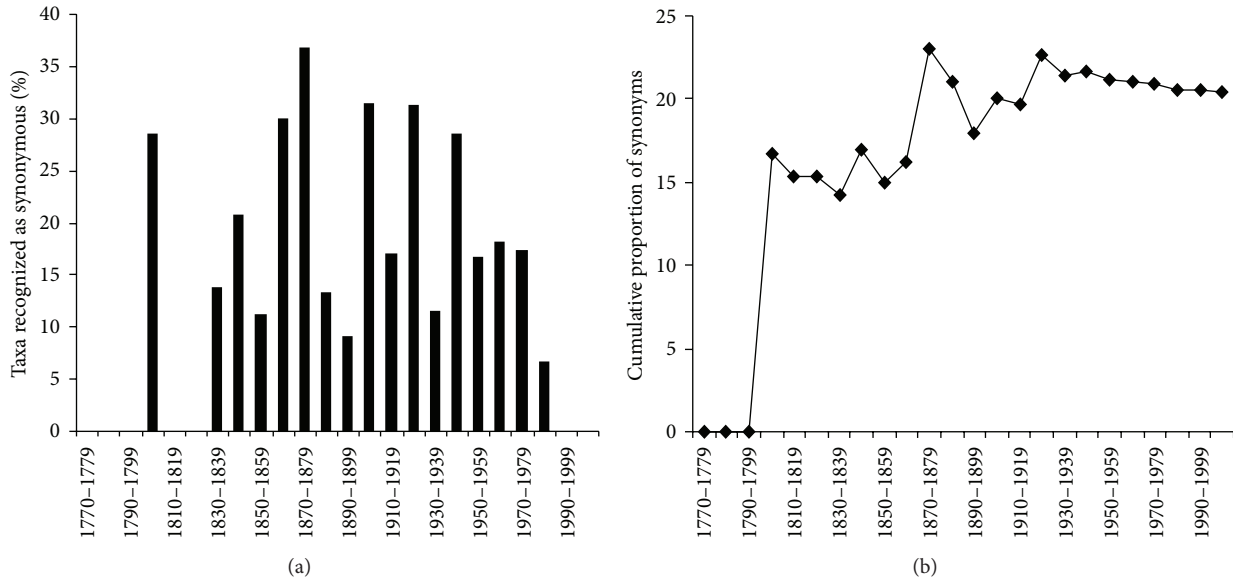


FIGURE 3: Percentages of synonymous taxa described in each decade (a) and their historical process of accumulation (b).

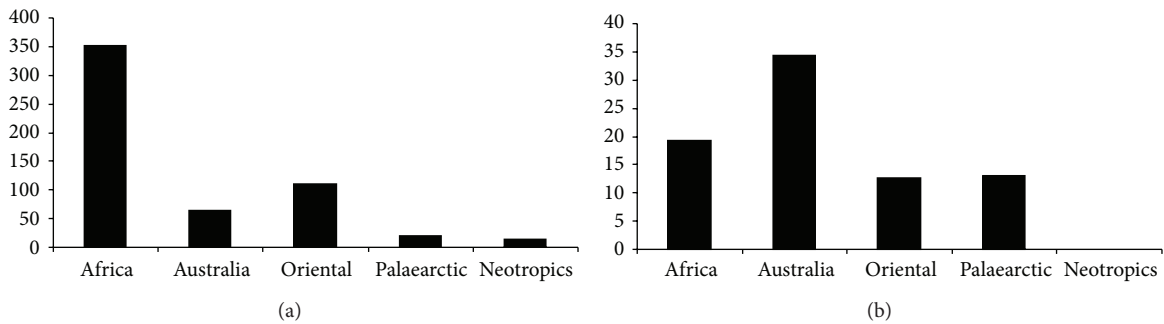


FIGURE 4: Number of valid species of Paussini per biogeographical region (a) and percentage of synonymous taxa of Paussini per biogeographical region (b).

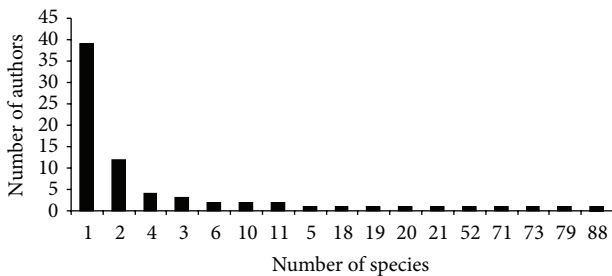


FIGURE 5: Number of authors in relation to the numbers of species of Paussini that they described.

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 redescrptions 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

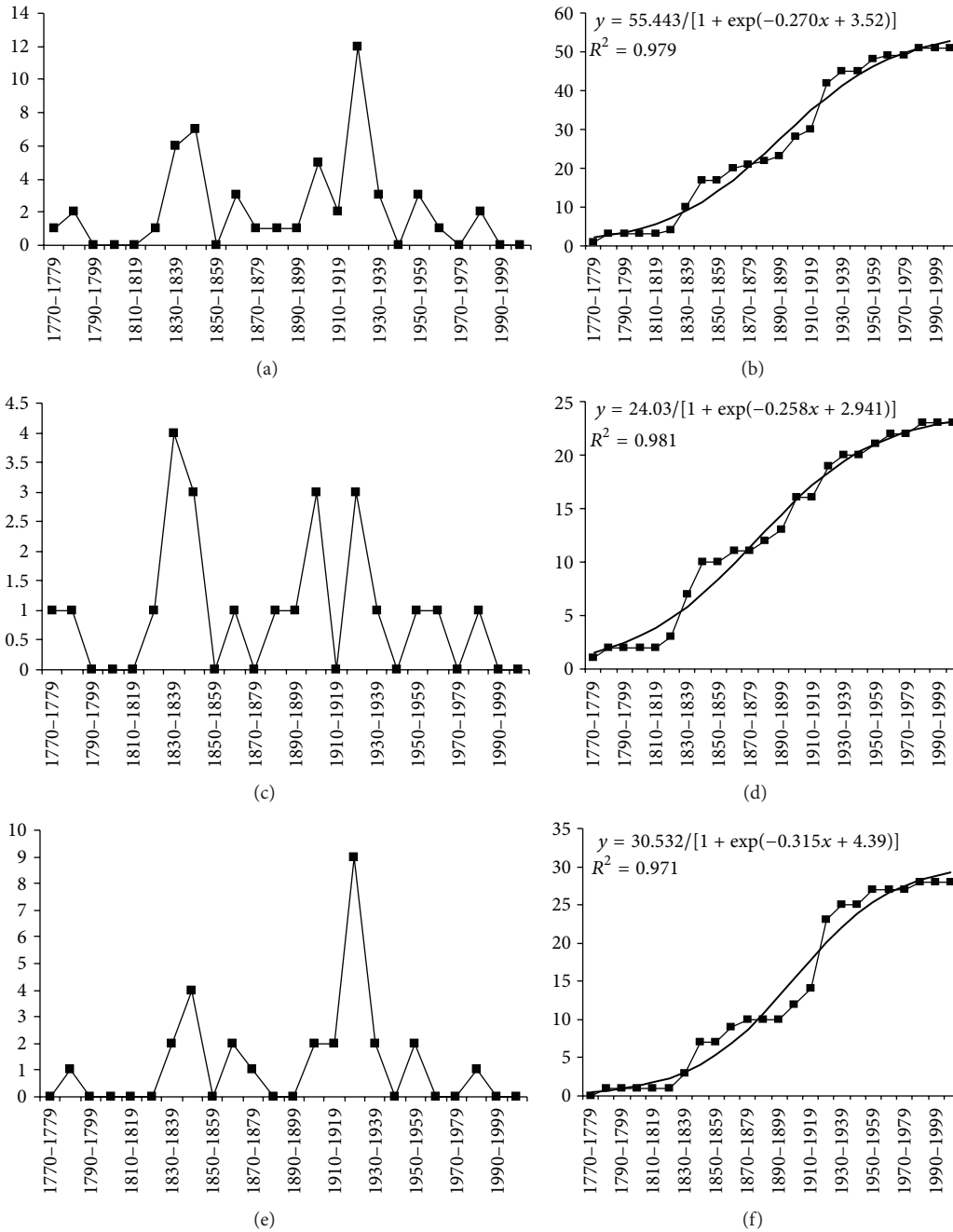


FIGURE 6: Numbers of total described genera (a, b), valid genera (c, d), and synonyms (e, f) of Paussini by decade. Figures (a), (c), and (e) report the absolute numbers, and Figures (b), (d), and (f) the cumulative numbers along with the equations of the fitted curves.

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

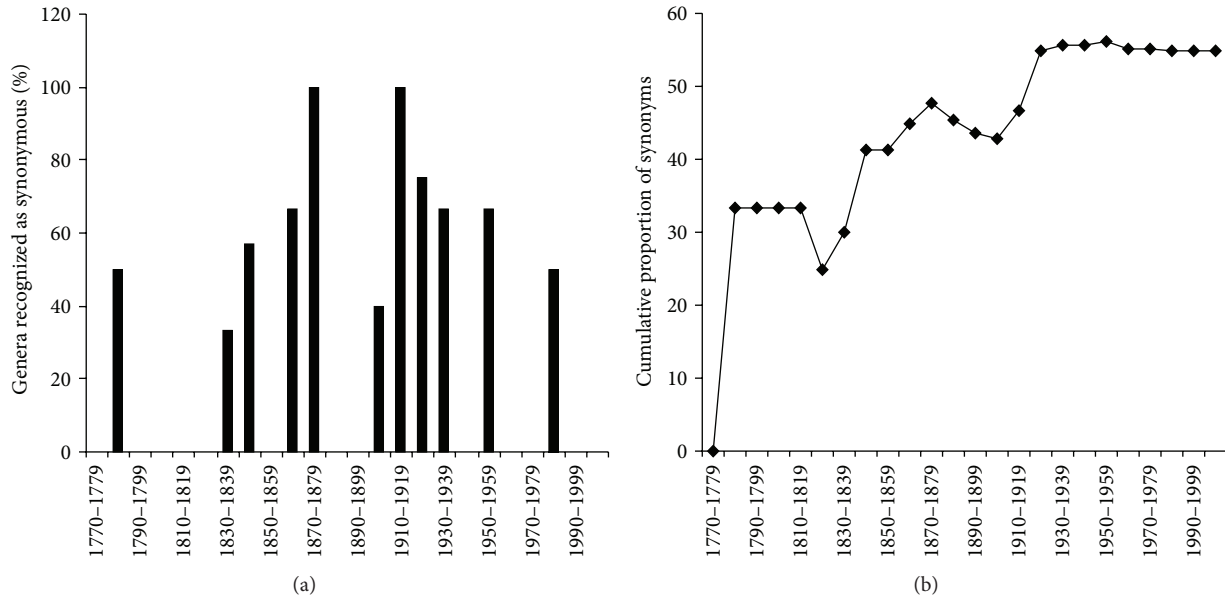


FIGURE 7: Percentage of synonymous genera described in each decade (a) and their historical process of accumulation (b).

(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 1880–1889, 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

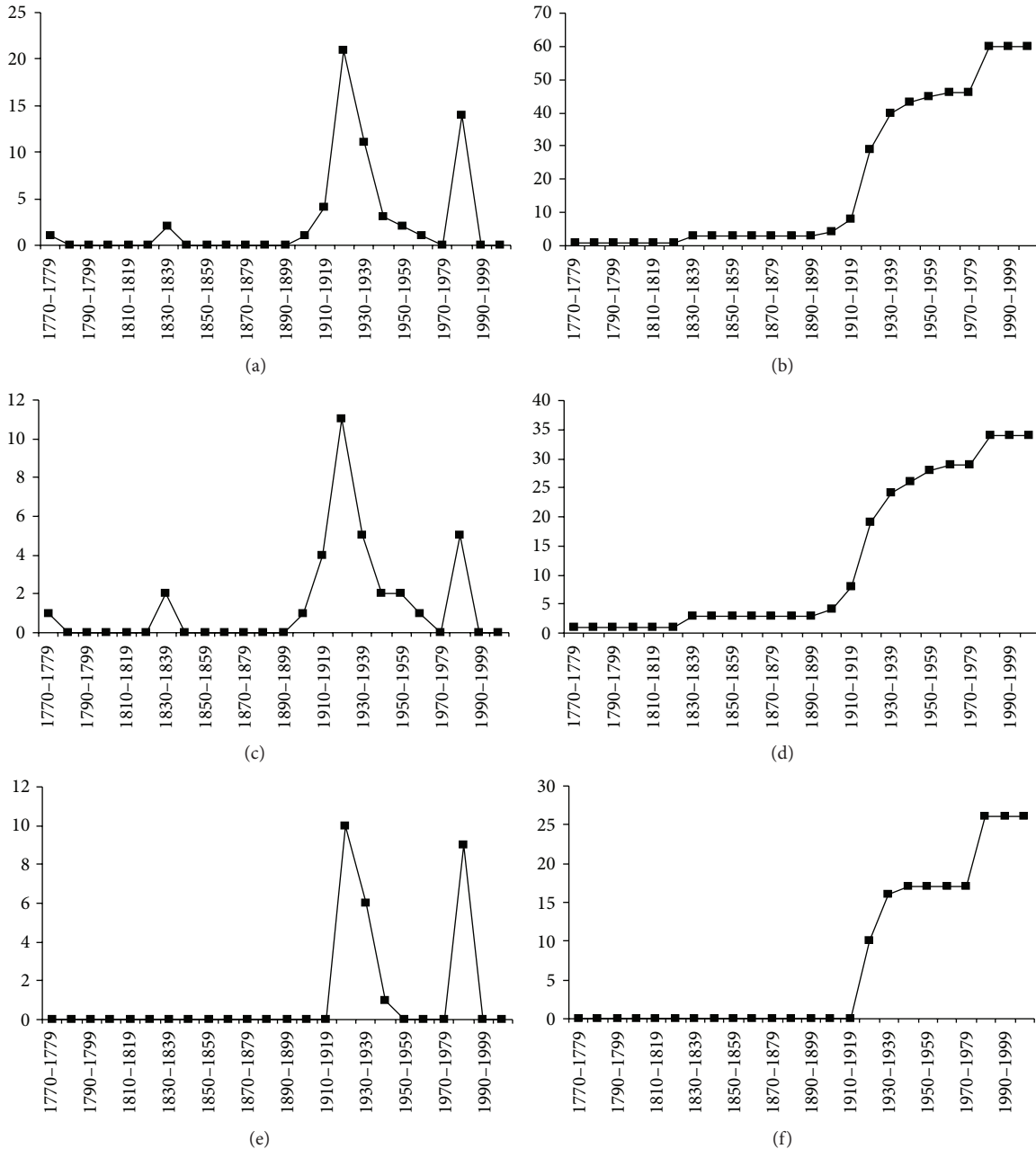


FIGURE 8: Numbers of total described subgenera (a, b), valid subgenera (c, d), and synonyms (e, f) of Paussini by decades. Figures (a), (c), and (e) report the absolute numbers, and Figures (b), (d), and (f) the cumulative numbers.

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

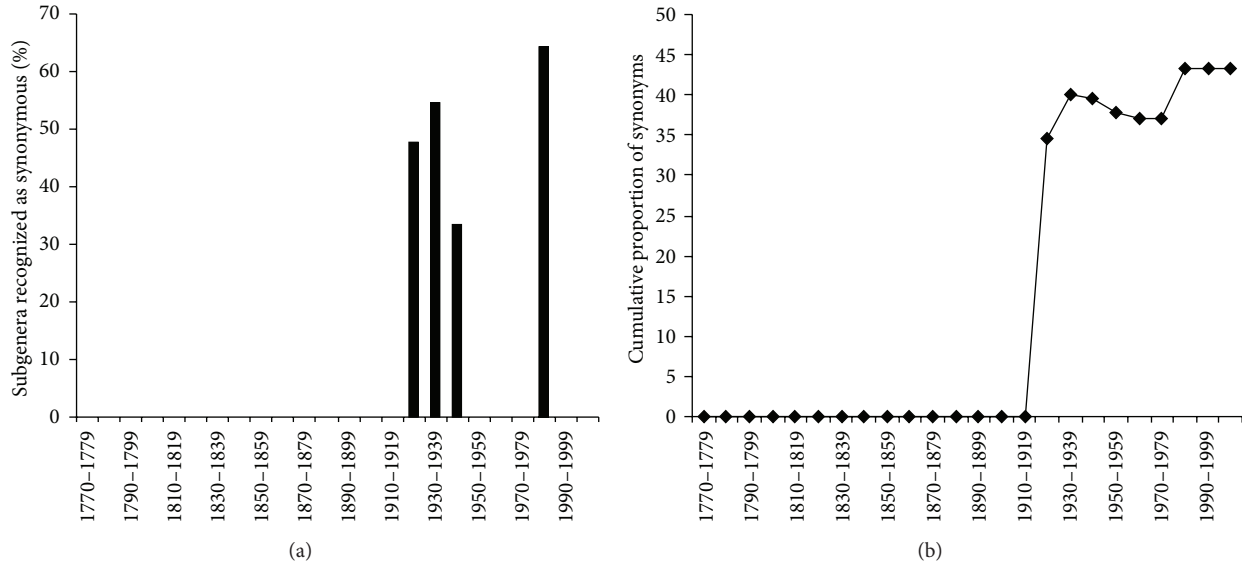


FIGURE 9: Percentage of synonymous subgenera described in each decade (a) and historical process of accumulation of percentage of synonyms over the total number of names in the Paussini, according to the date of their description (b).

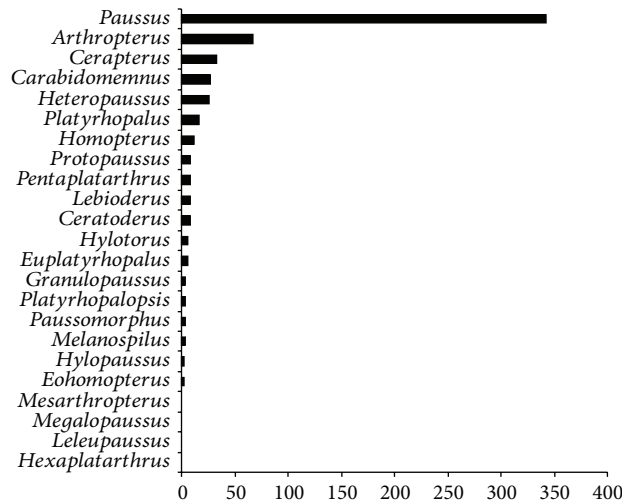


FIGURE 10: Number of species per genus in the tribe Paussini.

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

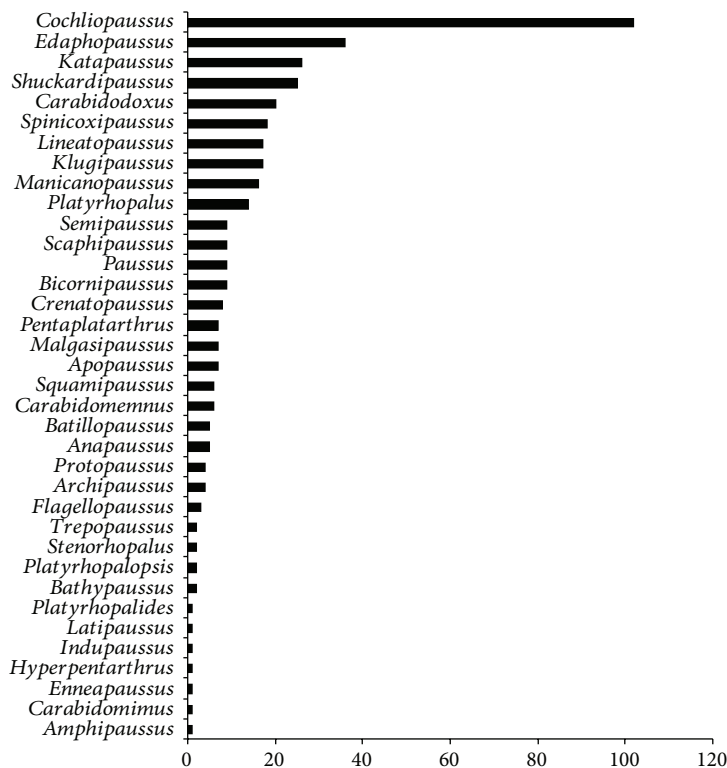


FIGURE 11: Number of species per subgenus in the tribe Paussini.

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.

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Review Article

Ant-Associated Beetle Fauna in Bulgaria: A Review and New Data

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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 antennae 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 merkliai* (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 merkliai* 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 | | | | |
| <i>Pausus turcicus</i> I. Frivaldsky von Frivald, 1835 | <i>Pheidole pallidula</i> (Nylander, 1849) | [19–21] [22] | Myrmecophile | |
| Histeridae | | | | |
| <i>Acritus nigricornis</i> (Hoffmann, 1803) | <i>Formica rufa</i> Linnaeus, 1761 | [23] | | |
| <i>Dendrophilus pygmaeus</i> (Linnaeus, 1758) | <i>Formica exsecta</i> Nylander, 1846 | [23, 24] | Myrmecophile | |
| | <i>Formica lugubris</i> Zetterstedt, 1838 | [23] | | |
| | <i>Formica rufa</i> Linnaeus, 1761 | [23] | | |
| <i>Haeterius ferrugineus</i> (Olivier, 1789) | <i>Formica fusca</i> Linnaeus, 1758 | [23] | Myrmecophile | |
| | <i>Formica cinerea</i> Mayr, 1853 | [25] | | |
| | <i>Lasius niger</i> (Linnaeus, 1758) | | | |
| <i>Sternocoelis merklii</i> (Schmidt, 1885) | <i>Messor structor</i> (Latreille, 1798) | [26] | Myrmecophile | Balkans |
| <i>Satrapes sartorii</i> (L. Redtenbacher, 1857) | <i>Tetramorium</i> cf. <i>caespitum</i> (Linnaeus, 1758) | New species for Bulgaria [27, 28] | Myrmecophile | |
| <i>Margarinotus ruficornis</i> (Grimm, 1852) | | [23] | | |
| <i>Onthophilus affinis</i> L. Redtenbacher, 1849 | <i>Formica fusca</i> Linnaeus, 1758 | [23] | | |
| | <i>Myrmica</i> sp. | [23] | | |
| Ptiliidae | | | | |
| <i>Pteridium pusillum</i> (Gyllenhal, 1808) | | [29] | | |
| <i>Ptilotum oedipus</i> (Flach, 1886) | <i>Formica pratensis</i> Retzius, 1783 | | New ant host record for Bulgaria | |
| <i>Ptilium myrmecophilum</i> (Allibert, 1844) | <i>Formica pratensis</i> Retzius, 1783 | | New species for Bulgaria | Myrmecophile |
| | <i>Formica rufa</i> Linnaeus, 1761 | | | |
| <i>Pteryx suturalis</i> (Heer, 1841) | <i>Formica rufa</i> Linnaeus, 1761 | | New ant host record for Bulgaria | |
| <i>Acrotichis atomaria</i> (De Geer, 1774) | <i>Formica pratensis</i> Retzius, 1783 | | New species for Bulgaria | |
| Leiodidae | | | | |
| Cholevinae | | | | |
| <i>Anemadus strigosus</i> Kraatz, 1852 | | [30, 31] | | |
| <i>Eocatops pelopis</i> (Reitter, 1884) | <i>Messor</i> sp. | [30, 32] [33] | Myrmecophile | |
| <i>Eocatops skopjensis</i> Karaman, 1957 | | [34] | Myrmecophile | Balkans |
| <i>Nemadus colonoides</i> (Kraatz, 1851) | | [30, 31] | Myrmecophile | |
| <i>Dreposcia umbrina</i> Erichson, 1837 | | [31, 34] | | |
| <i>Attaephilus arenarius</i> (Hampe, 1852) | | [30, 33] | Myrmecophile | |
| <i>Attaephilus</i> cf. <i>funebri</i> (Reitter, 1888) | <i>Messor structor</i> (Latreille, 1798) | [25], New record | | |
| <i>Attaephilus rambousecki</i> Jeannel, 1936 | <i>Messor</i> sp. | [25] | | Balkans |
| <i>Catopsimorphus marani</i> Roubal, 1936 | <i>Messor</i> sp. | [32, 33] | | Bulgaria |
| | | [33] | Myrmecophile | Bulgaria |

TABLE 1: Continued.

| Beetle families, genera, and species | Recorded ant hosts in Bulgaria | References | Ant-relation | Endemic beetles |
|--|---|------------------------------|------------------------------|-----------------|
| Staphylinidae | | | | |
| Pselaphinae | | | | |
| <i>Batrissodes buqueti</i> (Aubé, 1833) | <i>Lasius brunneus</i> (Latreille, 1798) | [25] [35] | Myrmecophile | |
| <i>Batrissodes delaporti</i> (Aubé, 1833) | <i>Lasius brunneus</i> (Latreille, 1798) <i>Lasius alienus</i> (Förster, 1850) | [36] [37] [25] | Myrmecophile | |
| <i>Batrissodes hubenthalii</i> Reitter, 1913 | | [25, 38] | Myrmecophile | |
| <i>Batrissodes oculus</i> (Aubé, 1833) | | [39] | Myrmecophile | |
| <i>Batrissodes sulcaticeps</i> Besuchet, 1981 | | [25, 40] | Myrmecophile | |
| <i>Batrissodes venustus</i> (Reichenbach, 1816) | | [25, 36, 38] | Myrmecophile | |
| <i>Batrissodes formicarius</i> Aubé, 1833 | | [35, 37, 41] | Myrmecophile | |
| <i>Claviger elysius</i> Reitter, 1884 | <i>Lasius brunneus</i> (Latreille, 1798) <i>Lasius alienus</i> (Förster, 1850) | [25] | Myrmecophile | Balkans |
| <i>Claviger engelii</i> Reitter, 1885 | <i>Lasius alienus</i> (Förster, 1850) | [25] | Myrmecophile | Balkans |
| <i>Claviger handmanni</i> Wasmann, 1898 | <i>Lasius flavus</i> (Fabricius, 1782) <i>Lasius alienus</i> (Förster, 1850) | [37] [25] | Myrmecophile | Balkans |
| <i>Claviger longicornis</i> P.W.J. Müller, 1818 | <i>Lasius alienus</i> (Förster, 1850) <i>Lasius niger</i> (Linnaeus, 1758) <i>Lasius brunneus</i> (Latreille, 1798) | [42] [37] [37] [37] | Myrmecophile | |
| <i>Claviger merkli</i> Reitter, 1885 | <i>Lasius niger</i> (Linnaeus, 1758) <i>Lasius alienus</i> (Förster, 1850) | [35, 43] [25] | Myrmecophile | Balkans |
| <i>Claviger testaceus</i> Preysslner, 1790 | <i>Lasius alienus</i> (Förster, 1850) | [25] | Myrmecophile | |
| <i>Euplectus frater</i> Besuchet, 1964 | <i>Lasius flavus</i> (Fabricius, 1782) | [25, 40] | Myrmecophile | |
| <i>Euplectus nanus</i> (Reichenbach, 1816) | <i>Formica rufa</i> Linnaeus, 1761 | [35, 44] [37] | | |
| <i>Euplectus signatus</i> (Reichenbach, 1816) | <i>Formica rufa</i> Linnaeus, 1761 | [39] | | |
| <i>Trichomyx sulcicollis</i> (Reichenbach, 1816) | <i>Formica pratensis</i> Retzius, 1783 <i>Formica</i> sp. | [37] [39] [37] [45] | | |
| <i>Trimium carpathicum</i> Saulcy, 1875 | <i>Lasius alienus</i> (Förster, 1850) | [36] | | |
| <i>Trimium puncticeps</i> Reitter, 1880 | <i>Formica rufa</i> Linnaeus, 1761 | [25] | | |
| <i>Bryaxis romaniae</i> Raffray, 1904 | <i>Formica pratensis</i> Retzius, 1783 | [35] | | |
| <i>Centrotoma bruckii</i> Saulcy, 1874 | <i>Formica rufa</i> Linnaeus, 1761 | [37] | | |
| <i>Centrotoma lucifuga</i> C. Heyden, 1849 | <i>Tetramorium caespitum</i> (Linnaeus, 1758) | [25] | Myrmecophile | Balkans |
| <i>Chennium bituberculatum</i> Latreille, 1807 | <i>Tetramorium caespitum</i> (Linnaeus, 1758) | [37] | Myrmecophile | Balkans |
| <i>Chennium steigerwaldi</i> Reitter, 1882 | <i>Tetramorium</i> cf. <i>caespitum</i> (Linnaeus, 1758) <i>Tetramorium ferox</i> Ruzsky, 1903 | [37] [25] | Myrmecophile Myrmecophile | |

TABLE 1: Continued.

| Beetle families, genera, and species | Recorded ant hosts in Bulgaria | References | Ant-relation | Endemic beetles |
|--|---|------------|--------------|-----------------|
| Tachyporinae | | | | |
| <i>Ischnosoma splendidum</i> (Gravenhorst, 1806) | <i>Lasius brunneus</i> (Latreille, 1798) | [46] | | |
| <i>Tachinus rufipes</i> (Linnaeus, 1758) | <i>Formica rufa</i> Linnaeus, 1761 | [46] | | |
| <i>Tachyporus hypnorum</i> (Fabricius, 1775) | <i>Formica pratensis</i> Retzius, 1783 | [46] | | |
| <i>Tachyporus nitidulus</i> (Fabricius, 1781) | <i>Lasius fuliginosus</i> (Latreille, 1798) | [46] | | |
| Aleocharinae | | | | |
| <i>Piochardia reitteri</i> (Wasmann, 1894) | <i>Cataglyphis nodus</i> (Brullé, 1833) | [46] | Myrmecophile | |
| <i>Lyprocorrhe anceps</i> (Erichson, 1837) | <i>Formica pratensis</i> Retzius, 1783 | [46, 47] | Myrmecophile | |
| <i>Notothecta flavipes</i> (Gravenhorst, 1806) | <i>Formica pratensis</i> Retzius, 1783 | [46, 47] | Myrmecophile | |
| | <i>Formica rufa</i> Linnaeus, 1761 | [46] | | |
| <i>Euryusa sinuata</i> Erichson, 1837 | <i>Lasius brunneus</i> (Latreille, 1798) | [46] | Myrmecophile | |
| <i>Anaulacaspis nigra</i> (Gravenhorst, 1802) | <i>Formica pratensis</i> Retzius, 1783 | [46] | | |
| <i>Oligota pumilio</i> Kiesenwetter, 1858 | <i>Formica pratensis</i> Retzius, 1783 | [46] | | |
| <i>Oligota inflata</i> (Mannerheim, 1830) | <i>Formica pratensis</i> Retzius, 1783 | [46] | | |
| <i>Lomechusa emarginata</i> (Paykull, 1789) | <i>Formica</i> sp. | [48] | Myrmecophile | |
| | <i>Myrmica</i> sp. | [25] | | |
| | <i>Myrmica rugulosa</i> Nylander, 1849 | [46] | | |
| | <i>Formica cinerea</i> Mayr, 1853 | [46] | | |
| <i>Drusilla canaliculata</i> (Fabricius, 1787) | <i>Tetramorium caespitum</i> (Linnaeus, 1758) | [46] | | |
| <i>Myrmocacia plicata</i> (Erichson, 1837) | | [49] | Myrmecophile | |
| <i>Pella funesta</i> (Gravenhorst, 1806) | | [39] | Myrmecophile | |
| <i>Pella hampei</i> (Kraatz, 1862) | | [50] | Myrmecophile | |
| <i>Pella humeralis</i> (Gravenhorst, 1802) | | [51, 52] | Myrmecophile | |
| | <i>Formica pratensis</i> Retzius, 1783 | [47] | | |
| <i>Pella laticollis</i> (Märkel, 1844) | | [39] | Myrmecophile | |
| <i>Pella limbata</i> (Paykull, 1789) | | [48] | Myrmecophile | |
| <i>Pella lugens</i> (Gravenhorst, 1802) | | [53] | Myrmecophile | |
| <i>Pella ruficollis</i> (Grim, 1845) | | [50] | Myrmecophile | |
| <i>Zyras collaris</i> (Paykull, 1789) | | [45] | | |
| <i>Zyras fulgidus</i> (Gravenhorst, 1806) | | [54] | | |
| <i>Zyras haworthi</i> (Stephens, 1832) | | [48] | Myrmecophile | |
| <i>Dinarda hagensii</i> Wasmann, 1889 | <i>Formica exsecta</i> Nylander, 1846 | [24, 46] | Myrmecophile | |
| <i>Dinarda maerkelii</i> Kiesenwetter, 1843 | <i>Formica pratensis</i> Retzius, 1783 | [46, 47] | Myrmecophile | |
| <i>Dinarda</i> sp. | <i>Formica sanguinea</i> Latreille, 1798 | [25] | Myrmecophile | |
| <i>Haploglossa gentilis</i> (Märkel, 1845) | <i>Lasius fuliginosus</i> (Latreille, 1798) | [55] | | |
| <i>Haploglossa pulla</i> (Gyllenhal, 1827) | | [51] | Myrmecophile | |
| <i>Oxyptoda formiceticola</i> Märkel, 1841 | <i>Formica rufa</i> Linnaeus, 1761 | [46] | | |
| | <i>Formica lugubris</i> Zetterstedt, 1838 | [46] | Myrmecophile | |
| <i>Oxyptoda haemorrhoa</i> (Mannerheim, 1830) | <i>Formica exsecta</i> Nylander, 1846 | [24] | Myrmecophile | |
| | <i>Formica pratensis</i> Retzius, 1783 | [46, 47] | | |
| <i>Oxyptoda pratensiscola</i> Lohse, 1970 | <i>Formica pratensis</i> Retzius, 1783 | [46, 47] | Myrmecophile | |
| <i>Oxyptoda rugicollis</i> Kraatz, 1856 | <i>Formica exsecta</i> Nylander, 1846 | [24, 46] | Myrmecophile | |
| | <i>Formica pressilabris</i> Nylander, 1846 | [46] | | |
| <i>Oxyptoda vittata</i> Märkel, 1842 | | [54, 56] | Myrmecophile | |

TABLE 1: Continued.

| Beetle families, genera, and species | Recorded ant hosts in Bulgaria | References | Ant-relation | Endemic beetles |
|--|---|----------------------------------|--------------|-----------------|
| <i>Thiasophila angulata</i> (Erichson, 1837) | <i>Formica rufa</i> Linnaeus, 1761 | [46] | Myrmecophile | |
| <i>Thiasophila canaliculata</i> Mulsant and Rey, 1875 | <i>Formica exsecta</i> Nylander, 1846 | [24, 46] | Myrmecophile | |
| <i>Thiasophila lohsei</i> Zerche, 1987 | <i>Formica pratensis</i> Retzius, 1783 | [46, 47] | Myrmecophile | |
| Scydmaeninae | | | | |
| <i>Euconnus chrysochomus</i> (Saulcy, 1864) | <i>Tetramorium</i> cf. <i>ferox</i> Ruzscky, 1903 | [25] | Myrmecophile | |
| <i>Euhiconus conicicollis</i> (Fairmaire and Laboulbène, 1854) | | [57] | | |
| <i>Microscydinus nanus</i> (Schaum, 1844) | | [39, 58] | | |
| <i>Neuraphes parvulus</i> Rambousek, 1909 | <i>Lasius fuliginosus</i> (Latreille, 1798) | [39] | | |
| <i>Sydmoraphes minutus</i> (Chaudoir, 1845) | | [58] | | |
| <i>Sydymaenus perrisi</i> Reitter, 1881 | | [39, 59] | | |
| Steninae | | | | |
| <i>Stenus aterrimus</i> Erichson, 1839 | <i>Formica pratensis</i> Retzius, 1783 | [46, 47] | Myrmecophile | |
| <i>Stenus heydeni</i> L. Benick, 1915 | <i>Formica rufa</i> Linnaeus, 1761 | [46] | | Balkans |
| Paederinae | | | | |
| <i>Lithocharis nigriceps</i> Kraatz, 1859 | <i>Formica pratensis</i> Retzius, 1783 | [47] | | |
| <i>Astenus gracilis</i> (Paykull, 1789) | <i>Formica pratensis</i> Retzius, 1783 | [46] | | |
| <i>Senius melanocephalus</i> (Fabricius, 1793) | <i>Formica rufa</i> Linnaeus, 1761 | [46] | | |
| <i>Scopaeus pusillus</i> Kiesenwetter, 1843 | <i>Formica pratensis</i> Retzius, 1783 | [46] | | |
| <i>Scopaeus sulcicollis</i> (Stephens, 1833) | <i>Formica rufa</i> Linnaeus, 1761 | [46] | | |
| Staphylininae | | | | |
| <i>Leptacinus formicetorum</i> Märkel, 1841 | <i>Formica pratensis</i> Retzius, 1783 | [47] | Myrmecophile | |
| | <i>Formica rufa</i> Linnaeus, 1761 | [46] | | |
| | <i>Formica exsecta</i> Nylander, 1846 | [46] | | |
| <i>Gyrophypnus angustatus</i> Stephens, 1833 | <i>Formica pratensis</i> Retzius, 1783 | [46] | | |
| | <i>Formica rufa</i> Linnaeus, 1761 | [46] | | |
| <i>Xantholinus linearis</i> (Olivier, 1795) | <i>Formica pratensis</i> Retzius, 1783 | [46] | | |
| <i>Gabrius splendidulus</i> (Gravenhorst, 1802) | <i>Lasius brunneus</i> (Latreille, 1798) | [46] | | |
| <i>Que dius brevis</i> Erichson, 1840 | <i>Formica rufa</i> Linnaeus, 1761 | [46] | Myrmecophile | |
| Monotomidae | | | | |
| <i>Rhizophagus bipustulatus</i> (Fabricius, 1792) | <i>Lasius niger</i> (Linnaeus, 1758) | New ant host record for Bulgaria | Myrmecophile | |
| <i>Monotoma conicicollis</i> Aubé, 1837 | | [29] | | |
| | <i>Formica pratensis</i> Retzius, 1783 | New ant host record for Bulgaria | | |
| Cryptophagidae | | | | |
| <i>Hypocoprus latridioides</i> Motschulsky, 1839 | | [54] | Myrmecophile | |
| | <i>Formica exsecta</i> Nylander, 1846 | [24] | | |
| | <i>Formica rufa</i> Linnaeus, 1761 | New ant host record for Bulgaria | | |
| | <i>Formica lugubris</i> Zetterstedt, 1838 | New ant host record for Bulgaria | | |
| Nitidulidae | | | | |
| <i>Amphotis marginata</i> (Fabricius, 1781) | <i>Lasius fuliginosus</i> (Latreille, 1798) | [29, 60, 61] | Myrmecophile | |
| <i>Amphotis orientalis</i> Reiche, 1861 | | [34, 62] | Myrmecophile | |
| Cerylonidae | | | | |
| <i>Cerylon histeroideus</i> (Fabricius, 1792) | <i>Lasius brunneus</i> (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 Jelezmitsa 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 *Oxyptoda* 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].

Hypocopus 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 Merophysinae (*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.

Myrmecixenus 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, Vitoshka 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, Vitoshka 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. Vitoshka 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 composed 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.

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Review Article

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

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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 araneid 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 classification 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 trophobiotic 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 | Syncoxeni “Synoeketes” | Passive/intranidal guests “Inside nest guests” | Les clients “Clients” | Accidental/preferred or obligate commensals | Specialized species | Nonintegrated species | Synocious myrmecophiles |
| Scavengers or predators, treated with hostility; defensive | Synechthrans | Cleptoxeni “Cleptoketes” | Passive/intranidal guests “Inside nest guests” | Les clients or les associates “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 associates “Associate\$” | 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 associates “Associate\$” | Obligate commensals | — | Integrated species | — |
| Penetrate body to feed on blood; parasite | Endoparasites | Parasitoxeni “Parasites” | Passive/intranidal guests “Inside nest guests” | Les associates “Associate\$” | Obligate commensals | — | Integrated species | — |
| Exchange of honeydew or nectar for protection | Trophobionts | Euxeni “True guests” | Active/extranidal guests “Outside nest guests” | Les associates “Associate\$” | Obligate commensals | — | Integrated species | — |
| Follow hosts on raids | Synechthrans | — | 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.”

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Review Article

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

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

TABLE I: Continued.

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

TABLE 1: Continued.

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

* Reported as "*Monomorium minutum* (Buckley)" by Greene [37, 64]. The valid name for 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 Microdentinae Associated with Ants? The larval habits remain unknown for the majority of microdentine 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 Microdentinae (Figure 1). *Spheginobaccha* de Meijere (tribe Spheginobacchini) is the sister group to all other Microdentinae (tribe Microdentinae), but the larvae of this taxon are presently unknown. Within the tribe Microdentinae (the remaining part of the tree), *Mixogaster* Macquart is the first genus to branch off (a strongly supported clade; see Reemer and Ståhls [28]),

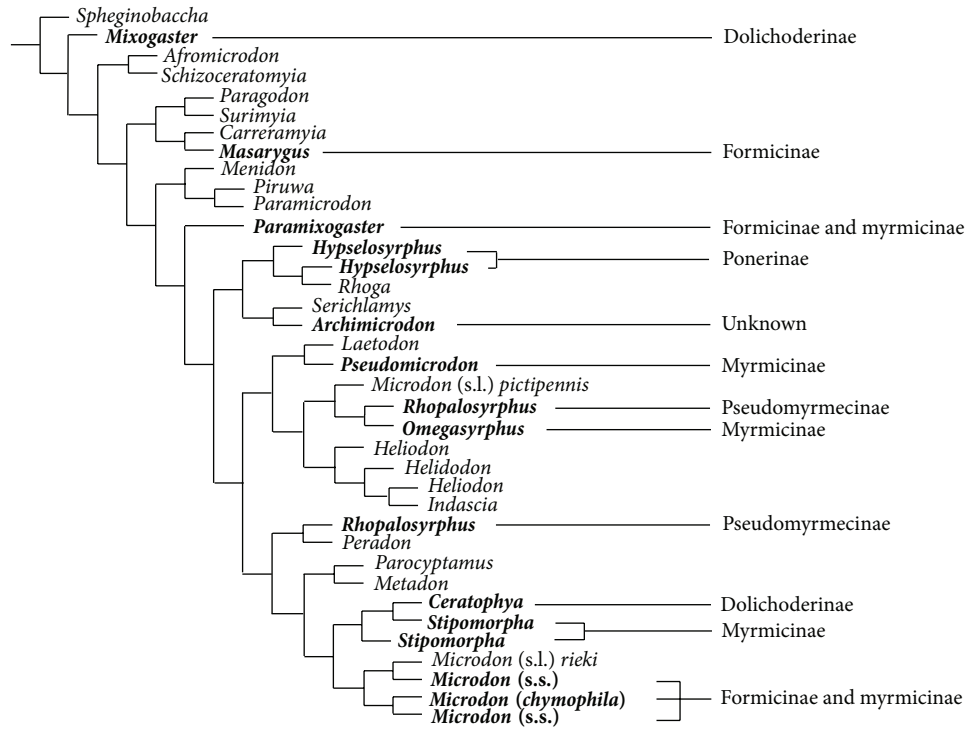


FIGURE 1: Phylogenetic hypothesis of 28 genera of Microdontinae (based on [28]), with indication of known associations with subfamilies of ants. Genera for which such associations are known are printed in bold. Note that several associations listed in Table 1 are lacking, because several taxa of Microdontinae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

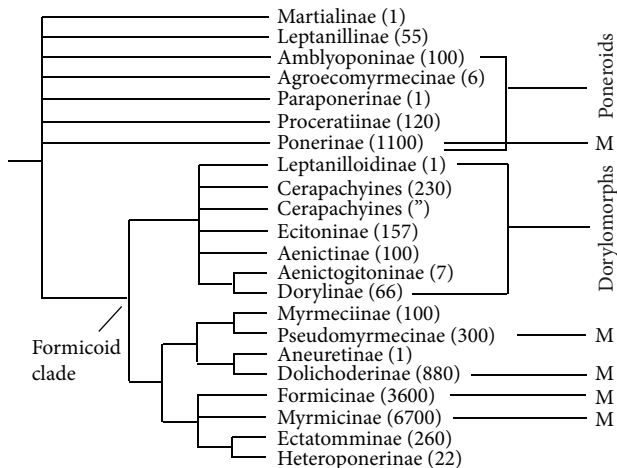


FIGURE 2: Phylogenetic tree summarizing well-supported relationships between extant subfamilies of ants (modified from [31]), with indication of known associations with Microdontinae ("M"). Numbers in parentheses are estimated numbers of described species per subfamily (based on [27, 31]).

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 association 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 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.

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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*

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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).

| Substances | <i>Z. collaris</i> | | <i>Z. haworthi</i> | |
|--|--------------------|-----------------|--------------------|-----------------|
| | 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 |

¹Numbers 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 $\mu\text{g}/\mu\text{L}$ or 10 $\mu\text{g}/\mu\text{L}$. Control filter paper balls were treated with 10 μL hexane. Each test solution was tested 20 times with different ant specimen. Hexane 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 $\mu\text{g}/\mu\text{L}$. For tests with 10 $\mu\text{g}/\mu\text{L}$, 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 $\mu\text{g}/\mu\text{L}$ or 10 $\mu\text{g}/\mu\text{L}$ 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.

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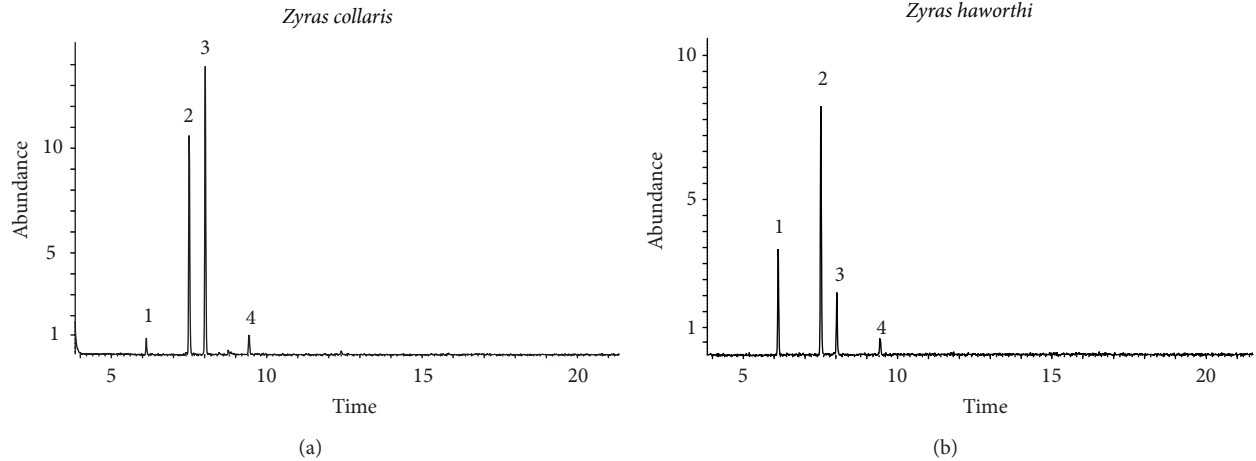


FIGURE 1: Gas chromatograms (TIC) of the tergal secretions obtained by stir bar irritation of *Zyras collaris* (a) and *Z. haworthi* (b). 1: α -pinene; 2: β -pinene; 3: myrcene; 4: limonene.

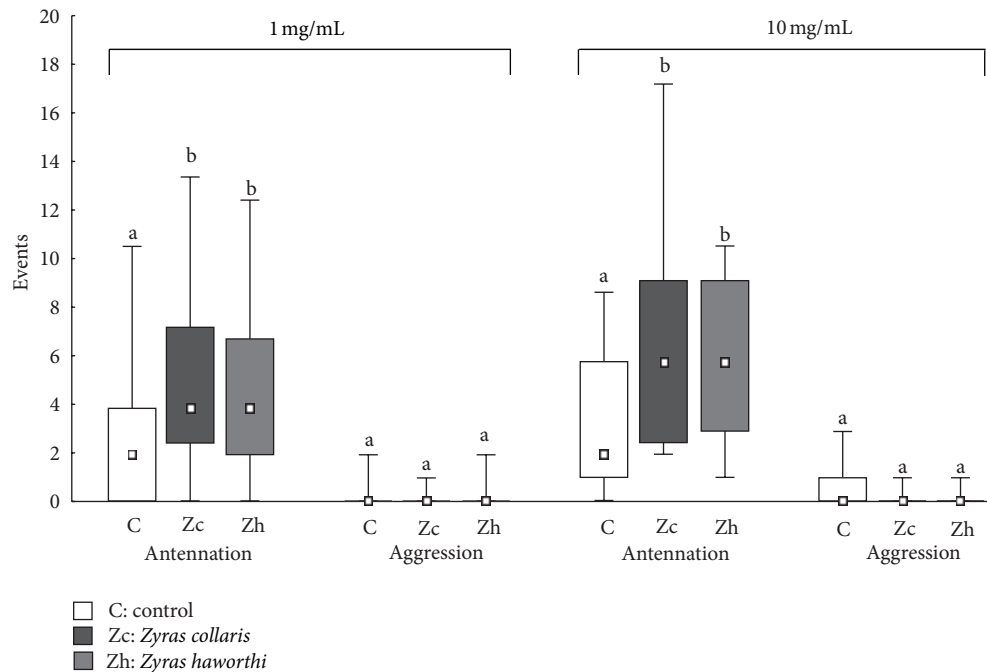


FIGURE 2: Antennation and aggressive behaviour (\square : median, boxes: 25–75 percentiles, whiskers: min.–max.) by *Lasius fuliginosus* ants in a laboratory experiment towards a filter paper ball treated with mixtures of substances (1 mg/mL and 10 mg/mL), which are present in the tergal gland secretion 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.

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Review Article

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

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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 symphylics 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 *Szelenyopria 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,

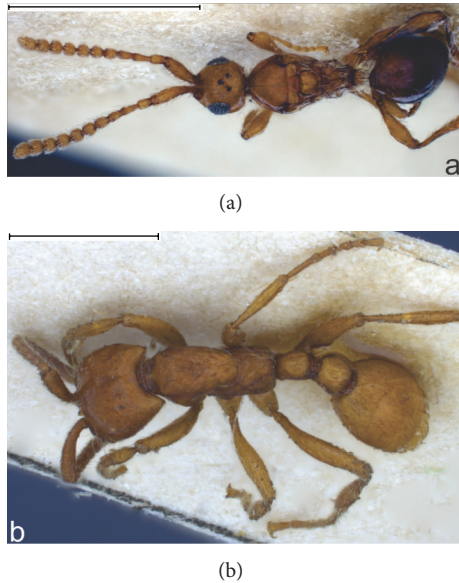


FIGURE 1: (a) *Asolenopsia rufa* female dealate in dorsal view. (b) *Neivamyrmex carettei*. Scale: 1 mm.

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*,

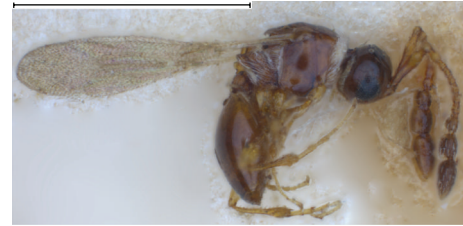


FIGURE 2: *Doliopria collegii* female in lateral view. Scale: 1 mm.

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-Ménéville) [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].

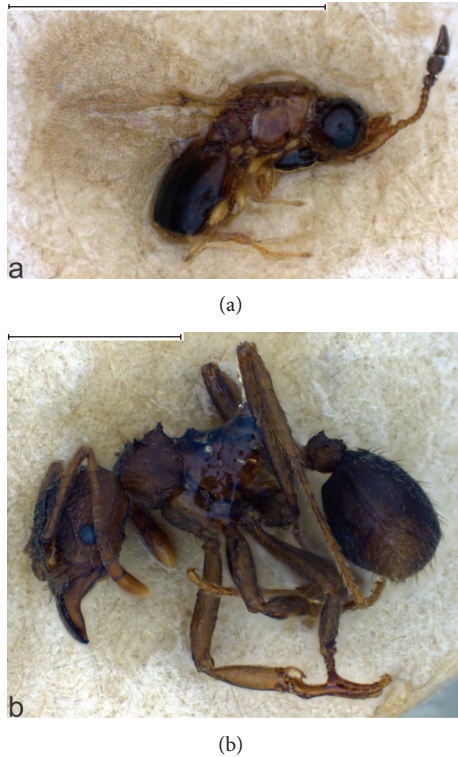


FIGURE 3: (a) *Doliopria myrmecobia* female in lateral view. (b) *Acromyrmex lundii*. Scale: 1 mm.

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. Loíacono [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 Loíacono, 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].

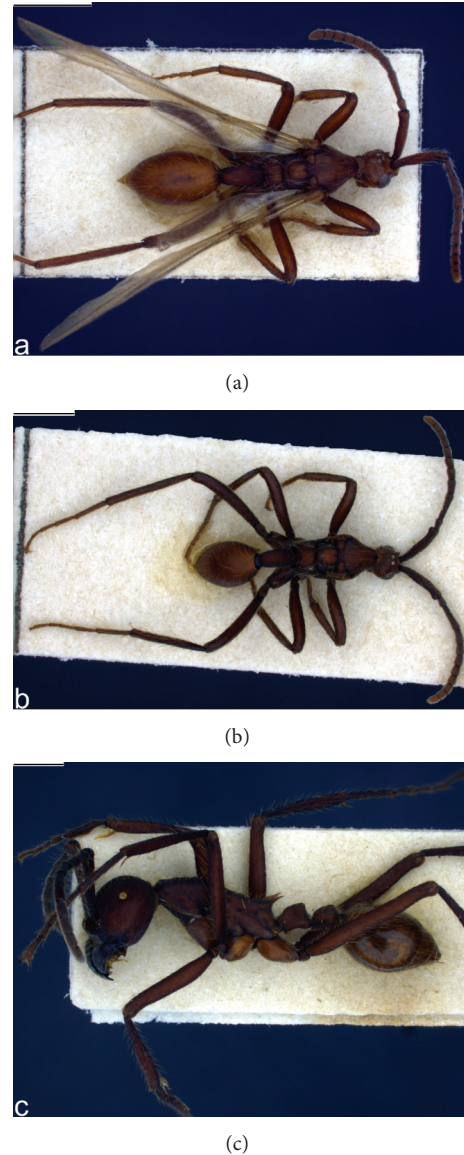


FIGURE 4: *Notoxoides pronotalis* female in dorsal view: (a) alate and (b) dealate specimens. Scale: 1 mm. (c) *Eciton dulcium* collected with *Notoxoides pronotalis*, in lateral view. Scale: 1 mm.

Material Studied. Syntype, female dealated, collected with *Eciton dulcium*, Argentina, Córdoba, Alta Gracia, 4-XII-1921, 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

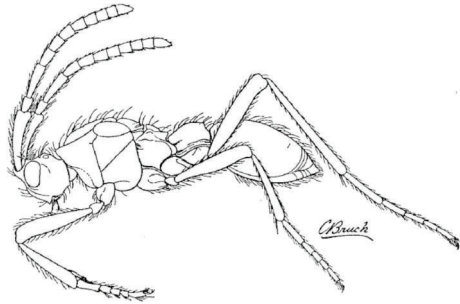


FIGURE 5: Original illustration of *Notoxoides pronotalis* female in lateral view, by Bruch.

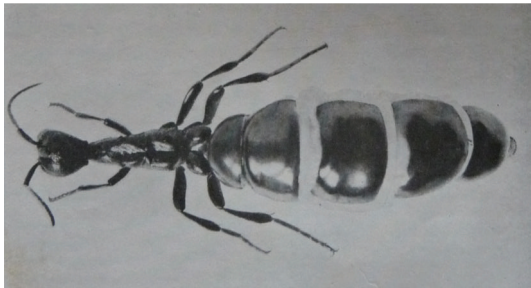


FIGURE 6: *Neivamyrmex pseudops*, gravid queen in dorsal view, photographed by Bruch.

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 diapiiid 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 diapiiid cuticle; similarly we observed the presence of peculiar neck hairs in *N. pronotalis* [20].



(a)



(b)

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

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

Gymnopria Loíacono, 1987: 130 [21].

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

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

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

Szelenyopria pampeana: Loíacono 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 reichenspergeri Ferrière, 1929: 165 [35].

Szelenyiopria reichenspergeri: 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*. World-

wide [5].

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

3.1.16. *Trichopria formicans* Loíacono, 2000 [4] (Figures 8(a) and 8(b)). *Trichopria formicans* Loíacono 2000 in Loíacono 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].

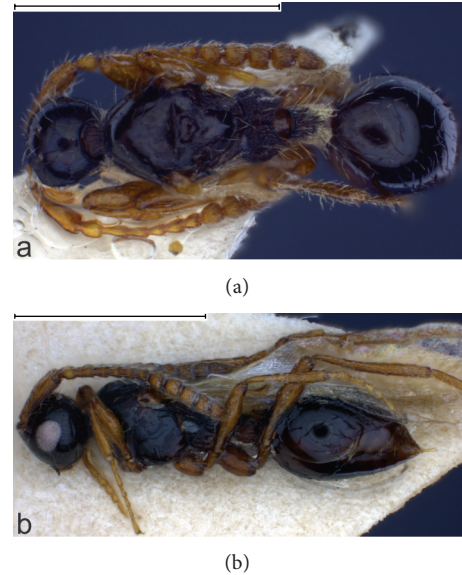


FIGURE 8: *Trichopria formicans* female (a) in dorsal view and (b) lateral view. Scale: 1 mm.

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 Misio- nes) [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.” Loíacono 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

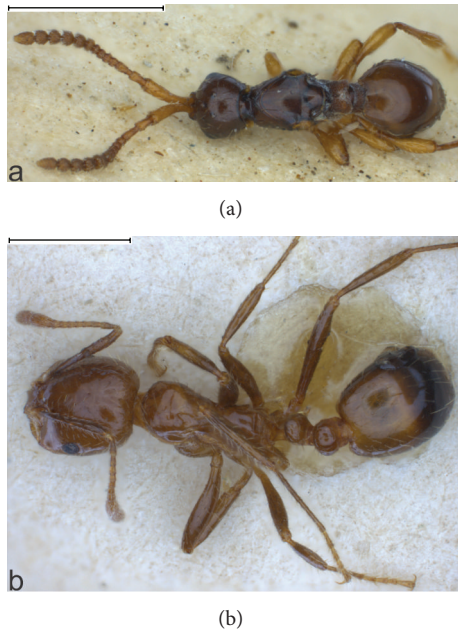


FIGURE 9: (a) *Bruchopria hexatoma* female dealate in dorsal view. (b) *Solenopsis richteri*. Scale: 1 mm.

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

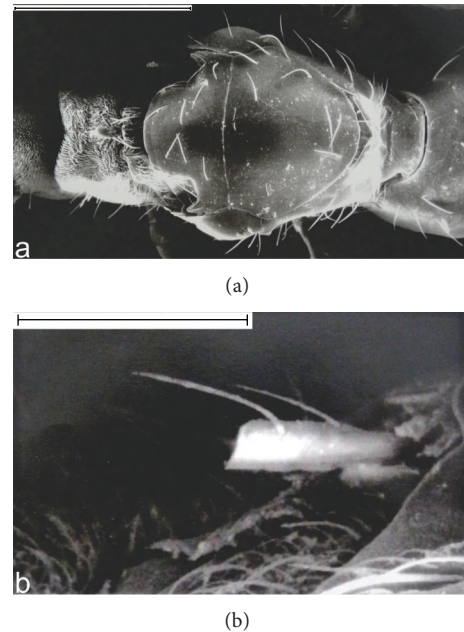


FIGURE 10: *Bruchopria hexatoma* female. (a) mesosoma and petiole in dorsal view, scale: 0.5 mm; (b) wing stump, scale: 0.1 mm [26].

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 |
|----------------|--|---|--------------------------|-------------------------|---|
| | <i>Asolenopsia rufa</i> | Córdoba, Entre Ríos, Santa Fe | Ecitoninae | Ecitonini | <i>Neivamyrmex carettei</i> |
| | <i>Basalys</i> sp. | Buenos Aires | Dolichoderinae | Dolichoderini | <i>Linepithema humile</i> |
| | <i>Doliopria collegii</i> | Buenos Aires, Misiones | Ecitoninae | Ecitonini | <i>Eciton burchellii</i> , <i>Eciton quadriglume</i> |
| | <i>Doliopria myrmecobia</i> | Buenos Aires, Misiones | Myrmicinae | Attini | <i>Acromyrmex lundii</i> |
| Diapriini | <i>Notoxoides pedissequus</i> | Córdoba | Ecitoninae | Ecitonini | <i>Neivamyrmex pseudops</i> |
| | <i>Notoxoides pronotalis</i> | Córdoba, Salta, San Luis, Santiago del Estero | Ecitoninae | Ecitonini | <i>Eciton dulcium</i> , <i>Neivamyrmex sulcatus</i> |
| | <i>Szelenyopria pampeana</i> | La Pampa | Myrmicinae | Attini | <i>Acromyrmex lobicornis</i> |
| | <i>Szelenyopria reichenspergeri</i> | Salta, Tucumán | Ecitoninae | Ecitonini | <i>Eciton quadriglume</i> , <i>Neivamyrmex legionis</i> |
| | <i>Szelenyopria</i> sp. | Córdoba | Ecitoninae | Ecitonini | Ecitonini sp. |
| | <i>Trichopria formicans</i> | La Pampa | Myrmicinae | Attini | <i>Acromyrmex lobicornis</i> |
| | <i>Trichopria myrmecophila</i> | Buenos Aires | Myrmicinae | Solenopsidini | <i>Solenopsis richteri</i> |
| | <i>Trichopria</i> sp. | Buenos Aires | Dolichoderinae | Dolichoderini | <i>Linepithema humile</i> |
| | <i>Bruchopria hexatoma</i> | Buenos Aires, Córdoba, Misiones | Myrmicinae Myrmicinae | Solenopsidini Attini | <i>Solenopsis richteri</i> <i>Acromyrmex lundii</i> |
| Spilomicrini | <i>Bruchopria pentatoma</i> | Córdoba | Myrmicinae | Solenopsidini | <i>Solenopsis richteri</i> |
| | <i>Pentapria</i> cf. <i>nodicornis</i> | Córdoba | Myrmicinae | Solenopsidini | <i>Solenopsis saevissima</i> |
| | <i>Spilomicrus</i> 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 *Szelenyopria* occurs in La Pampa

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

We considered that *Szelenyopria 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.

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Research Article

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

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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

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 <i>M. tetramorii</i> | |
|------------------|----------------------|--------------------|-----------------------|-----------------------------|---|
| Formicinae | <i>Camponotus</i> | <i>japonicus</i> | 8 | 0 | |
| | | <i>obscuripes</i> | 1 | 0 | |
| | <i>Formica</i> | <i>hayashi</i> | 4 | 0 | |
| | | <i>japonica</i> | 17 | 1 | |
| | | <i>sanguinea</i> | 1 | 0 | |
| | | <i>yessensis</i> | 1 | 0 | |
| | | <i>Lasius</i> | <i>capitatus</i> | 1 | 0 |
| | Myrmicinae | <i>Lasius</i> | <i>flavus</i> | 5 | 0 |
| | | | <i>fujii</i> | 3 | 0 |
| | | <i>Polyrhachis</i> | <i>japonicus</i> | 40 | 0 |
| | | | <i>nipponensis</i> | 7 | 0 |
| | | | <i>sakagamii</i> | 2 | 0 |
| | | | <i>spathepus</i> | 5 | 0 |
| | | | <i>orientalis</i> | 2 | 0 |
| | | | <i>umbratus</i> | 1 | 0 |
| | | | <i>lamellidens</i> | 1 | 0 |
| | | | <i>Polyergus</i> | <i>samurai</i> | 2 |
| Myrmicinae | <i>Aphaenogaster</i> | <i>japonica</i> | 1 | 0 | |
| | | <i>Myrmica</i> | <i>jessensis</i> | 1 | 0 |
| | <i>Pristomyrmex</i> | <i>kotokui</i> | 1 | 0 | |
| | | <i>punctatus</i> | 1 | 0 | |
| Termites | <i>Tetramorium</i> | <i>tsushimae</i> | 79 | 33 | |
| | | <i>speratus</i> | 1 | 0 | |
| Outside ant nest | | | 2 | 0 | |
| Total | | | 187 | 34 | |

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.

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

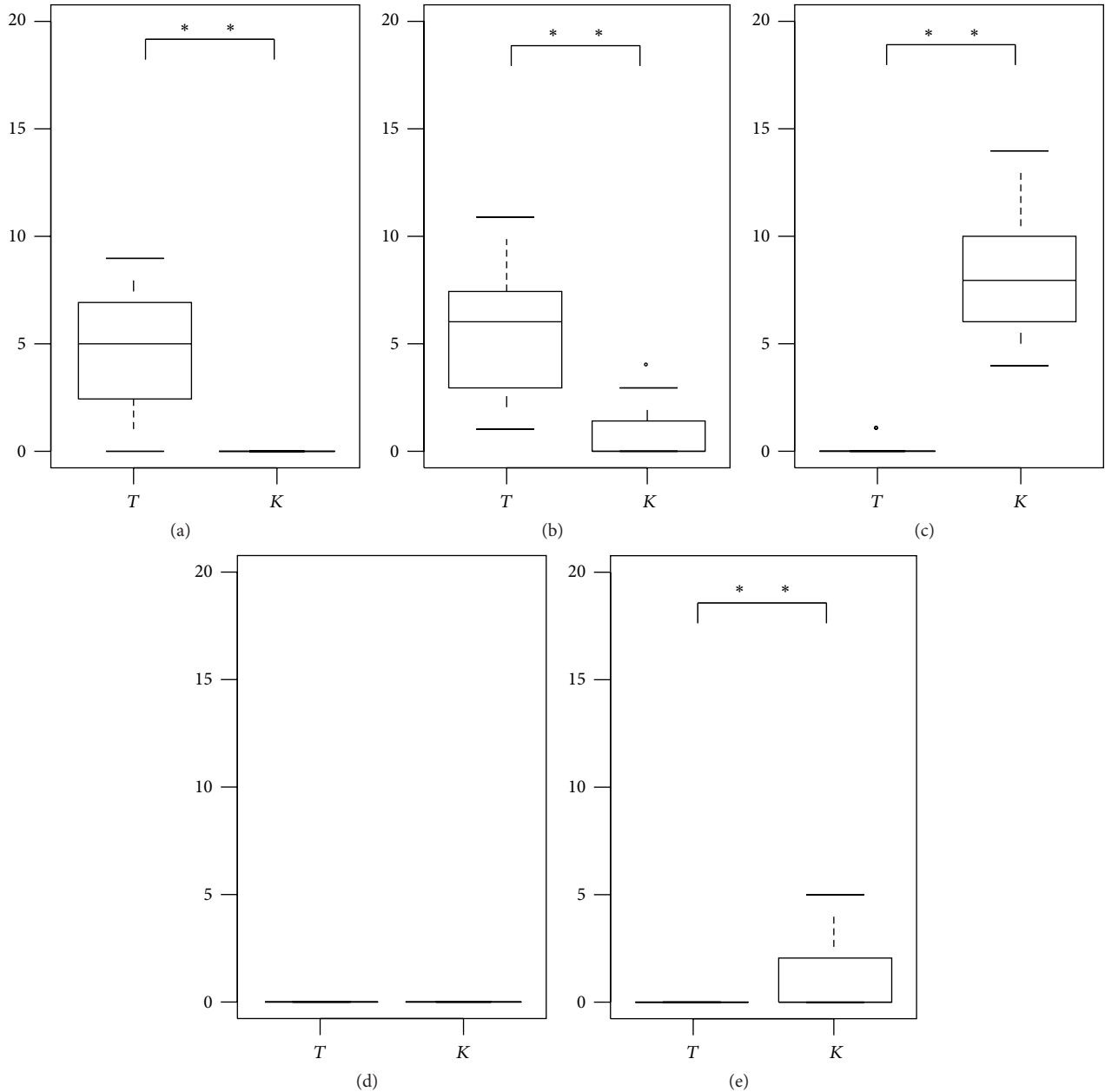


FIGURE 1: Behavior recognized in *M. tetramorii* (T) and in *M. kubotai* (K) in colonies of *T. tsushimae*. (a) Being attacked by ants and escaped from them immediately, (b) feed foods for themselves, (c) groom ant body, (d) muscle in trophallaxis between ants, (e) be done a feeding by direct mouth-to-mouth 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 outliers 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 \pm 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.

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