

Psyche

# Ants and Their Parasites

Guest Editors: Jean-Paul Lachaud, Alain Lenoir, and Volker Witte



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## Editorial

# Ants and Their Parasites

**Jean-Paul Lachaud,<sup>1,2</sup> Alain Lenoir,<sup>3</sup> and Volker Witte<sup>4</sup>**

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Ants accumulate and protect collective resources and, with the exception of nomadic species, live in a nest which is considered to be one of the bases of the evolution of eusociality. Resources and/or protective services provided by ant colonies are exploited in manifold ways by an amazing diversity of other organisms acting as guests and/or parasites: viruses, bacteria, fungi, sporozoa, amoeba, ciliates, nematodes, trematodes, cestodes, mollusks, diplopods, crustaceans, mites, spiders, and a large variety of parasitic or parasitoid insects. Such associations can be obligatory or facultative, permanent or temporary, harmful or beneficial for the host. Due to the diversity of interactions, an understanding of the nature of these relationships and the mechanisms of integration used by parasites as well as the defense strategies developed by their potential host remains a challenge. Parasites certainly increase specific selection pressures on colony phenotype, and they may also shape the composition and dynamics of ant communities. Over the past two decades there has been a growing interest in the impact of parasites on colony phenotype, and their role in the ecology and evolution of their hosts. Despite the apparent importance of ant parasites, detailed knowledge is lacking, for example, about their diversity and abundance or selection pressures imposed through parasitism on host reproductive strategies.

Parasitism in ants has attracted the attention of numerous scientists in the last two centuries (see the numerous publications on this topic by authors like E. W. Janson, A. Forel, C. Janet, E. Wasmann, H. Viehmeyer, J.-J. Kieffer, K. Escherich, W. M. Wheeler, H. St. J. K. Donisthorpe, W. M. Mann, C. Rettenmeyer, E. O. Wilson, B. Hölldobler,

D. Kistner, U. Maschwitz, and P. Schmid-Hempel among others). Since the synthesis of Kistner in 1982 [1] and of Schmid-Hempel in 1998 [2], only one complete review has been published [3] (but see also [4] for social parasites) and, in spite of the accumulation of much information in the last decades, this meaningful topic has not been brought together in a specific issue for a long time. This special issue, of course, cannot cover all possible ant parasites, but it examines a wide range of species: viruses, bacteria, fungi, nematodes, silverfishes, flies, butterflies, beetles, spiders, wasps, and ants themselves. It is divided into two main sections: (1) behavioral and ecological aspects of parasitism, in which existing associations are reviewed and discussed, some new associations are described, and some concepts are reanalyzed in a more up-to-date integrative vision; (2) infection, impact on ants and biological control, in which particular effort has been made to provide both an analytical review of the experimental material actually available and a proposal of guidelines for future research on the topic.

*Behavioral and Ecological Aspects of Parasitism.* Numerous species take advantage of the supplies of other organisms and cleptobiosis, a quite common phenomenon among animals, also occurs at ants' expenses. M. D. Breed et al. review concepts linked to food stealing in social insects, distinguishing cleptobiosis from some related phenomena, and place this knowledge in ecological and evolutionary contexts. In most cases, success in parasitizing ants depends largely on the degree of resemblance to the host, which varies considerably among the diverse mimetic parasites found in

ant societies. Different morphological, behavioral, and/or chemical adaptations allow cleptoparasites to deceive ant defense mechanisms. Reviewing more especially the use of the terms that describe the chemical resemblance between the cuticular chemical profiles of parasites and that of their hosts, C. von Beeren et al. give an overview of cases concerning adaptive chemical resemblance and propose a terminology more consistent with that generally used in biology: “chemical crypsis” when the operator (the host) does not detect the mimic as a discrete entity and “chemical masquerade” when the operator detects the mimic but misidentifies it as an uninteresting entity.

The reports of adaptive resemblance (visual, morphological, and/or chemical) are amazingly numerous. Spider-ant associations, for example, involving either myrmecomorphy, myrmecophily or myrmecophagy, or a combination of the three, are very common, and P. E. Cushing provides an excellent update of her important 1997 review [5] with a welcome summary of recent work testing the adaptational significance of these associations. Among spiders, myrmecomorphy is supposed to usually involve Batesian mimicry but such an assumption has not been demonstrated experimentally. X. J. Nelson provides the first evidence that salticid ant mimicry is truly Batesian mimicry. She shows experimentally both that ant mimicry is perceived by the predator and has a protective effect for the mimic, and how a predatory spider is affected by the degree of visual resemblance of ant mimics to ants.

Among the myrmecophiles, the Coleoptera are probably the most diverse group. In some cases, associations have been known for a long time but the behavioral adaptations allowing the integration of the parasite remain poorly studied. Through a quantitative analysis, E. Maurizi et al. show that the rewarding behavior, during which the ground beetle *Paussus favieri* provides attractive chemical substances to its host *Pheidole pallidula*, is one of the key factors for acceptance and full integration in the ant society. Another way of deceiving a host is through innate chemical mimicry (*sensu* C. von Beeren et al.), involving a change in the parasite’s chemical profile in accordance with the host nest odor. This is what A. Lenoir et al. report for a histerid and a staphylinid beetle, both of which parasitize *Aphaenogaster senilis*, whereas a silverfish that shares the same host shows only low quantities of host hydrocarbons, which are probably acquired passively. After isolation, the histerid beetles *Sternocoelis hispanus* keep their hydrocarbon quantity, showing that they are able to synthesize them and adjust their profile to the host colony via direct contacts. This is the first such demonstration in a myrmecophile beetle. In all the other cases studied, the hydrocarbons are provided by the host as occurs, for example, in *Malayatelura ponerophila*, a kleptoparasitic silverfish of *Leptogenys distinguenda* [6]. Various species, like many coccinellids, indirectly affect ants through their predation on trophobiotic Hemiptera. Possibly, frequent interactions with ants led some species to become myrmecophilous and to use chemical mimicry to get close to their prey. A. Vantaux et al. provide an overview of the evolution of myrmecophilous traits in ladybirds and discuss from an evolutionary perspective both costs and benefits of myrmecophily and even dietary shifts

to myrmecophagy, which occurred in a few species. By comparison, the myrmecophagy on leaf-cutter ant queens by dung beetles of the genus *Canthon* is a much better known phenomenon, even if behavioral studies are scarce. L. C. Forti et al. present in their thorough behavioral study many details on how *Canthon virens* attacks *Atta* queens.

Besides hemipterans, numerous butterflies species are associated mutualistically with ants, and species from the family Lycaenidae are among the best studied. Interestingly, associations also changed towards parasitic interactions several times. K. Fiedler presents a comprehensive review of the host ants of parasitic lycaenids and analyzes the macroecological patterns that could be related with the use of particular ant genera as hosts. His large-scale survey reveals that those ant genera in which associations with lycaenids are particularly numerous are also more likely to serve as hosts for parasitic species. Among lycaenids, associations between the genus *Maculinea* and the ant genus *Myrmica* are certainly the most investigated. F. Barbero et al. present an overview of the adaptations used by *Maculinea* butterflies to infiltrate and live as parasites in *Myrmica* ant colonies, and more particularly, based on a synthesis of recent research, how they mimic the acoustic communication of their hosts. Some other parasitic butterflies are less well known, and L. A. Kaminski and F. S. Carvalho-Filho describe and illustrate for the first time the immature stages of *Aricoris propitia* and uncover the diversity of life cycles in the still enigmatic butterfly family Riodinidae.

A large number of parasitoid species have been reported in association with ants but, most often, the true nature of their relationships is poorly known and few species are really highly specialized on ants in general. High specialization on ants is however the case in various genera of phorid flies and in numerous species of wasps from three superfamilies: Chalcidoidea, Ichneumonoidea, and Diaprioidea. B. V. Brown and S. M. Philpott describe three new species of *Pseudacteon* flies with some details on the natural history of the ant-fly interaction and provide a useful taxonomic key to the species. Successful parasitism by phorids involves utilization of multimodal cues to locate and recognize the host. K. A. Mathis and S. M. Philpott review some important components of phorid biology, and the variety of strategies and cues used by the three most common phorid genera attacking ants (*Apocephalus*, *Pseudacteon*, and *Neodohrniphora*). Apart from their direct parasitic effects on ants, phorid flies also affect their behavior, and H.-Y. Hsieh and I. Perfecto review the impact of parasitoid phorids on ants and other organisms that interact with ants. They focus both on the variety of mechanisms used by ants to cope with phorid parasitism and on the complexity of these interactions through trait-mediated indirect effects on other trophic levels. Finally, in an attempt to understand how parasitoids affect their host ants’ foraging success in a community framework involving species of different body size and behavioral dominance, E. B. Wilkinson and D. H. Feener Jr. examine how habitat structural complexity affects the foraging behavior of two species of the genus *Pheidole* by interacting with parasitoids of the genus *Apocephalus*. Hymenopterous parasitoids of ants also exhibit a wide

array of adaptations to attack such potentially dangerous hosts. Reports of parasitoid wasps associated with ants are numerous but real primary parasitoidism has rarely been proven. J.-P. Lachaud and G. Pérez-Lachaud review all of the cases for which such primary parasitoidism has reliably been established, providing an updated list of at least 138 species from 9 families. They report some new associations and focus both on the diversity of these parasitoid wasps and the diversity of the types of interactions they have formed with their ant hosts. Among these hymenopteran parasitoids of ants, the eucharitid family has been particularly under scrutiny for the last three decades, especially the genus *Kapala*, the most common in the Neotropics [7]. A. A. Vásquez-Ordóñez et al. present useful natural history information on the interactions between a *Kapala* species and the host ant *Ectatomma ruidum* in Colombia. Evaluating the effect of habitat type on eucharitid parasitism, they report a significantly higher prevalence of parasitism in host ant colonies in woodland compared to grassland habitat.

*Infection, Impact on Ants and Biological Control.* In numerous applied studies on ant parasitism, specific attention has been given to the ways in which parasite pressure may affect patterns of life history in ant hosts. For example, generalist entomopathogenic fungi could be used in biological control of pest ants as discussed by M. M. R. Ribeiro et al. in the case of *Beauveria bassiana* and *Aspergillus ochraceus* against the grass-cutting ant *Atta bisphaerica*, one of the most important pests of pastures and crops in Brazil. It is the first time that *A. ochraceus* is reported to infect *Atta* with a high prevalence. However, field experiments are necessary to test for their effect as biological control. Pathogens are difficult to identify because sick or dying ants are promptly removed from the nest or leave the nest themselves (see the recent review by Shorter and Rueppell [8]). Some entomopathogenic fungi are ant specific and X. Espadaler and S. Santamaria review what is known concerning the taxonomy, natural history, and/or ecology of ecto- and endoparasitic fungi specialized on ants throughout the Holarctic region. The fungi considered in this paper show a gradient of negative effects on the host, and their specificity does not seem to be always very strict since various fungi are known from a range of hosts (e.g., *Laboulbienna*; *formicarum* is hosted by 24 ant species belonging to 3 formicine tribes). Specificity is apparently higher both in the mutualistic basidiomycetous fungi cultivated for food by neotropical fungus-growing ants of the tribe Attini, and the specialized microfungus parasites which coevolved with these associations and have a negative impact on the fungus gardens. Recent research on this issue has provided novel insights into coevolution, antibiotic defense mechanisms, and behavioral interactions within symbiotic systems. F. C. Pagnocca et al. pulled together diverse literature and present a review of the microfungi associated with leaf-cutting ant gardens, while S. H. Yek et al. synthesize our current understanding on the evolution of specialized parasites of the attine fungus gardening system. Using a modified version of Tinbergen's four categories of evolutionary questions to structure their review, they focus on development, mechanism, adaptation, and evolutionary

history and suggest further directions for investigations of this symbiosis.

Various other organisms, in addition to entomopathogenic fungi, can affect the biology of their hosts. Bacteria of the genus *Wolbachia*, for example, are known to alter the reproductive capabilities of their hosts significantly, showing complex interactions with them, which, in some cases, have evolved to symbiotic associations. K. K. Ingram et al. examine possible parameters affecting the spread of *Wolbachia* infections in a newly established population of *Formica fusca*. Their results show that horizontal transmission of *Wolbachia* is apparently uncommon and that there are no marked fitness differences between infected and noninfected colonies. This is an additional illustration of the complex role of *Wolbachia* in ants which is not yet explained (see [9, 10]). Ants can also serve as hosts of a variety of internal or external parasitic nematodes from several families with more or less complex life cycles. Different entomopathogenic nematodes like *Steinernema* and *Heterorhabditis* have been suggested to control ants through inundative applications. The current state of knowledge regarding the occurrence, systematics, life history, and pathology of all described nematodes associated with formicids is summarized by G. Poinar Jr. through a richly illustrated review. Apart from including a simple key to the higher taxa of ant-infecting nematodes, he identifies the large gaps that exist in our understanding of this very interesting system.

The use of ant parasites as a means of biological control has been most heavily investigated in relation to one of the most important pests in the New World, the imported fire ants (*Solenopsis*). Focusing their review on research programs that have been carried out over 25 years in their laboratory, J. Briano et al. give a wide panorama of the natural enemies of fire ants (microsporidia, nematodes, viruses, phorid flies, eucharitid wasps, myrmecolacid strepsipteran, and social parasitic ants). They summarize published information and include many complementary unpublished observations. Among these natural parasites, a more special focus is given by S. M. Valles about the research on viruses through a compilation of the literature on fire ant viruses, and a review on the properties of three particular viruses infecting *S. invicta*. It is worth noting that viruses were unknown in any ant species before the first discover by Valles and colleagues in 2004 [11], and this topic will certainly be a central issue in fire ants control programs in the future. Finally, the evaluation of the use of *Pseudacteon* parasitoid flies as potential biological control agents of invasive *Solenopsis* fire ants is critically and exhaustively reviewed by L. W. Morrison. The sound conclusions of this review about the realities of biological control of fire ants by phorid flies contrast with most of the literature on that topic and emphasize the necessity of a battery of complementary natural enemies, in addition to the release of phorid flies, for potentially successful regulatory effects on fire ant populations. This points to a need for investing more effort into studies on other potential control agents.

More and more studies show a fascinating coevolution between parasites and their hosts. An accurate survey of this topic will provide useful information to refine our understanding of both the mechanisms involved and their

phylogenetical and evolutionary components. With the growing interest in biodiversity, we realize that we are far from concluding our assessment of existing forms of parasitism. Considering the increasing losses in biodiversity due to habitats restructuring and climatological changes, the urgent need for making such inventories is obvious. The world of the microorganisms is one of the most promising. For example, G. Poinar Jr. indicates that some 20,000 nematodes have been described, while their species diversity has been estimated to be as high as 10 million, and even if the proportion of known species associated with ants is low, their real number is surely much more impressive than actually suggested. A metagenomics approach will be useful in the future; it has already begun in honeybees [12] and in termites [13] and is just starting up in ants [14]. Parasites contribute to maintaining complex ecosystems and have a role in stabilizing mutualisms as observed in fungus-growing ants [15]. Their role as a “top-down” process, structuring ant communities and populations, is also considered to be important. Some authors, like Feener [16], suggest that the assembly of ant communities is mediated by parasitoids. Others [2, 17, 18] suspect that parasites and parasitoids may be involved in the emergence of alternative reproductive strategies such as polygyny and/or multiple mating (polyandry), by inducing an enhancement in the genetic diversity of the workers that would increase resistance to parasites and pathogens (but see [19]). Recent data on the evolution of elaborate mushroom bodies in the brains of hymenopteran insects even suggest that the neurobehavioral modifications linked to the capacity for associative and spatial learning during host-finding behavior in parasitoids may have served as preadaptations for central place foraging in social hymenopterans [20]. Ants (and, more generally, social insects) and their parasites are an exceptional model. In the next years, 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, will surely constitute a challenging and fascinating goal for us and many colleagues.

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Jean-Paul Lachaud

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## Review Article

# Trait-Mediated Indirect Effects of Phorid Flies on Ants

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This paper provides a synthesis of the ecological impact of phorid fly parasitoids on ants. We find the most important impact of phorids on ants to be trait-mediated effects. Phorids diminish the foraging activity of ants, frequently reducing the number and average size of foragers and reducing the amount of food retrieved by a colony. However, ants' coping mechanisms include changing foraging site and time. Phorids can also affect competition, especially through changes in the ability of the host to win in exploitative competition. Factors such as microclimate, resource size, and habitat complexity interact with phorids to change their effect on competition. By being highly specific and attacking ants high in the competitive hierarchy, phorids can alter the linear nature of the competitive transitivity, and by reducing the number of foragers, they can change the discovery-dominance tradeoff that is observed in some ant communities. Trait-mediated effects of phorids also cascade to other trophic levels. As an example, we discuss the trait-mediated cascade of phorids on the *Azteca instabilis* system in coffee. In this system, by reducing the foraging activity of *A. instabilis*, phorids reduce the direct and indirect biological control impact of the ant in the coffee agroecosystem.

## 1. Introduction

The best-studied family of ant parasitoids, Phoridae, has been recognized as an important mediator of ant community structure [1]. Indeed, over the past decades, there have been many studies on the impacts of phorid attacks on ants, from the effects on ant foraging activity, size of foragers, and amount of food retrieval, to the effects at the community level involving several interacting species at different trophic levels. What is clear from this literature is that the main consequences of phorid attacks on ants are not direct density effects but rather effects mediated by changes in the behavior of ants, the so-called trait-mediated indirect effects (TMIEs) [2]. Although many reviews have been written about TMIE generally [2–4], there has not been a review on how phorid flies impact ant communities through trait-mediated indirect interactions (TMII). Since phorid parasitoids attack mainly workers and parasitism rates tend to be very low, their direct impact on the colony is minor [1, 5]. However, attacking phorid flies elicit ant defensive behaviors that can have repercussions at the community

level. These trait-mediated effects have been shown to be important for understanding invasibility of ants [6] as well as the role of ants in biological control of agricultural pests [7].

In this paper we provide a synthesis of studies on the ecological impacts of phorids on ant assemblages and ecological networks focusing on TMII. The literature review focuses on studies published over the last ten years, since Feener's review [1]. However, we also use some of the older literature to support our conclusions. We first examine the effects of phorid parasitoids on ant foraging activity, including number of foragers, forager sizes, and amount of food retrieved. We then examine the evidence for the hypothesis that phorid parasitoids alter the outcome of competition among ants. More specifically, we examine evidence for the impact of phorid flies on exploitative and interference competition and for how parasitoids may alter competitive dominances among ant assemblages [1, 8–11]. Finally, we present evidence for trait-mediated effects that transcend ant assemblages and result in changes in the broader community including ant prey, ant mutualists, and

the predators of the mutualists. For this last section, we focus on our own work of *Azteca instabilis* in coffee plantations.

## 2. Direct Density Effects of Phorid Parasitization on Ants

Studies examining direct mortality due to phorid parasitism on ants have reported very low parasitism rates. For example, *Pseudacteon* parasitism on *Solenopsis geminata* has been reported to be only 3% [12], while *Apocephalus* parasitization on *Pheidole* has been reported to be 5% [13, 14]. Parasitization rates of ants in their introduced ranges can be even lower. Morrison and Porter [5] reported a 0.058% of average parasitism rate per colony of *Solenopsis invicta*, a host ant species of *Pseudacteon tricuspis* Borgmeier in a time-span of two years study, in northern Florida. In his review, Feener [1] also indicated that the effects of density-mediated interaction between phorid parasitoids and host ants are normally very low, with 1-2% as the likely magnitude of the effect of direct phorid parasitism on host ant density reduction. Since most phorid flies that parasitize ants are highly host specific, use ant pheromones to find their host [15], and attack workers while foraging, retrieving food resources, or performing other tasks outside their nest, they are bound to elicit specialized defensive behavior that can result in TMIE [1]. Most of the research on the effects of phorid flies on ants has focused on these trait-mediated interactions between phorids and their ant hosts.

## 3. Trait-Mediated Effects on Ant Foraging Activity, Resource Acquisition, and Defense

**3.1. Number of Foragers.** One of the first recognized TMIE of phorids on ants was their effect on foraging activity [16]. Most ant colonies show behavioral responses to attacks by phorids. The most common behavioral response is a reduction in the number of foragers. Most of the studies show that the number of foragers or ants recruited to a resource is reduced significantly in the presence of phorids (Table 1) [16–37]. In the case of *Azteca instabilis*, this activity reduction results from two actions on the part of the individual workers. First, some ants go inside their nest, and second, some ants acquire a defensive posture and stop moving [7]. This defensive stationary posture has also been observed in *S. geminata* [21]. It has been shown that some species of *Pseudacteon* that parasitize *A. instabilis* and *Solenopsis* species require movement of the host in order to oviposit [15, 38]. The combination of these two actions on the part of *A. instabilis* and *S. geminata* workers results in a reduction of 50% of the foraging activity in these two species [21, 30, 31]. Reductions in ant foraging activity in the presence of phorid flies have also been reported for *Linepithema* [32, 39], *Pheidole* [13, 14], and *Atta* [17, 40]. Ants can also respond to phorid attacks by increasing foraging activity during periods of time when phorids are not active, for example, at night. This seems to be the case for several species of the leaf cutter ants in the genus *Atta*

[16, 17, 26], for *Linepithema* [39], and for *A. instabilis* (de la Mora, unpublished data).

**3.2. Size of Foragers.** Phorid parasitoids also influence the size of foragers (Table 1). The pattern of worker size selection by ovipositing female phorid flies has been described for a few species of *Pseudacteon* on *Solenopsis* [12, 41–43], and *Neodohrniphora* on *Atta* [44, 45]. Mathis and Philpott [15] discuss ant size as a factor in host acceptance by phorid species. Differences in host size preferences within the fire ants are seen as an effective niche partitioning when several species attack the same host [36]. However, in general, phorid parasitoids tend to prefer larger than average workers. In these cases, the response of the ants to the presence of phorids is to reduce the average size of the foragers [17, 23, 25–28, 40, 46, 47]. Morrison and Gilbert [43] reported that the size of the emergent phorid was positively related to the size of the host worker with females emerging from a larger host. If ant colonies respond to phorid attacks by changing the size distribution of foragers, this can alter the phorid's sex ratio and can potentially affect the efficiency of phorid parasitoids in biological control of invasive ants [43].

**3.3. Acquisition of Food Resources.** The reduction in the number and size of foragers can have an effect on the ability of ants to obtain and defend food resources [16, 17, 22–28] (Table 1). Laboratory and field studies have reported up to 50% reduction in food acquisition by *S. invicta* in the presence of phorid flies [21, 23, 40]. In laboratory experiments, Mehdiabadi and Gilbert [22] showed that the presence of only one phorid fly per 200 workers of *S. invicta* reduced the number of large size workers 50 days later. In the same experiment, they demonstrated that the reduction in foraging and size of foraging workers resulted in a nearly twofold reduction of protein colony consumption. Reduction in the amount of food consumed in the presence of phorid flies has been reported for other ant genera including *Linepithema* [24] and *Pheidole* [20]. However, in another laboratory experiment with *S. invicta* and its *Pseudacteon* parasitoids, Morrison [23] showed that in control trials, where no phorids were present, food retrieval was intermediate to that of the phorid-no-phorid trials, suggesting that ants are foraging more in the no-phorid trials (of the phorid-no-phorid trials) to compensate for the reduction in food retrieval from the phorid-present trials. This kind of compensation can happen in the field if the ants forage more during periods of no-phorid activity, as discussed previously, or shift to forage underground when phorids are present, something that has been shown to happen in *Solenopsis* [48]. Furthermore, in a laboratory experiment, Ramirez et al. [49] reported that reduction in food retrieval was not observed when the trials were left running for a period of 72 hours. These experiments suggest that in the long run and under field conditions ants that are attacked by phorid parasitoids compensate for potential losses in the amount of food retrieved by foraging more at times when phorids are not active.

TABLE 1: Ant response to phorid parasitism.

<i>Research retrieval</i>			
Ant spp.	Phorid spp.	Measured ant response to phorid parasitism	References
<i>Atta cephalotes</i>	<i>Neodohniphora curvinervis</i>	Reduced resource retrieval	[16]
<i>Atta sexdens</i>	<i>Neodohniphora</i> sp.	Reduced number of loaded ants	[17]
<i>Atta sexdens</i>	<i>Neodohniphora</i> sp.	Reduced resource retrieval	[18]
<i>Azteca instabilis</i>	<i>Pseudacteon</i> sp.	Increased time for foragers to carry away resource	[19]
<i>Pheidole diversipilosa</i>	<i>Apocephalus</i> sp. 8	Increased resource turnover rate by competitor	[20]
<i>Solenopsis geminata</i>	<i>Pseudacteon browni</i> and <i>P. bifidus</i>	Reduced resource retrieval	[21]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced colony protein consumption	[22]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced resource retrieval	[23]
<i>Solenopsis richteri</i>	<i>Pseudacteon</i> sp. (multiple)	Reduced number of workers at resource	[24]
<i>Worker size</i>			
Ant spp.	Phorid spp.	Measured ant response to phorid parasitism	References
<i>Atta</i>	<i>Neodohniphora erthali</i>	Increased number of hitchhikers	[25]
<i>Atta cephalotes</i>	<i>Neodohniphora curvinervis</i>	Sent out workers in smaller size	[17]
<i>Atta cephalotes</i>	Unreported	Sent out workers in smaller size	[26]
<i>Atta laevigata</i>	<i>Apocephalus attophilus</i>	Sent out workers in smaller size	[27]
<i>Atta sexdens</i>	<i>Neodohniphora</i> sp.	Reduced forager mass	[17]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Sent out workers in smaller size	[22]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Sent out workers in smaller size	[23]
<i>Solenopsis invicta</i>	<i>Pseudacteon</i> sp.	Altered ratio of worker size	[28]
<i>Solenopsis richteri</i>	<i>Pseudacteon</i> sp.	Sent out workers in smaller size	[24]
<i>Ant activity</i>			
Ant spp.	Phorid spp.	Measured ant response to phorid parasitism	References
<i>Atta laevigata</i>	<i>Apocephalus attophilus</i>	Altered number of foragers	[27]
<i>Atta sexdens</i>	<i>Myrmosicarius grandicornis</i>	Reduced number of loaded ants	[29]
<i>Atta sexdens</i>	<i>Neodohniphora</i> sp.	Increased number of unloaded workers returning to nest & decreased number of loaded workers returning to nest	[17]
<i>Atta sexdens</i>	<i>Neodohniphora</i> sp.	Altered number of foragers	[17]
<i>Azteca instabilis</i>	<i>Pseudacteon</i> sp.	Reduced ant activity	[30]
<i>Azteca instabilis</i>	<i>Pseudacteon</i> sp.	Reduced number of ants	[31]
<i>Linepithema humile</i>	<i>Pseudacteon</i> sp.	Altered number of foragers	[32]
<i>Pheidole titanis</i>	<i>Pseudacteon</i> sp.	Reduced ant activity	[14]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced number of exposed ants	[20]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Altered recruited ant size	[22]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Altered forager size	[34]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reducers number of ants at baits	[23]
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced number of foragers	[35]
<i>Solenopsis invicta</i>	<i>Pseudacteon</i> sp.	Reduced number of ants at baits	[28]
<i>Solenopsis richteri</i>	<i>Pseudacteon</i> (multiple spp.)	Reduced number of ants at baits	[24]
<i>Solenopsis richteri</i>	<i>Pseudacteon</i> (multiple spp.)	Reduced various ant activity	[24]
<i>Solenopsis saevissima</i>	<i>Pseudacteon</i> sp.	Reduced ant activity	[36]
<i>Solenopsis</i> sp.	<i>Pseudacteon</i> sp.	Altered number of foragers	[37]

3.4. *Compensatory Factors.* Other factors can help host species compensate for the negative trait-mediated effects of phorid parasitoids. For example, habitat complexity in the form of leaf litter provides refuge from parasitoids for soldier

caste of *Pheidole diversipilosa* and *P. bicarinata* resulting in an increased number of foraging soldiers even in the presence of phorid parasitoids [50]. Habitat complexity, thus, allows these two species to balance foraging success with the

avoidance of parasitism. Likewise, the size and distribution of resources can have similar effects. In a field experiment, Wilkinson and Feener [51] demonstrated that the presence of multiple large resources allows colonies of *P. diversipilosa* to redistribute soldier ants from sites that have phorid flies to sites that do not have phorids, therefore maintaining overall numbers of foraging soldiers at the same levels as found in the absence of phorid parasitoids.

There is a gap in our knowledge about how many ant hosts mitigate the threat of phorid parasitism by altering regimes, altering posture of exposure, or by foraging on other resources. It is possible that we may be overestimating the population level impact of phorid parasitoids on ants by focusing on day time interactions or by not measuring other population level parameters such as density, occupancy, and colony migration.

#### 4. Phorid Parasitoids and Competition within Ant Assemblages

The kinds of behavioral changes described in the previous section can have important consequences for ant community structure. Since phorid parasitoids tend to be highly specific and attack only one or very few species of ants within a community, they can alter the competitive interactions and change ant community structure [1]. This effect can be especially important when the host ant is a competitive dominant species, which in the best-studied cases of ant-phorid interactions they frequently are, probably because dominant ant species are evolutionarily more conspicuous [1].

When phorid parasitoids are present, host species are faced with a tradeoff between defending themselves against parasitism and maximizing their competitive abilities. The outcome of this tradeoff is not always clear. Based on the evidence of the TMIE of phorid parasitoids on host ants, it is tempting to conclude that phorid parasitoids reduce the competitive ability of host species. However, this is not always the case. Indeed, competitive interactions among ants are complex and influenced by a variety of factors, and therefore, a generalized outcome of the effects of phorid parasitoids on ant competitive interactions is highly unlikely. What we see in the literature is a reflection of that complexity.

**4.1. Exploitative and Interference Competition.** Ants of different species engage in exploitative and interference competition with each other [20, 21, 34, 52–54]. Exploitative competition occurs when the removal of a limiting resource by one species makes it unavailable for other species, while interference competition involves direct aggressive interactions between individuals of different species. The presence of phorid parasitoids has been shown to influence both of these types of competition but this is, in no way, a universal phenomenon. For example, in laboratory experiments, phorid parasitoids were found to increase the exploitative competitive ability of *Forelius mccooki*, a competitor of the host species, *S. invicta*. However, phorids did not affect the direct aggressive interactions between the two species [34]. Furthermore, although the competitor of

the host species increased the number of foragers by a factor of two in the presence of phorid flies, that did not translate into higher colony growth. Similar results have been reported in field experiments. A study of the competitive interactions between *S. geminata* and *S. invicta* in the presence of phorid parasitoids of *S. geminata* found that the host species retrieved 50% less food than the nonhost species in the presence of phorid flies [21]. Much the same as in the lab experiment, in the field, phorid parasitoids had no effect on the interspecific aggression between *S. geminata* and *S. invicta* and did not affect the outcome of these interactions at resources. In the case of *A. instabilis*, phorids also seem to influence exploitative but not interference competition [31, 55]. In field experiments, competitors of *A. instabilis* were able to access bait resources 12 times more often in the presence of phorids and were able to take over baits only when phorid parasitoids of *A. instabilis* were present. However, in most cases, *A. instabilis* did not lose competitive interactions with other species [55]. The lack of an effect in the interference competition interactions between host and nonhost species could be due to the behavioral response of the ants engaged in the fight or a change in behavior of the phorid parasitoids. For example, *S. geminata* has been seen to ignore attacking phorids when engaged in fights with *S. invicta* [21]. But, phorid parasitoids have also been observed to lose interest or be distracted by ants that are engaged in active fighting with other ants. Feener [13] presented the first evidence for the TMIE of phorids on interference competition between the host species *Pheidole dentata* and its competitor, *Diplorhoptrum texanum* (referred to as *Solenopsis texana*). It is reported that parasitism by phorids was the factor that most strongly influenced the turnover of resources from *P. diversipilosa* to its competitors [20]. The same study also reports that phorid parasitoids reduce exploitative competitive abilities of *P. diversipilosa* [20]. On the other hand, Orr et al. [53] report that phorid parasitoids seldom influence exploitative competition between two *Linepithema* species and their nonhost competitors in Brazil. This field study joins others that have not been able to detect clear effects of phorids on ant competition [56].

**4.2. Factors That Interact with Phorid Parasitoids to Affect Competition.** The lack of a clear pattern on the effect of phorid's TMIE on ant competition has to do with the many other factors that are involved in determining the winners and losers of both exploitative and interference competition. Among the potential factors, here we will discuss four, for which there is some evidence in the literature: feedback loops caused by ant chemical pheromones, size and distribution of resources, habitat complexity, and abiotic factors such as temperature and humidity.

Phorid flies are known to use ant kairomones released by their host ant to locate them [15]. When an ant encounters a competitor, it is more likely to release alarm pheromones that can be used by their phorid parasitoids to find them more easily, causing a positive feedback that may result in a higher turnover rate of resources from host species to their competitors [20]. There are at least two cases where these

kinds of positive feedbacks have been documented. The parasitoid *Apocephalus* sp. discovers faster and arrives in greater numbers at recruitment events where its host species, *P. diversipilosa*, is engaged in competitive conflict than to recruitment events where the host is foraging alone or does not experience conflict [20]. Likewise, parasitoids of two species of *Linepithema* arrived significantly faster at resources where the host was with another ant species than when it was alone [39]. Furthermore, the rate at which phorid flies arrive at baits depends on the competitor species present and the type of response it elicits from *Linepithema*. Phorid parasitoids arrived faster at baits when the competitor elicits a chemical response versus baits where the competitor elicits primarily physical aggression [39]. If host ants engaged in direct competition with other species elicit faster and stronger responses from their phorid parasitoids than those that do not encounter competitors, phorids can have an even stronger effect on competition through this positive feedback. Moreover, if the feedback mechanism works for some competitors and not others, as in the case of *Linepithema* and its competitors, the impact of phorids on community structure and colony energetic will depend on these behavioral responses and will be different in different community contexts.

The effects of resource size and distribution and habitat complexity were discussed in the previous section in the context of compensation mechanisms for acquiring resources under the pressure of phorid attacks. These factors can also buffer the impacts of phorids on competitive interactions between host species and nonhost species [10, 20, 50, 51]. For example, habitat complexity, by allowing continued foraging even when phorids are present, can influence the competitive success of the host species [50]. Likewise, widely distributed resources may allow host species to redistribute their foragers to resources not monitored by phorids and continue succeeding in exploitative competition [51]. Recruitment to large resources, on the other hand, could increase the number of phorid attacks but the effect of resource size has not been well explored in the literature. In general, ants that recruit to resources tend to recruit more and larger workers to larger resources [20, 24]. Since phorid parasitoids show a density-dependent response to ants [55, 57], higher numbers of ants at a resource will attract higher numbers of phorid parasitoids. Therefore, a higher proportion of large resources at a particular site could represent a liability for those host species that recruit to large resources, which is the case for most species attacked by phorids. However, if a higher proportion of larger resources also results in greater availability of large resources to hosts, ant hosts would be able to switch to resources not monitored by parasitoids [50].

Temperature and humidity affect not only ants but also phorids [58–61]. These two variables could interact to lead to very different competitive outcomes under varying environmental conditions. For example, parasitoid habitat preferences (see [15]) have been shown to cause major differences on parasitism pressure on host ants and their interactions with competitors [11]. In laboratory experiments, Ramirez et al. [49] demonstrated that changes in

humidity interact with the presence of phorid parasitoids to alter the competitive outcome of encounters between the invasive *S. invicta* and the native species *S. xyloni*. They attributed the lack of establishment and spread of *S. invicta* in New Mexico to these interactions.

**4.3. Competitive Dominance Hierarchies and Species Coexistence.** Interspecific competition can have profound effects on the abundance, composition, and distribution of species. Communities structured by competition can be organized in a variety of ways that can greatly influence species coexistence and, therefore, the maintenance of diversity within a community. Competitive communities that are organized in a linear transitive dominance hierarchy will tend to have low species diversity because, at equilibrium, the competitive dominant species will exclude all others. On the other hand, intransitive hierarchies, a situation in which the competing species cannot be ranked in a perfect competitive hierarchy, can promote diversity [62–67]. Interspecific competition has been identified as an important factor in structuring ant communities, especially among ground foraging omnivorous ants that forage more or less for the same resources [52, 68–76]. However, to date, no competitive intransitivity has been convincingly demonstrated for any ant community. Rather, ant communities have been described to be organized in transitive dominance hierarchies [10, 11, 52, 69, 77, 78]. A question then emerges as to how ant communities are able to maintain species diversity under conditions of transitive dominant hierarchies. TMIE mediated by phorid parasitoids can provide a partial answer to this question [10, 13, 20], although other factors such as environmental variation [56, 75, 79] and size of resources [10, 51, 80, 81] have called into question the generality of the transitive dominance hierarchies among ant communities.

By being highly specific and attacking ants that tend to be high in the competitive hierarchy, phorids can alter the linear nature of the competitive transitivity. In a study of the ant community in pine-oak woodlands in Arizona, LeBrun [10] describes several distinct dominance hierarchies within the ant assemblage. However, the linearity of the dominance hierarchies was determined by the size of the resource and the presence of phorid parasitoids. When competing for fixed resources or for small nonfixed resources in the absence of phorids, the assemblage exhibits significant linear dominance hierarchies. In contrast, in the presence of phorids for both fixed and small resources, this linearity breaks down [10]. For example, on fixed resources, phorids caused the second dominant species to drop to the second most subordinate, and the third species dropped to the fourth position. These changes in the ranking of species dominance generated more indeterminacy in the outcome of individual paired interactions reducing the asymmetries underlying the dominance in the transitive hierarchy. It has been shown, at least theoretically, that when interactions take place locally, which is the case for ants competing for food resources, an increase in symmetry favors diversity [66], providing a potential mechanism for the maintenance of diversity in ant communities.

An alternative competitive structure to the dominant hierarchy that has been described for ant assemblages is the so-called dominance-discovery tradeoff [11, 68, 69, 72, 82–87]. The tradeoff implies that while some species are good at dominating resources, others are good at discovering the resource [54, 69, 74, 83, 85]. This tradeoff can also lead to species coexistence in much the same way that the competition-colonization tradeoff [88] and the virulence-transmission tradeoff [89] lead to species coexistence [10, 90]. Phorid-induced TMIE can play an important role in maintaining species within the dominance-discovery tradeoff curve, therefore promoting diversity [85]. For example, examining the same ant assemblage in pine-oak woodlands in Arizona, LeBrun and Feener found that the elimination of phorid parasitoids caused host species to become too dominant for their level of discovery ability, breaking down the dominance-discovery tradeoff. It has been proposed that this could be a mechanism for the spread of invasive ant species in areas where they are released from parasitoid pressure [1, 85]. However, examining the competitive interactions among an assemblage of ground foraging ants and *S. invicta* in its native range, Feener et al. [11] did not find strong support for this hypothesis. Rather, they proposed that both interspecific competition and TMIE of phorid parasitoids affect the success of *S. invicta* in its native range, but that these factors vary dramatically in different regions.

Overall, these studies suggest that phorid parasitoids can have a strong effect on competitive interactions within ant assemblages but these effects are, in no way, consistent and are mediated by many other factors that vary in time and space, such as microclimate, habitat heterogeneity, and size and abundance of resources.

## 5. Trait-Mediated Cascades: The Case of *Azteca instabilis* in the Coffee Agroecosystem

Ants are an important component of ecosystems in most regions of the world. Since they frequently constitute a great part of the animal biomass in ecosystems, are taxonomically diverse, and act as ecosystem engineers [91–93], they tend to interact with many other organisms. Given the strong TMIE of phorids on ants, and given the wide range of ecological interactions that ants form with other organisms, it should come as no surprise that these TMIEs cascade into other trophic levels of an ecological community. The best-documented case of these sorts of phorid-mediated cascading effects can be found in the *A. instabilis* system. For more than ten years, we have been studying the ecological interactions surrounding this ant species in coffee plantations in southern Mexico (for a review see [7]). Here we will describe the pivotal role that phorid parasitoids play in shaping these interactions.

*Azteca instabilis* is a dominant arboreal ant with a wide distribution in the Americas, from Brazil to Mexico [94]. On coffee plantations it is found nesting in shade trees and foraging on both shade trees and coffee plants. This species forms spatial clusters of nests that have a high genetic

relatedness (Remfert, unpublished data). The clusters appear to be the result of self-organization emerging from the internal dynamics of the system—short distance dispersal to adjacent trees and density-dependent mortality [95]. One of the main resources for *A. instabilis* in the coffee plantations is honeydew from *Coccus viridis*, the green coffee scale [96]. This mutualism plays an essential role in the distribution of the scale insect, which is a potential pest in coffee [96]. *Azteca instabilis* has been reported to prey on a variety of herbivores in coffee plantations contributing to the control of potential insect pests [7, 19, 96–100]. The effect of *A. instabilis* on deterring herbivores is not only through the direct action of preying or removing herbivores from plants, but also through an indirect effect in which some herbivores avoid plants that have been foraged on by *A. instabilis*, but were no actual ants where present when the herbivores arrive [99]. Additionally, it has been shown that through a complex network of ecological interactions *A. instabilis* is a keystone species that contributes to the regulation of insect pests and diseases in coffee [7, 101–103]. *Azteca instabilis* also competes with other arboreal ant species, especially twig-nesting species [104], and influences the abundance and diversity of ground nesting and arboreal ant species (Perfecto and Vandermeer, in review; Ennis, unpublished data) and spiders (Marin, unpublished data). The mutualism between *A. instabilis* and the scale insects consists of protection of scales from parasitoids and predators, especially the coccinellid beetle, *Azya orbiger*a [30, 101], and removal of sooty mold (Jha et al., in review).

Philpott et al. [19] published the first documented case of a phorid parasitoid attacking *A. instabilis*. At that time it was thought that only one species of *Pseudacteon* was responsible for the attacks. However, recently (see [105] in this issue) three species have been described attacking *A. instabilis*. *Pseudacteon* spp. have strong TMIE on *A. instabilis* [19, 31, 55], as reported previously. More importantly, these TMIEs cascade to other trophic levels within the community, with important implications for the biological control of insect herbivores and diseases of coffee [7, 101].

By reducing *A. instabilis* foraging activity, phorids disrupt the ability of the ants to remove insect pests from coffee [19]. In laboratory experiments, it was shown that phorids essentially cancel the ability of *A. instabilis* to deter coffee berry borer attacks on coffee fruits [100]. Likewise, we have demonstrated that the presence of phorids reduces the ability of ants to attack, carry away, and force off plants lepidopteran caterpillars that could be potential pests in coffee [19].

Higher-order cascading trait-mediated indirect effects have also been documented for this system [7, 30, 101]. The protection that the ants offer to their scale mutualist is the first level trait-mediated indirect effect—the ants disrupt the ability of the predatory beetle to kill and consume scale insects. By causing a reduction in the foraging activity of *A. instabilis*, phorids disrupt the ability of the ant to protect its mutualist, the green coffee scale [30]. This is the second order trait mediated indirect effect (Figure 1). When phorids are present, they essentially cancel the protective effect of ants against adults of *A. orbiger*a, the coccinellid predator. In laboratory experiments, in the presence of ants and phorids

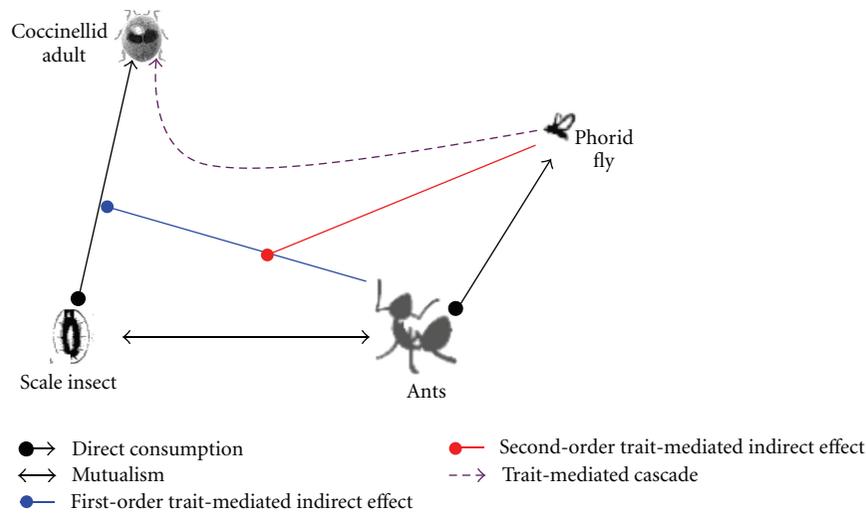


FIGURE 1: Diagrammatic representation of the cascading trait-mediated indirect interactions between *Pseudacteon* spp. and *Azya orbigera*. Arrows represent positive effects and solid circles represent negative effects. Black solid lines represent direct interactions, the blue solid line represents first level trait-mediated indirect interactions, the red solid lines represents the second level trait-mediated indirect interactions, and the dashed purple line represents the resulting cascading trait-mediated indirect interaction between the phorid flies and the coccinellid beetle.

adults of the predatory beetle were able to gain access to the scale and consume the same quantities as when no ants (and no phorids) were present. In other words, through these higher order cascading trait mediated indirect effects, the phorids facilitate the coccinellid beetle (Figure 1).

The complexity of this network of interactions increases when the larval stage of the coccinellid beetle is considered. The larva of *A. orbigera* is covered by waxy filaments that protects it from ant predation [106]. This means that larvae of the main predator of the scales are able to live in patches of high ant activity where the scale is abundant. Furthermore, the ants repel parasitoids in the vicinity of the scale insects, including any parasitoids of the coccinellid beetle, essentially protecting coccinellid larvae [106]. The presence of phorids could, potentially, eliminate this unintended protective effect of the ants on the coccinellid larvae, by reducing ant patrolling on clusters of scales. However, this interaction has not been yet documented.

Our research also shows that gravid female beetles of *A. orbigera* are able to eavesdrop on the “phorid-alert pheromones” (Hsieh, unpublished data) and oviposit under green coffee scales or other clandestine microsites that workers of *A. instabilis* and natural enemies of *A. orbigera* would have difficulty finding, removing, and preying. The natural history and interactions between *Pseudacteon* spp., *A. instabilis*, and *A. orbigera* can well explain why we can find high abundances of *A. orbigera* in the coffee agroecosystem. Since this is the main predator of the green coffee-scale, and it seems to require patches of *A. instabilis* for the successful development of its larvae, it can be argued that the maintenance of the *Azteca*-green coffee scale mutualism is essential for the successful biological control of the green scale at the level of the entire farm [7, 95].

Theoretically and empirically, parasitism in spatially distinct patches has been suggested to be an important driver of spatial self-organization of host-parasitoid dynamics [107]. The *Azteca* system in the coffee agroecosystem adds empirical evidence to the theory of spatial self-organization in host-parasitoid systems. We proposed that *Pseudacteon* spp. contributes to the spatial pattern formation of *A. instabilis* by acting as a density-dependent control mechanism [95]. Given the fact that the coccinellid beetle is able to capitalize on the trait mediated interaction between *Pseudacteon* spp. and *A. instabilis*, we suggest that adding trait-mediated cascades to theoretical models would increase our understanding of how complex systems might contribute to spatial self-organization and system stability. Furthermore, the *A. instabilis*-*Pseudacteon* spp.-*A. orbigera* system illustrates how trait-mediated cascades effect biological control in a spatially explicit complex ecosystems.

## 6. Conclusion

Phorid fly parasitoids influence ants mainly through trait-mediated indirect interactions. The presence of phorid flies results in a reduction of foragers, a change in the average size of foragers, mainly toward the smaller sizes, shifts in the time and places of foraging to avoid encounters with phorids, and reduction in the amount of food retrieved. These effects, independently or in combination, have important consequences for the way ants interact with other ant species and with other members of the interacting network within a community.

Through these TMIEs phorids can have important effects on competitive interactions among ants. When phorid parasitoids are present, host species respond behaviorally and

can impact their competitive abilities. However, since competitive interactions among ants are complex and influenced by a variety of factors, the outcome of the effects of phorid parasitoids on ant competitive interactions is highly variable. Phorids have been shown to reduce exploitative competitive abilities of some host species but not others. Likewise, they have been shown to affect the interference competition between host and nonhost, but this effect is not widespread among studies. It has been shown that phorid parasitoids can break a competitive hierarchy within ant assemblages by attacking the most competitive dominant species within the hierarchy. Phorids also can influence the dominance-discovery tradeoff that is found in some ant assemblages. The alteration of the competitive structure of ant assemblages could be important in understanding invasibility of ants to ranges where their phorid parasitoids are absent.

Trait-mediated effects of phorids on ants can also transcend the ant assemblage and have cascading effects on other trophic levels and other organisms linked to the host ant species through complex ecological networks. For example, phorid parasitoids can also influence the impact of ants on herbivores. If the host species is an important predator of an herbivore, the presence of phorids can release these herbivores from predation pressure from ants. This could be important in agroecosystems where ants have been shown to be important predators of insect pests. The study of *A. instabilis* in coffee plantations presents an excellent case study of these cascading trait-mediated indirect interactions and shows that they could be important in maintaining biological control.

Many areas of research remain open in the study of ant-phorid interactions. In particular there are very few studies that link TMIEs of phorids to population level consequences in ants and other organisms. Making and testing predictions regarding the TMIEs of phorids on population density, occupancy, colonization, and migration patterns across landscapes should be priority of future studies. The *Azteca* system described previously represents a step in the right direction to fill this gap in our knowledge of ant-phorid interactions. However, this system is only one example of the many complex ecological networks that could be influenced by phorid parasitoids. Future studies should focus on these kinds of complex ecological networks and on trait-mediated cascading effects that would be important in understanding the role of ants when they are embedded in complex ecological networks.

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## Review Article

# Spider-Ant Associations: An Updated Review of Myrmecomorphy, Myrmecophily, and Myrmecophagy in Spiders

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This paper provides a summary of the extensive theoretical and empirical work that has been carried out in recent years testing the adaptational significance of various spider-ant associations. Hundreds of species of spiders have evolved close relationships with ants and can be classified as myrmecomorphs, myrmecophiles, or myrmecophages. Myrmecomorphs are Batesian mimics. Their close morphological and behavioral resemblance to ants confers strong survival advantages against visually hunting predators. Some species of spiders have become integrated into the ant society as myrmecophiles or symbionts. These spider myrmecophiles gain protection against their own predators, live in an environment with a stable climate, and are typically surrounded by abundant food resources. The adaptations by which this integration is made possible are poorly known, although it is hypothesized that most spider myrmecophiles are chemical mimics and some are even phoretic on their hosts. The third type of spider-ant association discussed is myrmecophagy—or predatory specialization on ants. A table of known spider myrmecophages is provided as is information on their biology and hunting strategies. Myrmecophagy provides these predators with an essentially unlimited food supply and may even confer other protections to the spiders.

## 1. Introduction

The majority of spiders are solitary generalist predators of insects [1]. Most spiders, as with most arthropod predators, are averse to ant predation because ants are generally aggressive, some are venomous, and most are simply noxious for a variety of reasons [2]. Nevertheless, hundreds of arthropod species live in some level of proximity or association with ants [3–5]. The present paper supplements a review I published in 1997 [5] identifying and describing the biology of spiders that are found in association with ants. In the earlier article, I summarized what was then known about the biology and identities of ant-mimicking, or myrmecomorphic, spiders as well as spiders living in close proximity to or living within ant colonies, known as myrmecophiles. That review included tables listing known spider myrmecomorphs and myrmecophiles. The purpose of the present paper is not to replicate information contained in the 1997 article but, instead, to provide a summary of the extensive theoretical and empirical work that has been carried out in recent years testing the adaptational significance of the various spider-ant associations. Additionally, I summarize instances of a

different kind of spider-ant association—that of predator-prey relationships, or myrmecophagy—and provide a table of known species of spiders that feed on or specialize on ants.

## 2. Spider Myrmecomorphy

*2.1. Morphological and Behavioral Adaptations.* Morphological adaptations conferring mimetic resemblance to ants include color pattern similarities as well as more dramatic morphological changes such as abdominal constrictions and/or constriction of the cephalothorax, both of which give the illusion that the spider has more than two body parts [5–7] (Figures 1(a) and 1(b)). One recent paper demonstrated that some of these morphological adaptations may be synapomorphic for lineages [8], suggesting that at least some of the morphological adaptations associated with myrmecomorphy may be under phylogenetic constraint. Additional morphological adaptations seen in some spider myrmecomorphs include enlargement of the chelicerae or enlargement or other adaptations associated with the pedipalps or first legs. For example, males of some species of salticids in

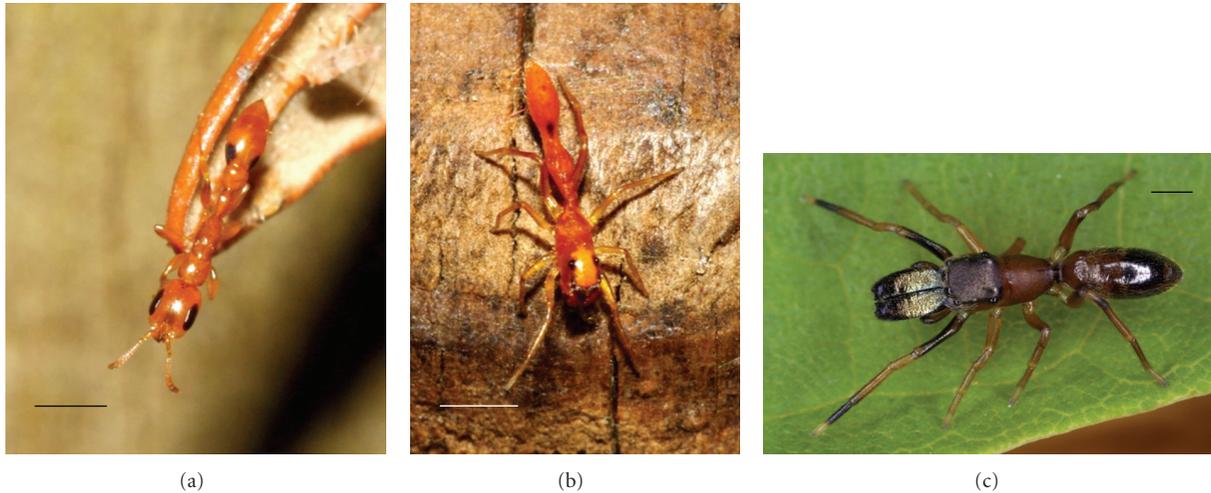


FIGURE 1: Myrmecomorphy in spiders. (a) The model ant *Pseudomyrmex simplex* (Smith) and its mimic, (b) *Synemosyna petrunkevitchi* (Chapin) (Salticidae). Photos © Lyn Atherton, used by permission. (c) *Myrmarachne formicaria* (De Geer) (Salticidae) showing the enlarged chelicerae of the male. Photo © Jay Cossey/PhotographsFromNature.com, used by permission. Scale bars = 1 mm.

the genus *Myrmarachne* have greatly enlarged chelicerae that extend anteriorly [9] (Figure 1(c)). These large chelicerae are thought to have evolved via sexual selection [10]. Recent research demonstrated that male *Myrmarachne* with enlarged chelicerae mimic encumbered ants (worker ants carrying items in their mandibles) [11, 12]. In the myrmecomorphic species in the family Corinnidae, *Pranburia mahannopi* Deeleman-Reinhold, the first pair of legs of males has a thick brush of setae around the distal part of the femora. When the spider is disturbed or alarmed, it brings the femora together and the brushes give the illusion of an ant head (i.e., the spider behaviorally and morphologically acquires a third body part [13]).

Spider myrmecomorphs resemble the model ants to varying degrees of accuracy. Some myrmecomorphs are, at least to the human observer, nearly perfect mimics; others generally resemble ants but no specific model species in the vicinity of the spider can be identified. The latter are termed “imperfect” or “inaccurate” mimics [14, 15]. Some species of myrmecomorphic spiders are polymorphic mimics, mimicking multiple species of ants found in the habitat (see [5, Table 1], and [9, 16–20]). One species of jumping spider (Salticidae), *Myrmarachne bakeri* Banks, is polymorphic in color patterns and individual spiders can even change patterns during the course their lives, even after molting to maturity [20]. Individuals can change their patterns even under constant environmental conditions and feeding regime [20]. Other myrmecomorphs are transformational mimics, mimicking different species of ants during their different developmental stages (see [5, Table 1], and [9, 16–18, 21]).

In addition to morphological resemblance to ants, most spider myrmecomorphs are also behavioral mimics (see citations in [5]). This behavioral mimicry includes erratic movement, much more akin to the movement of ants than the movement of spiders, and lifting the first or second pair of legs when moving through the environment as an antennal illusion [22]. Myrmecomorphic salticid spiders also hunt

their prey by lunging at and sometimes tapping the prey rather than by leaping on it as is common in most non-mimetic salticids [11, 22–24]. In other words, these spiders maintain their resemblance to ants even when hunting.

**2.2. General Adaptive Significance of Myrmecomorphy.** Myrmecomorphy has long been hypothesized to be an example of Batesian mimicry, conferring an adaptive advantage to the mimics against visually hunting arthropod predators that have either an innate or learned aversion to ants. Several studies have provided strong support for this hypothesis, demonstrating that myrmecomorphic spiders are less likely to be chosen as prey by visually hunting predators that would otherwise readily accept spiders [25–32]. In several of these studies, the predators used are naïve and have never encountered ants before, demonstrating that aversion to ants, at least in some arthropod predators, is innate rather than learned [27, 28, 30]. In order for myrmecomorphy to provide an adaptive advantage to the mimics, the mimics must live in close proximity to the models [33–38]. In addition, mimics should be rarer than models [15, 34, 36, 39, 40].

However, myrmecomorphic spiders, particularly those in the salticid genus *Myrmarachne*, often live in high concentrations within a given area. For example, *Myrmarachne melanotarsa* Wesolowska and Salm lives in aggregated groups in which their silken nest complexes are in close association with nests of their model ant, *Crematogaster* sp. [24]. Since ants live in often very large colonies, it has been hypothesized that aggregations of myrmecomorphs are an example of “collective mimicry” in which the myrmecomorphic spiders are, by living in aggregated groups, mimicking the colonial aspects of the models. Groups of mimics may be perceived by predators as more aversive than single individuals found in the habitat [24, 29]. A counter to this hypothesis is that the mimic may therefore outnumber the model in small areas of the habitat, making it more likely that predators will

sample and learn the patterns of the palatable mimics and making Batesian mimicry less effective [34]. In some visually hunting spider predators, such as the wasp *Pison xanthopus* (Brulle) (Sphecidae), individuals can develop search images for myrmecomorphic spiders and stock proportionally more mimics in their mud cells than would be expected if the wasp was randomly hunting spiders in the environment [41]. Therefore, some predators are capable of learning to search for myrmecomorphs. However, in a study of the mud-dauber *Sceliphron spirifex* (L.), Jocqué found no myrmecomorphic spiders among almost 600 spiders removed from mud nests, despite *Myrmarachne* species being common in the habitat suggesting that, at least for this wasp, ant mimicry does provide protection from visually hunting predators [42].

Yet, it has been pointed out that mimics can still confer protection against predators even when they are more abundant than the model if certain conditions exist: (1) if the model is very noxious, then the predators will avoid good mimics regardless of the relative proportions of models and mimics; (2) if the mimic has low nutritional value and is, therefore, not worth pursuing; (3) if very profitable alternative prey are present in which case the predator will avoid both model and mimic regardless of the relative abundance of each; or (4) if the relative perception of abundance is different, for example, if the predator perceives the model as more abundant than the mimic (perhaps because of the higher activity levels of the models) [37].

**2.3. Evolution of Polymorphic Mimicry.** In recent years, researchers have explored the adaptive basis and the conditions under which polymorphic mimicry might arise. Theoretically, a mimic species should converge on mimetic resemblance of the single model species found in that habitat, particularly for predators that learn to avoid the model [37]. Yet many instances of polymorphic mimicry among spider myrmecomorphs have been documented (see citations in Section 2.1). Several hypotheses have been proposed to explain the existence of polymorphic mimics. For example, Ceccarelli and Crozier [43] suggested that the evolutionary rates between different morphs of the salticid *Myrmarachne* and their presumed models differ [43]. These authors demonstrated that morphs of the mimics radiated rapidly leading to higher degrees of polymorphism and provided evidence of possible sympatric speciation. *Myrmarachne platalaeoides* (O. P.-Cambridge) mimics the weaver ant *Oecophylla smaragdina* (Fabricius). Borges et al. [19] showed that the different color morphs of *M. platalaeoides* may mimic different models in the habitat besides *O. smaragdina*. Males of each color morph showed greatest interest in the silk retreats of females of their own color morph. Disruptive selection may be maintaining the polymorphism in this population [19]. In addition, it has been proposed that polymorphic mimicry, in essence, provides a “moving target” for template learning among visually hunting predators that learn to avoid aversive prey [44]. Nelson [44] proposed that polymorphism in a myrmecomorphic species reduces the apparent number of mimics per model. Therefore, predators cannot easily distinguish palatable mimics from the unpalatable models

because the characteristics of the prey are continuously changing. The new mimetic form will be advantageous since it is rare, but if this morph increases too much in frequency within the habitat, it may lose its mimetic protection and be selected against [37]. This selective process itself may generate selection for new morphs [37].

Sexual dimorphism can be considered a type of polymorphism. In many cases of sexually dimorphic spider myrmecomorphs, the male is more mimetic than the female, such as in species of the Corinnidae genus *Castianeira* and the Oonopidae genus *Antoonops* [13, 45]. Such sexual dimorphism may be adaptive if the sexes are different in ecology and are thus exposed to different predation pressures and selective forces [46]. Joron [46] provides a model supporting this mode of evolution and selection for sexual dimorphism among mimetic species. Although mimics gain protection from the resemblance to noxious species, they are often more conspicuous in their color markings than related species that have evolved cryptic coloration. Thus conspicuousness can be considered a cost of Batesian mimicry [47]. A palatable species may be evolutionarily maximizing its level of protection for the smallest cost (in terms of conspicuousness) and this evolutionary balancing act may lead to sexual dimorphism in which the more active sex (which, in spiders, is typically the males) evolves mimetic resemblance to noxious models whereas the other sex remains relatively more concealed and camouflaged behaviorally and morphologically [47].

In some species of the salticid genus *Myrmarachne*, the males and females are both mimetic but the males have extraordinarily long chelicerae. This sex mimics ants carrying an object in their mandibles [11, 12]. The large chelicerae of males are thought to have evolved via sexual selection [10]. These large chelicerae are an encumbrance to males during prey capture; however, they make males much more efficient than females in breaking into other spiders' silken retreats and feeding on eggs or juveniles [10]. Consequently, in this case of sexual dimorphism, both sexes have maintained mimetic resemblance to the models, although the male is mimicking a slightly different type of model ant (an encumbered ant). Any costs incurred from the dimorphism may be outweighed by benefits in opening up a different trophic niche for the males (oophagy).

**2.4. Evolution of Imperfect or Inaccurate Mimicry.** It is well documented that many mimics are imperfect in their mimetic resemblance to the model. These species generally resemble the putative models but are not accurate mimics [14, 31, 37, 47, 48]. Some authors propose that poor mimics are just on an evolutionary trajectory towards perfection. This hypothesis is discussed by Edmunds [49] and Gilbert [37]. Gilbert [37] refutes this hypothesis saying, “*In my view it is better to assume that poor mimetic patterns have evolved to an equilibrium state, rather than being in the process of being perfected by constant directional selection*” since there is no experimental or theoretical support for the hypothesis that imperfect mimics are just mimics on their way towards perfection.

Recently, authors have instead proposed various evolutionary scenarios that may select for imperfect or inaccurate mimicry rather than explain this phenomenon away as “evolution in progress.” Many papers point out that if a model is extremely unpalatable, noxious, or difficult to capture, then even imperfect mimics will gain strong selective advantage from a general resemblance to this model and there may be no selective advantage or pressure for more accurate mimetic resemblance [34, 37, 39, 47, 50]. In fact, the fitness costs of close morphological resemblance (see Section 2.5) may select against accurate mimicry and may select for imperfect mimicry if either confers approximately the same selective advantage in terms of escape from predation. In a study by Duncan and Sheppard [50], the authors experimentally demonstrate that, when the model is very noxious, even imperfect mimics gain protection. However, when the model is only moderately distasteful, selection favors more accurate mimics. They showed that when the cost of making a mistake, attacking a distasteful model because it is mistaken for a palatable mimic, is high, the predator rejects a greater proportion of mimics and there is little selection for more accurate mimicry. When the penalty for making a mistake is low, tiny improvements in mimetic resemblance confer a selective advantage to the mimics, leading to more accurate mimicry [50]. In a study by Speed and Ruxton [47], the authors propose that if generalization by the selective agents (the predators) is narrow, selection towards accurate mimicry is predicted. If generalization by predators is relatively wide (e.g., in the case of a particularly noxious model), variations in mimetic forms may be selected for with both accurate and inaccurate mimics. Finally if generalization by predators is intermediate, then the rate of evolution selecting for accurate mimicry will be slow and polymorphic mimetic forms will be stable.

In situations in which the model either becomes rare or is weakly aversive and the incentive to attack and sample the models (by predators) is high, then close mimics may in fact be selected against. Kin selection among the mimetic population would select for less accurate mimics that diverge in their mimetic resemblance to the weakly defended model [15, 34, 37]. Inaccurate mimicry can also be favored in species with limited dispersal and high local abundance in which neighboring mimics are related (i.e., kin selection) [15].

A study by Kikuchi and Pfennig [39] provided experimental support for the hypothesis that evolution of accurate mimicry is a gradual process and depends on the relative abundance of the model. In this study, the authors found that in areas where the model was abundant, predators attacked cryptic (or camouflaged) prey, accurate mimics, and intermediate (or imperfect) mimics with the same low frequency. In other words, in areas where the model was abundant, predators generalize and imperfect mimics gain the same relative protection as more accurate mimics. In habitats where the model population was low, camouflaged species and mimics attained greater protection than imperfect mimics. Thus the authors showed that Batesian mimicry can evolve through gradual steps towards more accurate mimicry depending on conditions and context (particularly the abundance of models in the habitat) [39]. This study also suggests that

mimics may have evolved from cryptic or camouflaged ancestors.

Accuracy of the mimetic resemblance may depend largely on the visual acuity of the selective agent. If predators with keen vision serve as the primary selective agents, then these predators may select for more accurate mimicry [34]. Then again, mimicry may be in the eyes of the beholder. Arthropods that humans view as poor mimics were perceived by pigeons, in an experimental test, as very good mimics [14]. Dittrich et al. [14] also showed that slight changes in the morphology of the mimic led to sometimes dramatic improvements, from the perspective of the selective agent, in perceived mimetic resemblance. They further pointed out that discrimination between a good and a poor mimic occurs via multiple features (e.g., color, form, size), not a single characteristic [14]. Other authors have also suggested that selection for increasingly better mimetic resemblance can, in fact, be a gradual process through directional selection [50, 51].

Related to the hypothesis that mimetic accuracy is dependent on the visual acuity of the selective agent is the multi-predator hypothesis, which proposes that inaccurate Batesian mimics evolved as a result of selective forces from a suite of predators [52]. For example, model averse predators select for more accurate morphological mimics in a given habitat while specialist predators on the model (e.g., ant predators or myrmecophages) select for inaccurate mimicry or for secondary defenses in the mimic [52]. Secondary defenses may include fast evasive movements by the mimics (quickly dropping all pretense of behavioral mimicry) or signaling the predator in such a way as to communicate its true identity [52]. If both kinds of predators are present in a habitat, there may be selection for inaccurate mimics or for polymorphic mimicry [52].

One hypothesis explaining imperfect Batesian mimicry that has gained some momentum in recent years is the multi-model hypothesis. If many potential model species live in a given habitat (e.g., many different species of ants), then it may be adaptive for the mimetic species to evolve a general, imperfect resemblance—a gestalt resemblance—to all of them than to evolve a specific morphological resemblance to a particular model [33, 37, 49]. For example, a general ant-mimicking spider in such a habitat can then have a much greater range than a spider that resembles only one of the potential models. If it is an accurate mimic, then its range is limited to the range of that one species in order to be an effective Batesian mimic. In one study, the authors found that some species of accurate ant mimics were found in association with a single model (measured as the closest ant collected where the spider was found). Some imperfect mimics (by human standards) were collected in proximity to more than one species of ant, conferring some support for the multi-model hypothesis [33]. However, in this same study, the author also found habitats in which accurate and inaccurate mimics did not associate with the models as predicted.

*2.5. Trade-Offs Affecting the Evolution of Myrmecomorphy.* A close morphological resemblance to ants makes myrmecomorphs more attractive to ant predators or myrmecophages. Thus myrmecomorphs are faced with an evolutionary trade-off: they gain protection from general arthropod predators but risk predation from a completely different suite of predators ([11, 12, 53] and citations above under discussion of multi-predator hypothesis). Many spider myrmecomorphs confront a threat from a myrmecophage by completely dropping their behavioral mimicry. These spiders will stop their erratic ant-like movement and run away, drop on a silk thread, signal to the predator in a spider-specific manner, or otherwise communicate their true identity to the predator [11, 52, 54]. This strategy is effective in allowing the spider to escape from the myrmecophage (or from ants that may confront it directly) [11, 24, 54].

Myrmecomorphs face other costs that may affect their fitness, including (1) constraint of the circadian rhythm of the mimic since it must be active at the same time of day as the model for the resemblance to be adaptive; (2) an imposed limit to the myrmecomorph's trophic niche because it would only have access to prey that lived in the same habitat as the model; (3) a possible detrimental or costly effect on mating or reproduction since many myrmecomorphs must mate in a sheltered location, where their non-ant-like behavior will not "give the game away" or may mate for a shorter duration than non-mimetic relatives for the same reason; (4) a lowering of fecundity with the abdominal narrowing or constrictions often associated with myrmecomorphy and the resultant decrease in the number of eggs a female can produce [37, 55]. It has been documented that narrower abdomens in female spiders limit the number of eggs that can be produced in comparison to non-mimetic relatives [9, 18, 56–61]. In addition, there may be a cost associated with alteration in the prey capture behaviors, such as those seen in myrmecomorphic salticids that lunge rather than jump upon their prey, which may be a much less effective prey capture strategy.

Nevertheless, if the primary predators demonstrate an innate, rather than learned, aversion to ants, the circadian rhythm of the myrmecomorphs may not be greatly affected and they can be active at any time of day. The limitation of trophic niches may not apply to general ant mimics since these spiders can exist, according to the multi-model hypothesis, across a potentially broad range of habitats. It does seem though that most spider myrmecomorphs do share the same habitat as their models and are active at the same time of day. It has even been pointed out that no species of wolf spider (family Lycosidae) has been reported to be an ant mimic because most lycosids are nocturnal and not active when visually hunting arthropod predators are most active [4]. Researchers investigating the inaccurate myrmecomorphs *Lio-phurillus flavitarsis* (Lucas), *Phrurolithus festivus* (C. L. Koch) (both in the family Corinnidae), and *Micaria socialis* Kulczynski (Gnaphosidae) found that, in comparison to these species' closest relatives, the trophic niche of each was constrained by their resemblance to ants because they were limited to catching only small invertebrates found in the same habitat as the models. The circadian rhythms of these

myrmecomorphs were also constrained because the myrmecomorphs were all diurnal (as were the models) but the closest relatives were nocturnal. However, the reproductive traits were not constrained since the fecundity of the inaccurate mimics was about the same as the non-mimetic relatives and the myrmecomorphs mated out in the open on bark, not dropping their behavioral mimicry when copulating [55].

The evolution of close morphological and behavioral mimicry of ants is costly and these costs should be measured as fitness components [37]. In addition, more studies should attempt to identify the operators or selective agents selecting for mimetic resemblance since the visual acuity of these selective agents (if they can be identified) may affect the accuracy of the resemblance. All these costs, trade-offs, and constraints should be taken into account when testing or modeling the adaptive significance of myrmecomorphy. The relative measures of the costs and benefits of mimetic resemblance may have a significant impact on the accuracy of the resemblance. If, for a particular species, the fitness costs of close mimetic resemblance due to lower fecundity greatly outweigh the benefits, then imperfect or inaccurate mimicry may be selected for. For example, in a habitat where the primary selective agent is a predator with low visual acuity, increased mimetic accuracy may impose a higher cost in terms of fecundity than is gained in terms of escape from predation. In small species of spiders in which greater mimetic resemblance would lead to dramatically lower fecundity due to a narrowing of the female's abdomen, dimorphic mimicry may be selected for and males may show greater mimetic resemblance than females. Too few models take into account fitness costs of mimetic resemblance and the relative effect such trade-offs may have on the evolution of imperfect, polymorphic, transformational, and dimorphic mimicry.

### 3. Spider Myrmecophily

*3.1. Additional Records of Spider Myrmecophiles.* Myrmecophiles are defined as ant guests, arthropods that have evolved close associations with ant species, often living alongside the ants or within the ant colonies [2, 3, 5, 62]. Some, but not many, of these myrmecophiles are also myrmecomorphs. Recent work (cited below) has found that, among spider myrmecophiles, some are also myrmecophages.

An extensive table of spider myrmecophiles was presented by Cushing [5]. Table 1 supplements this earlier table and provides records of spider myrmecophiles not included in the previous table. Not as much work has been carried out exploring the natural history, adaptations, or evolutionary significance of spider myrmecophiles as has been done with spider myrmecomorphs and myrmecophages. Nevertheless, some significant research has been conducted recently that expands our understanding of the biology of these interesting ant associates and how this unique lifestyle may have evolved in a group of arthropods that otherwise includes primarily free-living, solitary predators.

*3.2. Adaptive Significance of Myrmecophily.* An ant colony, as pointed out by Hölldobler and Wilson [2], can be considered

TABLE 1: Spider myrmecophiles found in association with or inside ant nests. This table is meant to supplement the table of Araneae myrmecophiles found in Cushing [5]. Spider taxonomy according to Platnick [63]; ant taxonomy according to <http://antbase.org/>.

Spider myrmecophile	Ant host	Notes on biology	References
<b>Linyphiidae</b>			
<i>Diastanillus pecuarius</i> (Simon)	<i>Formica</i> cf. <i>fusca</i> L. and <i>F. lemami</i> Bondroit	Found under stone near ants.	[64, 65]
<i>Pseudomaro aenigmaticus</i> Denis	<i>Lasius flavus</i> (Fabricius)	Associated with nests.	[65]
<i>Syedra myrmicarum</i> (Kulczynski)	<i>Manica rubida</i> (Latreille) and <i>Formica</i> sp.	Found under stone near ants.	[64, 65]
<b>Oonopidae</b>			
<i>Dysderina principalis</i> (Keyserling)	<i>Labidus praedator</i> (Smith) (publ. as <i>Eciton praedator</i> )	Found inside nests.	[66]
<i>Gamasomorpha maschwitzi</i> Wunderlich	<i>Leptogenys processionalis distinguenda</i> (Emery) (publ. as <i>L. distinguenda</i> )	Found inside nests. Chemical mimic. Phoretic. Follows emigration trails of hosts. Builds webs inside nest.	[65, 67–69]
<i>Gamasomorpha wasmanniae</i> Mello-Leitão	<i>Eciton</i> sp.	Found inside nests.	[70]
<i>Xestaspis loricata</i> (L. Koch) (publ. as <i>G. loricata</i> )	<i>Myrmecia dispar</i> (Clark)	Found inside nests.	[71]
<b>Salticidae</b>			
<i>Cosmophasis bitaeniata</i> (Keyserling)	<i>Oecophylla smaragdina</i> (Fabricius)	Lives inside nest. Is chemical mimic of ant. Feeds on ant larvae by using tactile mimicry.	[72–76]
<i>Phintella piatensis</i> Barrion and Litsinger	<i>O. smaragdina</i>	Lives in proximity to ants.	[77]
<b>Theridiidae</b>			
<i>Eidmannella pallida</i> (Emerton)	<i>Atta sexdens</i> (L.)	Lives in old fungus chambers of nest.	[78]

an isolated ecosystem. Arthropods symbiotic with ant hosts typically experience a stable microclimate, plentiful food (either in the form of other symbionts, the hosts themselves, or other resources brought into the colony by the hosts), and protection from their own predators and parasites [5, 68, 77]. The degree of integration into the colonies varies greatly from species with just a loose affiliation or association with the ant nests to symbionts that spend their entire lives within the ant nests and fail to thrive when removed from this habitat [5, 79]. These symbionts can have a neutral, a positive, or a negative influence on the host colonies depending on their natural history. If the effect of the myrmecophile on the host is costly enough, there should be selection for the host to recognize and attack or remove these guests from the nest [69]. For example, the myrmecophile *Masoncus pogonophilus* Cushing (Linyphiidae) feeds on collembolans and other symbionts found in the colonies of the harvester ant, *Pogonomyrmex badius* (Latreille) [80] (Figure 2). Therefore, this spider may have a slightly negative effect on the colonies of these ants since the primary prey of the spiders, collembolans, graze fungal spores found inside the nest chambers, particularly the seed storage chambers [80], and thus keep fungal infestations low. However, populations of these spiders are so small within any given colony that their net effect on the host's success is probably negligible [79, 80]. Some evidence suggests that hosts can recognize and will attack these symbionts, particularly those introduced from

a neighboring nest [81, Cushing pers. obs.]. The myrmecophilic spider *Gamasomorpha maschwitzi* (Wunderlich) (Oonopidae) is found inside the nests and bivouacs of the army ant, *Leptogenys distinguenda* (Emery), where it apparently feeds on insects captured by the hosts. Therefore, this myrmecophile has a negative impact on host fitness as a kleptoparasite on the host's prey. However, as with *M. pogonophilus*, the abundance of spiders within any given colony is so low that its negative impact is likely negligible and these spider guests are either ignored or treated with only very low levels of aggression [67, 68]. Sometimes spiders are even groomed by the host ants [69]. The spider *Attacobius attarum* (Roewer) (Corinnidae) (originally published as the clubionid *Myrmecques attarum*) lives with *Atta sexdens* (L.) where it feeds on ant larvae and pupae [82] and thus also has a negative impact on host colonies. The hosts are known to antennate the spiders but do not show any aggression towards these myrmecophiles [82].

It has been noted that certain types of ant colonies are more open to invasion by myrmecophiles than others. Characteristics of host colonies that are most open to invasion by myrmecophiles include: colonies with multiple queens (polygynous colonies), colonies with multiple nest sites (polydomous colonies, which are often also polygynous), and very large colonies [83]. These societies tend to be more “loose, flexible, and dynamic” than monogynous colonies and tend to have less social cohesion leading to increased

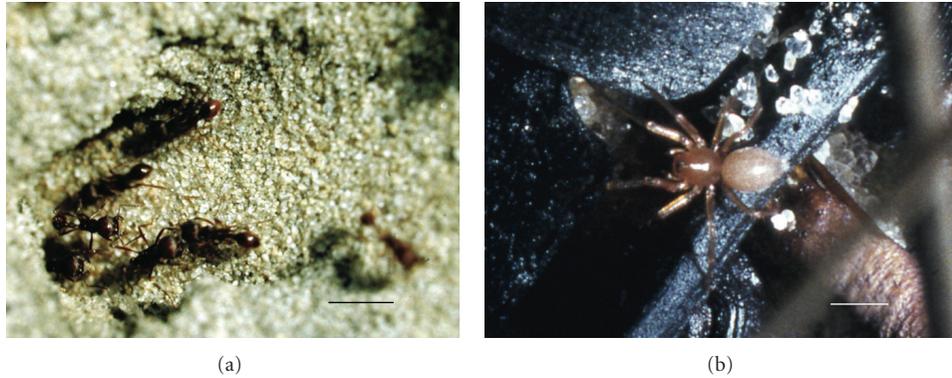


FIGURE 2: Myrmecophily in spiders. (a) The host ant *Pogonomyrmex badius* (Latreille) at the nest entrance. (b) The myrmecophilic spider, *Masoncus pogonophilus* Cushing on the surface, walking along the emigration trail of the host ant. Scale bar in (a) = 8 mm, scale bar in (b) = 1 mm. Photos © author.

vulnerability to invasion by myrmecophiles [83]. In general, myrmecophile populations tend to occur in one of the following distinct patterns: (1) a myrmecophilic species is found in many colonies at certain locations throughout a host species' range but not at other locations (i.e., high infestation but low transmission), (2) a myrmecophilic species is found throughout the host's range but only within a few colonies at any given locality (i.e., low infestation but high transmission), or (3) the myrmecophile is found in only a few colonies at any one locality and not throughout the host's range (i.e., low infestation and low transmission) [83]. Population size of myrmecophiles is often quite low within a colony, but this depends on the type of myrmecophile. Spider myrmecophiles that have been studied in any depth, in general, tend to have small populations within a colony [67, 68, 79]. Intraspecific aggression between spider myrmecophiles within a colony has been reported [69] and may be one factor in keeping populations small.

### 3.3. General Adaptations Facilitating Integration into Colonies.

Close integration within ant colonies seems to be more common in certain families, such as the Linyphiidae and Oonopidae [5]. These spiders have several characteristics (morphological and behavioral) that may serve as preadaptations to a symbiotic lifestyle inside ant nests [67]. For example, both families include very small spiders (typically less than 5 mm); the species are often found in moist, humid microhabitats such as leaf litter, under rocks or logs, or under bark; and many species in these families (particularly oonopids) have morphological adaptations such as hard sclerotized scuta covering their abdomens that may provide some protection against attacks by host ants. Witte and colleagues point out that some species of oonopids may scavenge insect remains in the webs of other spiders [67]. All these behavioral and ecological characteristics may preadapt spiders to a myrmecophilic lifestyle within ant colonies. Smaller body sizes allow them to "sneak" inside the nests and become integrated. Protective scuta (and small sizes) may provide some protection against attacks from the hosts. A scavenger lifestyle may be considered a preadaptation to stealing food (insects

or ant brood) from workers. The constant temperature and humidity of an underground ant nest may be an attractive environment to species otherwise restricted to similar temperature and humidity regimes.

Once integrated into colonies, spider myrmecophiles certainly have evolved dramatic host-specific adaptations allowing them to become even more integrated into various aspects of the host's life cycle. These adaptations, in turn, place severe constraints on the geographical distribution of these inquilines or ant guests; the symbionts are restricted to the range of that host species [83]. This may explain why such inquilines are very localized or rare and may be subject to frequent extinctions [83]. Adaptations common to myrmecophiles include evasive devices such as behaviors, morphological structures, or chemical signals used to appease hosts or to mimic hosts; protective morphological structures such as sclerotized cuticular "shields" or plates; mechanisms to communicate with hosts via chemical cues, tactile cues, or even auditory cues [83].

### 3.4. Chemical Mimicry.

Among spider myrmecophiles, besides the preadaptations mentioned above, many have evolved the capacity to absorb, biosynthesize, or otherwise mimic the host ant's cuticular hydrocarbon colony odor. To survive inside the host colony, the guest must be considered a nest mate by the hosts and should, therefore, have somehow acquired the chemical odor of the hosts via either biosynthesis of the key compounds or by passively acquiring the chemical cues [84]. Thus far, no research has definitively documented glandular secretions that spider myrmecophiles might use to biosynthesize the compounds. If such glands are documented, then it is likely that the association between the host and the myrmecophile is an ancient association and the myrmecophile and host coevolved [85, 86]. However, biosynthesis may evolve rapidly in myrmecophile populations if the compounds biosynthesized can be easily manufactured by co-opting an already existing chemical pathway or if the guest can re-purpose an already existing compound [86].

The chemical signature of ant colonies may change over time [2]. Thus intruders (guests) into colonies must be able

to update their profiles constantly in order to avoid detection and attack. If the myrmecophile's chemical profile does not match the host's closely enough then it will become more difficult for the guest to approach the host in order to update its profile, making social integration into the colonies a "well-balanced and potentially fragile system" [69]. Myrmecophiles can acquire colony odors by rubbing against the host ants, associating with nest materials, or by eating the ant's brood (larvae or pupae) [84]. All these mechanisms are seen in spider myrmecophiles. It may be that these myrmecophiles do not need to acquire an exact chemical match to the host's hydrocarbon profile, but need only one or two key constituents that are biologically most important in nest recognition and acceptance by the hosts [86].

For example, the oonopid, *G. maschwitzi*, found with the army ant, *L. distinguenda*, has a cuticular hydrocarbon profile that includes only compounds also seen in the host ant's profile but not all the compounds seen in the host's profile [69]. These spiders crawl on top of workers, moving their legs actively over the cuticle of the host, perhaps as an adaptation to acquire the host's chemical odor [68, 69, 78]. The hydrocarbon profile of the myrmecophilic spider matches that of the host's to a high degree; however, colony-specific matching was not evident [69]. Nevertheless, ants of *L. distinguenda* from different colonies did not show high levels of intercolony aggression; therefore, it may not matter that the myrmecophile's profile lacks these colony-specific compounds but just generally matches the gestalt odor of the species (i.e., has key chemical constituents that identify it as an ant and a member of the same species) [68, 69]. Research has also demonstrated that the phoresy displayed by *G. maschwitzi* may also function as a behavioral mechanism for the spider to acquire food (ant larvae, pupae, or insects being carried by the workers) via kleptoparasitism [68]. The spider riding on the back of the ant snatches the food item directly from the host's mandibles. In fact, these spiders have not been observed to hunt prey on their own [67] so this kleptoparasitic lifestyle may be another example of extreme adaptation related to their symbiotic life with these ants.

The salticid *Cosmophasis bitaeniata* (Keyserling) lives inside the colonies of the weaver ant, *Oecophylla smaragdina* (Fabricius), where it feeds on the larvae of the host ant [72–75]. The spider is more often found in and around older nests that have lots of larvae [72]. The spider touches the antennae and head of minor workers with its front legs, stimulating the workers to release the larva that the worker is carrying [72]. The spider otherwise avoids direct contact with the worker ants [72, 75]. The spider is a chemical mimic of the host [73–76]. It has been shown that the spider acquires the colony specific hydrocarbon profile by handling and eating the ant larvae [74, 76]. The hydrocarbon profile of the spider is colony specific but does not match the profile of the major workers [75]. Larvae from different colonies do not elicit aggressive responses from the host; thus spiders that mimic the hydrocarbon profile of the larvae rather than the workers may be more easily accepted by both their own hosts as well as those of neighboring colonies [76].

The spider *Attacobius attarum* that lives inside the nests of the leaf cutter ant, *Atta sexdens* (L.) rides on the dorsa of

workers and alates [78, 82, 87]. The spiders may disperse to new colonies via the alates [78, 82, 87]. *Attacobius attarum*, like *G. maschwitzi* and *C. bitaeniata*, is a kleptoparasite; the spider feeds on ant larvae and pupae and can steal the brood directly from the mandibles of workers [82]. The ants antennate the spiders and the spiders reciprocate by "antennating" the ants with their front legs, possibly providing mimetic tactile cues [82]. No aggression towards these kleptoparasites has been reported [82].

The theridiid spider, *Eidmannella pallida* (Emerton) (published as *Eidmannella attae*), also lives with *A. sexdens* where it is found in unused fungus chambers that the ants use to store refuse and dead ants [78]. Likewise, the linyphiid, *M. pogonophilus*, lives in seed chambers and empty chambers of the seed harvester ant, *P. badius* [79, 80]. Both these spider myrmecophiles may acquire host colony odor passively via the nest materials. Neither has been reported as phoretic, as kleptoparasitic, or as a predator of the hosts or their brood. Thus passive integration and acquisition of colony odor is likely for these symbionts.

**3.5. Ability to Follow Chemical Cues of the Hosts.** *Cosmophasis bitaeniata* can distinguish between nestmate and non-nestmate major workers and shows less tendency to try and escape when confined with nestmates, demonstrating that these myrmecophiles are not only chemical mimics but are also able to interpret chemical cues provided by the hosts [74]. Data suggests that the ability to interpret chemical signals of the hosts may be a general characteristic of spider myrmecophiles that are closely integrated into ant colonies. Research on *M. pogonophilus* and *G. maschwitzi* showed that spiders are able to follow trail pheromones laid by the ants [67, 68, 79, 80]. In controlled tests, Witte et al. found that *G. maschwitzi* is sensitive to high concentrations of naturally laid ant trail pheromones [67]. I found *M. pogonophilus* in the emigration trails of *P. badius* when the hosts emigrated to new nest sites [79, 80] (Figure 2(b)).

Spider myrmecophiles may use ant trail pheromones as a means of dispersing to new colonies. In a given habitat, it is not uncommon to find spider myrmecophiles in all or nearly all the nests of a given host, even if the host is not polygynous or polydomous [68, 79]. Thus in at least these instances, dispersal to new colonies must be occurring. Only one study has attempted to examine the population structure of a myrmecophilic spider, *M. pogonophilus*, which was found in nearly all colonies of *P. badius* in a given habitat (i.e., 10 colonies out of 12 that were excavated) [79]. *Pogonomyrmex badius* colonies are established by single inseminated queens [88] that can live for at least 15 years [89]. I hypothesized that spider populations might be considered metapopulations [90], made up of isolated demes, or local populations, with very low per-generation migration between populations resulting in low genetic diversity between individuals within populations (i.e., myrmecophiles within an ant nest) and higher genetic heterogeneity between populations (i.e., between populations of spiders found in different colonies) due to genetic drift [79]. Instead, I found that genetic diversity among individual spiders within populations (within a

colony) was greater than the genetic diversity between populations from neighboring ant nests suggesting that spiders do disperse to new nests frequently enough to maintain high intra-population differentiation and low inter-population differentiation [79]. Although tests of the spiders' ability to follow trail pheromones (naturally laid and artificial trails) were inconclusive, I further hypothesized that spiders were able to locate new nests by following trail pheromones. They were found to emigrate with their hosts to new nest sites (see above), thus they may, during emigration, get "side-tracked" onto the foraging trail of a neighboring *P. badius* colony [79].

**3.6. Life Cycle of Spider Myrmecophiles.** Very little is known about the life cycle of any spider myrmecophile. Even for one of the best studied species, *G. maschwitzi*, no spiderlings have ever been detected in the emigration trails nor inside the nests [67, and Volker Witte, pers. communication]. *Masoncus pogonophilus* builds prey capture webs inside nest chambers and females deposit small silken egg sacs each containing up to seven eggs in depressions in the walls of the chambers [80]. The salticid, *C. bitaeniata* also deposits its egg sacs within the nest chambers of *O. smaragdina* [72]. A *G. maschwitzi* female was collected with one large egg in the abdomen and another with five smaller eggs [67]. Both *M. pogonophilus* and *C. bitaeniata* have female biased sex ratios [72, 80].

**3.7. Future Directions.** A great deal more research needs to be done to understand the basic biology of spider myrmecophiles. Questions and directions for future research include the following.

- (i) How closely integrated are spider myrmecophiles with their host ants?
- (ii) How do these spiders reproduce inside the ant colonies or does reproduction occur outside the nests?
- (iii) How do they disperse to colonize other nests?
- (iv) Is chemical integration a widespread phenomenon among spider myrmecophiles?
- (v) Can any spider symbiont biosynthesize chemical compounds that act to appease or mimic the hosts?
- (vi) Are spider myrmecophiles generally able to interpret the chemical signals of their hosts?
- (vii) Is there evidence of a co-evolutionary relationship between symbionts and hosts?
- (viii) How closely related are spider myrmecophiles within a colony and do these patterns of relatedness explain the female-biased sex ratios seen in some species?

## 4. Spider Myrmecophagy

**4.1. Species Records.** Spiders, like other arthropod predators, generally avoid preying upon ants. However, ants have been documented as part of the diet for well over 100 species of spiders (Table 1). Fossil evidence of spider myrmecophagy dates back 30–50 mya in Baltic amber specimens including one containing an inclusion of spider silk with an ant that

had been fed upon as well as another showing a spider with an ant in its chelicerae [91]. Myrmecophagic spiders exist on a continuum from euryphagous to stenophagous predators [92]. Huseynov et al. [92] propose five categories of spider myrmecophages: (1) non-acceptors of ants (the majority of spider species); (2) reluctant acceptors that do prey on ants but prefer other prey; (3) indifferent acceptors that feed indiscriminately on ants and other prey; (4) facultative ant choosers that prefer ants to other prey; (5) obligatory ant choosers that feed exclusively on ants (unless severely food deprived). In Table 2, the various spider myrmecophages that have been documented from the literature are categorized as (R) Reluctant acceptors, (I) Indifferent acceptors, (F) Facultative ant choosers, or (O) Obligatory ant choosers based upon information about their biology provided in the literature. If researchers have only documented that the particular species eats ants but provide no other information about the hunting behavior or prey preference of the spiders, the species is categorized as (Unk) Unknown. However, these spiders are likely to turn out to be either reluctant or indifferent acceptors of ants in the diet. Details of the predatory biology of spider myrmecophages are also included in the table.

**4.2. Evolutionary Costs and Benefits of Myrmecophagy.** Spider myrmecophagy is a high risk hunting strategy. Risks for myrmecophages include being attacked by the prey, living in close proximity to dangerous prey, being attacked when mating, having the prey attack and destroy one's eggs if nesting and oviposition occur close to the ant nests [58, 143, 175]. However, a spider that evolves strategies for overcoming an ant's defenses and aggression faces relatively little competition for a nearly unlimited food resource [114, 143] (Figure 3(a)).

One study demonstrated that myrmecophagic spiders may actually derive protection against attacks from their own prey: when myrmecophagic, myrmecophilic, myrmecomorphic, and non-ant associating salticids were trapped with ants, the myrmecophagic spiders showed the highest survival rate followed by the myrmecomorphs and myrmecophiles, suggesting that ant associates may signal the ants in such a way that the ants show little aggression towards these spiders [176]. Thus not only are myrmecophagic spiders obtaining a nutrient rich, unlimited food supply through their specialized diet, but they may also be deriving protection from the ants, just as myrmecophilic and myrmecomorphic spiders do.

Although it has not been suggested that spider myrmecophages are chemical mimics of ants, as has been demonstrated for spider myrmecophiles, there is some evidence that certain species of myrmecophages may either be releasing chemical compounds that appease their potential prey or may be able to "read" chemical cues released by ants. For example, Lubin suggested that the thomisid, *Tmarus stoltzmanni* Keyserling, may use its 1st and 2nd pairs of legs to detect chemical or tactile cues from the ants [148]. *Habronestes bradleyi* (O. P.-Cambridge) (Zodariidae) waves its front legs around when hunting and, when the legs are amputated, the spider has a difficult time locating ant prey

TABLE 2: Spider myrmecophages. \*Categories (defined in text) include R: Reluctant myrmecophage; I: Indifferent acceptor; F: Facultative ant predator; O: Obligatory ant predator; Unk: cannot be determined from information about their biology presented in the literature (these are most likely R or I myrmecophages). Spider taxonomy according to Platnick [63]; ant taxonomy according to <http://antbase.org/>.

Spider myrmecophage	Category of myrmecophage*	Notes on biology	References
Araneidae			
<i>Metepeira gosoga</i> Chamberlin and Ivie	Unk	Author suggests that spiders may feed on ants found only on cholla where spider is also found.	[93]
<i>Metepeira</i> sp.	Unk	Reported feeding on <i>Crematogaster opuntiae</i> Buren.	[93]
Deinopidae			
<i>Deinopis</i> sp.	Probably I	Throws web over ants passing below.	[94]
Eresidae			
<i>Seothyra</i> sp.	F	Lives in silk lined burrows. Mouth of burrow covered by prey capture web. Captures mostly ants. Male spider runs on ground during day and is myrmecomorph and behavioral mimic of <i>Camponotus</i> sp. and mutillid wasps (dimorphic mimicry).	[95]
Gnaphosidae			
<i>Callilepis nocturna</i> (L.)	May be F	Feeds on <i>Formica</i> spp. and <i>Lasius</i> spp. Actively searches for ants and may enter nests to hunt workers. Approaches ant and bites on base of antenna. Antennae seem to act as stimulus to trigger attack.	[96–98]
Linyphiidae			
<i>Frontinella communis</i> (Hentz)	I	Occasionally preys on ants.	[99]
Oecobiidae			
<i>Oecobius annulipes</i> Lucas	O	Main food is <i>Plagiolepis pygmaea</i> (Latreille) but other ants (e.g., <i>Lasius flavus</i> (Fabricius)) accepted in lab. Bites at base of antenna. Swaths ant in silk and encircles it. Sometimes uses last pair of legs as well as spinnerets to direct silk over prey. Reduced chelicerae and enlarged gnathocoxae may be adaptations to myrmecophagic lifestyle.	[100]
<i>O. cellariorum</i> (Dugès)	O	Feeds on <i>Plagiolepis pygmaea</i> (Latreille). Bites at base of antenna.	[100]
<i>O. templi</i> O. P.-Cambridge	O		[100]
Oonopidae			
<i>Triaeris stenaspis</i> Simon (publ. as <i>T. patellaris</i> )	Unk	Reported attacking <i>Cyphomyrmex costatus</i> Mann.	[101]
Oxyopidae			
<i>Oxyopes apollo</i> Brady	Unk	Eats ants.	[102]
<i>O. globifer</i> Simon	I/F	Ants constitute large % of prey.	[99, 102]
<i>O. licenti</i> Schenkel	Unk	Eats ants.	[102]
<i>O. salticus</i> Hentz	Unk	Eats ants.	[102]
<i>O. scalaris</i> Hentz	I	Occasionally eats ants.	[99, 102]
<i>O. sertatus</i> L. Koch	Unk	Eats ants.	[102]
<i>Peucetia viridans</i> (Hentz)	Unk	Eats ants.	[103]
Pholcidae			
<i>Crossopriza lyoni</i> (Blackwall) (publ. as <i>Crossopriza stridulans</i> )	Unk	Feeds on fire ants, <i>Solenopsis invicta</i> Buren.	[104]
Salticidae			
<i>Aelurillus aeruginosus</i> (Simon), <i>A. cognatus</i> (O. P.-Cambridge), and <i>A. kochi</i> Roewer	F	Prefer ants over other prey. Innately recognize ants even if ants are not moving. Attack from front unless ant is passing (then switch to rear attack). Use different hunting behavior for ants than for other prey. If hungry, show no preference for ants over other prey.	[105]

TABLE 2: Continued.

Spider myrmecophage	Category of myrmecophage*	Notes on biology	References
<i>Aelurillus m-nigrum</i> Kulczyński	F	Prefers ants over other prey; 85% of diet in field consists of ants. Uses different hunting behaviors for ants than for other prey: lunges, attacks from front, bites, releases, bites again.	[92]
<i>Aelurillus</i> spp.	F	Species in genus prefer ants over other prey. Use different hunting behaviors for ants than for other prey.	[106]
<i>Anasaitis canosa</i> (Walckenaer) (publ. as <i>Corythalia canosa</i> or as <i>Stoidis aurata</i> )	F	Prefers ants over other prey. Uses different hunting behaviors for ants than for other prey: attacks from front, holds forelegs away from struggling ant. Also stilts body off ground.	[107, 108]
<i>Anasaitis</i> spp.	F	Species in genus prefer ants over other prey. Use different hunting behaviors for ants than for other prey.	[106]
<i>Chalcotropis</i> spp.	F	Use different hunting behaviors for ants than for other prey: some attack from rear, some head-on, then lunge, bite, release, and wait.	[106, 109]
<i>Chrysilla lauta</i> Thorell	F	Prefers ants. Uses different hunting behaviors for ants than for other prey: attacks from rear, bites gaster (not appendages), retreats and waits, may lunge and strike several times. When ant quiescent, spider approaches, bites again, and carries it away.	[110]
<i>Chrysilla</i> spp.	F	Species in genus prefer ants over other prey. Use different hunting behaviors for ants than for other prey.	[106]
<i>Cosmophasis</i> sp.	Unk	Feeds on ants and is myrmecomorph.	[59]
<i>Euophrys</i> spp.	F	Use different hunting behaviors for ants than for other prey: some attack from rear, some attack head-on, then lunge, bite, release, and wait.	[106]
<i>Evarcha albaria</i> (L. Koch)	I/F	Robs ants of their prey and of their brood (eggs and larvae) that workers carry (kleptoparasites).	[111]
<i>Habrocestum pulex</i> (Hentz)	Some F Some I	Some individuals prefer ants over other prey; some prefer other prey over ants. Myrmecophagic individuals use different behaviors for ants than for other prey: lunge or leap onto petiole or thorax, bite, release, repeat (up to 6 times). Keep front legs off ground away from ant. Reported preying on <i>Crematogaster</i> spp.	[112–114]
<i>Habrocestum</i> spp.	F	Species in genus prefer ants over other prey. Use different hunting behaviors for ants than other prey.	[106]
<i>Hasarius adansoni</i> (Audouin)	Probably I	Will feed on ants.	[115]
<i>Hentzia palmarum</i> (Hentz) (publ. as <i>Eris marginata</i> )	Unk	Reported feeding on workers of <i>Myrmica</i> sp.	[113]
<i>Icius</i> sp.	Unk	Reported feeding on small brown ants.	[113]
<i>Menemerus fulvus</i> (L. Koch) (publ. as <i>Menemerus confuses</i> )	I/F	Robs ants of their prey and of their brood (eggs and larvae) that workers carry (kleptoparasites).	[111]
<i>Myrmarachne foenisex</i> Simon	F	Regularly feeds on weaver ant ( <i>Oecophylla</i> ) larvae. Also mimics weaver ants.	[59]
<i>Natta horizontalis</i> Karsch (publ. as <i>Cyllobelus rufopictus</i> )	F	Prefer ants. Uses different hunting behaviors for ants than for other prey: attacks from rear, bites gaster (not appendages), retreats, and waits, may lunge and strike several times. When ant quiescent, spider approaches, bites again, and carries it away.	[110]
<i>Natta</i> spp.	F	Species in genus generally prefer ants. Use different hunting behaviors for ants than for other prey: attack from rear, bite gaster (not appendages), retreat and wait, may lunge and strike several times. When ant quiescent, spider approaches, bites again, and carries it away.	[106, 110]

TABLE 2: Continued.

Spider myrmecophage	Category of myrmecophage*	Notes on biology	References
<i>Phidippus johnsoni</i> (Peckham and Peckham)	I	Occasionally eats ants.	[99, 116]
<i>Plexippus setipes</i> Karsch	I/F	Robs ants of their prey and of their brood (eggs and larvae) that workers carry (kleptoparasites).	[111]
<i>Siler cupreus</i> Simon (publ. as <i>Silerella vittata</i> )	F/O	Eats ants. Spider population increases in areas infested with Argentine ants, <i>Linepithema humile</i> (Mayr). Also robs worker ants of brood including eggs, larvae, and pupae being carried by workers (kleptoparasitism).	[117–120]
<i>Siler semiglaucus</i> (Simon)	F	Prefer ants. Uses different hunting behaviors for ants than for other prey; bites gaster (not appendages), retreats and waits, may lunge and strike several times. When ant quiescent, spider approaches, bites again, and carries it away.	[110]
<i>Siler</i> spp.	F	Use different hunting behaviors for ants than for other prey: some attack from rear, some from head-on, lunge, bite, release and wait.	[106, 109]
<i>Tutelina formicaria</i> (Emerton)	F	Also myrmecomorph. Preys on red and black ants.	[121]
<i>Tutelina similis</i> (Banks)	F	Preys primarily on ants and is also a myrmecomorph. Uses different hunting behaviors for ants than for other prey: bites quickly, releases, retreats, carries paralyzed prey to safe area.	[99, 113]
<i>Tutelina</i> spp.	F	Other species of <i>Tutelina</i> found on mound of <i>Pogonomyrmex salinus</i> Olsen (publ. as <i>P. owyheeii</i> ) feeding on worker ants.	[113]
<i>Xenocytaea</i> spp.	F	Species in genus prefer ants over other prey. Use different hunting behaviors for ants than other prey.	[106]
<i>Zenodorus durvillei</i> (Walckenaer), <i>Z. metallescens</i> (L. Koch), and <i>Z. orbiculatus</i> (Keyserling)	F	Prefer ants over other prey. Feed on ants caught in other spider's webs—but only if spiders can approach safely without getting caught. Ambush ants; hang upside down and lunge at ant while releasing dragline. Repeatedly bite larger ants. Do not hold onto injured ant.	[106, 108]
<i>Zenodorus</i> spp.	F	Species in genus prefer ants over other prey. Use different hunting behaviors for ants than other prey.	[106]
Scytodidae			
<i>Scytodes</i> sp.	Unk	Feeds on fire ants, <i>Solenopsis invicta</i> Buren.	[104]
Theridiidae			
<i>Achaearanea</i> spp.	Unk	Feed on “carpenter ants.” Ants become entangled in gum footed sticky thread attached to substrate. Movement of ant causes thread to snap and ant is lifted off ground.	[93]
<i>Argyrodes</i> sp.	Unk	Reported feeding on <i>Pogonomyrmex rugosus</i> Emery.	[93]
<i>Asagena fulva</i> (Keyserling) (publ. as <i>Steatoda fulva</i> ) and <i>A. pulcher</i> (Keyserling) (publ. as <i>S. pulcher</i> )	Unk	Feed on <i>Pogonomyrmex badius</i> (Latreille) and <i>P. subnitidus</i> Emery. When ant workers captured in webs, major workers (patrollers) may attempt to free them but become caught in webs themselves.	[93, 122]
<i>Cryptachaea riparia</i> (Blackwall) (publ. as <i>Theridion saxatile</i> and as <i>Acaeoraneae riparia</i> )	F	Captures ants with above-ground web that has sticky threads attached to substrate. Webs built in areas of high ant activity or traffic. Greater than 88% of diet made up of ants (mostly <i>Formica</i> spp.). Ant gets tangled in sticky silk, struggling causes line to snap, ant is suspended, spider responds to vibrations, bites ant several times in legs and antennae while wrapping in silk, cuts paralyzed ant, and carries it to sand-covered tube retreat.	[123, 124]
<i>Dipoena punctisparsa</i> Yaginuma	Unk	Feeds on small ants in genus <i>Lasius</i> .	[125]

TABLE 2: Continued.

Spider myrmecophage	Category of myrmecophage*	Notes on biology	References
<i>Enoplognatha ovata</i> (Clerck) (publ. as <i>Theridion lineatum</i> or <i>T. lineamentum</i> )	Unk	Feeds on <i>Pogonomyrmex barbatus</i> (Smith). Builds webs in grass near colony. Ants crawling up into grass or passing below get entangled.	[126]
<i>Euryopsis californica</i> Banks	I/F	Reported feeding on <i>Pogonomyrmex rugosus</i> Emery.	[93]
<i>Euryopsis coki</i> Levi	I/F	Preys on <i>Pogonomyrmex salinus</i> Olsen (publ. as <i>P. owyheeii</i> ). Spider captures ant on the mound by trapping ant against ground with sticky silk. Bites on leg. Ant swings off ground on thread. When paralyzed, spider drags it away using a web sling attached to the ant and to the spinnerets.	[127]
<i>Euryopsis episinoides</i> (Walckenaer) (publ. as <i>E. acuminata</i> )	I/F	Feeds on ants. Attacks <i>Crematogaster</i> ants and transports each attached to spinnerets.	[128]
<i>Euryopsis formosa</i> Banks	I/F	Captures and carries workers of <i>Pogonomyrmex salinus</i> Olsen. Carries ant across ground. One attack described: spider bit gaster, released ant, moved to front and waited, reapproached paralyzed ant, climbed onto ant and began dragging across ant nest using web sling.	[129]
<i>Euryopsis funebris</i> (Hentz)	F/O	Reported feeding on <i>Camponotus castaneus</i> (Latreille). Throws adhesive silk over ant passing by on tree trunk and fastens it to tree. Encircles ant, throwing silk. Bites leg. Cuts paralyzed ant free and carries it to crack or crevice or drops on line to feed.	[130, 131]
<i>Euryopsis scriptipes</i> Banks	I/F	Feeds on ants.	[132]
<i>Euryopsis texana</i> Banks	I/F	Female reported preying upon moving line of small ants.	[133]
Other <i>Euryopsis</i> spp.	I/F	Prey on ants. Throw adhesive silk over ants and fasten to trees.	[131–133]
<i>Latrodectus corallinus</i> Abalos	Unk		[93, 134]
<i>Latrodectus hesperus</i> Chamberlin and Ivie	Probably I	Feeds on <i>Pogonomyrmex rugosus</i> Emery. Builds web on colony mound over foraging trail. Spider throws silk on ant that gets caught in gum threads. Spider approaches ant from above, bites posterior femur, retreats, returns after ant paralyzed, and pulls ant to retreat or to hidden part of web. Also feeds on other species of ants.	[93]
<i>Latrodectus mactans</i> (Fabricius)	I/F	75% of prey in cotton fields in Texas made up of fire ants, <i>Solenopsis invicta</i> Buren. Also reported feeding on <i>Pogonomyrmex badius</i> (Latreille) and <i>P. barbatus</i> .	[89, 126, 135]
<i>Latrodectus mirabilis</i> (Holmberg)	Unk	Feeds on <i>Acromyrmex</i> spp. and <i>Camponotus</i> spp. Builds webs over colony entrances.	[93, 134]
<i>Latrodectus pallidus</i> O. P.-Cambridge	F	Primary prey are ants. Feeds on <i>Monomorium semirufus</i> ( <i>nomen dubium</i> , but probably <i>Messor semirufus</i> (André)). Females build webs over foraging trails. Capture ants from above with trip line attached to substrate and pull prey into retreat. Spiders can also descend to ground and catch ants running on trails.	[136–138]
<i>L. quartus</i> Abalos	Unk	Feeds on <i>Acromyrmex</i> spp. and <i>Camponotus</i> spp. Builds webs over colony entrances.	[93, 134]
<i>Latrodectus revivensis</i> Shulov	Unk	Remains of <i>Messor</i> sp. found in webs.	[136]
<i>Latrodectus tredecimguttatus</i> (Rossi)	Unk	Remains of <i>Messor</i> sp. found in webs.	[136, 137]
<i>Latrodectus</i> spp.	Unk	Members of genus may generally be myrmecophages. Reported feeding on <i>Monomorium</i> sp. and <i>Messor semirufus</i> (André).	[136–138]
<i>Parasteatoda tepidariorum</i> (C. L. Koch) (publ. as <i>Achaearanea tepidariorum</i> )	Unk	Feeds on fire ants, <i>Solenopsis invicta</i> Buren.	[107]

TABLE 2: Continued.

Spider myrmecophage	Category of myrmecophage*	Notes on biology	References
<i>Phycosoma mustelinum</i> (Simon) (publ. as <i>Dipoena mustelina</i> )	Unk	Captures various species of ants of wide range of sizes.	[125]
<i>Steatoda albomaculata</i> (De Geer)	I	Feeds on ants; ant remains found in webs.	[139]
<i>Steatoda fulva</i> (Keyserling)	I/F	Reported building webs near nest entrance of colonies of <i>Pogonomyrmex badius</i> (Latreille).	[122]
<i>S. triangulosa</i> (Walckenaer)	I	Feeds on fire ants, <i>Solenopsis invicta</i> Buren.	[104]
<i>Yaginumena castrata</i> (Bösenberg and Strand) (publ. as <i>Dipoena castrata</i> )	Unk	Mostly feeds upon <i>Camponotus</i> sp. and <i>Lasius</i> sp. and most individual spiders feed upon single type of prey. The larger the spider, the larger the ant it can attack.	[125]
Thomisidae			
<i>Amyciaea albomaculata</i> (O. P.-Cambridge)	O	Myrmecomorph of <i>Oecophylla smaragdina</i> (Fabricius) (publ. as <i>O. virescens</i> ). Adult spiders with eye spots on abdomen. Juvs. yellow and mimic other species of yellow ants (transformational mimics). Spider waits near foraging trail of ant, attacks from behind, bites back of body, drags paralyzed ant to edge of vegetation, drops down to feed.	[140]
<i>Aphantochilus rogersi</i> O. P.-Cambridge (publ. as <i>Cryptoceroides cryptocerophagum</i> )	O	Also a myrmecomorph of <i>Cephalotes pusillus</i> (Klug) (publ. as <i>Zacryptocerus pusillus</i> ). Attacks from behind. Holds dead ant as “protective shield.” Females oviposit near ant nest and defend egg sacs against worker ants.	[141–143]
<i>Aphantochilus</i> spp.	Unk	Feed on cephalotine ants.	[57, 141–143]
<i>Bucranium</i> spp.	Unk	Feed on cephalotine ants. Hold dead ants as protective shield against attacks from other ants.	[57, 141–143]
<i>Mecaphesa californica</i> (Banks) (publ. as <i>Misumenops californicus</i> )	Unk	Feeds on <i>Pogonomyrmex rugosus</i> in vegetation near ant nests.	[93]
<i>Mecaphesa coloradensis</i> (Gertsch) (publ. as <i>Misumenops coloradensis</i> )	Unk	Feeds on alate females of <i>Pogonomyrmex maricopa</i> Wheeler and <i>P. desertorum</i> Wheeler after they have removed their wings and while resting on bushes waiting for temperatures to drop in order to dig new nest chambers.	[144]
<i>Mecaphesa lepida</i> (Thorell) (publ. as <i>Misumenops lepidus</i> )	I	Occasionally feeds on ants.	[99]
<i>Misumenops argenteus</i> (Rinaldi)	Probably I	17% of prey are ants; mostly ants that get caught in trichomes of plant <i>Trichogoniopsis adenantha</i> (OC), where spider spends most of its time.	[145]
<i>Runcinioides argenteus</i> Mello-Leitão (publ. as <i>Misumenops argenteus</i> )	Unk	Includes ants in diet.	[146]
<i>Saccodomus formivorus</i> Rainbow	May be F or O	Builds a basket-like web that appears to attract wandering <i>Iridomyrmex</i> ants. Spider also uses behavioral tactics-tapping ant with its own legs before attacking.	[4, 147]
<i>Thomisus onustus</i> Walckenaer	I	42.8% of diet consists of ants.	[147]
<i>Tmarus stoltzmanni</i> Keyserling	O	Feeds exclusively on ants; but only those without stings such as dolichoderine and formicine ants. Uses frontal attacks. May have sensory structures on 1st or 2nd pair of legs to detect chemical or tactile cues from ants.	[148]
Other <i>Tmarus</i> sp. (from Australia)	Unk	Includes ants in diet.	[148, 149]
<i>Xysticus californicus</i> Keyserling	Unk	Attacks harvester ants in California (cites unpubl. work of Snelling).	[148, 149]
<i>X. loeffleri</i> Roewer	R	Ants comprise only a minor part of diet.	[150]
Other <i>Xysticus</i> spp.	I/F	30–35% of diet of some spp. of <i>Xysticus</i> comprised of ants. One spider seen preying on <i>Pogonomyrmex salinus</i> Olsen. Spider seen on back of ant where it rode around, biting ant until paralyzed. Spider bit at base of petiole.	[129, 150]

TABLE 2: Continued.

Spider myrmecophage	Category of myrmecophage*	Notes on biology	References
<i>Zodariidae</i>			
<i>Diores</i> spp.	Probably F or O	Feed on ants.	[151]
<i>Habronestes bradleyi</i> (O. P.-Cambridge)	O	Spider also myrmecomorph. Waves front legs around when hunting ants. When legs are amputated, spider has difficult time locating prey ( <i>Iridomyrmex purpureus</i> (Smith)).	[152–154]
<i>Lachesana insensibilis</i> Jocqué	I	Polyphagous but will eat ants smaller than themselves. Uses different hunting behaviors for ants than for other prey: bites, releases, re-approaches, bites again.	[155]
<i>Lachesana tarabaevi</i> Zonstein and Ovtchinnikov	F	Preys mostly on harvester ants in genus <i>Messor</i> and on isopods.	[156]
<i>Pax islamita</i> (Simon)	I	Polyphagous but will eat ants smaller than themselves. Uses different hunting behaviors for ants than for other prey: bites, releases, re-approaches, bites again.	[155]
<i>Trygetus sexoculatus</i> (O. P.-Cambridge)	O	Paralysis latency longer for male and juvenile attacks than for female attacks.	[157]
<i>Trygetus</i> spp.	O	Paralysis latency longer for male and juvenile attacks than for female attacks.	[155, 157]
<i>Zodariellum asiaticum</i> (Tyschchenko)	O	Specializes on formicine ants. Attacks other kinds of ants readily but there is shorter paralysis latency for formicine ants suggesting biochemical specificity of venom for certain kinds of ants.	[155]
<i>Zodariellum</i> spp.	Probably all O	Feed on ants.	[155]
<i>Zodarion cyrenaicum</i> Denis	O	Shows cooperative foraging behavior. But some individuals steal prey from others (kleptoparasitism). Paralysis latency longer for male and juvenile attacks than for female attacks.	[157–159]
<i>Zodarion frenatum</i> (Simon)	O	Feeds on <i>Cataglyphis bicolor</i> (Fabricius). Locates nests at night (maybe via odor cues?). Sometimes builds retreats near nest. Digs open closed nest entrances, which triggers ants to come out and repair. Spider sometimes enters nest. Bites ant's legs and carries paralyzed ant away from nest. Also kills ants in morning when they emerge from nest.	[158, 160, 161]
<i>Zodarion germanicum</i> (C. L. Koch)	O	Myrmecomorph as well as myrmecophage. Waves 1st legs as antennal illusion. Holds dead ant in chelicerae and presents dead ant to approaching live ant while “antennating” live ant with its own forelegs. Presumably presenting both odor and tactile cues to living ant to deceive it and avoid attack. Attacks <i>Cataglyphis bicolor</i> (Fabricius).	[162, 163]
<i>Zodarion jozefienae</i> Bosmans	O	Females and juveniles actively hunt ants. Mature males are kleptoparasites on females' prey (spend energy on mate searching, not prey capture). Sexual size dimorphism (females larger).	[161, 164, 165]
<i>Zodarion lutipes</i> (O. P.-Cambridge)	O	Paralysis latency longer for male and juvenile attacks than for female attacks.	[157]
<i>Zodarion nitidum</i> (Audouin)	O	Paralysis latency longer for male and juvenile attacks than for female attacks.	[157]
<i>Zodarion rubidum</i> Simon	O	Myrmecomorph as well as myrmecophage. Waves 1st legs as antennal illusion. Holds dead ant in chelicerae and presents dead ant to approaching live ant while “antennating” live ant with its own forelegs. Presumably presenting both odor and tactile cues to living ant to deceive it and avoid attack.	[163, 166–168]

TABLE 2: Continued.

Spider myrmecophage	Category of myrmecophage*	Notes on biology	References
<i>Zodarion</i> spp.	O	All species obligate myrmecophages. Species also imperfect myrmecomorphs. Documented hunting various species. Do not survive well on non-ant diet. Seem to be behaviorally adapted to hunt ants and seem to have evolved nutritional limitations (non-ant prey do not provide required nutrients). Attack from rear, bite legs, retreat, may repeat, re-approach, pick up, and carry away paralyzed ants. Move front legs while hunting. Have femoral organ that may secrete chemical involved in prey capture.	[49, 98, 151, 157, 158, 160, 161, 166, 168–174]

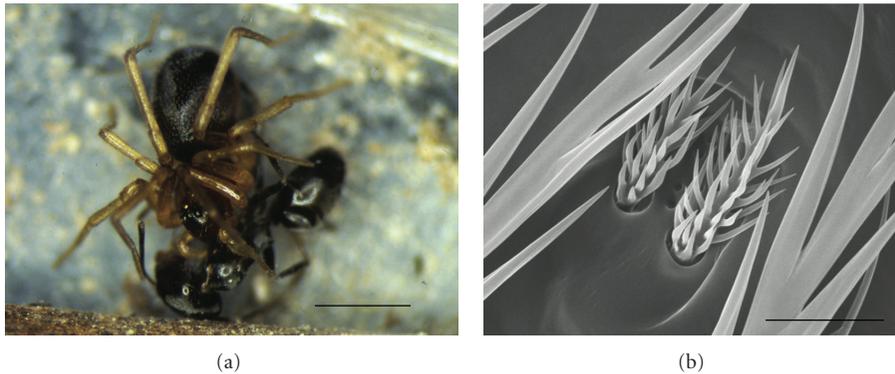


FIGURE 3: Myrmecophagy in spiders. (a) *Zodarion rubidum* Simon eating an ant. (b) Femoral organ on *Z. rubidum*. Note the pore openings in the chitin between the two specialized setae of the femoral organ. Scale bar in (a) = 1 mm, scale bar in (b) = 10  $\mu$ m. Photo of spider © author, SEM of femoral organ © Catherine Tuell, used by permission.

suggesting that the spider may have organs on its front legs that pick up chemical cues from ants [152, 153]. When these spiders detect chemical cues left by ants, they adopt prey capture posture and behavior [153]. Zodariid spiders in the genus *Zodarion* have a structure on the dorsolateral distal tip of the first femora called the femoral organ (Figure 3(b)). The organ consists of pores surrounded by specialized setae with secretory cells beneath the cuticle [171]. It is hypothesized that the femoral organ may release chemicals that somehow subdue the ants upon which the spiders prey (the setae may facilitate dispersion of the secretion) [171]. *Zodarion rubidum* Simon (and other species in the genus) move their front legs around while moving through the environment, similar to the antennal illusion of myrmecomorphs. The spiders seem to use the legs (perhaps via the femoral organs) to pick up cues about ants and conspecifics the spiders may encounter [166]. Recent work by Pekár and Jiroš [177] tested whether various species of myrmecomorphs including one myrmecophage, *Zodarion alacre* (Simon), were also chemical mimics of ants. They found little overlap in the chemical signature of the spiders and ants. Only a weak similarity in profiles was seen for the myrmecophage. The authors hypothesized that the femoral organ of *Zodarion* may be used to synthesize the compounds responsible for the similarity.

The family that includes the most specialized (stenophagous) myrmecophages is the Zodariidae (Table 2). Plesio-

morphic representatives of this family, *Lachesana insensibilis* Jocqué and *Pax islamita* (Simon), are polyphagous but will eat ants and hunt ants differently from other prey [155]. Thus these plesiomorphic representatives of zodariids have behavioral preadaptations for hunting ants [155]. Pekár hypothesized that obligatory myrmecophagy may be a derived behavior because, within the Zodariidae, it is only seen in more recent taxa; primitive representatives of the family seem to be polyphagous [98].

#### 4.3. Specialized Hunting Behaviors of Spider Myrmecophages.

The majority of reluctant or indifferent myrmecophages will accept ants in the diet but typically show no specialized hunting behavior for these potentially dangerous predators, whereas the majority of facultative and obligatory myrmecophages have evolved specialized hunting strategies to subdue ants with minimum risk to themselves. It has been pointed out that “when predators evolve prey-specific capture behaviour for use against dangerous prey, they also tend to evolve distinct preferences for these dangerous prey” [114, 178]. Hunting dangerous but abundant and/or high quality prey seems to select for behavioral plasticity in hunting behavior [105]. Such behavioral flexibility, or using different hunting strategies depending on the identity of the prey and on the circumstances, is common to both myrmecophagous and araneophagous spiders [11, 105, 179].

Many myrmecophagic spiders, particularly facultative or obligatory predators, live in close proximity to ant colonies, often building their webs directly over nest entrances or foraging trails or establishing retreats close to or adjacent to nest mounds [44, 93, 122, 124, 126, 127, 130, 131, 134, 136–138, 140, 143, 147, 158, 160, 161, 180]. In addition to living in close proximity to their prey, these spiders also show specialized hunting behaviors as predicted for stenophagous predators hunting dangerous prey. Web-building myrmecophages (largely in the family Theridiidae, see Table 2) often build webs directly over ant foraging trails where they extend sticky silk strands down to the substrate. When an ant contacts the sticky strand, the ant is catapulted into the air and into the aboveground portion of the web where the spider waits [4, 93, 124, 127, 136–138, 140]. The spider then typically bites the ant one or more times and, each time, the spider retreats until the ant is paralyzed or moribund [93, 130, 131]. The spider then typically carries the ant to a secluded retreat to feed or may even drop on a line to feed [93, 124, 130, 131, 136–138] (possibly to avoid detection from worker ants that may be attracted to alarm pheromones released by the captured ant). When catching non-ant prey, theridiids and other web building spiders do not typically retreat after biting the prey and may or may not carry the paralyzed prey to a different part of the web.

Non-web-building spiders, such as zodariids and salticids (the other families with large numbers of myrmecophagic species), show similar specialized hunting behaviors when attacking ants. For example, zodariids typically attack quickly from the rear of an ant, bite a leg, retreat, and may repeat this sequence several times until the ant is paralyzed. The spider then lifts the moribund ant and carries it to a secluded place to feed (Table 2 and [98, 158, 162, 167, 168]). It has been suggested that the paralyzed ant is used as a shield and a decoy to protect the zodariid from attacks by living ants; the paralyzed ant provides pheromone cues to a curious worker ant that passes by and may provide tactile cues as well [163, 166, 167]. Additional tactile cues are provided by the zodariid, which holds and waves its first pair of legs in front of its body like antennae [163]. The crab spider, *Aphantochilus rogersi* O. P.-Cambridge (Thomisidae), also uses the paralyzed ant as a shield, presumably protecting it from attacks by living ants [142, 143].

Many salticids lunge, rather than jump, at ant prey, then quickly bite, release, and bite again, each time retreating. Even nonmyrmecomorphic ant-eating salticids hunt ants by lunging. This is quite different from the usual stalk and pounce behavior shown to non-ant prey. Myrmecophagic salticids are much more cautious in their approach of ants and much more deliberate in where they bite the prey; some nearly always position themselves in front of the ant and bite the petiole or thorax [92, 105, 106, 108, 109, 114]. Others nearly always attack ants from the rear, lunging at the gaster (not the appendages), but always retreating and waiting until the ant is quiescent before carrying it away [99, 106, 109, 110]. Many salticids keep their front legs extended off the ground when attacking an ant, away from the ant's mandibles [108, 113]. The salticids do not show these behaviors when hunting non-ant prey. The salticids, *Zenodorus durvillei*

(Walckenaer), *Z. metallescens* (L. Koch), and *Z. orbiculatus* (Keyserling), are all facultative myrmecophages that feed on ants caught in other spiders' webs, but only if there is a safe way to capture these prey [106]. These species of *Zenodorus* will walk across a line of detritus to the captured ant or will even hang upside down above the ant and lunge at the prey caught in the web [106]. Some spider myrmecophages, particularly *Callilepis nocturna* (L.) (Gnaphosidae), and species of *Oecobius* (Oecobiidae) aim for the ant's antenna when hunting then retreat and wait as is seen in nearly all other species of myrmecophages [96, 97, 100].

**4.4. Nutritional Costs of Myrmecophagy and a Stenophagous Diet.** It has recently been demonstrated that at least some obligatory myrmecophages do not survive well on an ant-poor diet; some even starve rather than hunt non-ant prey [173]. Thus obligatory myrmecophages show both behavioral limitations (i.e., spiders are reluctant to hunt non-ant prey) and nutritional limitations (i.e., non-ant prey do not provide required nutrients for survival) [173]. In fact, in order to obtain the necessary nutrients for survival, these spiders selectively consume particular parts of the bodies of their ant prey suggesting that “specialist predators can use a behavioral strategy to balance nutrient intake by selective exploitation of different prey body parts” [174]. These authors found, for example, that *Zodarion rubidum* preferentially fed on the foreparts of the ant body, which were richer in proteins, than on the gaster, which is higher in lipids but also contains possible toxins such as formic acid. These obligatory myrmecophages may take their specialization a step further by feeding primarily on one or two types of their preferred prey. For example, *Zodarion* species possess more effective venoms against particular groups of ants, such as formicine ants rather than myrmicine ants [151, 157, 170]. *Zodarion germanicum* (C. L. Koch) does better, in terms of growth and survival, on a diet that includes the preferred formicine ants than on a diet restricted to myrmicine ants [172].

## 5. Discussion

Research on spider myrmecomorphs has demonstrated, unequivocally, that these spiders are Batesian mimics and that the mimicry confers strong adaptive advantages to their survival. Some research has also tested how and why polymorphic and imperfect mimicry evolved. Future research on myrmecomorphic spiders should focus on the costs, trade-offs, and constraints inherent in the evolution of close morphological (and behavioral) resemblance to ants. These factors may have a significant impact on the accuracy of the resemblance. It is also important to identify the selective agents involved in this type of mimetic resemblance since the characteristics of the selective agents (e.g., the visual acuity of the selective agents and whether there is more than one actor in the drama) may explain the phenomena of polymorphic and imperfect mimicry.

Research on spider myrmecophiles has not been extensive in the years since the first review article. Nevertheless, the research that has been carried out, particularly on the species

*Gamasomorpha maschwitz* and *Cosmophasis bitaeniata*, is fascinating and demonstrates that the biology of these symbiotic spiders is closely linked to the lifestyle and biology of the host ants. From my earlier review article [5], and from Table 1, it is clear that many more species of myrmecophilic spiders can be studied and details of their biology explored. In the section on spider myrmecophiles, I suggest additional directions for future research such as: What adaptations are involved in colony integration? How do myrmecophiles disperse to neighboring colonies? Do all spider myrmecophiles mimic colony odors? To what extent can myrmecophiles interpret the chemical cues released by the hosts? What is the population structure of spider myrmecophiles (i.e., is the spider population within a single nest made up of close relatives)?

I also provide a summary of what is known about spider myrmecophages and present an extensive table listing all (I hope) records of spider myrmecophages from the literature. Recent research on these specialist predators has revealed the evolutionary costs and benefits of this stenophagous diet. It has also highlighted the extraordinary morphological and behavioral adaptations that have evolved enabling spiders to specialize on such dangerous prey.

Although spiders and ants seem unlikely co-evolutionary partners given ants' territorial aggressiveness and spiders' solitary lifestyles, it is clear that hundreds of species of spiders have evolved close relationships with ants. The information on spider myrmecomorphs, myrmecophiles, and myrmecophages included herein supplements information presented in the 1997 review [5]. The present paper includes the first comprehensive summary of the extensive research on myrmecophagic spiders. In addition, it presents an overview of the research carried out since 1997 that examines the evolutionary costs and benefits of the various spider-ant associations. One of my primary goals has been to provide ideas for new or expanded avenues of research on these fascinating arthropod relationships.

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## Research Article

# ***Pseudacteon* Parasitoids of *Azteca instabilis* Ants in Southern Mexico (Diptera: Phoridae; Hymenoptera: Formicidae)**

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Three new species of the genus *Pseudacteon* are described, all from Chiapas, Mexico, and all of which are parasitoids of the ant *Azteca instabilis*. Sternite 6 of *Pseudacteon dorymyrmecis* Borgmeier is illustrated for the first time, and *P. confusus* Disney is synonymized with this species. The natural history of the *Azteca-Pseudacteon* interaction is described.

## 1. Introduction

The species of the phorid fly genus *Pseudacteon* Coquillett have been under intense scrutiny lately because of their potential to control invasive species of fire ants (*Solenopsis invicta* and *S. saevissima* in North America; *S. geminata* elsewhere) [1–3]. Other lesser-studied species of *Pseudacteon*, many of them undescribed, attack different hosts, including species of *Crematogaster*, *Lasius*, *Liometopum*, *Nylanderia*, *Pseudolasius*, and other small ants.

In a series of papers [4–7], a new host record, with *Azteca instabilis* Fr. Smith, has been documented from southern Mexico. Below, the so-far-known species of *Pseudacteon* associated with these ants are described, their identification clarified, and natural history summarized.

## 2. Materials and Methods

Specimens were collected into 70% alcohol and dried using hexamethyldisilazane [8]. They were deposited in the following collections:

CEET: El Colegio de la Frontera Sur, Colección de Insectos Asociados a Plantas Cultivadas en la Frontera Sur, Tapachula, Chiapas, Mexico,

LACM: Natural History Museum of Los Angeles County, California, USA,

MCZC: Museum of Comparative Zoology, Harvard University, Massachusetts, USA,

MUCR: Universidade de Costa Rica, San Jose, Costa Rica,

USNM: Smithsonian Institution, Washington, DC, USA.

## 3. Systematics

*Pseudacteon* Coquillett [9]; full synonymy in Borgmeier, 1968 [10]; type species. *Pseudacteon crawfordi* Coquillett, original designation.

Note on gender: the word *Actaeon* is a Greek name for a (male) hunter; thus, the name *Pseudacteon* means “false *Actaeon*” and is masculine in gender.

*Pseudacteon laciniosus* new species (see Figures 1(a) and 2(a)).

**Diagnosis.** The last general key to adults of *Pseudacteon* is that of Borgmeier [11]. This new species keys to couplet 10, where a user is given the alternatives of “ovipositor lanceolate” versus “ovipositor subcylindrical, tapering at apex.” Since the protruding stylet could fool users into accepting the first alternative, these flies could key out to either *P. dorymyrmecis* Borgmeier in the first lead or *P. onyx*

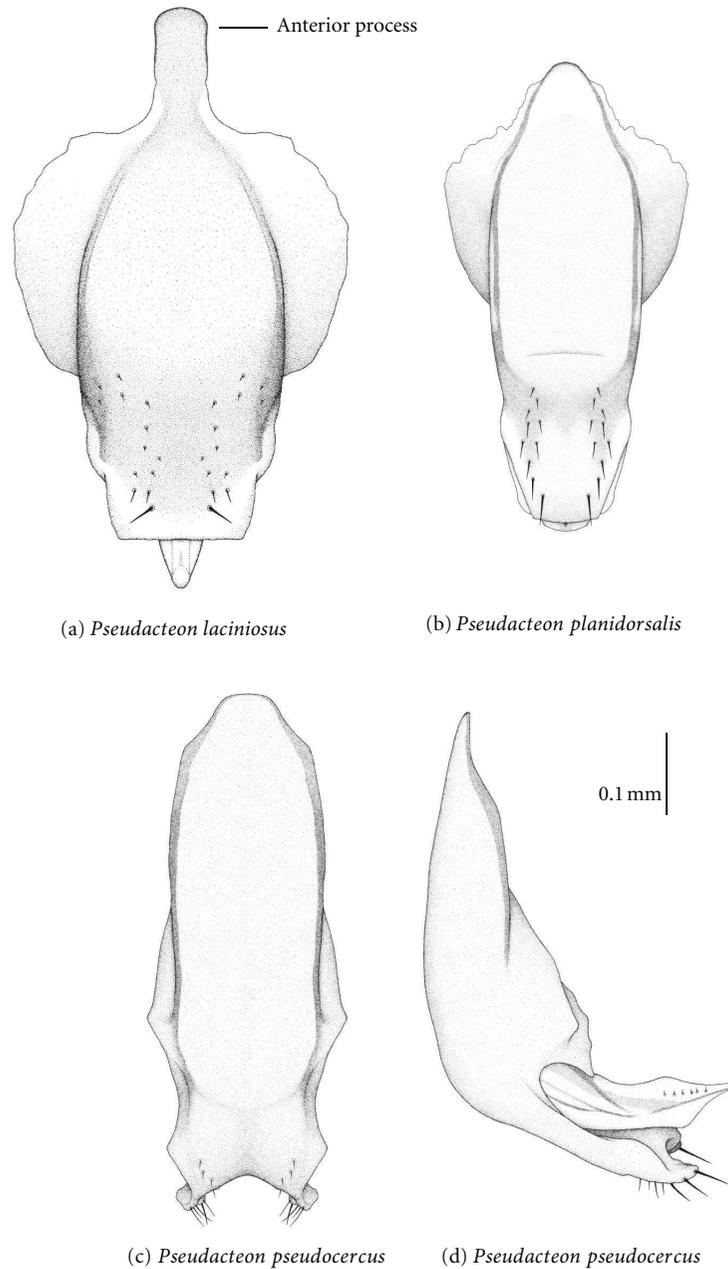


FIGURE 1: *Pseudacteon* species, female oviscapes, dorsal and right lateral (Figure 1(d)).

Steyskal in the second. Both of these species are markedly smaller than *P. laciniosus*, and both have narrower oviscapes. Further, based on examination of the holotype specimen, sternite 6 of *P. dorymyrmecis* has a long pair of medial setae originating basally on the segment (see Figure 2(b)). The oviscape of *P. onyx* was illustrated by Borgmeier [12] and is much more ventrally curved than that of *P. laciniosus*. Finally, unlike both of the other species, *P. laciniosus* has a dark brown body that strongly contrasts with its yellowish legs.

Disney (in [13]) described a new species, *Pseudacteon confusus*, that also keys to *P. dorymyrmex*. We examined a

paratype female of *P. confusus*, comparing it to the holotype female of *P. dorymyrmex*, and conclude the two are the same species. Therefore, *P. confusus* is a junior subjective synonym of *P. dorymyrmex* (new synonymy).

*Description. (Female)* Body length 1.2–1.5 mm (mean = 1.3). Frons dark brown, with 2-4-4-4 setae and one pair of proclinate supraantennal setae. Flagellomere 1 dark brown, rounded, flat; length of arista about two times that of flagellomere 1. Palpus light brown, setulae thick. Thorax dark brown. Scutellum with two pairs of large setae, anterior

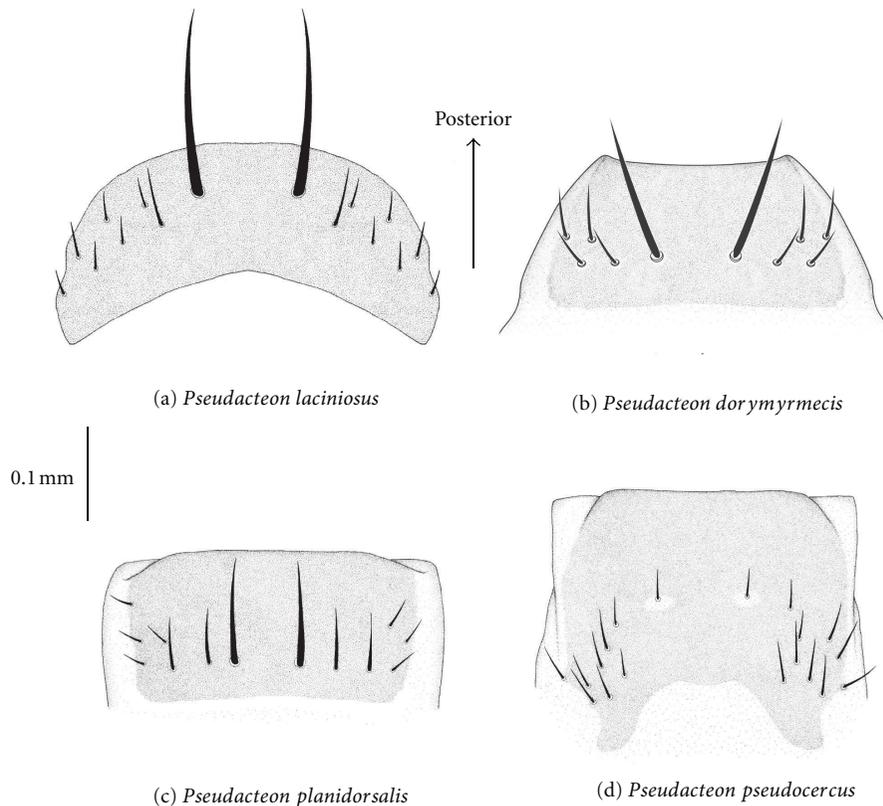


FIGURE 2: *Pseudacteon* species, venter of segment six.

pair 0.8 length of posterior pair. Legs yellowish brown, except forecoxa lighter, mid- and hind coxae darker brown. Wing with mean costal length 0.36 wing length, range 0.35–0.37. Halter yellow. Abdominal tergites dark brown, ventral abdominal membrane dark gray. Tergite 6 with large posterior emargination and lateral pair of setae. Sternite 6 with one large (0.18 mm) pair of slightly divergent setae; more lateral pair much smaller (0.06 mm), but larger to subequal in size to median pair on one or both sides in some specimens; with scattered setulae (see Figure 2(a)). Oviscape (Figure 1(a)) with narrow anterior process, convex dorsally, with large lateral flanges anteriorly, terminating bluntly, although preserved specimens often with stylet protruding, making oviscapes appear pointed. Minute setae on dorsal surface of oviscape arranged in laterally concave lines, with larger, divergent, apical pair.

*Holotype*. ♀, Mexico: Chiapas: Finca Irlanda, 15°11' N, 92°20' W, July 2010, S. Philpott, over *Azteca instabilis* (barcode LACM ENT 139561) (CEET).

*Paratypes*. 16♀, same data as holotype (CEET, LACM, MCZC, USNM).

*Etymology*. The specific epithet is from a Latin word for “fringed,” referring to the enlarged lateral margin of the oviscape.

*Pseudacteon planidorsalis* new species (see Figures 1(b) and 2(c))

*Diagnosis*. The species is similar to *Pseudacteon dorymyrmecis*, but differs in the presence of longer setae flanking the relatively shorter medial pair on sternite six.

*Description*. (*Female*) Body length 1.1–1.4 mm (mean = 1.3). Frons dark brown, with 2-4-4-4 setae and one pair of proclinate supra-antennal setae. Flagellomere 1 dark brown, rounded, flat; length of arista about two times that of flagellomere 1. Palpus yellow, setulae thick. Thorax brown. Scutellum with two pairs of large setae, anterior pair 0.47–0.60 length of posterior pair. Legs yellowish brown, except forecoxa lighter. Wing with mean costal length 0.38 wing length, range 0.37–0.39. Halter yellow. Abdominal tergites dark brown, ventral abdominal membrane dark gray. Tergite 6 with large posterior emargination and lateral pair of setae. Sternite 6 with one large (0.09 mm) pair of large setae and smaller lateral setae in basal transverse row; smaller lateral setae scattered more posteriorly (see Figure 2(c)). Oviscape (Figure 1(b)) lacking narrow anterior process, flat dorsally, with large lateral flanges anteriorly, terminating in rounded point (which is difficult to see in Figure 1(b) because the oviscape is downturned apically). Minute setae on dorsal surface of oviscape arranged in laterally convex lines, with slightly larger apical pair.

*Holotype*. ♀, Mexico: Chiapas: Finca Belen, 15°15' N, 92°23' W, 8.ii.2003, S. Philpott, over *Azteca instabilis* (barcode LACM ENT 294148) (CEET).

*Paratypes.* 3♀, same data as holotype (LACM).

*Etymology.* The specific epithet is from a Latin word for “flat backed,” referring to the surface of the oviscapae.

*Pseudacteon pseudocercus* new species (see Figures 1(c), 1(d) and 2(d))

*Diagnosis.* This species can be recognized by the strongly downturned, lightly sclerotized oviscapae with the pair of cercuslike apical processes. Other species of *Pseudacteon* with a bilobed oviscapae have the structure strongly sclerotized and dark brown in color.

*Description. (Female)* Body length 1.2 mm. Frons dark brown, with 2-4-4-4 setae and one pair of proclinate supra-antennal setae. Flagellomere 1 dark brown, rounded, flat; length of arista about two times that of flagellomere 1. Palpus light brown, setulae small, thin. Thorax brown. Scutellum with two pairs of large setae, anterior pair 0.47–0.60 length of posterior pair. Legs yellowish brown, except forecoxa lighter. Wing with costa 0.40 wing length. Halter yellow. Abdominal tergites brown, ventral abdominal membrane gray. Tergite 6 with large posterior emargination, pair of lateral setae near midline, and longer, thicker seta more laterally. Sternite 6 anteriorly emarginate, with scattered small setae (see Figure 2(d)). Oviscapae (see Figures 1(c) and 1(d)) without narrow anterior process, convex dorsally, apically downturned with pair of cercuslike lobes.

*Holotype.* ♀, MEXICO: Chiapas: Finca Belen, 15°15' N, 92°23' W, 8.ii.2003, S. Philpott, over *Azteca instabilis* (barcode LACM ENT 294147) (CEET). No other specimens preserved.

*Etymology.* The specific epithet is from Latin words for “false circus,” referring to the apex of the oviscapae.

#### 4. Natural History

Little is known about the life cycle of *P. lacinosus*, *P. planidorsalis*, and *P. pseudocercus*, as the three species are only known from adults. Further, most natural history information available treats the flies as a genus, rather than as individual species, so more work will be necessary to distinguish between them. Adults have been observed in a range of shaded coffee plantations varying in canopy cover from ~25 to 100% in the Soconusco region of Chiapas, Mexico. Specifically, *P. lacinosus*, *P. planidorsalis*, and *P. pseudocercus* have been observed in Finca Irlanda, Tapachula municipality (15°11' N, 92°20' W), between 800–1100 m elevation, Finca Hamburgo, Tapachula municipality, between 800 to 1100 m elevation (15°10' N, 92°19' W), and in Finca Belen, Huixtla municipality, between 800 to 1200 m elevation (15°15' N, 92°23' W). The shade coffee habitats from which the phorids have been seen range from shaded monocultures with relatively low levels of canopy cover, tree

diversity, and density to rustic coffee plantations with a high diversity and density of shade trees and nearly 100% canopy cover [14]. Preliminary work indicates that the relative abundance of the three species in a range of coffee agroecosystems is similar, even as canopy conditions change [15].

Within the shade coffee habitats, females of *P. lacinosus*, *P. planidorsalis*, and *P. pseudocercus* have only been observed when hovering over or ovipositing in the host ant species, *A. instabilis*, or flying out of leaf litter collected from the ground or on tree trunks and branches near to *A. instabilis* nests. Males of the three species have not been collected or identified. As a group, the flies are attracted to the host ant by an alarm pheromone (1-acetyl-2-methylcyclopentane) released from the dorsal section of the ant gaster, but they do not attempt to oviposit without ant movement [16]. Work is underway to determine whether visual and similar chemical cues are used by each species in host location and host selection processes. Once a female fly locates host individuals, it will remain in the area for up to several minutes, closely hovering over and following moving ant individuals. Individual flies have been observed to attempt to oviposit at least a dozen times before disappearing from view; actual oviposition has not yet been quantified. Several individuals of the three species (up to 8–10) have been observed simultaneously around the same *A. instabilis* nest.

Phorids strongly modify the behavior of the *A. instabilis* ants and thereby indirectly affect other insects in coffee agroecosystems. *Azteca instabilis* is an aggressive, canopy-dominant ant that has important impacts on many members of the coffee insect food web [17]. In the presence of *Pseudacteon* flies, *A. instabilis* ant foraging is reduced (by about 50%) for up to 90 min. after the first appearance of the phorid [4]. Once the *Pseudacteon* arrives near an *A. instabilis* nest, the ants will either (1) run back to their nest, or to hiding places under tree bark or (2) remain motionless with their heads tilted back [17]. This reduction in ant activity allows other species of ants to gain access to food resources [3, 5] and reduces the predatory effects of ants on lepidopteran larvae [4] and the coffee berry borer (*Hypothenemus hampei* Ferrari) [18]. Furthermore, *A. instabilis* normally prevent adults of the coccinellid beetle *Azya orbiger* Mulsant from feeding on scale insects (*Coccus viridis* Green), a keystone mutualist of the ant. When the *A. instabilis* are under attack by the phorids, *A. orbiger* greatly increase their feeding rates [19] and oviposition rates (Hsieh and Perfecto, unpublished data). The host ant, *A. instabilis*, is patchily distributed within coffee agroecosystems, and one force maintaining this distribution and relative abundance of colonies within sample areas may be attacks from the phorid flies [7]. Thus the *Pseudacteon* flies, through their influence on the activity and distribution of this keystone species, likely have widespread impacts on the coffee insect food web. This result contrasts with conclusions of studies with *Pseudacteon tricuspis* Borgmeier and *P. curvatus* Borgmeier and fire ants (*S. invicta*). At least some studies have concluded that phorids attacking *S. invicta* do not have long-term impacts on the ants or associated arthropods (e.g., [20]).

Phorids that attack *A. instabilis* do not lower population sizes, similar to findings with *P. tricuspis* and *P. curvatus*, but likely impact coffee food webs due to their role in maintaining a similar number of colonies and reducing ant behavior.

Field evidence and observations suggest that *P. lacinosus*, *P. planidorsalis*, and *P. pseudocercus* adults probably live near their host, in leaf litter on the ground. Data suggest that the fly population is likely distributed in a density dependent manner, as number of attacks on *A. instabilis* individuals are more frequent and more numerous where *A. instabilis* densities are greater [6, 7]. Likewise, field evidence indicates that *Pseudacteon* adults are usually located within the leaf litter on the ground because time to first oviposition attempt on *A. instabilis* adults placed on the ground is much less than for ants placed at 1.5 m above ground [6]. However, more rapid arrival on the ground could mean that the phorids primarily search for hosts at ground level.

### 5. Key to Females of *Pseudacteon* Attacking *Azteca* in Southern Mexico

This key is intended for ant ecologists who need to identify phorid parasitoids from known ant hosts. A new general key to New World *Pseudacteon* is needed, as researchers currently must use a combination of Borgmeier [11], Porter and Pesquero [21], Plowes et al. [1], the key below, and reference to species not covered in the previously listed keys [22–26].

- (1) Apex of oviscapae with lobelike processes (Figures 1(d) and 2(a)); venter of segment 6 with short setae only (Figure 2(d)).....*P. pseudocercus* new species  
 - Apex of oviscapae without lobe-like processes (Figures 1(a) and 1(c); venter of segment 6 with some long setae.....2
- (2) Oviscapae anteriorly with narrow process, dorsally domelike, with small setulae scattered posteriorly, but longer pair near apex; apex of oviscapae truncate (Figure 1(a)); enlarged ventral setae of segment 6 placed posterior to midpoint of sternite, much longer than other ventral setae (see Figure 2(b)).....*P. lacinosus* new species  
 - Oviscapae anteriorly with broad apex; dorsally flattened on apical third (except for downturned tip); small setulae in laterally convex rows; apex of oviscapae pointed; enlarged ventral setae of segment 6 placed anterior to midpoint of sternite, only slightly longer than those directly lateral (see Figure 2(d)).....  
 .....*P. planidorsalis* new species

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## Review Article

# The Host Genera of Ant-Parasitic Lycaenidae Butterflies: A Review

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Numerous butterfly species in the family Lycaenidae maintain myrmecophilous associations with trophobiotic ants, but only a minority of ant-associated butterflies are parasites of ants. *Camponotus*, *Crematogaster*, *Myrmica*, and *Oecophylla* are the most frequently parasitized ant genera. The distribution of ant-parasitic representatives of the Lycaenidae suggests that only *Camponotus* and *Crematogaster* have multiply been invaded as hosts by different independent butterfly lineages. A general linear model reveals that the number of associated nonparasitic lycaenid butterfly species is the single best predictor of the frequency of parasitic interactions to occur within an ant genus. Neither species richness of invaded ant genera nor their ecological prevalence or geographical distribution contributed significantly to that model. Some large and dominant ant genera, which comprise important visitors of ant-mutualistic lycaenids, have no (*Formica*, *Dolichoderus*) or very few ant-parasitic butterflies (*Lasius*, *Polyrhachis*) associated with them.

## 1. Introduction

Associations between ants and butterfly species in the families Lycaenidae and Riodinidae have attracted the interest of naturalists since more than 200 years. Building upon an ever-increasing number of field records and case studies (summarized in [1]) these interactions with their manifold variations and intricacies have developed into a paradigmatic example of the evolutionary ecology and dynamics of interspecific associations [2]. Interactions with ants are most well developed during the larval stages of myrmecophilous butterflies. To communicate with ants, myrmecophilous caterpillars possess a variety of glandular organs and often also use vibrational signals that may modulate ant behaviour [3, 4]. Essentially, interactions between myrmecophilous caterpillars and visiting ants comprise a trade of two commodities. The caterpillars produce secretions that contain carbohydrates and amino acids [5]. In turn, the ants harvest these secretions, do not attack myrmecophilous caterpillars and the presence of ant guards confers, at least in a statistical sense, protection against predators or parasitoids (reviewed in [2]). Thus, such interactions are basically mutualistic in

nature, even though the extent of benefits accruing to both partners may be asymmetric and manipulatory communication (by means of mimicking chemical or vibrational signals of ants) is not uncommon. In certain cases, especially if butterfly-ant associations are obligatory (from the butterfly's perspective) and involve specific host ants, interactions may extend into other life-cycle stages of the butterflies, such as pupae (if pupation occurs in ant nests or pavilions built by ants to protect their trophobiotic partners), adults (if egg-laying or nutrient acquisition occurs in company with ants), or eggs.

The vast majority of known butterfly-ant interactions are mutualistic or commensalic in nature. In the latter case the butterfly larvae benefit from their association with ants, while no costs accrue to the ants. Some few butterflies, however, have evolved into parasites of ants [6]. These unusual associations have served as models for host-parasite coevolution [7]. Ant parasitism requires very precise tailoring of the chemical and mechanical signals employed to achieve social integration into ant colonies. Accordingly, ant-parasitic lycaenid butterflies are highly specific with regard to their host ant use, which also renders them extraordinarily

susceptible to the risk of coextinction [8]. Indeed, many ant-parasitic lycaenids are highly endangered species [9], and the well-studied Palaearctic genus *Maculinea* is now regarded as a prime example of insect conservation biology [10].

In this essay, I will focus on the ant genera that serve as hosts of parasitic butterflies. First, I summarize which ant genera in the world are known to be parasitized by butterflies. I then discuss whether this host ant use reflects the macroecological patterns seen in mutualistic butterfly-ant associations. Finally, I will explore if the observed host use patterns allow for generalizations and testable predictions, for example, with regard to expected host ant affiliations in underexplored faunas. Specifically, I expected that the number of associated parasitic lycaenids per host ant genera increases with their ecological prevalence, geographical distribution, and species richness.

## 2. What Constitutes an Ant-Parasitic Butterfly Species?

I here use a rather restrictive definition of ant parasitism. I regard a butterfly species as a parasite of its host ants only if (a) the butterfly caterpillars (at least from some developmental stage onwards) feed on ant brood inside ant nests (“predators”) or (b) the caterpillars are being fed through trophallaxis by their host ants (“cuckoo-type” parasitism). Both these types of parasitism occur in *Maculinea* [11], but the extent of the nutrient flow from the ant colony to the caterpillars may vary across species. For example, in some lycaenid species feeding through trophallaxis apparently occurs only as a supplementary mode of nutrient acquisition. Yet include such cases here as parasites of ants, since the respective behavioural and communicative strategies are in place.

In contrast, I exclude two types of “indirect” parasitism. First, there are a few myrmecophilous lycaenid species that feed obligately on myrmecophytic ant plants. The best documented examples are certain SE Asian *Arhopala* species on ant-trees of the genus *Macaranga* [12, 13]. These caterpillars cause substantial feeding damage to the ant-trees and thereby likely inflict costs to the *Crematogaster* ants that inhabit these trees. *Arhopala* caterpillars on *Macaranga*, however, possess a nectar gland and secrete nectar at rates typical for ant-mutualistic lycaenids (K. Fiedler, unpublished observations). They are also not known to elicit trophallaxis or even to prey on ant brood. Accordingly, I did not score these associations as parasitic, but rather as competitors of ants for the same resource (namely, the ant-tree). Analogous cases are known, or suspected, to occur in other tropical lycaenid butterflies whose larvae feed on obligate myrmecophytes, such as various *Hypochrysops* species in Australia and New Guinea on *Myrmecodia* ant plants [14, 15].

Similarly, I do not include those lycaenid species (notably in the subfamily Miletinae) whose larvae prey upon ant-attended honeydew-producing homopterans and often also feed on homopteran honeydew [16–20]. In analogy to the case of myrmecophytes, these butterflies compete with ants for the same resources (here: trophobiotic homopterans), but

as a rule the caterpillars neither prey on ant brood nor elicit trophallaxis. Some species of the Miletinae, however, are known to supplement their diet through ant regurgitations, and these are included below since they show the behavioural traits considered here as essential for parasitism with ants.

Two further restrictions are (1) cases where trophallaxis or predation on ant brood have so far only been indirectly inferred, but not be confirmed through direct observational evidence, are largely excluded. This relates to a couple of tropical lycaenid species for which only old, or very incomplete or vague, information on their life cycles is available. In these cases, new data are needed, before any conclusions become feasible. (2) The butterfly family Riodinidae is also excluded. Ant-associations occur in at least two clades of Neotropical Riodinidae (tribes Eurybiini and Nymphidiini, see [21, 22] for many case studies and [23] for a tentative phylogeny). Circumstantial evidence exists that in at least one genus within the Nymphidiini (*Aricoris*) the larvae may feed on trophallaxis received from *Camponotus* host ants [21], but otherwise the existence of ant-parasitic life habits in the Riodinidae (though not unlikely to exist amongst Neotropical riodinids) must await confirmation.

## 3. Data Sources

Butterfly life-history data were compiled from a large variety of sources, ranging from faunal monographic treatments across hundreds of journal papers to databases in the Internet. The data tables in [1] formed the initial basis, and they have been continually extended and updated ever since [24, 25]. Here, I focus on that subset of sources where (a) the butterfly species qualifies as a parasite of ants according to the restrictions stated above and (b) the host ant has been reported at least at genus level. Three reasons justify the choice of the ant genus level for the subsequent comparisons. (1) For most ant genera, no modern revisions are available. Thus, proper species identifications are often impossible, especially in tropical realms. (2) Ant genus delimitations are quite stable and recognizable on a worldwide basis ([26], see also <http://www.antweb.org>). Accordingly, records (often reported by lepidopterists and not myrmecologists) should usually be reliable on this level. (3) Most ant-parasitic lycaenids are not bound to one single ant species, but are affiliated with a couple of congeneric ant species. For example novel *Myrmica* host ant species continue to be discovered in Eastern Europe for butterflies in the genus *Maculinea* [27, 28]. Therefore, I performed all analyses on the taxonomic level where the highest reliability can be achieved. Data on species richness of ant genera was extracted from the website [antweb.org](http://www.antweb.org) (as of 9 October 2011).

A complete bibliography of the evaluated literature would extend beyond the scope of this essay. For ant-parasitic Lycaenidae, many sources have been detailed in [6]. Full information on data sources is available upon request from the author.

#### 4. Summary of Ant Genera That Are Confirmed as Hosts of Parasitic Lycaenid Butterflies

Of the 54 ant genera known to attend lycaenid larvae on a worldwide basis ([24], only *Liometopum* has been added to this list since) just 11 genera are for certain recorded as hosts of parasitic butterflies.

##### 4.1. Subfamily Formicinae

**4.1.1. *Camponotus*.** This is one of the globally most prevalent ant genera in terms of species richness (>1050 described species) as well as ecological significance. It is also the numerically leading ant genus with regard to the number of associated parasitic lycaenid species. At least 9 species of the large Afrotropical genus *Lepidochrysops* have been recorded from nests of either *Camponotus niveosetosus* or *C. maculatus*. *Lepidochrysops* larvae have a life cycle similar to the *Maculinea-Phengaris* clade. They initially feed on flowers of plants (mostly in the families Lamiaceae, but also Verbenaceae and Scrophulariaceae). At the onset of their third instar they are adopted by *Camponotus* workers into the ant colonies where they turn into predators of ant brood. There are more than 125 described *Lepidochrysops* species [29]. Many of them are microendemics of high conservation concern [30]. Presumably all *Lepidochrysops* species are parasites of *Camponotus* ants. The small South African genus *Orachrysops* is the closest relative of *Lepidochrysops*. *Orachrysops* larvae are not parasites of ants, but live in close association with *Camponotus* ants as leaf, and later root, herbivores of various Fabaceae plants [31]. *Orachrysops* species may therefore be seen as models for the evolutionary transition between “normal” phytophagous ant-mutualistic lycaenids and species that are parasites of ants.

The East Asian *Niphanda fusca* is an obligate cuckoo-type parasite of various *Camponotus* ants [32]. Unusual for ant-parasitic lycaenids, larvae of this species retain a fully functional nectar gland whose secretions are tuned towards the gustatory preferences of their host ants [33]. Life histories of other *Niphanda* species, that all occur in East and South-East Asia, are unknown. Within the genus *Ogyris* (13 species in New Guinea and Australia) most species maintain obligate mutualistic associations with ants, but two are reported to occur inside nests of *Camponotus* species, namely, *O. idmo* and *O. subterrestris* [15, 34, 35]. Finally, for at least two representatives of the aphytophagous African genus *Lachnocnema* (*L. bibulus*, *L. magna*) there is evidence that caterpillars supplement their diet by eliciting trophallaxis from *Camponotus* ants (in *L. bibulus* reportedly also from *Crematogaster* ants). The major nutrient source of *Lachnocnema* larvae, however, is preying on homopterans and drinking their honeydew excretions.

**4.1.2. *Oecophylla*.** The two species of weaver ants in the genus *Oecophylla* are extremely dominant insects in their habitats in tropical Africa, southern and south-eastern Asia, Australia, and New Guinea. Two lycaenid genera are specialist parasites of weaver ants. *Liphyra* (*L. brassolis*, *L. grandis*)

are predators of the brood of *Oecophylla smaragdina* in the Oriental region [15, 25], while African *Euliphyra* (*Eu. mirifica*, *Eu. leucyania*) are cuckoo-type parasites of *Oe. longinoda* by means of trophallaxis and also steal prey items of their host ants [36]. Many more lycaenid species are associated with weaver ants, including striking examples of obligate and specific interactions, but these all appear to be mutualistic associations.

**4.1.3. *Polyrhachis*.** Even though this large ant genus (>600 described species) ranks rather high in the visitors list of lycaenid caterpillars, only one of its reported associated 27 myrmecophilous butterfly species is a parasite. The rare *Arhopala wildei* in Australia and New Guinea preys on brood in nests of *Polyrhachis queenslandica* [37, 38].

**4.1.4. *Lasius*.** Ant species of this moderately rich genus (>100 species) are frequent visitors of lycaenid caterpillars, especially in the Palaearctic realm [25]. *Shirozua jonasi* from East Asia is the only ant-parasitic butterfly known to be affiliated with *Lasius* ants (*L. spathepus*, *L. fuliginosus*, and *L. morisitai*). The caterpillars apparently receive occasional trophallactic regurgitations, but their principle mode of feeding is to prey on a variety of homopterans and to drink their honeydew excretions [39].

**4.1.5. *Lepisiota*.** Butterflies of the South African genus *Aloeides* all have an obligate relationship to ants. *Lepisiota capensis* is their major host ant [40]. As far as known, most *Aloeides* species are phytophagous ant mutualists (host plants in the Fabaceae and more rarely the Malvaceae, Zygophyllaceae and Thymelaeaceae), but older larvae of *A. pallida* have been observed to feed on ant eggs and appear to be completely aphytophagous [40].

**4.1.6. *Anoplolepis*.** Another endemic South African butterfly genus is *Thestor*, with about 27 recognized species [41]. The life histories of these butterflies are still very incompletely known, but for sure they are essentially aphytophagous, as is the rule in the Miletinae to which this genus belongs. Younger larvae prey on various homopterans, and in at least 3 species (*Th. yildizae*, *rileyi*, and *basutus*) older larvae live inside ant nests where they feed on brood of the ant *Anoplolepis custodiens*. It is suspected that all *Thestor* species share this habit [41].

##### 4.2. Subfamily Dolichoderinae

**4.2.1. *Papyrius*.** The small endemic Australian butterfly genus *Acrodipsas* can be divided into two clades [42]. Larvae of one of these, comprising the species *A. brisbanensis* and *A. myrmecophila*, are obligate parasites of *Papyrius nitidus* [35] from their first instar onwards, that is, without a phytophagous phase as in *Lepidochrysops* or the *Maculinea/Phengaris* clade. *Papyrius* species are highly dominant components of Australian ant assemblages and serve as mutualistic partners for some additional Australian lycaenids [34].

### 4.3. Subfamily Myrmicinae

4.3.1. *Crematogaster*. This diverse ant genus (>450 described species) ranks second in terms of associated ant-parasitic lycaenid butterflies. In the lycaenid tribe Aphnaeini (about 260 species, of which >90% occur in Africa) caterpillar-ant associations are nearly always obligatory, and the predominant host ant genus is *Crematogaster*. Few Aphnaeini species, however, are well established to be parasites of *Crematogaster* ants. Only one of these is a brood predator (*Cigaritis acamas* [43]), whereas in other cases trophallactic feeding has been reported (e.g., *Aphnaeus adamsi*, *Chrysoiritis (Oxychaeta) dicksoni*, *Spindasis takanonis*, and also *S. syama*; [40, 44]). Beyond the tribe Aphnaeini, parasitic relationships occur in the Australian *Acrodipsas* of which three species (*A. cuprea*, *illidgei*, and *aurata*) are predators of *Crematogaster* ants [35, 42, 45]. According to one old account caterpillars of the aphytophagous African *Lachnocnema bibulus* (which essentially prey on homopterans and drink their honeydew exudates, see above) also supplement their diet by trophallaxis obtained from *Crematogaster* ants [46].

4.3.2. *Myrmica*. This genus is famous as being the host of the ant-parasitic *Maculinea* butterflies in temperate regions of Eurasia. *Maculinea* comprises about 10–15 species, depending on the status allocated to local forms and cryptic lineages detected through recent sequence analyses [47]. All *Maculinea* species are either brood predators or cuckoo-type parasites [11] of *Myrmica* ants. Host specificity was initially thought to be generally high [48], but research over the past two decades has revealed more complex, locally to regionally variable patterns of host specificity [27]. Especially in previously underexplored regions of central and east Europe many new local host associations have been elucidated through thorough field work [28]. Caterpillars of the closely related East Asian butterfly genus *Phengaris* also parasitize *Myrmica* species [44, 47].

4.3.3. *Aphaenogaster*. There are two *Maculinea* species from East Asia (*M. arionides*, *M. teleius*) for which the use of *Aphaenogaster* ant species as hosts has been recorded. Both these butterfly species are known to parasitize mainly *Myrmica* host ants. It remains to be shown to what degree *Aphaenogaster* ants really qualify as valid hosts. Alternatively, these records might be based on misidentifications or represent rare affiliations that only occur under exceptional circumstances (see the discussion about primary and secondary hosts in [27]).

4.3.4. *Rhoptromyrmex*. Representatives of this small Oriental ant genus have been observed to attend a range of lycaenid caterpillars in a mutualistic manner. Besides, trophallactic feeding does occur in one unusual case, the Miletinae species *Logania malayica*. *L. malayica* larvae prey essentially on homopterans and drink their honeydew exudates, but young larvae also elicit regurgitations from *Rh. wroughtonii* ants, with which the butterflies are closely and specifically associated over their entire life cycle [17, 49].

## 5. Macroecological Patterns of Host Ant Use among Ant-Parasitic Lycaenidae Butterflies

Myrmecophilous associations between lycaenid butterflies and ants are confined to that subset of ant genera which maintain trophobiotic interactions [24]. Trophobiotic ants form a highly significant fraction in terms of their ecological prevalence as well as species diversity. They essentially derive liquid nutrients from extrafloral plant nectar [50, 51] and from the excretions (“honeydew”) of sap-sucking homopterans [52, 53]. Lycaenid and riordinid butterfly species that offer nectar-like secretions in exchange for protection largely “hitch-hike” on the behavioural and ecological syndromes which are associated with ant trophobiosis. Harvesting nutrient-rich liquids requires specialized anatomy [54] and behaviour in ants (e.g., trophallactic exchange of liquid food within the colony), with trophobiosis demanding a more complex suite of morphological and behavioural traits than licking-up plant nectar [55].

Ant-parasitic lycaenids form a very small subset of myrmecophilous ant-attended species in that butterfly family. Not surprisingly, the host ants parasitized by them constitute a small subset of ant genera known to visit and attend caterpillars in mutualistic associations. In two earlier studies the ecological prevalence and geographical distribution of ant genera were shown to be the best predictors for their representation in mutualistic lycaenid-ant associations [24, 25]. For parasitic interactions, this pattern changes according to a similar analysis. In analogy to [24], I constructed a multiple linear regression model, with the number of recorded ant-parasitic lycaenids as response variable and the species richness (log-transformed), representation in lycaenid-ant interactions (log-transformed), ecological prevalence, and geographical distribution of ant genera as predictors. Geographical distribution was scored on a rank scale (from 1 to 10) as the number of faunal regions from which an ant genus is known, using the following 10 regions: West Palaearctic region (Europe eastwards up to the Ural mountains, including Africa north of the Sahara, Asia Minor, and the Near East); East Palaearctic region (Asia east of the Ural mountains, including Japan and Taiwan); India; South East Asia (comprising Thailand, the Malay Peninsula, and the large islands of the Sunda shelf like Sumatra, Borneo, and Java); New Guinea; Australia; Central Africa (south of the Sahara to approx. 15° southern latitude); Southern Africa (mainly comprising South Africa, Namibia, Botswana, and Zimbabwe); North America (north of Mexico); Central and South America. Ecological prevalence (sensu [56]) was scored on a rank scale from 1 to 5 (Table 1).

The linear model revealed that only the number of associated lycaenid species had a significant and positive relationship with the number of recorded cases of lycaenid-ant parasitism in an ant genus (see Table 2 for full documentation). All three other potential predictors were far from having any significant effect. Inspection of residuals confirmed that the model assumptions were met with reasonable accuracy. Moreover, application of a Ridge correction (with  $\lambda = 0.1$ ) to account for collinearity among predictors did not change the overall model outcome (data

TABLE 1: Classification of ant genera known to associate with Lycaenidae caterpillars into prevalence groups. Ant genera are classified into that group which corresponds to the dominance status of its most dominant component species involved in butterfly-ant associations. For example, *Formica* is scored as “top dominant” since many (but not all) *Formica* species are territorial key-stone ant species in their respective habitats and communities, adapted from [24].

Class	Score	Criteria	Genera
Top dominant	5	Dominant ants in habitat; defend territories and resources intra- as well as interspecifically; monopolize resources against all heterospecific competitors	Myrmicinae: <i>Pheidole</i> ; Formicinae: <i>Formica</i> , <i>Oecophylla</i> ; Dolichoderinae: <i>Anonychomyrma</i> , <i>Azteca</i> , <i>Forelius</i> , <i>Froggattella</i> , <i>Iridomyrmex</i> , <i>Papyrius</i>
Second-order dominant	4	Subordinate relative to top dominants, but may become dominant in the absence of these; monopolize resources <sup>†</sup>	Myrmicinae: <i>Crematogaster</i> , <i>Meranoplus</i> , <i>Monomorium</i> , <i>Myrmecaria</i> , <i>Solenopsis</i> , <i>Tetramorium</i> ; Formicinae: <i>Anoplolepis</i> , <i>Camponotus</i> , <i>Polyrhachis</i> , <i>Lasius</i> , <i>Lepisiota</i> , <i>Myrmecocystus</i> ; Dolichoderinae: <i>Dolichoderus</i> , <i>Linepithema</i> , <i>Liometopum</i> , <i>Ochetellus</i> , <i>Philidris</i> ;
Submissive	3	Subordinate to both classes of dominants; usually opportunistic species with generalized feeding habits; rarely defend and monopolize resources against heterospecific ants	Myrmicinae: <i>Acanthomyrmex</i> , <i>Aphaenogaster</i> , <i>Myrmica</i> , <i>Rhoptromyrmex</i> ; Formicinae: <i>Echinopla</i> , <i>Notoncus</i> , <i>Paratrechina</i> , <i>Prolasius</i> ; Dolichoderinae: <i>Dorymyrmex</i> , <i>Tapinoma</i> , <i>Technomyrmex</i> ; Ponerinae: <i>Ectatomma</i>
Solitary	2	Foraging individually; rarely monopolize resources	Myrmeciinae: <i>Myrmecia</i> ; Myrmicinae: <i>Cataulacus</i> ; Ponerinae: <i>Gnamptogenys</i> , <i>Odontomachus</i> , <i>Rhytidoponera</i> Pseudomyrmecinae: <i>Tetraponera</i> , <i>Pseudomyrmex</i>
Cryptic	1	Minute species foraging on the ground or in leaf litter; inferior to all other ants in direct confrontation	Myrmicinae: <i>Leptothorax</i> ; Formicinae: <i>Brachymyrmex</i> , <i>Plagiolepis</i> ; Dolichoderinae: <i>Bothriomyrmex</i>

<sup>†</sup> Includes many species that become dominant in disturbed habitats or when introduced as alien species into non-adapted ant communities.

not shown). In a stepwise forward model selection, again only the frequency of nonparasitic associations remained as significant predictor. Likewise, using Poisson-type (instead of Gaussian) error distributions did not affect the outcome of this analysis (data not shown).

Hence, it is not the ecological or geographical prevalence that is decisive for the establishment of parasitic relationships between lycaenid butterflies and ants. Rather, the more butterfly species do interact with a given ant clade, the more likely it is that some of these interactions may turn, in evolutionary time, into parasitic relationships.

This also becomes evident when the incidence of ant-parasitism is plotted against the rank the ant genera have in interactions with lycaenid caterpillar species (Figure 1). Instances of social parasitism are more likely amongst those ant genera that are numerically more important in lycaenid-ant associations in general, whereas again species richness of the respective ant genera had no significant influence (Table 3).

A number of ant genera (e.g., *Pheidole*, *Dolichoderus*, *Formica*, and *Iridomyrmex*) that are ecologically dominant in

TABLE 2: Results of general linear model relating the number of parasitic lycaenid species associated with an ant genus to its species richness (log-transformed), ecological prevalence, geographical distribution, and importance in nonparasitic lycaenid-ant associations (log-transformed). Given are standardized regression coefficients  $\beta$ , and the  $F$  and  $p$  scores for each variable. SS: sum of squares; MS: mean of squares. Overall model fit:  $R = 0.5394$ ,  $R^2_{\text{corr}} = 0.2332$ ,  $F_{4;49} = 5.0288$ ;  $P = 0.0018$ .

	SS	df	MS	$\beta$	$F$	$p$
constant	12.78	1	12.78		2.607	0.113
dominance	0.35	1	0.35	-0.0370	0.072	0.789
associated lycaenid spp.	47.54	1	47.54	0.5051	9.698	0.003
species richness	3.44	1	3.44	0.1201	0.702	0.406
geographic regions	0.018	1	0.018	-0.0097	0.004	0.952
error	240.21	49	4.90217			

their habitats and serve as hosts for many well-integrated myrmecophilous ant parasites from other insect groups (e.g., [55]) are thus far completely missing in the host list of

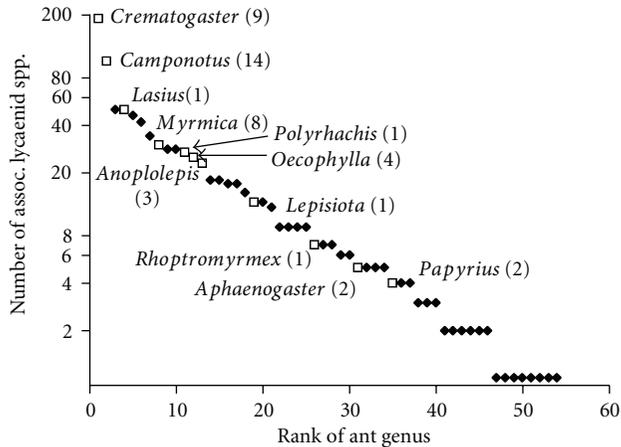


FIGURE 1: Rank-frequency plot of ant genera of the world involved in myrmecophilous associations of Lycaenidae butterflies, based on 927 record pairs of 497 butterfly species with 54 ant genera. Rank 1: ant genus with largest number of associated lycaenid species reported. Ranks 47 to 54: ant genera with only one associated lycaenid species known thus far. Filled diamonds: ant genera only known to be involved in mutualistic interactions with butterflies; open squares: ant genera that also serve as hosts for ant-parasitic Lycaenidae larvae (with genus name included; figure in parentheses: number of confirmed ant-parasitic lycaenid species). Note log-scale of y-axis.

TABLE 3: Results of a bivariate logistic regression, modelling the incidence of ant-parasitic associations within an ant genus ( $N = 54$  ant genera), in relation to species richness and number of nonparasitic associations with lycaenid immatures (both log-transformed) per ant genus. Given are the regression coefficients  $b_i$ , their standard errors and corresponding  $t$  and  $p$  values. Overall model score:  $\chi^2_{2df} = 6.577$ ;  $P = 0.0373$ .

	$b_i \pm 1 \text{ SE}$	$t$	$p$
Constant	$-3.0864 \pm 1.1160$	2.766	0.008
Number of associated lycaenid species	$0.9105 \pm 0.3936$	2.313	0.025
Species richness of genus	$-0.1799 \pm 0.2327$	0.773	0.443

ant-parasitic lycaenids. Even considering that trophobiosis is an important evolutionary prerequisite for the establishment of lycaenid-ant interactions (thereby excluding nontrophobiotic ants such as army ants, leaf-cutter ants or harvester ants as potential hosts), the discrepancy in host use between ant-parasitic lycaenids, and other well-integrated myrmecophilous parasites remains striking.

Only two ant genera, *Camponotus* and *Crematogaster*, have been the target of multiple evolutionary trajectories towards parasitic life habits amongst the Lycaenidae. Even though complete phylogenetic analyses are still lacking for the family Lycaenidae, there can be no doubt that parasitism of *Camponotus* through the butterfly genera *Lepidochrysops*, *Niphanda*, *Ogyris*, and *Lachnocnema* has evolved independently—these four butterfly genera are far apart from each other in all systematic accounts of the family Lycaenidae, and they represent all three potential pathways

to ant-parasitism suggested earlier [6]. Likewise, parasitism of *Crematogaster* ants by *Acrodipsas* is certainly unrelated in phylogenetic terms to the multiple (and probably again: independent) occurrences amongst single species of Aphnaeini that all belong to larger genera where the majority of species is nonparasitic (*Cigaritis*, *Spindasis*, and *Aphnaeus*).

Overall, the scattered occurrence of ant-parasitism amongst the Lycaenidae gives evidence that such interactions have evolved multiple times, rather independently from another, and under quite different circumstances [6]. Only few such cases have given rise to moderate or even substantial radiations, most notably in the African genus *Lepidochrysops* (over 120 species) and in the Eurasiatic *Phengaris-Maculinea* clade (some 10–20 species). The host ant use of the latter remains a mystery in terms of its evolutionary and ecological roots. *Myrmica* ants are visitors of only a moderate number of ant-mutualistic lycaenids in the Holarctic region (recorded with 22 species thus far). Moreover, *Myrmica* ants usually neither form very large colonies nor are they territorial and ecologically dominant in most habitats where they occur today. Hence, they lack typical characters of other host ants of parasitic myrmecophiles. On the other hand recent phylogenetic evidence [47] strengthens the notion that evolution of parasitic associations with *Myrmica* ants occurred just once, at the base of the *Phengaris-Maculinea* clade. Similarly, the affiliation with *Camponotus* ants in parasitic *Lepidochrysops* as well as mutualistic *Orachrysops* suggests that specialization on *Camponotus* hosts predated the evolution of parasitism in that butterfly lineage.

Host shifts among ant-parasitic butterflies from one ant host genus to another have apparently rarely occurred in the Lycaenidae. One well-documented case is the Australian genus *Acrodipsas*, where some species parasitize *Papyrius* ants, but one clade subsequently shifted to *Crematogaster* hosts [42]. This rare case even implies a switching of hosts across ant subfamily boundaries. In contrast, the significance of *Aphaenogaster* recorded as host ants of some East Asiatic *Maculinea* needs to be rigorously addressed. In all likelihood, these are stray (or even erroneous) records rather than an indication of host shifts beyond ant genus boundaries.

## 6. Which Cases of Ant-Parasitism Might Await Detection amongst the Lycaenidae?

Starting from the patterns of host-ant use among ant-parasitic Lycaenidae, and in combination with other information on life-history traits of lycaenid butterflies, I here finally outline a few expectations in which butterfly clades and biomes further instances of parasitic interactions might most likely be uncovered. These expectations are amenable to testing by systematic assembly of further life history data or by evaluating earlier inconclusive reports.

One major group of lycaenid butterflies where a larger number of instances of trophallactic feeding by ants can be expected is the subfamily Miletinae. Miletinae larvae are essentially predators of homopterans. Since many homopterans are attended by ants and since quite a number of Miletinae larvae also drink honeydew, it would not come as

a surprise to see more cases of trophallaxis with ants being documented in the future. Particularly likely candidates are those Miletinae species that are specifically adapted to spend their entire life cycle (including adult feeding on homopteran honeydew) with individual ant species. This is the case for *Logania malayica* with *Rhoptromyrmex wroughtonii*, and analogous candidate species occur in tropical SE Asia (*Miletus* spp. with *Dolichoderus* spp.; *Allotinus unicolor* with *Anoplolepis longipes*; [18, 20]). In two cases (*Allotinus apries* with *Myrmecaria lutea* [17]; *Logania hampsoni* with *Iridomyrmex* [15]) parasitic interactions have explicitly been suspected to exist, but until now these cases remain unsupported by direct observations of parasitic behaviours of the lycaenid caterpillars (*A. Weissflog*, personal communication for *A. apries*). As stated above, it is also quite likely that most, if not all *Thestor* species in South Africa will turn out to maintain parasitic relationships to *Anoplolepis custodiens* and allied ants [41]. Such cases of ant-parasitic relationships may also occasionally shift from the lower trophic level of cuckoo-feeding to the higher trophic level of brood predation (as in the genera *Liphyra* and *Euliphyra*). However, certain Miletinae do not interact intensively with ants that attend their homopteran prey [17, 57–59]. It is unlikely that traits required to entering into host-specific parasitic butterfly-ant interactions have evolved here. All further examples of ant-parasitism derived from predation on homopterans would obviously fall into the “Miletinae type” [6].

Another lycaenid clade where further cases of ant-parasitism can surely be expected to occur is the tribe Aphnaeini. Even though the few confirmed cases of ant-parasitism are rather isolated incidences nested within larger clades of ant-mutualists (e.g., *Chrysoritis dicksoni* in the genus *Chrysoritis* [60]), further species may show up to depend on nutrients derived from their close association with ants, as has been speculated many times in the literature (for critical reviews see [40, 61, 62]). Most additional instances of ant-parasitism in the Aphnaeini are expected to involve *Crematogaster* ants (the prevalent ant partner in mutualistic Aphnaeini species), but in *Aloeides* also further incidences of *Lepisiota*-parasitism may be found.

Other obvious candidates to furnish more ant-parasitic lycaenids are the genera *Lepidochrysops* (with *Camponotus*), *Maculinea*, and *Phengaris* (hitherto undescribed host associations in East Asia expected to refer to *Myrmica*), and *Niphanda* (probably with *Camponotus*). Beyond that, no valid extrapolations seem feasible at present. For example, the parasitic association between *Arhopala wildei* and *Polyrhachis queenslandica* does not seem “predictable” in a phylogenetic framework [13]. The most likely candidates for the discovery of novel ant-parasitic lycaenids of the “Aphnaeini type” are clades where a number of butterfly species show intimate host-specific mutualistic relationships towards specific host ants.

From the ant perspective, two genera which account for a very substantial fraction of records with lycaenids (namely, *Lasius* and *Formica*) score strikingly low as hosts of ant-parasitic butterflies. The only confirmed case with *Lasius* involves a species (*Shirozua jonasi*) whose larvae obtain most of their nutrient income from preying on homopterans and

drinking their honeydew. This hairstreak species is ecologically similar to Miletinae butterflies and does not enter into *Lasius* nests to prey on ant brood. Possibly, the lack of brood being present in *Lasius* nests over winter poses a constraint in the evolution of ant-parasitism in temperate-zone climates. This would also explain why so far no case of ant-parasitism has been confirmed from the genus *Formica*. In East Asia, larvae of *Orthomiella rantaizana* have been found in *Formica* nests (Shen-Horn Yen, personal communication), but whether these are parasites, commensals, or mutualists of ants remains to be uncovered. Clearly, *Lasius* as well as *Formica* species serve as hosts for a large range of well-integrated myrmecophiles [55], but the majority of these parasites have evolved from detritivorous or predacious ancestors, and not from herbivores.

Two other sociobiological traits of ant colonies that have been suggested to be related to the evolution of parasitic myrmecophily are the level of polygyny or polyandry, and the brood cycle. With regard to the latter, as already noted above the absence of winter brood may have prevented the intrusion of Holarctic lycaenids as parasites into *Lasius* and *Formica* colonies. With regard to ants from the humid tropics, however, seasonal fluctuations in brood availability are less likely to constrain the evolution of lycaenid butterflies into parasites of ants, so that this factor (if valid at all) would have to be restricted to seasonal climates. Genetic intracolony heterogeneity, which can result from the presence of multiple queens and/or the occurrence of multiple matings during their nuptial flight, may facilitate the intrusion of social parasites as well as of parasitic myrmecophiles [63]. It is presently impossible to rigorously test these two hypotheses, since data on the colony structure and population demography of many tropical and subtropical ants that are parasitized by lycaenids are too scant. Polygyny seems to be common among ants that serve as hosts [64], but in at least one instance (*Camponotus japonicus*, the host ant of *Niphanda fusca*) monogyny and claustral colony foundation have been confirmed [65].

## 7. Perspective

Ant-parasitic lycaenid butterflies are a bewildering evolutionary outcome: carnivores or cuckoo-type feeders in an otherwise phytophagous clade of insects. The communication modes required for integration into their host colonies, the phylogenetic roots, and population genetic consequences of their unusual interactions with ants, and their repercussions into conservation biology [66, 67] will continue to attract the interest of scientists. However, these parasitic interactions encompass only a small minority of myrmecophilous Lycaenidae butterfly species. Also the ant genera involved comprise but a small minority as compared to the range of trophobiotic ants that could potentially be parasitized. For sure, some further extensions can be expected, especially in hitherto underexplored tropical regions or in butterfly clades whose life histories are thus far very poorly documented. Most known ant-parasitic lycaenids occur in seasonally cold and/or dry regions [6], where

both the butterfly and the ant faunas are comparatively well covered. It has even been suggested, though not yet rigorously tested, that avoidance of unfavourable seasons might have promoted the entering of ant nests as safe places for lycaenid caterpillars. The detection of additional cases of butterfly-ant parasitism in these regions in all likelihood will not radically turn the robust patterns described here upside down. For tropical faunas, some more unexpected incidences of ant-parasitism may await discovery, yet it does not seem likely that many instances of butterfly caterpillars living in brood chambers of ant nests would have gone undetected thus far. Rather, future progress will be made in uncovering the microevolutionary steps that drive host-parasite co-evolution [7]. It will also be rewarding to rigorously assess the macroevolutionary pathways leading to ant-parasitism in a phylogenetically controlled manner. To achieve this goal, besides elucidating the phylogenetic relationships of lycaenids and their ant hosts, more bionomic data on both of these players, but especially a better documentation of the sociobiology and ecology of the host ants (beyond the well-studied *Myrmica* case) will be essential.

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## Research Article

# Chemical Integration of Myrmecophilous Guests in *Aphaenogaster* Ant Nests

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Social insect nests provide a safe and favourable shelter to many guests and parasites. In *Aphaenogaster senilis* nests many guests are tolerated. Among them we studied the chemical integration of two myrmecophile beetles, *Sternocoelis hispanus* (Coleoptera: Histeridae) and *Chitosa nigrita* (Coleoptera: Staphylinidae), and a silverfish. Silverfishes bear low quantities of the host hydrocarbons (chemical insignificance), acquired probably passively, and they do not match the colony odour. Both beetle species use chemical mimicry to be accepted; they have the same specific cuticular hydrocarbon profile as their host. They also match the ant colony odour, but they keep some specificity and can be recognised by the ants as a different element. *Sternocoelis* are always adopted in other conspecific colonies of *A. senilis* with different delays. They are adopted in the twin species *A. iberica* but never in *A. simonellii* or *A. subterranea*. They are readopted easily into their mother colony after an isolation of different durations until one month. After isolation they keep their hydrocarbons quantity, showing that they are able to synthesize them. Nevertheless, their profile diverges from the host colony, indicating that they adjust it in contact with the hosts. This had never been demonstrated before in myrmecophile beetles. We suggest that the chemical mimicry of *Sternocoelis* is the result of a coevolution with *A. senilis* with a possible cleaning symbiosis.

## 1. Introduction

Ant colonies often host microcosms of myrmecophile guests, mostly arthropods that take advantage of ant nest favourable environment and food resources [1–3]. The largest known association is the army ant *Eciton burchellii* with more than 300 guest species [4]. Interactions with ants range from true predators, commensals that live on ant food remains, mutualists, and parasites [3, 5, 6]. In order to get accepted they must break the ant colony “fortress” which is based on a chemical recognition system by which ant workers are able to recognize and exclude aliens. More precisely, a colony-specific mixture of cuticular hydrocarbons has been shown to constitute the recognition pheromone of most ant species [7, 8]. Several strategies have been described for the chemical

integration of myrmecophiles into ant colonies. Chemical mimicry is achieved either in a few cases by biosynthesising the same hydrocarbons as their host (but this is rare) or more generally by acquiring them through cuticular contacts and/or grooming (e.g., the guest actively licks the host's cuticle; see reviews by [9–11]). Myrmecophiles like woodlice, mites, phorid flies, and snails can also be “chemically insignificant,” that is, their cuticle bears very small amounts of hydrocarbons as it has been shown in *Leptogenys* [6]. Similarly, callow ants are chemically insignificant which allows them to get accepted in alien colonies during the first hours after emergence (see [12]). Another possibility of integration has been discovered recently in social insects: guests and parasites can be chemically “transparent” if they have only saturated hydrocarbons, which are not involved

in recognition [13]. Nevertheless, some myrmecophiles like *Pella* in *Lasius fuliginosus* colonies do not present chemical mimicry, simply escaping from the ants or using appeasement or repelling behaviour [14].

In the present study we conducted a survey of all arthropods living in the nest of the gypsy ant *Aphaenogaster senilis* in southern Spain. Then, we compared the chemical integration of two myrmecophile beetles (*Sternocoelis hispanus* and *Chitosa nigrita*) with that of an undetermined silverfish. We hypothesized that guests specialized with only one host (like *Sternocoelis*) have coevolved with it and biosynthesize the hydrocarbons while host-generalists like silverfish would mimic passively their hosts and can shift easily to different host species. To test for host specificity and relate it to chemical distance, we designed adoption experiments with *Sternocoelis* in conspecific colonies and congeneric species. We then analysed the mechanisms of chemical mimicry looking at the effects of separation of the beetles from their host. After two weeks, the exogenous hydrocarbons of the myrmecophile beetle *Myrmecaphodius* begin to disappear [15]. Therefore, after one-month isolation, we supposed that all exogenous hydrocarbons acquired by contact with ants had disappeared. As *Sternocoelis* was frequently observed licking the ant larvae, we investigated possible roles of these beetles in larval predation or prophylaxis. If the beetles fed on larvae by piercing the cuticle (haemolymph feeding on larvae by ant workers is known in *Amblyopone* [16]), larvae were supposed to decline. On the contrary, if the beetles fed only by licking the cuticle, larvae will maintain their wellbeing.

## 2. Material and Methods

**2.1. Inventory of Guests in *A. senilis* Colonies.** We completely excavated 57 nests between February 2008 and December 2009 on the banks of Guadalquivir near Sanlúcar de Barrameda to list and count all the guests, mites, silverfish, soubugs, staphylinids, and histerid beetles.

### 2.2. The Guest Studied

**2.2.1. *Sternocoelis* (Coleoptera: Histeridae).** This genus regroups myrmecophile beetles that live in ant nests of several species within the genera *Aphaenogaster*, *Cataglyphis*, *Formica* and *Messor* [17]. They are frequently found on the brood pile on which they were thought to feed (Figure 1(h)). According to Lewis [18] *Sternocoelis* feed on larvae and dead adult ants. Otherwise, little is known on their biology and reproduction. Larvae and pupae are unknown [17]. *Sternocoelis hispanus* (Figures 1(a) and 1(b)) occurs in central and southern Portugal and Spain, as well as in northern and central Morocco (see [19] for details). In the Iberian Peninsula it has been found living only in *A. senilis* colonies [17, 19]. On the other hand, in Morocco it was found in at least four different species of *Aphaenogaster* with more than 30 specimens in some nests (Lackner, unpublished). After exposing the colony by turning the stone under which they live, some *S. hispanus* immediately headed for the security of the nest searching for the nearest gallery, whereas the other attempted to “hitch a ride” by clinging

onto the ants (Figures 1(c) and 1(e)). The histerids, rather than the ants (as is the case in *Chennium bituberculatum* observed in eastern Slovakia; Lackner, unpublished), always actively seek out the ants in order to be transported into the nest. This phenomenon of *Sternocoelis* riding the ants has so far been observed only in four *Sternocoelis* species: *S. hispanus*, *S. slaoui*, *S. arachnoids*, and *S. espadaler* (Lackner, unpublished). As very few is known on *S. hispanus* biology, we measured the length, width, and weight of the *Sternocoelis* to search for sex differences.

**2.2.2. *Chitosa* (Coleoptera: Staphylinidae) and Silverfish (*Thysanura*).** They are very active insects moving rapidly into the nest. Very little is known on their biology. Since they are associated with various ant species, they are apparently host generalists. *Chitosa nigrita* is a rare myrmecophilous species known only from Spain and Morocco [20] (Figure 2). We collected *C. nigrita* in two colonies of *A. senilis* (1 and 4). From colony 4 we also collected two silverfish. Silverfish are known to move freely within the entire nest [6]. *Chitosa* and silverfish were only used for chemical analyses.

**2.3. Ant Colonies.** In November 2008, 43 *S. hispanus* beetles were discovered in an *A. senilis* colony (hereafter, colony 1) in Andalusia, Doñana National Park (Las Beles, 36°58.53'N, 6°29.11'W, sea level). Three other colonies were collected, colony 2 and 4, just a few meters from colony 1, and a fourth one (colony 3) collected 60 km apart, near Aznalcazar in a pine forest (37°14.77'N, 6°12.17'W, 36 m). For adoption experiments we used four colonies of different *Aphaenogaster* species: one colony of *A. simonellii* (Egine's island, Greece; 37°45.22'N, 23°31.46'E, 580 m), one colony of *A. subterranea* (Cévennes, France; 44°02.57'N, 3°49.68'E, 370 m) and two colonies of *A. iberica* (Sierra Nevada, Spain; 37°08.42'N, 3°28.34'E, 1370 m). Like *A. senilis* and *A. simonellii*, *A. iberica* belongs to the subgenus *Aphaenogaster*, while *A. subterranea* belongs to a different subgenus (*Attomyrma*), suggesting it is phylogenetically more distant from *A. senilis* than the other two.

Colonies were maintained in the laboratory in large plastic boxes and fed at libitum with live maggots, pieces of orange, sliced *Tenebrio* larvae, and a commercial solution for bumblebees (Beehappy).

**2.4. Behaviour of *Sternocoelis*.** We performed a behavioural repertoire of the beetles using scan sampling method: during 3 days, we recorded during 50 sequences the behaviour of all beetles that were visible in colony 1 (total number of observations 741). Behaviours were the followings: isolated in the colony (either immobile or moving), on larvae, on prey, on a worker, licked by a worker (see Figure 1).

As the *Sternocoelis* were observed frequently on the ant larvae, we made small nests with 6 *A. senilis* nurse worker ants, 6 beetles, and 6 larvae of different developmental stages. The behaviour of the beetles and the number and aspect of larvae were observed during 30 days.

**2.5. Adoption Experiments.** We observed the behaviour of the *Sternocoelis* beetles and examined whether the beetles can be



FIGURE 1: *Sternocoelis* beetles (Coleoptera: Histeridae). (a, b): *Sternocoelis hispanus* morphology, sex unknown (photo (b) by Martin Švarc and Peter Koniar); (c): two *Sternocoelis slaoui* riding on an *Aphaenogaster* worker (Photo Martin Švarc and Peter Koniar, Larache, Morocco, February 2010); (d): *Sternocoelis hispanus* beetles feeding on mealworm larvae; (e): *S. hispanus* jumping on an ant worker; (f, g): aggressive behaviour against allocolonial *S. hispanus* in the foraging arena-transport by an *Aphaenogaster* worker (f): aggression; (g): *S. hispanus* cleaning ant larvae. All photos unless (b) and (c) by Alain Lenoir.



FIGURE 2: *Chitosa nigrita* (Coleoptera: Staphylinidae) (photo by Alain Lenoir). Determination by Munetoshi Maruyama.

adopted by another *A. senilis* colony or a colony of another species. Adoption tests were conducted on small colony fragments ( $n = 6$  for *A. senilis*) containing 120 workers and a brood kept in small flat plaster nests covered with a glass. Observations were realized through a red plastic sheet. The nest communicated with a foraging arena, made of a plastic box where the beetles were introduced. These experimental colonies have been acclimated in the experimental setup for at least 24 h before the adoption experiments were conducted. Consecutive experiments were separated by at least one week.

One or two beetles were introduced in the foraging arena of each *Aphaenogaster* experimental colony. We then measured the following variables:

- (i) Latency to the first Contact with the beetle (LC);
- (ii) Total time of Contact between the beetle and ants in the External area (TCE);
- (iii) total time of TRansport of the beetle by ants into the nest (TR);
- (iv) total time of Contact between the beetle and ants In the Nest (CIN);
- (v) the sum of these four durations, the Total Time until Adoption (TTA). In some cases the beetle was again aggressed inside the nest and we added this duration to the first TTA.

If the beetle was left in the foraging arena during one week and always neglected, it was considered not adopted and returned to its original nest. *Sternocoelis* beetles were introduced either into a fragment of their own colony (controls, colony 1;  $n = 10$  beetles), a different colony of *A. senilis* (colony 2 and 3;  $n = 10$  beetles per colony), a colony of *A. simonellii* ( $n = 4$  beetles), a colony of *A. iberica* ( $n = 12$  beetles), or a colony of *A. subterranea* ( $n = 4$  beetles).

In order to evaluate the chemical integration of *Sternocoelis* into colonies of *A. iberica*, we made 5 more trials: 3 were adopted and used for chemical analysis, 2 disappeared, probably killed by foragers.

**2.6. Isolation Experiments.** To observe the effect of separation from the *Aphaenogaster* hosts, groups of 5 *Sternocoelis* were isolated in a small glass tube with water and food. Individual isolation was not possible as the beetles died rapidly. They were reintroduced into their original nest after 1, 3-4, 5-6, 7-8, and 30 days of isolation ( $n = 4$  for each and  $n = 6$  for 30 days) and we measured the readoption time. These data were compared to controls retrieved directly into the host nest ( $n = 8$ ). We performed chemical analysis of the hydrocarbons on the 8 controls, 5 beetles isolated for 4 and 8 days, and 6 beetles isolated 30 days (see Section 2.7).

**2.7. Chemical Analyses.** In a first step we used the whole ants and the whole myrmecophiles. The animals were frozen at  $-18^{\circ}\text{C}$  and immersed in 200  $\mu\text{L}$  of pentane during one hour, and the extract stored at  $-18^{\circ}\text{C}$  until analysis. Substances were identified by combined gas chromatography/mass spectrometry (TurboMass system, Perkin-Elmer, Norwalk, CT,

USA, operating at 70 eV) using a nonpolar DB-5HT apolar fused silica capillary column (length: 25 m; ID: 0.25 mm; film thickness: 0.25  $\mu\text{m}$ ). Samples injections were performed in splitless mode for 1 minute, a temperature program from 100 $^{\circ}\text{C}$  (2 min initial hold) to 320 $^{\circ}\text{C}$  at 6 $^{\circ}\text{C min}^{-1}$  with 5 min of final hold. A mixture of 10 linear hydrocarbon standards (from C20 to C40) was injected at regular time intervals in order to recalibrate retention times. To analyze the effects of social isolation, as we had only a few beetles, we used SPME: the live beetle was held in forceps and rubbed gently on the dorsal and lateral surfaces with a polydimethylsiloxane (PDMS, 7  $\mu$  fused silica/SS, Supelco, color code green) fiber for 3 minutes. The fiber was immediately desorbed in the GC-MS in the same conditions of pentane extracts. It has been shown that the profiles obtained with SPME and classical solvent extraction are qualitatively identical [21, 22], but a precise quantitative analysis showed that the proportions of compounds are slightly different [23], so we made also SPME controls for ants. SPME were made on 4, 8, and 30 days of isolation. An internal standard (eicosane) was added to the extract or deposited on the fiber to measure the hydrocarbon quantities. Hydrocarbons of *A. senilis* were previously identified [24, 25] and we added some new compounds present in very small quantities.

**2.8. Statistics.** ANOVA was performed on behavioural data for adoption experiments, Kruskal-Wallis on hydrocarbon quantities of isolated beetles.

Statistical analysis of the chemical profiles was done using all peaks that were identified. To determine the level of similarity of the CHC profile of the beetles and their hosts, isolated beetles and between species we used hierarchical cluster analysis (Euclidean distances, Ward's method) to construct a single-linkage dendrogram (see, e.g., [26, 27]). The Nei index of similarity was used to compare the chemical profiles of the species when large qualitative differences are observed (see, e.g., [27–29]).

### 3. Results

**3.1. Inventory of Guests in *Aphaenogaster* Nests.** Figure 3 shows the frequency of nests in relation to the number of guests. Many nests did not contain any guest; for example, 51% of nest were free of mites, 98% of histerids beetles. Out of the 57 nests excavated, 23 contained at least one individual of the staphylinid *Chitosa nigrita* (mean  $\pm$  SE: 1.1  $\pm$  0.3; range: 0–9). Silverfish were present in 14 colonies (mean  $\pm$  SE: 0.9  $\pm$  0.3; range: 0–14) while sowbugs were found in 15 colonies (mean  $\pm$  SE: 0.8  $\pm$  0.3; range: 0–13). In Doñana National Park *Sternocoelis* beetles were rare. With the exception of two colonies that contained 43 and 30 individuals (resp., in November 2008 and July 2011), only five *Sternocoelis* beetles were found in four different colonies among more than 300 colonies. The mean length of *Sternocoelis* was 2.01 mm (SE = 0.01; min 1.79, max 2.24,  $n = 42$ ) and the width 1.29 mm (SE = 0.01, min 1.06, max 1.39,  $n = 42$ ). The weigh was 2.25 mg (SE = 0.18, min 1.5,

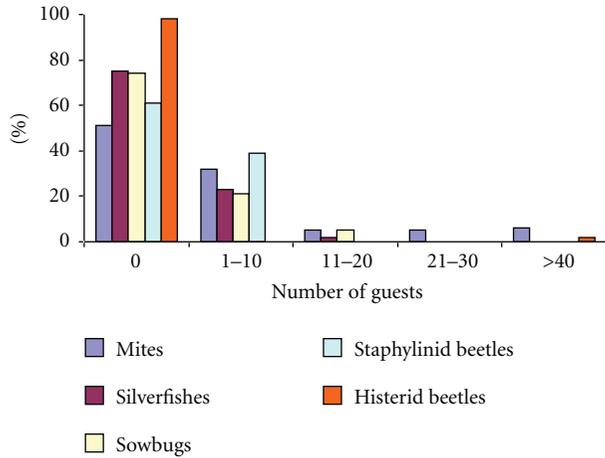


FIGURE 3: Frequency of colonies containing 0, 1 to 10, 11–20, 21–30, and > 40 guests in *A. senilis* colonies.

max 2.80,  $n = 8$ ). The distributions were unimodal, and therefore no sexual dimorphism appeared.

**3.2. Behaviour and Longevity.** Beetles stayed isolated or moved freely inside the host nest (28% of observations; it is a raw indication of the time budget of the beetles). From time to time they clutched to a worker's leg, jump on it, and stayed there (34.55% of observations) (Beetle on the ant body: Figures 1(c) and 1(e)). As observed by Yélamos [17], they were frequently found near or on the larvae (31.6%) (Figure 1(h)) and fed directly on pieces of *Tenebrio* (4.3%) (Figure 1(d)). They were occasionally licked by a worker (1.35%). It is possible that in the nests there is competition for food. Beetles had a rather long life, since one year later 18 of them were still alive. We never observed any sexual behaviour nor found any *Sternocoelis* larvae, so we do not know how these beetles reproduce.

In the small nest experiments with larvae we always observed at least 3 beetles on the ant larvae while the others were moving around searching for food. On the larvae they were either immobile or licking the cuticle. After one month, the observations were stopped because we did not observe any larval mortality and the larvae appeared to maintain normally. We never observed any brown spot, which would indicate a piercing of the cuticle.

We did not quantify the behaviour of the other guests, but observations indicated that *Chitosa* beetles and silverfish had a very different behaviour compared to *Sternocoelis*: they had very few interactions with the host, moving frequently in the nest. Silverfish were very fragile and died in less than 24 hours in the laboratory nests.

### 3.3. Adoption Tests in Alien Colonies

#### Behaviour

- (i) when deposited into the foraging arena of the alien colony, the beetle spent some time without contact with ants either because they did not meet it or because they did not perceive their presence. They

also simply stopped and inspected the ants, and continued their way;

- (ii) at their first contact with the beetle, ants behaved aggressively. They seized them in their mandibles, maintained them on the ground and inspected them with their antennae. They made short attacks with their mandibles (Figure 1(g)). The beetles are difficult to seize with the mandibles in account of their hard, smooth and rounded surface [17, 18] (Figure 1(f)). Some ants stopped after this initial inspection and continued their way;
- (iii) thence, the ants grasped the beetles by their legs. They were transported either inside the nest, or, on the contrary, farther from the nest. Sometimes the beetle held a prey and it was therefore more difficult to seize. It could also cling to the legs and thus be transported passively. Alternatively, it could cling to the antennae of the ant, which would try to shake it off;
- (iv) when the beetle reached the nest, it was maintained by ants and received a mixture of aggression and grooming. Sometimes it was transported again into the foraging arena, which indicates a rejection, at least provisory. The adoption was considered successful when the beetle was neglected and moved freely. Once adopted inside the nest, it searched rapidly for the chambers with larvae.

In *A. senilis* alien colonies adoption was almost systematically a success except for colony 2 which rejected one beetle (=5% total rejection). All but 2 beetles introduced in *A. iberica* were adopted, 2 were rejected and died ( $n = 17$ , = 11%). The duration of the adoption phases is given on Table 1. The latency without contact (LC) and duration of contacts (TCE) in the foraging arena, the transport times (TR) are longer in colony 2. The total adoption times (TTA) is 20 minutes in controls, more than one hour in colony 3 and very long in colony 2 where it attains 38 hours as the beetle is seized and aggressed many times. In *A. iberica* the duration of contacts in the nest is longer, the total adoption time is also longer (3 hours) but the difference is not significant (Table 1). When the adopted beetles were reintroduced into their *A. senilis* mother colony, they were aggressed but readopted rapidly.

In *A. simonellii* ( $n = 4$ ) and *A. subterranea* ( $n = 4$ ), the beetles were maintained always in the foraging arena and no adoptions occurred.

**3.4. Adoption of Isolated Beetles.** When reintroduced into their mother colony, isolated beetles were rapidly readopted in half an hour versus 22 minutes in controls. There is a small tendency to increase the adoption time with the isolation duration but none of the measures were significant (ANOVA,  $n = 30$ , Lambda de Wilk = 0.242,  $F_{(25, 86.943)} = 1.615$ ,  $P = 0.054$ —details not shown). At 30 days, 2 about 10 (i.e., 20%) died during the isolation.

**3.5. Chemical Profile of the Beetles and Hosts.** *Sternocoelis*, *Chitosa* and silverfish had the same hydrocarbons as their host *Aphaenogaster senilis* (Figure 4 and Table 2).

TABLE 1: Duration of different behavioural phases of adoption of *Sternocoelis* beetles (mean  $\pm$  SE). LC: latency of the first contact with ants in the external area; TCE: time contacts in the external area; TR: time of transport of the beetle into the nest; CIN: time of contact with ants in the nest; TTA: total time of the adoption. *A. iber*: *A. iberica*.

	LC		TCE		TR		CIN		TTA	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Col 1 (control)	6.5	2.0	2.0	0.8	2.2	0.3	12.3	3.4	22.9	3.1
Col 2	<b>73.3</b>	42.5	<b>154.9</b>	33	<b>44.7</b>	16.3	73.4	17.3	<b>2491.1</b>	479.1
Col 3	20.6	8	23.7	14.6	7	3.7	19.6	3.5	70.8	22.6
<i>A. iber</i>	6	2	12.7	2.6	2.2	0.5	<b>158.2</b>	21.3	179.3	20.5

ANOVA, Wilk = 0.006,  $F = 28.45$ ,  $df = 15$ ,  $P < 0.00001$ ; in bold significant differences for each column with post hoc Neumann-Keuls ( $P < 0.001$ , all other with  $P > 0.15$ ).

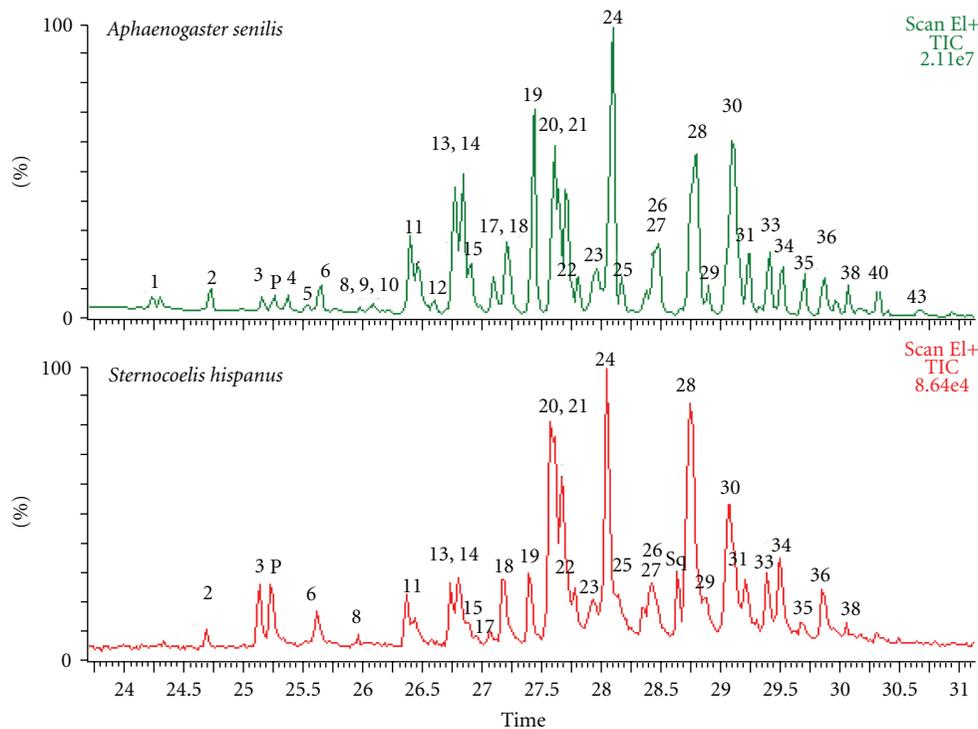


FIGURE 4: Chromatograms of *Aphaenogaster senilis* and *Sternocoelis hispanus*. Numbers refer to hydrocarbons in Table 2. P: phthalate and Sq: squalene pollutants.

*Aphaenogaster iberica* and *A. simonellii* also had the same hydrocarbons as *A. senilis* (Nei indexes were close: *A. senilis*/*A. iberica* = 0.75; *A. senilis*/*A. simonellii* = 0.65; *A. iberica*/*A. simonellii* = 0.88). *A. subterranea* had a very different profile with very small quantities of hydrocarbons (using total peak areas) and 20% of unsaturated alkanes which were absent in all other species. Surprisingly, it has also a lot of heavy hydrocarbons (25.8% had more than 32 carbons) that were not found in other species. This species is mostly subterranean and lacks saturated hydrocarbons protecting against desiccation. The Nei index between *A. subterranea* and the other *Aphaenogaster* species is very low (0.211), indicating a high chemical disparity. Therefore, this ant species has not been included in the following analyses.

In the first analysis we constructed a dendrogram of chemical distances between the guests and their *Aphaenogaster* host. It appeared clearly that the four *A. senilis*

colonies had different profiles (Figure 5), confirming previous analyses [30]. All the beetles, both *Sternocoelis* and *Chitosa*, were grouped with their host colony, indicating a chemical mimicry fitting the colonial signature. Nevertheless, beetles aggregated distinctly from their host. The chemical distance between colonies did not depend on their geographical distance, and was not linked to the beetle adoption time. Colonies 2 and 3 were equally chemically distant to colony 1 but accepted the beetles more or less rapidly. *Aphaenogaster iberica* and *A. simonellii* were close to *A. senilis* colonies 2 and 3 (data not shown) but the first species accepted the beetles whereas the second did not (but only 4 adoption trials). On the contrary, the silverfish did not match the host colony. Interestingly, *Sternocoelis* adopted in *A. iberica* were close to their new host but did not match completely to the new colony. *A. subterranea* is very different and as expected never adopted the beetles.

TABLE 2: Hydrocarbon quantities (mean  $\pm$  SE) in *Aphaenogaster simonellii*, *A. subterranea*, *A. senilis*, *A. iberica*, *Sternocoelis hispanus*, *Chitosa nigrata*, and a silverfish. Blanks indicate the absence of the substance or that it is present only as not quantifiable traces.

Peakno.	Name	<i>Aph simonelli</i>		<i>Aph subterranea</i>		<i>Aph senilis</i>		<i>Aph iberica</i>		<i>Sternocoelis</i>		<i>Chitosa</i>		Silverfish	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	C25:1	0.19	0.07			0.33	0.08	0.07	0.04	0.77	0.32	1.10	0.52	1.08	0.20
2	C25	1.43	0.20	19.86	3.47	0.29	0.04	1.38	0.31	0.81	0.13	0.67	0.19	4.26	0.37
3	11+13C25 (+7C25)	1.38	0.34	1.04	0.40	1.08	0.17	6.51	1.26	4.70	1.21	0.57	0.16	0.98	0.02
4	5C25	0.33	0.06	0.15	0.15	0.18	0.06	1.41	0.84	0.46	0.08	0.20	0.07	0.83	0.05
5	9,15C25					0.04	0.02			1.21	0.58	1.39	0.52		
6	3C25	0.92	0.11	2.26	0.40	1.01	0.18	1.30	0.21	5.05	1.63	1.22	0.38	3.72	0.25
7	5,9C25					0.14	0.02	0.82	0.23	0.49	0.07	0.17	0.07		
8	C26	4.31	0.91	2.45	0.49	0.16	0.04	5.04	0.62	0.24	0.07	0.10	0.03	0.75	0.01
9	4,6C25					0.12	0.05			0.85	0.33	0.08	0.02		
10	10+12C26	10.67	2.03	0.49	0.13	2.21	0.20	6.48	0.71	2.39	0.27	1.34	0.34	4.91	0.78
11	6+8C26	1.93	0.35			1.76	0.19	0.41	0.12	1.65	0.21	1.06	0.11	0.45	0.17
12	4C26	0.41	0.07	0.29	0.19	1.06	0.18	0.59	0.11	0.36	0.05	0.31	0.07	2.82	0.22
13	10,14C26	2.14	0.28	0.11	0.11	4.49	1.19	1.91	0.30	2.44	0.35	3.23	0.98	5.36	0.10
14	8,12C26					2.07	0.57	0.42	0.09	4.17	0.52	3.23	0.72		
15	C27:1			1.46	0.45										
16	6,10C26					0.65	0.14	0.05	0.05	1.03	0.19	0.21	0.07		
17	4,8C26					0.22	0.12			1.11	0.19	0.94	0.31		
18	C27	15.59	3.19	11.27	2.19	3.82	0.37	13.05	1.38	2.83	0.16	0.76	0.04	6.47	0.19
19	4,8,12C26					5.77	0.79	0.20	0.09	2.97	0.30	6.98	0.60		
20	9+11+13C27	8.36	1.78	1.43	0.21	18.84	2.08	17.04	1.08	11.01	0.49	9.00	1.84	5.84	0.14
21	7C27	1.15	0.46	0.07	0.07	3.65	0.49	1.03	0.19	3.64	1.02	4.64	1.37	2.12	0.06
22	5C27	2.29	0.39	0.18	0.08	1.50	0.34	0.87	0.22	1.48	0.39	1.83	0.58	1.10	0.05
23	9,13C27					0.43	0.24			3.12	0.20	2.43	0.80		
24	3C27	24.55	2.82	1.81	0.54	15.64	1.50	17.53	2.05	7.21	1.15	10.02	0.90	38.77	0.51
25	5,9C27					1.07	0.16			1.96	0.41	0.87	0.22		
26	C28	6.17	1.37	2.48	0.21	1.00	0.32			0.67	0.24	0.11	0.04	1.07	0.03
27	3,7+3,9+3,11C27	6.09	0.97			1.32	0.47	7.30	0.65	2.47	0.83	5.35	0.28	3.53	0.04
28	10+12C28	5.70	1.56	1.29	0.74	7.32	0.70	3.40	0.22	10.32	1.57	8.56	0.56	3.33	0.09
29	6C28	1.21	0.29			0.61	0.05			1.02	0.28	0.75	0.06	0.57	0.08
30	4C28+10,14C28	1.08	0.12	0.44	0.21	6.11	0.96	2.32	0.31	8.41	0.50	10.31	0.65	4.79	0.15
31	6,10C28	0.64	0.17			2.01	0.16	0.52	0.24	2.67	0.47	3.40	0.18	1.16	0.07
32	C29:1			1.06	0.40										
33	4,8+4,10C28	0.11	0.08			1.89	0.15	0.27	0.06	2.76	0.26	6.01	0.68	0.82	0.05
34	C29	1.14	0.21	9.43	1.09	2.39	0.35	0.48	0.08	3.22	0.51	1.27	0.06	1.78	0.05
35	TM C28					1.38	0.08			1.33	0.04	3.32	0.36		
36	11C29	0.79	0.23	2.86	0.65	3.90	0.49	4.12	0.53	2.24	0.30	1.95	0.14	0.49	0.03
37	7C29	0.19	0.05			2.04	0.49	1.65	0.16	0.45	0.12	0.62	0.20	1.00	0.15
38	5C29	0.86	0.12			1.79	0.34	0.32	0.08	0.48	0.24	1.55	0.35	2.03	0.01
39	11,15C29					0.14	0.06			0.52	0.20	0.26	0.06		
40	7,xC29					0.18	0.10			0.76	0.13	1.80	0.64		
41	3C29			2.36	0.25										
42	5,9C29	0.26	0.02	0.00	0.00	0.17	0.02	0.72	0.24	0.16	0.06	0.42	0.08		
43	C30	0.13	0.05	1.14	0.13	0.08	0.03	0.00	0.00	0.24	0.09	1.07	0.47		
44	10+12C30					0.11	0.02	1.14	0.42	0.17	0.07	0.43	0.24		
45	10,14+10,16+12,14C30					0.17	0.04	0.86	0.16	0.17	0.08	0.25	0.08		
46	C31:1			3.32	0.58										
47	4,8+4,10+4,12C30					0.01	0.01	0.03	0.02			0.01	0.00		
48	C31			1.15	0.06										
49	?			0.00	0.00	0.05	0.04					0.01	0.01		
50	11+13C31			2.54	0.46	0.20	0.07	0.19	0.09			0.10	0.09		

TABLE 2: Continued.

Peakno.	Name	<i>Aph simonelli</i>		<i>Aph subterranea</i>		<i>Aph senilis</i>		<i>Aph iberica</i>		<i>Sternocoelis</i>		<i>Chitosa</i>		Silverfish	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
51	11,15+13,xC31			3.23	0.63	0.58	0.20	0.31	0.07			0.08	0.05		
52	C32														
53	10C32			2.19	0.44			0.26	0.06						
54	11C33			2.17	0.54										
55	11,15C33			3.23	0.75										
56	12C34			3.16	0.72										
57	11+12C35:1			9.08	2.87										
58	C35:1			6.01	1.68										
	Total	100		100		100		100		100		100		100	
	<i>n</i> =	5		5		16		11		4		9		2	

11+13C25: 11-MethylC25 + 13-MethylC25; 9,15C25: 9,15-DiMethylC25; TMC28: TriMethylC28.

In the second analysis, we constructed the dendrogram of isolated *Sternocoelis* beetles (Figure 6). It revealed that they did not match completely the colony odour in a few days compared to controls maintained in their host colony. Some beetles after 4 or 8 days had always their colony profile—in a red ellipse on Figure 6—indicating a progressive change. Nevertheless, these changes were not sufficient to induce the rejection of the beetle.

We also measured the quantities of hydrocarbons on the cuticles. In the pentane rinses, the hydrocarbon quantities of *Aphaenogaster senilis* workers were 1099 ng/worker ( $\pm 860$ ,  $n = 5$ ), 446 ng ( $\pm 542$ ,  $n = 5$ ) for *Sternocoelis*, and 1567 ng ( $\pm 1270$ ,  $n = 5$ ) for *Chitosa*. These beetles were not chemically insignificant. On the contrary, silverfish had only 34 ng (30.1 and 37.7;  $n = 2$ ) indicating that these insects are insignificant and not protected against desiccation and explains why they die very rapidly after collection. For isolated *Sternocoelis* we retrieved by SPME only a very small quantity of hydrocarbons (1 to 5 ng/beetle, see medians in Figure 7), but the profile was comparable to liquid extracts. There were no differences between 4, 8, and 30 days isolated beetles compared to the controls (Kruskal-Wallis Chi-square = 3.91,  $df = 3$ ,  $P = 0.27$ ). It shows that the beetles maintained their hydrocarbons quantities independently of their host.

#### 4. Discussion

The three guest species mimic chemically their host: they have the same hydrocarbons (chemical mimicry sensu lato). This explains why they are tolerated inside the nest without being aggressed the ants and they have the host colony odour. This was predictable for *Sternocoelis*, which lives intimately with brood in the colony, but it was more surprising for *Chitosa* which has very few interactions with the host workers. Nevertheless, both species maintain some chemical specificity into the host colony (Figure 5), they are probably recognised as a different though tolerated element. This can be compared to social parasites that also keep their own identity into the host colony [11, 31]. It indicates that chemi-

cal mimicry is not sensu stricto, it means that ant workers have a double template: they must know and recognize both their nestmates and their guests. The queen also has a slightly different chemical profile and is recognized by workers (see reviews [7, 21, 27]). Recently, Vantaux et al. [27] described chemical mimicry between predatory larvae of a *Diomus* coccinellid and the little fire ant *Wasmannia auropunctata*. The myrmecophile larvae of *Diomus* also segregate separately in the clusters made on hydrocarbons, indicating that it may be general [27]. On the contrary, silverfish while matching the host hydrocarbons also, do not have an *A. senilis* colony-specific odour. They escape host aggression by avoiding contacts. They probably get their hydrocarbons directly by transfer from the host ant as it has been demonstrated in *Malayatelura* silverfish and their host *Leptogenys* using radioisotopes [32].

Adoptions of *Sternocoelis* are possible in all colonies of *A. senilis* with different delays. *Aphaenogaster* colonies are not completely closed [30], favouring the adoption of beetles that bear the same hydrocarbons in different proportions. The differences in adoption times can probably be explained by the fact that *A. senilis* colonies are very different in aggression levels [30]. Differences between *A. iberica* (acceptance) and *A. simonellii* (rejection) are difficult to explain. They may be due at least partly to the chemical distances with *A. senilis* being more important to *A. simonellii* (0.65) compared to *A. iberica* (0.75). We can also hypothesize that *A. iberica* are less aggressive, they do not have alkaloids in the venom gland, which may repel the beetles [33]. It may also simply not representative as we had only one small colony of *A. simonellii* and 4 adoption trials.

We discovered that *Sternocoelis* beetles kept their hydrocarbon quantities even after one-month isolation. Therefore, chemical mimicry by biosynthesis, rather than camouflage, may explain the host tolerance. If the hydrocarbons were transferred from the host passively, they should have disappeared in a few days because of the rapid turnover of these substances on the cuticle. For example, *Myrmecaphodius* isolated from their *Solenopsis* host colony lose their hydrocarbon profile in two weeks [15] and silverfish *Malayatelura*

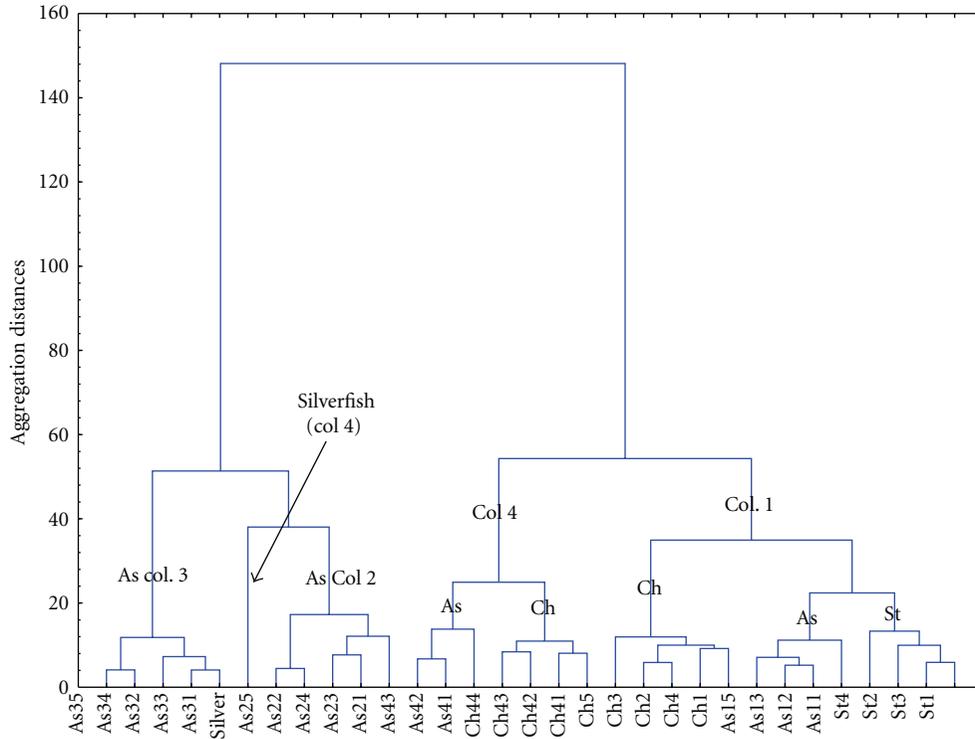


FIGURE 5: Dendrogram of chemical distances (Ward method, Euclidian distances). As: *A. senilis* colonies (colony 1 As11 to As15; col. 2 As21 to 24; col. 3 As31 to 35; col. 4 As 41 to 43). Ch: *Chitosa nigrita* (colony 1 Ch1 to 4; col. 4 Ch41 to 44). St: *Sternocoelis hispanus* (col 1 St1 to St4). Silverfish form col. 4 (mean of 2 individuals).

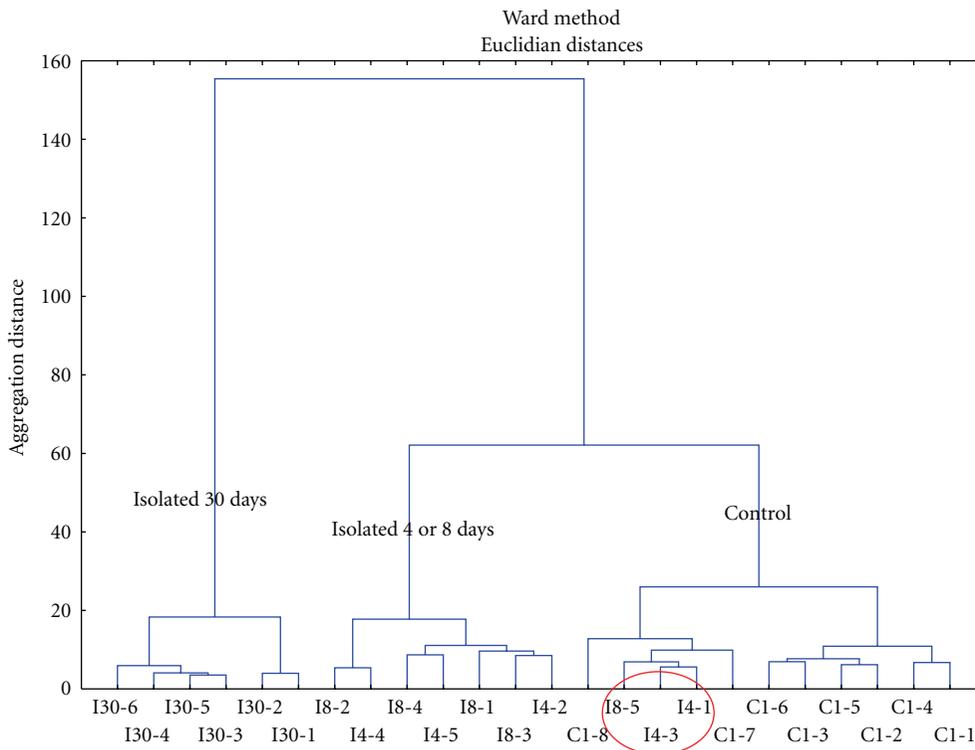


FIGURE 6: Dendrogram of the isolated *Sternocoelis* beetles (Ward method, Euclidian distances). Controls: C1 ( $n = 8$ ); I4 ( $n = 5$ ), I8 ( $n = 5$ ), I30 ( $n = 6$ ) Isolated during 4, 8, and 30 days. In the red ellipse, isolated individuals having kept completely their colony odour.

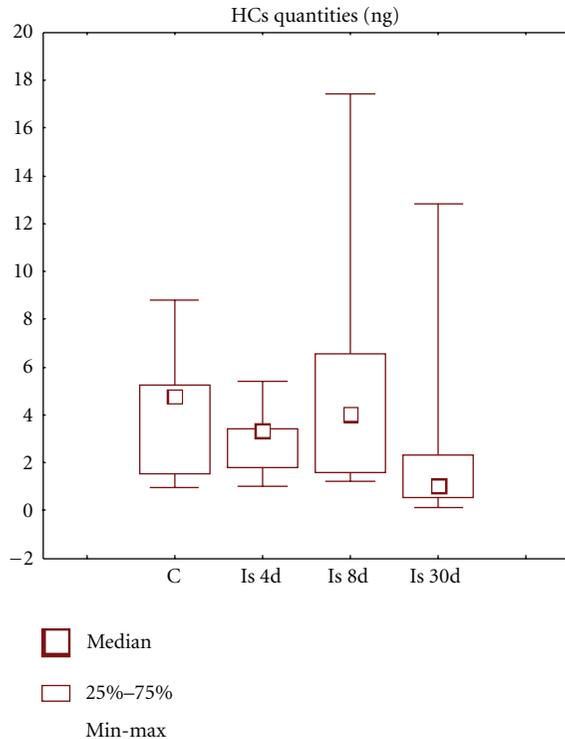


FIGURE 7: Hydrocarbon quantities (ng/individual; median, quartiles, Min-Max) retrieved by SPME on *Sternocoelis* beetles. C = Controls (beetles in the host nest,  $n = 8$ ), I4 ( $n = 5$ ), I8 ( $n = 7$ ), and I30 ( $n = 17$ ): beetles isolated from the hosts during 4, 8, and 30 days.

after six or nine days separation from their host *Leptogenys* showed reduced chemical host resemblance and received more aggression [32]. This suggests that *Sternocoelis* beetles are able to biosynthesize the host hydrocarbons and adjust their profile to the host colony by contacts. It explains why they change a little their profile after isolation, but are always accepted. This is an indication of a coevolution with the host, with a species-specificity of the association. We could not determine whether the chemical mimicry of the *Chitosa* staphylinid is an active or passive camouflage, but it is probably the latter as the beetle has very few direct interactions with the host and these beetles are not species-specific (Maruyama, pers. comm.). The silverfish have very few hydrocarbon quantities and are chemically insignificant, as observed in a species living in *Aenictus* colonies (but no details are given in the paper, [34]). Nevertheless they also have the host odour, probably acquired simply by contact with the nest material (see above V. Witte pers. comm.), but it is not colony-specific. It is interesting to note that the inside nest material odour is not colony specific as shown in *Lasius niger* [23]. This may explain why silverfish are killed in *Leptogenys* experimental colonies [6].

Chemical mimicry has been studied only in a few beetles in social insects and all the situations are possible. Biosynthesis has been demonstrated using radio-labelling  $^{14}\text{C}$ -acetate. It was shown to occur in two species of thermophilic Staphylinidae with their host *Reticulitermes* [35, 36].

Hydrocarbons are also biosynthesized by the larvae of the fly *Microdon* that are transported in the ant nest [37, 38]. The larvae of the butterfly *Maculinea rebeli* use a double mechanism: they first synthesize the hydrocarbons of the ant brood and later acquire additional hydrocarbons from the ants enhancing the mimicry [39]. Concerning the association of larvae of *Diomus* coccinellid and the little fire ant the authors suggested mimicry by biosynthesis, but they do not prove it [27]. In all the other cases studied, the myrmecophile mimics passively its host (see [9, 10]).

Is the presence of *Sternocoelis* beetles costly for the ant colony? In the army ant *Leptogenys distinguenda* workers are able to recognize and kill the intruders (and possibly eat them) to various degrees, which is the mark of a counterstrategy of the ant [6]. Nevertheless, *Leptogenys* are nomadic ants without a permanent nest, and the situation is different in ants that build a nest and mark it with the colony odour. Inside the nest, all individuals including guests are considered as friends as it was first hypothesized by Jaisson [40] and chemically explained in *Lasius niger* [23]. Apparently, the cost of *Sternocoelis* is insignificant for the host, but some competition for food is possible as beetles and ant larvae feed in the same chambers and beetles can be very numerous. On the opposite, the beetles licking the ant larvae may benefit if they protect them against parasites and infection. We suggest that this may mean a cleaning symbiosis as known in vertebrates, for example, between a cleaner fish and a client [41]. This symbiosis is probably weak as the *Sternocoelis hispanus* cleaners are rare, at least in some places. Therefore, our data suggest that *Sternocoelis* beetles cannot be considered as parasites and that an arms race with the ants will not occur like *Maculinea* larvae with their host ants [42].

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## Review Article

# Towards a Better Understanding of the Evolution of Specialized Parasites of Fungus-Growing Ant Crops

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Fungus-growing ants have interacted and partly coevolved with specialised microfungal parasites of the genus *Escovopsis* since the origin of ant fungiculture about 50 million years ago. Here, we review the recent progress in understanding the patterns of specificity of this ant-parasite association, covering both the colony/population level and comparisons between phylogenetic clades. We use a modified version of Tinbergen's four categories of evolutionary questions to structure our review in complementary approaches addressing both proximate questions of development and mechanism, and ultimate questions of (co)adaptation and evolutionary history. Using the same scheme, we identify future research questions that are likely to be particularly illuminating for understanding the ecology and evolution of *Escovopsis* parasitism of the cultivar maintained by fungus-growing ants.

## 1. Introduction

**1.1. The Attine Fungus-Growing Ants.** Fungus-growing ants (Hymenoptera: Formicidae: Attini) form a monophyletic tribe of primarily tropical ants that obligately depend on fungal cultivars (Agaricales: mostly Lepiotacea: Leucocoprineae). The ants provide the fungus with optimal growth conditions, and in return, the fungus serves as the main food source for the ants [1, 2]. The symbiosis between fungus-growing ants and their fungi originated about 50 million years ago [3–6] from a single ancestor that was most likely a generalist forager [3]. Subsequently, the Attini have diversified to encompass approximately 230 described species, distributed across 14 ant genera [4, 7]. Colonies of fungus-growing ants are typically founded by a single queen, who carries a piece of the fungus cultivar from her natal colony in the infrabuccal pocket [8] during her nuptial flight [9–11]. The Attini are divided into the “higher attine” and the phylogenetically basal “lower attine” genera based on their cultivars [5]. Lower attine cultivars are largely unmodified and resemble free-living Leucocoprine, whereas higher attine cultivars (including those of leaf-cutting ants) are highly derived [12].

The clonal propagation of cultivars through vertical transmission [2, 13] predicts ancient association and congruence between the ant and fungal cultivar phylogenies. High degrees of congruence have indeed been found at the deep phylogenetic levels in both higher [14, 15] and lower attines [12, 14]. However, the phylogenetic interaction specificity breaks down within, and occasionally between, ant genera and their cultivar strains, indicating that switches and/or reacquisitions of new garden cultivars have occurred (e.g., [12, 16–19]). While the higher-attine fungi no longer persist outside the symbiosis, lower attine fungi have free-living close relatives, which is likely to facilitate gene flow and reacquisitions of symbionts [12]. Cultivar switches can be induced in the laboratory, including the formation of chimeric gardens [20–22]. However, consistent with predictions from host-symbiont conflict theory [23], mature individual colonies appear to consistently maintain a single fungus clone, at least in leaf-cutting ants (*Atta* and *Acromyrmex*) where this has been best studied [24, 25].

Clonally propagated monoculture crops are expected to be particularly prone to infection with parasites and pathogens [26], because they represent an attractive resource that should be easy to exploit. This “Red Queen” logic

[27, 28] assumes that parasites and hosts are involved in evolutionary arms races, in which unpredictable genetic heterogeneity, due to sexual recombination, is the most powerful defence against parasites that have short generation times relative to their hosts [28–30]. Single asexual cultivar clones thus seem to represent a liability for the farming symbiosis [25] that needs to be overcome by active protection by the host ants (see below). Colony-level monoculture does not imply population-wide monoculture, as is often the case in modern human crops that are vulnerable to disease. With the possible exception of some species, there is likely to be considerable strain diversity across neighbouring colonies [16, 18] that should discourage the spread of infections between colonies.

*1.2. Specialised Coevolved Parasites.* Microfungal parasites in the genus *Escovopsis* (anamorphic Hypocreales) have been known for more than a century to overgrow fungus gardens of laboratory colonies [1, 13, 31], but the formal status of *Escovopsis* as a disease was confirmed only just over a decade ago when Currie et al. [32] showed that *Escovopsis* fulfils Koch's four defining postulates [33] for causative disease agents. This included evidence that *Escovopsis* (i) is found in abundance in diseased but infrequently in apparently healthy colonies, (ii) can be isolated from diseased colonies, (iii) can cause disease when colonies are artificially infected, and (iv) can be reisolated from diseased experimental colonies [32]. It was also shown that *Escovopsis* has a directly negative impact on the ant cultivar [32, 34, 35] through the secretion of compounds that break down the cultivar mycelium [36]. As fungus-growing ants rely on healthy fungus gardens for growth and reproduction, this implies that *Escovopsis* is a potentially serious threat to ant fitness [35].

Deep-level phylogenetic congruence has been found between the fungus-growing ants, their cultivars, and *Escovopsis* parasites, suggesting a long history of codivergence within the attine agricultural systems [37]. However, cophylogenies at lower levels appear to be punctuated with occasional host switching of the parasites [38], consistent with ongoing arms races [37], although null hypotheses of genetic drift in isolated parasite populations can usually not be dismissed.

Even within ant genera, there is some evidence for ant-cultivar-*Escovopsis* pairing specificity. Four morphologically and genetically distinct *Escovopsis* types parasitize the cultivars maintained by *Apterostigma*, a basal fungus-growing ant genus [39]. These have so far been categorised as “brown,” “yellow,” “pink,” and “white,” but are genetically distinct and likely different subspecies or species (cf. [39]). Even within these groupings, there is evidence for specificity: “pink” *Escovopsis* appears to infect only G3 cultivars and (rare) “white” *Escovopsis* only G2 cultivars, whereas “brown” and “yellow” *Escovopsis* commonly coinfect G2 cultivars (cf. Table 1 in [39]). Current evidence suggests that these pathogen lineages display patterns of phylogenetic congruence with their fungal host [39], maintained by chemotaxis and host resistance in nonnative (i.e., not naturally occurring) combinations [40]. A similar scenario

of association specificity is apparent for the lower attine genus *Cyphomyrmex*, where subclades of a single *Escovopsis* morphotype (pink) are phylogenetic congruent with corresponding clades of cultivar host genotypes [41]. In the higher attine ants, *Trachymyrmex* and *Sericomyrmex* are infected by specific *Escovopsis* parasites that are phylogenetically distinct from the two clades that parasitize *Atta* and *Acromyrmex* leaf-cutting ants [37, 38]. Within the leaf-cutting ant genera, *Escovopsis* infections are nonspecific [38], confirming the high degree of ant-cultivar specificity of all extant leaf-cutting ants to a single species of *Attamyces* symbiont [42].

*1.3. Defence Strategies against Escovopsis.* Fungus-growing ants, especially the leaf-cutting ants, have elaborate prophylactic fungus grooming and weeding behaviours to keep their cultivar free from parasites [44, 45]. In *Acromyrmex*, minor workers are particularly efficient at restricting spore germination [45], and major workers appear to recruit minor workers to infected sites, thereby potentially increasing the efficiency of disease suppression [46]. If spores manage to escape the attention of minor workers and germinate, major workers appear to perform the task of removing infected garden pieces (weeding) [45]. Task specialization between castes thus appears to make hygienic policing more efficient in general, which has been proposed to be normally sufficient for eliminating generalist fungal parasites, but not for completely eradicating *Escovopsis* infections [44].

To control *Escovopsis* infections, fungus-growing ants may also use metapleural gland secretions, which contain an array of compounds with antibiotic properties [48, 69]. In a seminal study, Fernández-Marín et al. [47] described highly coordinated and challenge-specific foreleg movements along the metapleural gland opening (metapleural gland grooming), which allowed *Atta* and *Acromyrmex* ants to precisely target the application of antibiotic secretion to their gardens. In combination with metapleural gland grooming, fungus-growing ants utilize their infrabuccal pocket (located in the oral cavity) as a further filtering and sterilising device. After grooming, the ants collect *Escovopsis* spores in this pocket, where they are sterilised by an as-of-yet unknown mechanism (potentially metapleural gland compounds), after which the infrabuccal pellet is expelled on the colony refuse pile [47, 49, 70].

The cuticle of major garden workers is often covered with a thick white growth of Actinobacteria [50, 51], which produce antimicrobial compounds that aid in the protection of the fungal cultivar from *Escovopsis* [49–51, 64, 71] and possibly other parasites [65]. These beneficial Actinobacteria are reared by the ants and housed in cuticular crypts, tubercles, or other modifications associated with subcuticular exocrine glands [52]. Most work on the Actinobacteria has focused on specifically associated lineages of *Pseudonocardia* [51, 55]. *Pseudonocardia* appears to be vertically transmitted by default [50], but phylogenetic evidence indicates that events of horizontal transfer and incorporation of free-living *Pseudonocardia* to the symbiosis have occurred [55–57]. Recent studies have further shown that other Actinobacteria

genera (mainly *Streptomyces*) are often also present [57–61], but their degree of specificity with the symbiosis is less clear. There is little doubt that cuticular Actinobacteria cultures serve active defence functions in the symbiosis, but clarifying the relative importance of predominantly vertically transmitted *Pseudonocardia* and horizontally transmitted other defensive microbes will need much further work.

Ant cultivars, the hosts of *Escovopsis* parasitism, are able to launch defences themselves by secreting chemical compounds that suppress *Escovopsis* growth. This has been tested in the *Apterostigma* and *Cyphomyrmex* [41, 43], where antifungal compounds secreted by the cultivar appeared to be more effective in suppressing the growth of *Escovopsis* strains that are unknown to infect them in nature, but less effective against their native *Escovopsis* strains [41, 43]. Such cultivar responses towards novel *Escovopsis* strains might result in limitations for *Escovopsis* host switching outside the agricultural system that they are adapted to. Overall, therefore, the defences of the ants, the Actinobacteria, and the cultivar appear to reinforce each other in suppressing *Escovopsis* infection and proliferation within attine ant fungus gardens (see e.g., Figure 10.1 in [22]).

**1.4. Trade-Offs between Alternative Defence Functions.** Over the course of millions of years of selection on the interaction between fungus-growing ants and *Escovopsis*, different ant genera have diversified in their specific utilization and combination of alternative defence mechanism to reduce the impact of *Escovopsis*. This has been best studied in species of the leaf-cutting ant genera *Atta* and *Acromyrmex*. *Escovopsis* infections appear to be more prevalent in *Acromyrmex* than *Atta* colonies [35], possibly due to differences in the efficiency of alternative defensive strategies. First, the chemical compounds in the metapleural glands differ between *Acromyrmex* than *Atta*, reviewed in [53], making it inevitable that compounds with different antimicrobial properties are produced (cf. [48]). Second, Actinobacteria are abundant in *Acromyrmex* and essentially absent in *Atta* [54]. Third, the rate of metapleural gland grooming differs in a contrasting manner, with *Atta* increasing grooming rates after *Escovopsis* infection and *Acromyrmex* maintaining a constantly low rate of metapleural gland grooming [54].

Differences in metapleural gland chemistry, grooming rate, and Actinobacteria coverage indicate that trade-offs between these alternative defensive strategies are likely, conceivably because these defences are known to be costly [72, 73]. Different defences may target the same parasite, but with different modes of action. For example, in *Acromyrmex*, metapleural gland secretions kill *Escovopsis* spores but show limited effect on hyphae [48], while Actinobacteria secretions suppress hyphal growth but do not kill spores [64]. A similar scenario has been proposed for two other genera of higher attine ants, *Trachymyrmex* and *Sericomyrmex*, as certain species from the former genus have abundant Actinobacteria cover and low frequencies of metapleural gland grooming, while *Sericomyrmex* has very few Actinobacteria and a higher frequency of metapleural gland grooming [72].

## 2. Using Tinbergen's Four Quadrats to Structure Attine-*Escovopsis* Research

Nikolaas Tinbergen was a Dutch ethologist and ornithologist who received a Nobel Prize in Physiology or Medicine in 1973 together with Karl von Frisch and Konrad Lorenz for their joint work on the organization and elicitation of individual and social behaviour in animals [74]. Tinbergen's four categories of evolutionary questions were originally developed to obtain an integrated explanation for animal behaviour, based on complementary understanding of proximate mechanisms (1) and ontogenetic developments (2), as well as ultimate selection forces resulting in adaptive evolution of individuals (3) and long-term evolutionary change of populations or higher-level clades (phylogenetic history) (4) [75]. Tinbergen's framework has since been used in many research programs throughout the life sciences [76–78] but has, to our knowledge, not been applied to host-parasite interactions. For the purpose of the present paper, we modify Tinbergen's framework to encompass a classification of questions that have been (Table 1), or could be (Figure 1), addressed to better understand the evolutionary ecology of attine ant-*Escovopsis* interactions.

Table 1 summarizes how studies available so far can be grouped into Tinbergen four quadrats framework. This was relatively straightforward for the ultimate questions of adaptive evolution and phylogenetic history, but not always for the proximate ontogeny and mechanism categories, because available research tools have so far not allowed much understanding of the (epi)genetics behind developmental pathways and phenotypic plasticity. It is, therefore, also arguable that the questions addressed in our ontogeny and mechanism categories are rather ambiguous, in being both technologically challenging and relatively imprecise in their fit to a single Tinbergen quadrat. We nonetheless felt that making a first attempt to structure a research agenda was worthwhile and have chosen to group questions of *Escovopsis* specialization in the ontogeny quadrat and questions of cultivar utilization and defences by the ants and fungal symbionts in the mechanism quadrat. In the sections below, we utilise these groupings to formulate how new experimental work, combined with the increasing availability of genome sequences, may allow novel insights in *Escovopsis* parasitism.

## 3. Tinbergen's Ontogeny Quadrat

**3.1. *Escovopsis* Recognition of Cultivars.** *In vitro* assays have shown that *Escovopsis* can recognize native cultivar hosts through chemotaxis, followed by directed growth of the parasite towards the cultivar, the secretion of parasite enzymes breaking down cultivar cells, and absorption of cultivar cell contents [36]. In contrast, *Escovopsis* is not able to utilize nonnative cultivar strains and can even be inhibited by them [41, 43]. The mechanisms and genes underlying parasite differentiation between native and nonnative host cultivars remain unknown, that is, we neither know the identity or the evolution of the chemicals (what does *Escovopsis* recognize?) nor the genes coding for the chemicals produced and their

TABLE 1: Overview of available studies on *Escovopsis* virulence in gardens of fungus-growing ants, and our assortment of these studies into the four Tinbergen quadrats.

Quadrat	Study focus	References
Ontogeny	Pathology, impact, and prevalence	[32, 34, 35]
	Genetic and chemical basis of <i>Escovopsis</i> recognition of cultivars	[36, 38–41, 43]
Mechanism	Ant behavioural defences	[44–47]
	Chemical defences	[47, 48]
	Actinobacteria defences	[49–52]
	Cultivar defences	[40, 41, 43]
Phylogeny	Population-level specificity	[38–41, 43]
	Cross-phylogeny specificity	[38, 51]
Adaptation	Susceptibility/resistance to metapleural gland compounds	([48], reviewed in [53, 54])
	Degree of Actinobacteria specificity	[55–63]
	Susceptibility/resistance to Actinobacteria secretions	[50, 58, 60, 61, 64, 65]
	Host cultivar use	[38–41, 43]

		Objects of explanation	
		Development/Historical <i>Progression in current form</i>	Single form <i>What organisms need to function and why those functions arose</i>
Questions	Proximate <i>How organisms work by describing their developmental and functional traits</i>	Ontogeny (i) <i>Escovopsis</i> recognition of cultivars (ii) Genetic basis for <i>Escovopsis</i> recognition by the ants (iii) Trade-offs between alternative defences	Mechanism  (i) <i>Escovopsis</i> transmission between colonies (ii) Colony-level virulence
	Evolutionary <i>How evolution has shaped organisms to acquire their extant forms</i>	Phylogeny (i) Origin and diversification of the association (ii) Phylo-geographic patterns, coevolutionary interactions, and dispersal	Adaptation  (i) Evolutionary potential of <i>Escovopsis</i> as a parasite (ii) Evolutionary consequences of host-parasite interactions

FIGURE 1: Tinbergen’s four quadrat framework applied to evolutionary questions about *Escovopsis* parasitism of fungus-farming ant crops. Ontogeny refers to the description of development, from DNA to progressive phenotype, mechanism refers to the physiological and cellular processes that organisms have available, phylogeny refers to the idiosyncratic evolutionary history of a lineage, and adaptation refers to traits that acquired their extant function because of specific selective advantages, modified from [66–68].

evolutionary history. Ongoing genome sequencing of cultivars and *Escovopsis*, as well as efforts to isolate the chemicals involved, will thus allow considerable progress to be made.

Two evolutionary explanations for the maintenance of *Escovopsis*-cultivar utilization patterns seem possible. The nonadaptive explanation would hold that *Escovopsis* strains (or species) would be subject to consistent genetic drift in isolated populations, so that they would lose adaptations to allopatric hosts by chance. The alternative adaptive explanation would hold that populations are mostly panmictic, so that genes coding for innovative pathogen traits and defensive recognition and resistance traits of cultivars would tend to coevolve. If so, *Escovopsis* would track cultivar evolution in continuous, but variable, arms races reminiscent of a geographic mosaic of coevolution [79]. If the latter is the case, expectations are that positive selection on specific gene complexes (e.g., recognition or resistance genes) will likely

have left signatures of enhanced dN/dS ratios compared to housekeeping and neutral genes, while nonsignificant dN/dS ratios would make the nonadaptive null hypothesis more likely. In general, it seems unlikely that *Escovopsis* populations are highly structured (see also below), but solid empirical evidence on this is lacking.

**3.2. Genetic Basis for *Escovopsis* Recognition by the Ants.** Ants are able to discriminate between *Escovopsis* and other fungi and behave accordingly [44, 45]. Natural selection in the ant host is expected to select for genes involved in the recognition and removal of *Escovopsis* from the fungus garden, as this is predicted to provide a selective advantage. Further, *Escovopsis* has the potential to be much more virulent than any general fungal weeds of attine ant colonies, at least in the higher attine system where virulence has been studied, implying stronger selection on *Escovopsis* recognition pathways in the

ants compared to pathways mediating the recognition of weed fungi. The genetic basis of *Escovopsis* recognition has not been explored, but genomic tools will make this possible in the years to come [80, 81]. For example, two leaf-cutting ant genomes are now published [82, 83] and a sequenced *Escovopsis* genome will soon follow (anonymous reviewer, personal communication), providing the tools necessary for such new approaches to studying behavioural recognition mechanisms. Recognition of, and concomitant behavioural responses to, *Escovopsis* infection are faster and last longer than the response to general fungal pathogens [44, 47], leading to the prediction of higher levels of recognition gene expression in the presence of *Escovopsis*. However, it is conceivable that the mechanism of recognition of *Escovopsis* and other fungi by the ants does not differ but that responses do, so that it is rather genes underlying behavioural removal responses that are differentially expressed.

**3.3. Trade-Offs between Alternative Defences.** Defences against *Escovopsis* include behavioural removal (including self- and allo-grooming), glandular secretions, cultivar defensive compounds against nonnative *Escovopsis*, and compounds with antibiotic properties derived from Actinobacteria. These defences all involve interactions on the ant cuticle and are expected to require coordinated interactions to avoid negative interference. In *Acromyrmex octospinosus*, the metapleural gland secretions do not appear to harm the Actinobacteria, so that both defences can be freely expressed [54]. Further, complementarity is expected to maximize cost-benefit ratios of defences as well as to avoid redundancies. It is conceivable that differences in Actinobacteria cover between closely related ant species, such as *A. octospinosus* and *A. echinator* [72], reflect more recent adjustments (trade-offs) in the relative importance of defences between the species.

Explorations of defence trade-offs have only been done in some higher attines, leaving questions of this kind unexplored in most of the fourteen extant fungus-growing ant genera. We propose that utilizing the phylogenetic framework of structural modifications over the course of the association between fungus-growing ants and Actinobacteria [52] would offer a good basis for future work to understand the dynamics of defence components across the attine tribe. The relative usage of metapleural gland grooming and the chemistry of glandular and bacterial secretions in *Acromyrmex/Trachymyrmex* versus *Atta/Sericomyrmex* exemplify how such comparative approaches can be insightful [54]. However, considering the vast diversity of cultivar usage, Actinobacteria communities, substrate choice, and ant life-history traits, it is conceivable that defence strategies and trade-offs in unstudied attine ants might be different from those found in the higher attines.

## 4. Tinbergen's Mechanism Quadrat

**4.1. Escovopsis Transmission between Colonies.** The success of parasitism is tightly linked to the transmission frequency between host colonies [84]. The most common transmission for fungal spores is passive dispersal through the air, but this

is unlikely to be the case for *Escovopsis* because it sporulates inside colonies and has wet spores [35]. The mechanism of *Escovopsis* transmission, therefore, continues to be enigmatic, with untested hypotheses of commensal garden arthropods vectoring spores between colonies, or foraging ants picking up spores via encounters outside the nest as reasonable leads [41, 84]. Both mechanisms could be further facilitated by attine colonies nesting in each others close proximity. Culture-based attempts to isolate *Escovopsis* from potential vectors are, therefore, needed for a better understanding of transmission modes. Expectations are that *Escovopsis* is more likely to be transmitted between colonies by commensal arthropods. This is so, because foragers presumably rarely, if ever, enter other colonies, and are therefore unlikely to pick up *Escovopsis* spores from nonnative infected colonies, and because workers are efficient at recognizing and removing *Escovopsis* spores from their cuticle (e.g., [85, 86]). In contrast, commensal arthropods moving between colonies are not expected to have evolved such avoidance behaviours towards *Escovopsis*.

**4.2. Colony-Level Virulence.** The within-nest dynamics of *Escovopsis* infections remain a frontier awaiting exploration. *Escovopsis* can coexist with other nonmutualistic filamentous fungi within colonies without colonies displaying signs of infection [62, 87–89]. However, it is not known if infection sets out shortly after *Escovopsis* introduction, or if *Escovopsis* spores remain dormant in the colonies until an outbreak of mycelial growth is triggered by external factors. To begin to understand these dynamics, two essential questions need to be addressed. Firstly, we need a better understanding of the level of metabolically active spores and hyphae of *Escovopsis* in normally functioning and apparently healthy colonies. This could be obtained through quantitative PCR approaches, so that amounts of *Escovopsis* biomass and levels of metabolic activity, measured as gene expression, can be estimated. Ideally, this should be explored over time to also determine temporal variation. Only when we have a better idea of such dynamics, we can begin to explore the role of the ants in mediating these threats. Secondly, if spore-dormancy is the rule, work should address what factors trigger within-colony outbreaks. One approach that could potentially address this is long-term field surveys of natural colonies to better understand the interplay between ecological fluctuations, (e.g., temperature, rainfall, and food availability), intrinsic factors (e.g., loss of queen, imbalance of worker to garden ratio, and emergence of reproductives), and infection dynamics.

## 5. Tinbergen's Phylogeny Quadrat

**5.1. Origin and Diversification of the Association.** The apparent presence of *Escovopsis* throughout the fungus-growing ants suggests that an ancestral *Escovopsis* was present as a parasite in the first ant cultivars that were domesticated ca. 50 million years ago (cf. [37, 90]). However, an alternative scenario is that *Escovopsis* parasitism originated shortly after the early attine ants had become irreversibly committed to farming. The latter would indicate that *Escovopsis* parasitism

was not merely a passive carry-over process, but that the highly peculiar garden phenotype of domesticated fungi created a novel niche to parasites like *Escovopsis*. Finding that *Escovopsis* parasitism would also occur in free-living relatives of lower attine garden symbionts would make an origin predating ant fungiculture more likely, but several lines of indirect evidence suggest that the “new garden niche” model is more likely to apply. First, Actinobacteria cultures on the cuticle of attine ants arose also shortly after these ants became farmers [52], and it would be hard to imagine that the origin of this costly biocontrol habit was not somehow related to *Escovopsis* infections. Second, the impact of *Escovopsis* on fungus-growing ant cultivars is likely to be particularly high because colonies keep a high density of cultivar mycelium without sufficient own defences. Third, it is striking that the only clade of attine ants that secondary developed a radically different and much less conspicuous garden phenotype, the yeast-rearing *Cyphomyrmex*, have secondary lost *Escovopsis* as a parasite [4].

To date, there are two described species of *Escovopsis*, with *E. weberi* from a Brazilian *Atta* species thought to be the monotypic species of the genus [91]. Later, a morphologically distinct *E. aspergilloides* was isolated from *Trachymyrmex ruthae* in Trinidad [92]. Both large scale (cf. [37, 38]) and lower-level lineage diversity [39–41] are considerable, suggesting that there are more *Escovopsis* species associated with fungus-growing ants. Molecular species delineation based on conserved genes such as EF-1 $\alpha$  and 18S rRNA is unlikely to distinguish lineages that diverged recently, so that more sensitive marker studies are needed. Recent multilocus sequence analyses (MLSAs) have provided the opportunity to estimate divergence dates for crucial nodes in phylogenetic trees [4, 19] and would thus also offer novel insights when applied to an *Escovopsis* phylogeny [37]. Approaches of this kind will ultimately allow conclusions about the origin of *Escovopsis* parasitism (before or after attine ants became farmers) and the rates of *Escovopsis* evolution in different host clades.

**5.2. Phylo-Geographic Patterns, Coevolutionary Interactions, and Dispersal.** Coevolutionary theory predicts that genotypic and phenotypic variation across the geographic range of a host-parasite association can lead to parasite adaptations to locally available host genotypes, while becoming maladapted to nonnative genotypes [93]. A prerequisite for such coevolutionary interactions is that host populations are genetically structured, so that gene flow between populations remains limited [93]. In fungus-growing ants, only a single study has attempted to explore such coevolutionary dynamics (in the ant species *Apterostigma dentigerum* [43]). This showed the presence of six distinct host genotype clusters across Central America, while structuring was essentially absent in the parasite, indicating that *Escovopsis* genotypes are not tightly tracking those of the host [43].

We would expect that other fungus-growing ant-cultivar-*Escovopsis* interactions will mirror the findings in *Apterostigma*, since cultivars are vertically transmitted by default while *Escovopsis* is horizontally transmitted. Therefore, the population structure in *Escovopsis* could be

explainable if their sticky spores would use vectors for long distance dispersal that are not available to dispersing ants. It would be tempting to speculate that other arthropods living in attine nests might have this vector function, but examples of such long distance flyers vectoring spores are presently lacking. Alternatively, wind dispersal of small leaf fragments with *Escovopsis* spores would also seem a realistic mechanism for parasite populations to become less viscous than host populations. Future studies addressing relative dispersal efficiencies of partners in the attine ant symbiosis would seem most informative if they could span geographic areas that would be large enough to include natural barriers that would differentially affect *Escovopsis* spores and dispersing ant queens transmitting fungus-garden symbionts.

## 6. Tinbergen’s Adaptation Quadrat

**6.1. Evolutionary Potential of *Escovopsis* as a Parasite.** As already mentioned, *Escovopsis* has probably persisted as a parasite of fungus-growing ant gardens since the origin of ant fungiculture 50 million years ago [4, 37]. If that is so, “Red Queen” like arms races with the ant and fungal hosts may have at least periodically occurred, so that genetic diversity of the parasite is likely to be substantial [26–29]. However, the sexual “teleomorph” of *Escovopsis* has never been observed so that *Escovopsis* may not have sexual reproduction, similar to many other Ascomycetes [94]. Lack of sex would not necessarily preclude the integration and exchange of genetic material between different anamorphous mycelia within nests, provided that coinfections occur with some frequency. This is because asexual Ascomycetes can undergo genetic exchange between strains after hyphal merging (anastomosis) and parasexual heterokaryosis (the exchange of cell nuclei) [95]. If such exchanges lead to mitotic crossovers, then there is potential for recombination between genetically different strains [95]. It will be very interesting to investigate whether the *Escovopsis* genome still shows signs of such genetic recombination.

The presence of coinfections within individual nests is a prerequisite for such genetic exchanges. Both *Atta* and *Acromyrmex* leaf-cutting ants appear to frequently harbour genetically distinct *Escovopsis* strains, including ones appearing in two separate phylogenetic clades [63]. Similarly, in the paleoattine genus *Apterostigma*, fungus gardens are infected by four distinct *Escovopsis* morphotypes “brown,” “yellow,” “pink,” and “white” [39]. This implies the potential for exchange of genetic material between coinfecting strains within colonies. By explicitly addressing this question, we could gain insight both in the dynamics of coinfections (e.g., facilitation, inhibition, the role of the order of infection precedence) within colonies and in the putative species status of different *Escovopsis* morphotypes.

**6.2. Evolutionary Consequences of Host-Parasite Interactions.** A common question in the evolutionary study of host-pathogen interactions is whether coevolutionary arms races are almost continuous or relatively rare. This is partly because of the difficulty of testing such dynamics when exploring

biological systems in real time. Fungus-growing ants have evolved extensive complementary defences to deal with *Escovopsis*, but the parasite nevertheless prevails at relatively high population-level frequencies, ranging from 27–75%, depending on the ant genus and geographic location (e.g., [32, 88]). This finding suggests that *Escovopsis* continues to exert selection pressure on the ant hosts, potentially leading to concomitant changes in ant defences. All this is suggestive of, but not decisive evidence for, antagonistic coevolution (cf. [96]).

The efficiency of behavioural defences (grooming/weeding) in attine ants is known to have an impact on the virulence of *Escovopsis* [44, 45]. Under a coevolutionary scenario, expectations are that *Escovopsis* has exerted selection pressures on the ants to optimize their behavioural response towards native parasite strains. Such a scenario would predict that infections with (avirulent) nonnative strains of the parasite would not elicit the same efficient response from the ants. Similarly, if metapleural gland grooming behaviour and chemistry have been shaped by coevolutionary interactions with *Escovopsis*, then we would expect that the grooming rate and the chemical secretion cocktail would be adapted to inhibit native parasite strains more than nonnative strains.

The coevolutionary patterns arising from interactions between *Escovopsis* and the Actinobacteria are inevitably different from those between *Escovopsis* and direct defences by the ants. Two, perhaps nonmutually exclusive, scenarios derived from Red-Queen dynamics in relation to Actinobacteria defence have been proposed. The first scenario suggests that Actinobacteria in the genus *Pseudonocardia* evolve in response to antibiotic resistance evolving in *Escovopsis*. Evidence supporting the potential for this to be the case comes mainly from observations of variation in the propensities of different *Pseudonocardia*-derived antibiotics, including the presence of *Escovopsis* strains that are resistant [55, 64]. Phenotypic variation is a prerequisite for such dynamics to be maintained, as this is what natural selection can act on. However, no studies have as yet shown that changes in *Pseudonocardia* genes for antibiotic production do indeed change in response to *Escovopsis* susceptibility. A second possible scenario is that attine ants frequently acquire strains of bacteria from the environment that have novel antibiotic properties against *Escovopsis*, be it either *Pseudonocardia* [55] or other Actinobacteria [57, 61, 65]. Evidence for such acquisitions comes from survey data showing that free-living *Pseudonocardia* are phylogenetically interspersed with ant-associated clades [55], and that additional Actinobacteria with antibiotic properties (mainly *Streptomyces*) can be obtained from the ant cuticles or gardens of colonies. We expect that characterizations of the antibiotic profiles produced by the major clades of *Pseudonocardia* that associate with fungus-growing ants will clarify the role that these alternative acquisition mechanisms have played in maintaining a successful *Pseudonocardia*-defence against *Escovopsis*. Further studies will also benefit from a more explicit emphasis on exploring how and to what extent such horizontal acquisitions of novel Actinobacteria occur, and whether they have a selective advantage for ant colony fitness.

## 7. Conclusions

Since the discovery of *Escovopsis* parasitism of fungus-growing ants less than 15 years ago, we have obtained a broad understanding of prevalence, impact, role, and coevolution of the parasite with the attine ant-fungus symbiosis. Nevertheless, many fundamental questions remain unanswered, including the origin of the host-parasite association, its presence and potential role outside attine ant nests, parasite transmission between colonies, and within-colony disease dynamics. We know that *Escovopsis* is attracted to specific ant cultivars in some cases, but the generality of this phenomenon and the underlying recognition mechanisms are unknown. Several defences against *Escovopsis* are known, including prophylactic behaviours, metapleural gland grooming and compounds, and Actinobacteria symbionts, which all contribute to reducing the impact of *Escovopsis*. However, we know little about the context-specific efficiency of these alternative and complementary defences, and only in some cases do we have a crude understanding of the potential trade-offs involved. More detailed phylogenetic studies of the association specificity of ants, fungal cultivars, *Escovopsis*, and Actinobacteria are needed to improve our interpretations of reciprocal interactions observed. Although the Tinbergen framework did not allow us to do full justice to the complexity of this host-parasite interaction, we feel that it does provide a useful structuring device for the research agenda that will be required to make further progress in understanding this unique genus of crop-pests of fungus-growing ants.

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## Research Article

# Sporadic Infection of *Wolbachia* in a Recently Established Population of *Formica fusca*

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This study examines the distribution and invasion dynamics of *Wolbachia* in a recently established *Formica fusca* population. Preliminary data revealed the intermittent infection of *Wolbachia* across colonies, providing the opportunity to test for ecological factors affecting the acquisition and spread of the parasite. Only 35% of colonies are infected in this population. Both infected and noninfected nests have similar dispersion patterns that approximate a random distribution, suggesting that transmission of *Wolbachia* between adjacent colonies is not common. There is no difference in the infection rate between workers and brood, indicating that workers are not actively eliminating the infection. Our results show no significant association between *Wolbachia* infection and nest size; however, infected colonies tend to be larger than noninfected colonies. Finally, *Wolbachia* infection was not associated with queen number. Overall, our results suggest no large fitness differences between infected and noninfected colonies, although small fitness effects cannot be ruled out for this population.

## 1. Introduction

*Wolbachia* is common endosymbiotic bacteria of arthropods, crustaceans, mites, and nematodes that induces a variety of effects on their hosts to promote their own spread within the host population [1–6]. It is estimated that *Wolbachia* is present in 20 to 75% of all arthropods [2, 7] including more than 90 species of ants [2, 8–14]. Within ant species, high levels of multiple *Wolbachia* infection are documented, including up to 4 strains of *Wolbachia* in single individuals [10, 13].

*Wolbachia* transmission normally occurs through vertical maternal transmission [5, 14, 15]. The parasite has been shown to increase transmission via manipulation of reproduction and the sex ratio of the host using a number of different mechanisms (reviewed in [16–19]). *Wolbachia* infection can benefit host females through positive fitness effects or via cytoplasmic incompatibility (CI) [4]. CI prevents infected males from successfully mating with a noninfected female or with a female infected with a different strain of *Wolbachia* [19]. Other mechanisms by which *Wolbachia* can bias host sex ratio in favor of infected females include male killing,

parthenogenesis, and feminization [19]. In social insects, worker control of sex allocation requires *Wolbachia*-mediated manipulation of worker's behavior to result in a favorable sex ratio [20]. To date, studies of sex ratio in ants have provided little evidence for *Wolbachia*-induced manipulations of sex ratio in ants [14, 15, 20].

*Wolbachia* can also spread via horizontal transmission of the parasite between species [13, 14, 21–23]. Occasional horizontal transmission has been documented and occurs most frequently between related species [5, 24]. In addition, the presence of multiple *Wolbachia* strains within a species shows evidence for horizontal gene transfer between host species or recombination events among *Wolbachia* strains [3, 10]. Less is known about the infection dynamics of *Wolbachia* within a single host species. Across several ant species, *Wolbachia* infection prevalence appears near fixation within some populations [5, 10, 14]. However, other populations vary in the prevalence of infection across colonies. Even within infected colonies, not all workers harbor the infection [14, 22], suggesting that *Wolbachia* is not transferred readily between workers. There is also evidence that infection rates of workers are

lower than infection rates of worker brood [14] suggesting a loss of infection with age.

We studied the distribution and infection dynamics of the *Wolbachia* parasite in a recently established *Formica fusca* population. *F. fusca* is a pioneering species and rapidly colonizes open environments prior to competition from other species [25, 26]. Within a single population, this species can establish both monogynous and polygynous colonies [25, 27]. A preliminary study suggested that *Wolbachia* is present in this population, but that only a subset of colonies is infected, contrasting previous findings of near fixation prevalence rates in related ants species [5, 14, 15, 20]. We surveyed the population to determine the prevalence of *Wolbachia* infection across colonies and to test whether infection is associated with nest size, a proxy for colony size [28], nest location, production of sexuals, and queen number.

## 2. Methods

**2.1. Study Population.** The isolated study population of *Formica fusca* inhabits a disturbed meadow of grasses and goldenrod that borders a temperate conifer forest in Hamilton, New York (N 42° 48.134 W 075° 30.343). While the exact colonization date is not known, estimates of the appearance of nest mounds at the site range from 10 to 15 years ago. Other ant species present at or near the site include *Formica* species, *Leptothorax longispinosus*, *Tapinoma sessile*, *Camponotus americanus*, *Lasius* species, *Myrmica punctiventris*, and *Monomorium minimum*. *Formica fusca* ants were the most common ants found at the site. We did not check for the presence of *Wolbachia* in other ant species.

**2.2. Nest Characteristics.** All nests within the study site were mapped with GPS coordinates using Google Earth and ArcView (Figure 1). The Clark-Evans nearest neighbor method was used to infer dispersion of *Wolbachia* across colonies [29], with  $R = 1$  indicating random dispersion and  $R = 0$  indicating clumped dispersion. In order to test whether  $R$  was significantly different from 1, a critical value,  $c$ , was calculated according to Clark and Evans [29] using a  $t$ -distribution. Significant differences between infected and non-infected colonies were tested by comparing  $R$ -values using the  $F$  distribution.

The size of the nest mound was measured in two directions across the nest entrance; the longest diameter and the one perpendicular to the longest. Measurement extended to the edge of the raised mound. The area of the nest mound was calculated as the area of an ellipse with the two perpendicular measures halved as radii. The average nest mound area was calculated, and mean nest mound area of infected and noninfected nests was compared with a two-tailed  $t$ -test. Nests were designated as either “small,” nests smaller than the mean nest area, or “large,” nests larger than the mean. A Fisher’s Exact Test was used to determine association between infection and nest size. Across infected colonies, the proportion of infected individuals was compared to nest area using Kendall’s coefficient of rank correlation. Nest size was used as a

proxy for colony size following the association described in Tuzzolino [28].

**2.3. Sample Collection.** Workers, worker brood, and reproductive brood were collected from all 35 colonies within the boundaries of the sampling site from late June to early August, 2011, during the period when reproductives are most abundant (unpublished data, [28]). Samples were collected from nests in both shady and sunny locations during late morning hours. Temperature during collection averaged between 24 and 29°C. Nests were watered with approximately 10 liters of water in the afternoon preceding collection to facilitate the sampling of reproductives [28]. During collection, small areas were probed with trowels to determine location of brood chambers and workers and brood were aspirated into vials with minimum disturbance to the nest. When no brood chambers were found, shovels were used to extract more dirt from the surface to collect workers. The duration of collection was limited to 20 minutes. A Fisher’s Exact Test was used to compare the number of reproductives obtained during this sampling period in infected and noninfected colonies.

Worker and brood samples were immediately frozen at  $-20^{\circ}\text{C}$ . DNA was extracted from all samples using 100  $\mu\text{L}$  of a 10% Chelex solution (Bio-Rad), and samples were boiled for 15 min and spun for 1 min at 13,000 rpm. The supernatant from worker samples was placed directly into a PCR reaction; the supernatant from brood samples was diluted 1 : 10 with water.

We sampled 20 colonies for presence of *Wolbachia*; for one of these colonies, microsatellite data was not available, resulting in a sample size of 19 colonies for comparisons of infection with queen number.

**2.4. Population Survey of *Wolbachia*.** All samples were amplified with 18S primers (18SF1 and 18SR1; [30]) to confirm that the DNA extractions were successful. Each sample was then amplified twice with *Wolbachia* specific primers (wsp 81F and wsp 691R; [31]) to confirm presence or absence of *Wolbachia* infection. Detection rate of *Wolbachia* infection was estimated at greater than 99%. For all reactions, samples were run in 25  $\mu\text{L}$  of the following reaction mixture: 1X of 10X PCR buffer, 0.2 mM each of dNTPs, 1.5 mM  $\text{MgCl}_2$ , 0.5  $\mu\text{M}$  each of primer, 1 unit of Taq polymerase (5 U/ $\mu\text{L}$ ), and 1  $\mu\text{L}$  DNA. Samples were run on a Bio-Rad DNAengine PTC thermocycler with the following protocol: 94°C for 5 min, 47 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 1:30 min, hold at 72°C for 7 min. Amplified products were run on 1.5% agarose gels and analyzed as presence/absence of a 610 bp band. Samples with no band after two runs were designated as noninfected samples.

**2.5. Microsatellite Analysis.** We genotyped 20 workers per colony from a total of 34 colonies at 5 microsatellite loci: FE42 [32], FL12, FL29, [33], FY7, FY15 [34]. PCR amplifications were performed in a 25  $\mu\text{L}$  final volume containing 1 X of 10X PCR buffer, 0.2 mM each of dNTPs, 1.5 mM  $\text{MgCl}_2$ , 0.5  $\mu\text{M}$  each of primer, 1 unit of Taq polymerase (5 U/ $\mu\text{L}$ ), and 1  $\mu\text{L}$  DNA. Samples were run on a Bio-Rad DNAengine



FIGURE 1: Map of the study site showing all colonies in the population. Infected colonies: red circles; noninfected colonies: blue circles; untested colonies: yellow circles.

PTC thermocycler with the following protocol: 94°C for 5 min, 27 cycles of 94°C for 30 s, 48/55°C for 30 s, 72°C for 45 s, hold at 72°C for 3 min. Amplified fragments were analyzed on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) and sized using GeneMapper 4.1 and 400ROX size standard from Applied Biosystems. All allele calls were manually verified.

Effective queen number was estimated from pairwise worker-worker relatedness values between colonies using Relatedness 4.2 according to the equation outlined in Krieger and Keller [35].

### 3. Results

In this population, 35% (7/20) of colonies were infected with *Wolbachia*. In infected colonies, the average proportions of individuals that were infected per colony ( $\pm$ SD) were workers,  $0.62 \pm 0.31$ ; worker brood,  $0.83 \pm 0.32$ ; reproductive brood, 1.00. In infected colonies, there was no difference between the proportions of infected workers per colony versus the proportions of infected worker brood per colony ( $t =$

$0.82$ ,  $P = 0.44$ ). Noninfected colonies were no more likely to have reproductives than infected colonies ( $P = 0.53$ ). Only one of the seven infected colonies and three out of 13 non-infected colonies produced sexuals, so it was difficult to test associations between infection and colony sex ratio.

The ratio ( $R$ ) of average distance to nearest neighbor to the expected distance based on density was 1.08 and 0.83, respectively, for noninfected and infected colonies, suggesting that both noninfected and infected nests occur in a random distribution. The  $R$ -value for infected colonies was not significantly different from one ( $R = 0.83$ ,  $t = 0.43$ ,  $P = 0.85$ ). The distribution of infected nests was not significantly different from noninfected nests ( $F = 0.24$ ,  $P = 0.70$ ).

The average nest mound size was 2203 cm<sup>2</sup>. Prevalence of infection is not associated with large nest size when comparing small and large nests ( $P = 0.12$ ). Mean nest size of infected colonies was nearly double the size of noninfected colonies, but this difference was not significant (infected = 3101 cm<sup>2</sup>; noninfected = 1632 cm<sup>2</sup>;  $t = 1.16$ ,  $P = 0.26$ ). Among infected colonies, nest area was not related to the proportion of infected individuals within a colony ( $\tau = 0.41$ ,  $P = 0.249$ ).

The population has a high level of genetic diversity with the number of alleles per locus ranging from 6 to 14 and expected heterozygosities for each locus ranging from  $H_e = 0.44$  to 0.83. There is significant genetic structure between nests in the population ( $F_{ST} = 0.20 \pm 0.13$ ), and there is no evidence for isolation by distance across nests ( $y = 9E^{-06}x + 0.28$ ;  $R^2 = 0.014$ ), suggesting that dispersal occurs primarily via mating flights and not by budding of queens and workers to adjacent nest sites (unpublished data).

Of the 19 colonies for which both *Wolbachia* infection and queen number were tested, 40% of infected colonies were monogynous and only 9% of noninfected colonies were monogynous, but this difference was not significant ( $P = 0.30$ ). The average queen number in infected colonies was  $2.37 \pm 1.06$  and was not significantly different from the average queen number in noninfected colonies,  $2.17 \pm 0.86$  ( $t = 0.46$ ,  $P = 0.65$ ).

#### 4. Discussion

The results from this study reveal a snapshot of early *Wolbachia* infection in a recently established *Formica fusca* population. The recent introduction of this population offers the unique opportunity to test for ecological correlates of *Wolbachia* infection and spread. Infected nests in the population were broadly scattered throughout the study site, and the probability of infection was not predicted by closest neighbors, indicating little or no transmission of *Wolbachia* between colonies. In one area, three infected nests are closely clumped together, but these nests are likely satellite nests of the same colony due to their close proximity. This result contrasts previous studies of other *Formica* species where *Wolbachia* seems to infect a high proportion of colonies within populations [5, 10, 14]. In addition, a survey of 32 species of *Formica* found multiple strains of *Wolbachia* infection in all species and sharing of parasite haplotypes across distant host mtDNA haplotypes, suggesting historical horizontal transmission between species [5].

One possible explanation for these differences is that many *Formica* species tend to form long-term, stable populations and these species may have enhanced opportunities for horizontal transmission. *Formica fusca* is an ephemeral species that invades relatively open spaces and establishes colonies that later may be outcompeted by more aggressive species [25]. These short-lived populations may not persist long enough to permit extensive horizontal transmission. Alternatively, the difference in prevalence rate among populations may be due to the fact that a population that spreads from an initial infected foundress or group of infected queens may also have complete transmission of infection to all nests via vertical transmission. For example, the fact that three closely spaced nests are infected likely resulted from vertical transmission of *Wolbachia* preceding the split into satellite colonies. Our results suggest that this newly established population was founded by multiple introductions of *Formica fusca*, some of which were infected with *Wolbachia* and others that were not. If *Wolbachia* is transmitted between colonies in this host population, it has not had time to spread. Determining

whether the current infections across this population represent the same *Wolbachia* strain or represent introductions of separate strains of the parasite will help disentangle the history of *Wolbachia* infection in this population. An additional possibility is that newly established colonies in this population were infected with *Wolbachia* via horizontal transmission of neighboring ant species. A parallel study of *Wolbachia* infection in other ant species within the population would provide the data necessary to test this hypothesis.

Although no significant association between nest size and infection prevalence was found, 9 out of 12 “small” nests do not harbor infections and mean nest size of infected nests is nearly double that of noninfected nests. The trends suggest that nest size may be positively associated with the prevalence of *Wolbachia* given a larger sample size. Such a finding would contradict the argument, as proposed and rejected by Wenseleers et al. [14], that *Wolbachia* may have deleterious effects on the colony via reducing worker biomass. Alternatively, because nest size is correlated generally with colony age, older colonies may have been established from foundress queens that emigrated from an infected population and the newly established, smaller colonies represent a recent introduction from a noninfected population. Multiple introductions and/or recent acquisition of the *Wolbachia* parasite might also explain the low prevalence rate of *Wolbachia* in this population (34%), which contrasts previous findings of near fixation of the parasite [5, 10, 14]. Interestingly, in other species, *Wolbachia* infections are not common across introduced populations [11, 36]. In Argentine ants and fire ants, selective pressures of colonization may impede the ability of an infected population to successfully colonize [11, 36]. This effect is not seen in this *Formica* population.

Within colonies, the infection rate can vary between workers, brood, and reproductives. In our study population, 100% of reproductives, 87% of worker brood were infected and 63% of workers were infected. This pattern resembles that seen in *Formica truncorum* species (95% in sex M, 94% sex F, 87% worker brood, and 45% workers) [14], but the differences in infection rate in this study were not significant. Thus, our results provide little support to the hypothesis that adult workers may be able to rid themselves of infection. There is no strong selective pressure inhibiting the loss of infection in workers because workers produce only males and are essentially an evolutionary dead-end for this parasite.

Overall, the results of this study show no large deleterious fitness effects of infection on colony size or longevity; infection does not appear to decrease longevity of the colony, at least within the time scale of this population expansion. *Wolbachia* infection could also cause a decrease in the number of reproductives produced, limiting the reproductive success of colonies. In this population, few colonies produce sexuals, but the production of sexuals does not appear to be linked to the presence of infection as both infected and noninfected colonies produce sexuals. It is important to note that the infected colony that produced sexuals produced only 4 males, compared to the greater than 16 reproductives produced in noninfected colonies. This result does support the finding of reduced biomass of sexual brood in infected *Formica truncorum* colonies [14]. Differences in sex ratios of infected

colonies could also result from infections [14, 15, 20, 37], but the small numbers of reproductives produced in this population make it difficult to test sex ratio predictions.

Finally, *Wolbachia* infection is not correlated with queen number in this population of *Formica fusca*. In native populations of fire ants, monogyne colonies harbor a higher frequency of *Wolbachia* infection than polygyne colonies. The difference in prevalence rate may be due to a reproductive advantage to monogyne colonies because these queens are less likely to produce diploid males when founding new colonies [11]. This pattern was not seen in our study or in studies of related species of *Formica* ants [8, 20]. Both studies show no difference in the infection rate of monogyne versus polygyne colonies. Thus, *Wolbachia* infection does not appear to be associated with queen number in *Formica* ants and is not likely to affect the genetic diversity of *Formica fusca* colonies.

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## Review Article

# **Myrmica Ants and Their Butterfly Parasites with Special Focus on the Acoustic Communication**

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About 10,000 arthropod species live as ants' social parasites and have evolved a number of mechanisms allowing them to penetrate and survive inside the ant nests. *Myrmica* colonies, in particular, are exploited by numerous social parasites, and the presence of their overwintering brood, as well as of their polygyny, contributes to make them more vulnerable to infestation. Butterflies of the genus *Maculinea* are among the most investigated *Myrmica*inquilines. These lycaenids are known for their very complex biological cycles. *Maculinea* species are obligated parasites that depend on a particular food plant and on a specific *Myrmica* species for their survival. *Maculinea* larvae are adopted by *Myrmica* ants, which are induced to take them into their nests by chemical mimicry. Then the parasite spends the following 11–23 months inside the ants' nest. Mimicking the acoustic emission of the queen ants, *Maculinea* parasites not only manage to become integrated, but attain highest rank within the colony. Here we review the biology of *Maculinea/Myrmica* system with a special focus on some recent breakthrough concerning their acoustical patterns.

## **1. Butterflies and Ants**

Most myrmecophiles are commensals or mutualists, which live undisturbed or even actively protected within the foraging areas or territories of ants [1–3]. Their functional and evolutionary ecology, as well as their truly amazing diversity, have been reviewed by Wasmann [4], Donisthorpe [5], Hinton [6], Malicky [7], Hölldobler and Wilson [1], DeVries [8, 9], Fiedler [10, 11], Pierce et al. [12], and others.

The interactions that have evolved between insects and ants range from loose facultative associations to obligate dependency (as concerns butterflies, see [3, 11, 13, 14]). The nests of eu-social arthropods, including insects such as ants, bees, wasps, or termites, are aggressively defended from predators and intruders alike. As a consequence, these nests provide very safe havens for any roughly ant-sized organism having evolved the necessary adaptations to penetrate them and to become accepted as “self” by the workers' caste [4, 5, 15]. Around 10,000–15,000 insect morphospecies have evolved as social parasites of ants, thus accounting for a significant proportion of the world's biodiversity. Yet, despite the many species, most ant social parasites are exceedingly rare or localized, in comparison to the abundance and

distribution not only of their ant hosts but also of other symbionts, which loosely interact with ants [1, 16, 17].

Myrmecophily is widespread among Lepidoptera, most particularly as concerns the Riodinidae and Lycaenidae [9, 12], which are often globally referred to as “lycaenoids” [10], and which make up approximately 30% of all known Papilionoidea [18]. Their relationships with ants can be mutualistic or parasitic and vary from facultative to strictly obligate. In the case of facultative myrmecophiles, the survival of butterfly larvae does not depend on the presence of attendant ants, and associations are unspecific. In other words, these lycaenoids can use ants belonging to several different species, or even subfamilies [11, 12]. On the contrary, in obligate ant associations, butterfly immatures are dependent on ants' presence, at least in some part of their life cycle and interactions are much more species specific [11, 12].

Achieving a myrmecophilous life style requires evolving numerous special adaptations, which are necessary for avoiding ant aggression and for communicating with ants. The cuticle of many myrmecophilous butterfly larvae is thicker than in other groups of Papilionoidea and the head can be retracted under a sclerotized plate [7, 19]. Frohawk [20] was the first to observe that most myrmecophilous butterfly

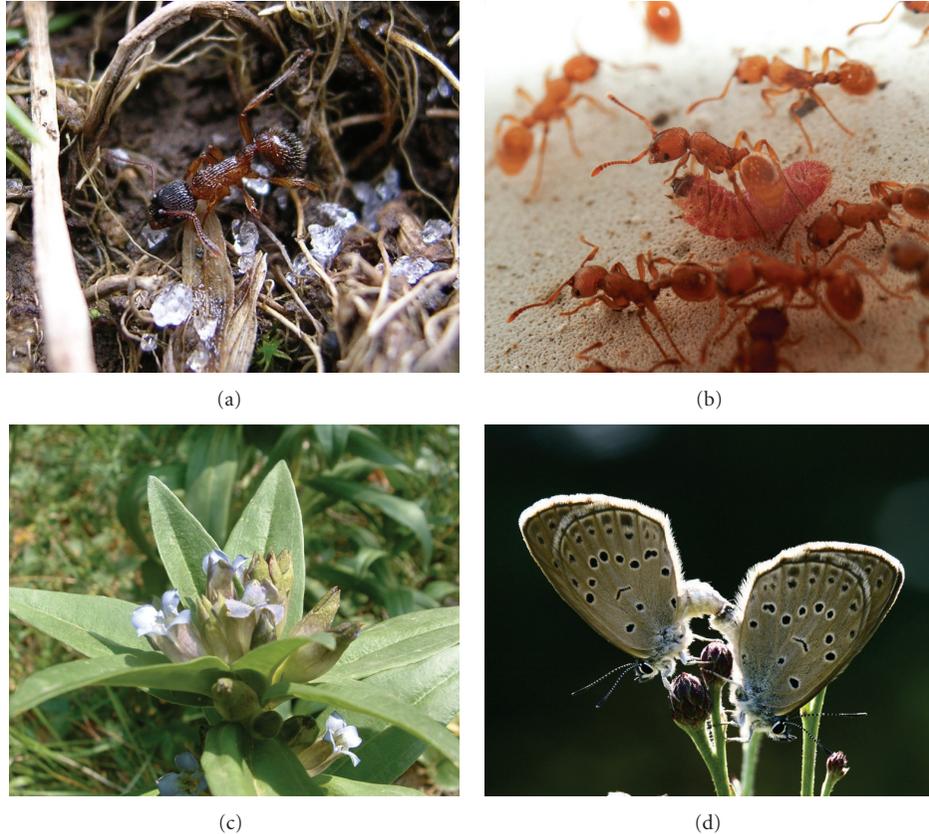


FIGURE 1: (a) Forager worker of *Myrmica* ant. (b) Trophallaxis between attendance worker and *Maculinea* larva. (c) *Maculinea rebeli* foodplant: *Gentiana cruciata*. (d) Mating of *Maculinea* butterflies.

larvae have dorsal nectar organs (DNOs), whose “honeydew” secretion attracts and pacifies ants, and plays an essential role in the maintenance of ant attendance [12]. Additionally, many lycaenoid caterpillars possess specialized epidermal glands, pore-cupola organs and tentacle organs, whose secretions are apparently not directly used by ants, but can somehow manipulate their behaviour [21–23]. Moreover, some butterfly species produce cohorts of other chemical and/or acoustical signals, which are involved in their interactions with ants [12].

## 2. The Parasites: *Maculinea* Butterflies

One of the most intensively studied systems in which both the communication channels are investigated concerns parasitic *Maculinea* butterfly larvae and their *Myrmica* host ants (Figures 1(a) and 1(b)) [24–26]. During the past decades butterflies of genus *Maculinea* (Figure 1(d)) have become “flagships” of European biodiversity conservation [24] and are perceived as umbrella species covering many grassland communities [27–29].

Some recent publications [30–32], based on both molecular and morphological data, have shown that species of *Maculinea* and *Phengaris* form a monophyletic group, where the three Chinese *Phengaris* species are basal. According to

Eric et al. [32] *Maculinea* Van Eecke, 1915 should be considered a junior subjective synonym of *Phengaris* Doherty, 1891. Possible alternatives are that *Maculinea* is, as subjectively, considered subgenus of *Phengaris*, or a distinct genus in its own right.

On the other end the obligate myrmecophilous life style of *Maculinea* has attracted a vast number of studies, many of which appeared in leading scientific journals. *Maculinea* is a model organism for studies on the origin and evolution of parasitic interactions and of host-parasite communication channels [11, 24–26, 30, 33].

*Maculinea* have also attracted a great deal of attention from a conservationist’s point of view [34–37]. For this reason some of the authors have asked the International Commission on Zoological Nomenclature to conserve the name *Maculinea* against *Phengaris* in all cases when the two are considered subjective synonyms. The decision by the ICZN is still pending and we will continue to use *Maculinea* rather than *Phengaris*, at least for the moment.

Another point is that no molecular evidence is available to distinguish *Maculinea rebeli* from *Maculineaalcon* and some authors have argued that the first of them is an ecotype of *M.alcon* [32]. Also in this case we have decided to stick to the traditional interpretation that *M.alcon* and *M.rebeli* represent separate clades (species) and in this paper we

will use the name *Maculinea rebeli* to designate what might represent the xerophilous ecotype of *M. alcon*.

European *Maculinea* species need urgent conservation actions, indeed four are mentioned in the European Red List of Butterflies and three of them are included in the Annex IV of the Habitats Directive [38, 39]. These lycaenids are known for their very complex biological cycles. *Maculinea* species are all obligated parasites that depend on a particular food plant and on a specific *Myrmica* species for their survival. After having spent 10–15 days feeding on a species-specific host plant (Figure 1(c)), the 4th instar larvae of all *Maculinea* species drop to the ground and wait until they are found and carried into an ant nest by a *Myrmica* worker [40–44]. Once in the ant colony, *Maculinea* species differ in their alimentary strategy: (i) *Maculinea alcon* and *Maculinea rebeli* utilize a “cuckoo” strategy, and are mostly fed directly by attending workers (trophallaxis) [42] (Figures 1(a) and 1(b)), they are known for experiencing “contest” competition at high densities [45], (ii) *Maculinea arion* and *Maculinea teleius* are “predatory species” and directly prey on ant brood, experiencing “scramble” competition when overcrowded in the host colony [46], while (iii) the alimentary strategy of *Maculinea nausithous* has not yet been fully clarified, with some authors suggesting the coexistence of both “cuckoo” and “predatory” strategy and others considering it as a “cuckoo” species [24, 47]. *Maculinea* larvae spend 11 or 23 months inside their host colonies. In many populations two separate cohorts of larvae spending either one or two years inside the ants’ nest are known to exist [33, 48–50]. The polymorphic growth pattern found in *Maculinea* populations is likely to have evolved for ergonomic, or perhaps hedge-betting reasons.

Two are the key moments in the life cycle of these butterflies: (i) the choice of an optimal food-plant on which to lay eggs and (ii) the first direct interaction with the host ants. The place where females lay their eggs is crucial for a myrmecophilous butterfly, to ensure its brood the chance to be adopted by a specific host ant. Because the worker ants’ foraging range is limited, selecting an “ideal” oviposition site requires that both the phenological stage of the larval food plant (short-term larval fitness) and the presence of suitable host ants (long-term larval fitness) are taken into account. The female’s selection of a valuable oviposition plant is influenced by a variety of factors. Plants are generally selected by females on the basis of their buds’ phenology, while the presence of the host ants in the near surroundings of the plant may be variously insured depending on local situations and perhaps on the species. In some cases the host-plant and the *Myrmica* ant share a similar ecological niche, so that their overlap ensures population persistence [51–54]. In other cases, however, female butterflies mostly choose those plants which occur in the ants’ foraging range [55–59]. To the best of the authors’ knowledge, nothing is known about the mechanism providing butterfly females with the ability to discriminate among host plants placed inside/outside the foraging range of a *Myrmica* colony.

The other hot point of research on *Maculinea* butterflies is their host specificity with ants, both for its relevance in

coevolutionary dynamics and as a background for conservation strategies. While *Maculinea* caterpillars induce workers of any *Myrmica* species to retrieve them by chemical and acoustical deception [26, 60], their survival till the adult stage will depend largely on which ant-species has found the larva [41–44, 61].

Before the 1970s a nonextensive study of *Maculinea* host specificity led scientists to consider all *Myrmica* species and, in some cases other ant’s genera (e.g., *Lasius*), as potential host of these butterflies. In the following decades Thomas et al. [61] revealed a clear host specificity pattern involving each of the five European *Maculinea* species. In their work authors demonstrated that the survival of every *Maculinea* species was linked to single and different *Myrmica* ant species, while the adoption by a non-host species caused a large decrease in the survival rate of these butterflies. More recently, the large amount of data collected by many researchers all across Europe, confirmed these general guidelines, but demonstrated that host specificity patterns are much more complex and hosts may vary geographically all along the range of each *Maculinea* butterfly. The only species that apparently keeps a single host is *M. nausithous* [34, 62, 63], which shows a clear adaptation to *Myrmica rubra* all over its distribution [47, 61, 62, 64]. The only known exception to this occurs in Transylvania, where it exploits *M. scabrinodis* as alternative host [65]. Data on other *Maculinea* species show a much more complicated pattern, which demonstrates that host specificity occurs at the population or, at least, at the regional scale. Several works have shown that *M. teleius*, *M. arion*, *M. alcon*, and *M. rebeli* may be locally adapted to some *Myrmica* species previously considered as nonhost [29, 50, 64–72] and in the case of the latter two species have developed the ability to successfully exploit more than one host species in the same site creating real multiple host populations [25, 73].

### 3. The Host: *Myrmica* Ants

*Myrmica* ants are hosts of *Maculinea* butterflies, but their colonies are infested by numerous other social parasites such as the larvae of the hoverfly *Microdon myrmicae* (Diptera Syrphidae; see [74, 75]), or by parasitic ant species of the same genus [76]. Reasons for this apparent asymmetry are unclear, but may be related to the biological cycle of these ants. The genus *Myrmica* has a Holarctic distribution. Most of the species, however, are found in Europe and Asia, while a smaller proportion occurs in North America [77]. Colonies are widespread and can be found in various kinds of habitat, such as meadows, forests, steppes, or mountains [76]. Although the biology of many *Myrmica* species has not been studied in detail, it seems that a general life style is common to all ants of this genus [76]. Most colonies contain on average 200–500 workers, as well as from one to many functional queens [78, 79]. New nests can be either funded by a single newly mated queen or, more often, by budding pre-existing colonies [45]. Oviposition starts in early spring and lasts throughout the summer, while it stops in autumn when temperature is decreasing [76]. Part of the larvae develop rapidly but others enter diapause and overwinter. The latter

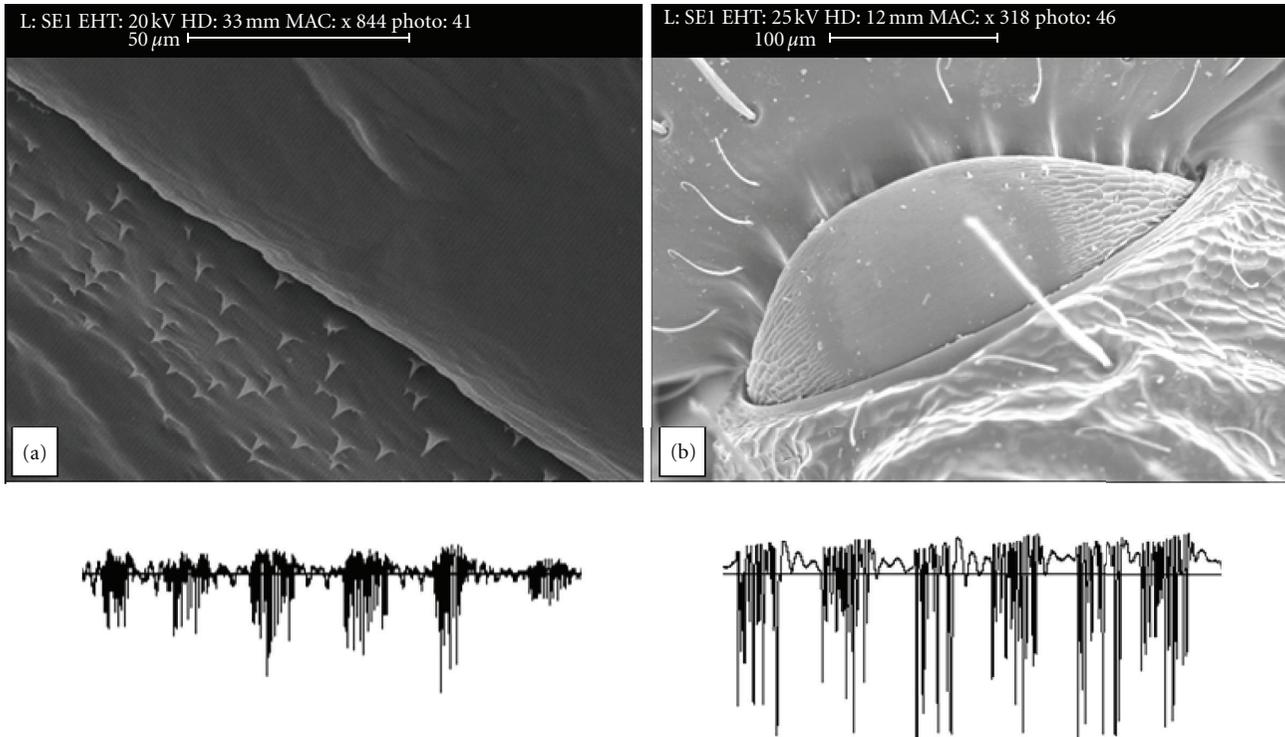


FIGURE 2: Morphology (upper part) and sounds (lower part) of the acoustical organs of (a) *Maculinea rebeli* pupa and (b) *Myrmica schencki* queen.

group includes both workers and all the gyne-potential larvae [80]. Some of these life history traits of *Myrmica* ants make them more vulnerable to infestations by social parasites. One of the most important is presence of overwintered ant larvae particularly essential for survival of the predatory *Maculinea* larvae, which start their intensive growth inside host colony at the beginning of spring and use overwintered ant brood as their food resource [49, 81]. Another significant trait that make *Myrmica* ants a proper host for many social parasites is that many *Myrmica* species live in polygynous colonies and some of them such as *M. rubra*, *M. ruginodis*, or *M. rugulosa* may contain a relatively high number of workers [76, 77]. This results in lower relatedness among worker nest mates [78, 82]. Many studies [83–85] showed that high genetic variance may be beneficial for social insects colonies, but it can also increase the likelihood of being infested by social parasites, because of the greater variance in nest mate recognition cues. It was indicated that *Microdon mutabilis* (Linnaeus, 1758) (Diptera: Syrphidae), a social parasite of *Formica lemmani* ants, more often infests host colonies where genetic relatedness is lower [86]. A similar situation was found for colonies of *M. rubra* infested by *M. alcon* [87]. Therefore, a cost of polygyny existing in most of *Myrmica* species is that their colony communication signals (e.g., chemical or acoustical) tend to be broader and more heterogeneous than in monogynous ant species and their colonies can be more easily invaded by cheats that mimic these signals.

#### 4. Acoustical Pattern in the *Maculinea-Myrmica* System

The more fine-tuned the host-parasite relationship is, the more intriguing studying how the host's deception can be achieved is. The communication of social insects is mainly based on chemical cues [1], but also the acoustic channel is used, thus it is clear that the parasite has to bypass the host's chemical and acoustical system to enter and live in its colonies [88].

Cuticular hydrocarbons have long been assumed to play a fundamental role in the nest mate recognition of social insects. All individuals living in the same society share a bouquet of chemicals, which serves as a “colony odour” and enables them to discriminate between nest mates and strangers. Additional variation in hydrocarbon pattern is associated with differences in sex, caste, and developmental stage [89, 90]. The fact that caterpillars of *Maculinea* butterflies use chemical mimicry to become adopted and to infiltrate colonies of their hosts was first proposed by Elmes et al. [42], while the first experimental evidence was produced by Akino et al. [43], who found that the chemical profile of *Maculinea rebeli* resembles that of its host more than those of other *Myrmica* species.

Even though sound production is not usually the dominant strategy, acoustic communication plays a fundamental role in some groups of insects [91]. Depending on the taxon, sound productions may have a number of functions, ranging

from mate attraction to courtship, aggression, defence, or recruitment of foragers, at least in social insects. Recently, it has been suggested that sounds play a role in the modulation of other signals. This was demonstrated to occur at least in honey bees [92–96].

The role of stridulations in ant communication was underestimated for a long time [8, 26], also because of our scant understanding of the structures involved in the production and the reception of the acoustic signals. Stridulations, however, have long been known to occur in 4 ant subfamilies [97, 98]. In these ants, sounds are produced by a minutely ridged stridulating organ (*pars stridens*) positioned on the middle-dorsal part of the 4th “abdominal” segment and by a spike (*plectrum*) jutting from the postpetiole’s rear margin [26, 99–103] (Figure 2(b)). When an ant moves its abdomen, the two parts rub on each other and emit a series of “chirps” [1, 103, 104]. Stridulations are variously defined depending on the transmitting medium. They are sounds, when transmitted by air, or vibrations, if transmitted by substrate. Myrmecologists have long believed that ants cannot “hear” the aerial component of a stridulation but perceive substrate-transmitted vibrations [105]. This notion was based on experience obtained in the early 20th century [106, 107], and has been indirectly confirmed ninety years later by the discovery of a subgenual organ in *Camponotus* ants [108]. More recently, however, a seminal paper by Hickling and Brown [105] provided fresh impulse to studies on the possible perception of air-transmitted sounds heating the debate on this subject [109, 110]. Hickling and Brown [105] maintain that ants cannot perceive the aerial component of sounds over a long distance (i.e., 1 m), but largely use short range acoustic communication (i.e., 1 cm).

Acoustic communication plays a wide range of roles in the ants’ social behaviour, from reciprocal attraction to intercaste interactions. In most cases, these stimuli are effective only at small range and are mainly used as signals of alarm, for foragers’ recruitment, mating requests, intimidation, and aposematic “threatening”, as well as to modulate other kinds of signals [1, 92, 111–118].

Functions of stridulations have been intensively surveyed in *Atta* ants, where foragers’ calls are most frequent when leaves of the highest quality for fungal cultures are found [119]. *Myrmica* workers frequently stridulate during trophallaxis, particularly the receiving worker, when food decreases [120, 121]. Intercaste acoustical communication has been recorded in only a few instances. Mating queens of *Pogonomyrmex badius* stridulate to signal to males when their spermathecae are full [111] whereas, in *Atta*, leaf-cutting workers stridulate when they are ready to return to the nest. This behaviour induces individuals of the smallest “minim” caste to climb onto the leaf fragment where from there they protect their larger sisters from attack by phorid flies during the journey home [117]. Until recently, there was no direct evidence that different members of an ant society produced distinctive caste-specific sounds to induce appropriate patterns of behaviour either in fellows or in other castes. At least two studies, however, suggested that different castes produce distinctive signals: the major workers of *Atta cephalotes* make sounds that are more intense and

carry further than those of their smaller nest mates [122], while the space between the ridges of the *pars stridens* of queens exceeds that of workers in four *Messor* species [102].

Our own findings demonstrated that *Myrmica schencki* queens generate distinctive sounds that elicit increased benevolent responses from workers, thereby reinforcing their supreme social status [26, 123]. These findings demonstrated that acoustical communication within the vast subfamily Myrmicinae (to which *Messor* spp. and *Myrmica* spp. belong) is more variable and conveys more social information within ant colonies than was previously recognized. In this group, stridulations also fulfil the strict adaptationist definition of biological communication, in which both the signal and the response are adaptive [26, 124, 125].

Since acoustic signals convey quite complex information, not only between worker ants while outside the colony (e.g., during foraging), but also within the nest and between castes, we started research aimed at understanding whether some social parasites, such as butterfly larvae, could interfere with this communication system. Lycaenid larvae, in fact, have long been known to be able to emit stridulations even if their life cycle is not linked at any degree to the ant presence, but sounds produced by myrmecophilous species are more complex and frequent than those emitted by nonmyrmecophilous species [22]. More in general, however, studies aiming at clarifying the function of interspecific acoustic communication in myrmecophilous Lepidoptera are scarce. Most of these studies considered butterfly larva stridulations as a merely defensive signals [6, 126] or, more rarely, as aggregation messages [127]. Sounds produced by lycaenid pupae and caterpillars originate from different organs; the former from tooth-and-comb stridulatory organs between the fifth and sixth segments [12, 126, 128, 129] (Figure 2(a)), whereas caterpillar sounds may emanate from muscular contraction and air compression through the tracheae [130]. The acoustics of mutualistic lycaenid species does not obviously mimic ant stridulations, and ants attraction has been demonstrated only in the pupae of one extreme mutualist species (i.e., *Jalmenus evagoras* see [12, 131]). On the contrary, the larval calls of four *Maculinea* species are similar in pulse rate and band width to those of their hosts, although the level of apparent mimicry is to the genus *Myrmica* rather than to individual host ant species [132]. The same study showed that *Myrmica* larvae are mute, suggesting that in this trait *Maculinea* caterpillars are mimicking an adult ant cue, but no direct cause-and-effect relationship was revealed (recordings by DeVries et al. [132] were restricted to distressed worker ants and caterpillars, and were not played back to the ants). Studying the *Maculinea rebeli/Myrmica schencki* system, we recently demonstrated the first case of acoustical mimicry in an ant social parasite [26]. In particular we demonstrated that *Maculinea rebeli* larvae and pupae are able to mimic the sounds produced by *Myrmica schencki* queens (Figures 2(a) and 2(b)), thus obtaining a high status in the host colony hierarchy. Queens, that never come out of the nest, produce peculiar stridulations, which attract workers. Ethological experiments revealed that the acoustical signals produced by *Maculinea rebeli* larvae elicit the same benevolent responses

in the worker ants as those emitted by their queen(s). When recordings of unstressed adult *M. schencki* were played back to laboratory cultures of workers, the sounds of both castes induced benign responses including aggregation and antennation at the speaker. Moreover, when workers were played their queen's sounds, they stood "on guard" on the speaker to a much greater extent than when worker sounds were played, each holding the characteristic posture adopted by a *Myrmica* worker when protecting an object of high value to the colony [26]. *Maculinea rebeli* caterpillars are rescued ahead of the ant brood when a colony is disturbed, and are fed in preference to host ant larvae when food is scarce [48]. Neither chemical mimicry nor their begging behaviour explains why *M. rebeli* caterpillars are treated in preference to host ant brood. Instead, we have suggested that acoustical cues are employed [26].

Thus it is possible that acoustical mimicry does not occur in *Maculinea rebeli* only, but rather provides another route for the infiltration of other *Maculinea* species, as well as for other myrmecophilous insects [26]. Acoustical mimicry can also be related to the level of interaction between host and parasite, or may play a role in host-specificity. In particular, in the *Maculinea/Myrmica* system the level of host's integration within the colony results from the two distinct parasites' foraging strategies. In the so-called "cuckoo" species, *Maculinea* larvae become perfectly integrated members of the colony, as they need to be tended by worker ants. Larvae of predator species, in contrast, will prey on the ants' brood and spend much of their life hidden in the remote chambers of the nest. DeVries et al. [132] showed that also the caterpillars of the predatory *Maculinea* species produce sounds that appear to mimic *Myrmica* (worker) stridulations, although in nature they are less closely integrated with their host's society [14], so that they might be less perfect acoustical mimics of their hosts. We tested [124] this hypothesis by comparing the acoustics of unstressed *Maculinea arion* caterpillars and pupae with those of the queens and workers of its host ant, *Myrmica sabuleti*, and with data obtained for *Maculinea rebeli* and *Myrmica schencki*, but found no evidence that *M. rebeli* is a closer mimic of *M. schencki* than *M. arion* is to *M. sabuleti* [26]. We also compared the worker and queen sounds of *M. sabuleti*, and those of two other ants, *Myrmica scabrinodis* and *M. schencki*, to determine whether the distinctive acoustical communication system occurring in the different castes of *M. schencki* exists in its congeners.

We found that stridulating queens from two additional *Myrmica* species (i.e., *M. sabuleti* and *M. scabrinodis*) make distinctive sounds from those of their workers by using morphologically distinct organs [124]. Interestingly, the calls produced by queen from the three *Myrmica* species were indistinguishable from each other, as were workers' stridulations even at a less extent. This suggests that acoustics plays little or no part in the cues used by *Myrmica* to distinguish between kin and nonkin, or other species of ant and members of their own society. Indeed numerous studies demonstrate the predominant role of chemical cues and the gestalt odour in colony recognition or between physiological states within an ant society [1]. However, our recent results suggest that acoustical communication, in isolation, is capable of

signalling at least the caste and the status of a colony member, as well as of inducing appropriate behaviour towards it by the attending workers [124]. In other words, acoustical mimicry is genus rather than species specific, as DeVries et al. [132] concluded. We have not yet studied whether different castes of *Myrmica* ants responded differently when played the same sounds, although this seems probable, because *Myrmica schencki* queen respond aggressively when introduced to *Maculinea rebeli* pupae (which mimic queen sounds) whereas the workers tend them gently [26].

## 5. Concluding Remarks

To our knowledge, although 10,000 species of ant social parasites may exist [24] particularly among the Coleoptera, Diptera and Lepidoptera [1], acoustical mimicry has rarely been examined outside the case of *Maculinea*. Together with Di Giulio and his collaborators, we recently surveyed the acoustical emissions of *Paussus favieri* (Coleoptera, Paussinae), a myrmecophilous paussine beetle which lives in the nests of the ant *Pheidole pallidula* [133]. The presence of stridulatory organs in members of the myrmecophilous ground beetles tribe Paussini has long been known. However, due to the rarity of these beetles and the challenges in rearing them in captivity, sounds emitted by these organs have never been investigated, as well as their biological significance. The complexity of *P. favieri*'s sound repertoire suggests that it has an important role in its interaction with *P. pallidula*.

We strongly believe that the implementation of studies on acoustic communication will bring about significant advances in our understanding of the complex mechanisms underlying the origin, evolution and stabilisation of host-parasite relationships. To improve our understanding of how important and how generalised acoustic mimicry is we also need to clarify which sensory structures are involved in sound perception processes, both in queen and worker ants. Nobody, so far, has ever investigated the possibility that the larvae and pupae of myrmecophilous lycaenids may perceive the sounds emitted by conspecifics, or by their host ants. In this respect it is worth noticing that some of the most important research on the role of filiform hairs in sound perception (e.g., [134, 135]) were carried out on *Barathra brassicae* (Lepidoptera: Noctuidae). The larvae of this moth, indeed, are able to detect the vibrations produced by a parasitoid wasp, by their thoracic hairs.

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## Review Article

# Diversity of Species and Behavior of Hymenopteran Parasitoids of Ants: A Review

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Reports of hymenopterans associated with ants involve more than 500 species, but only a fraction unambiguously pertain to actual parasitoids. In this paper, we attempt to provide an overview of both the diversity of these parasitoid wasps and the diversity of the types of interactions they have formed with their ant hosts. The reliable list of parasitoid wasps using ants as primary hosts includes at least 138 species, reported between 1852 and 2011, distributed among 9 families from 3 superfamilies. These parasitoids exhibit a wide array of biologies and developmental strategies: ecto- or endoparasitism, solitary or gregarious, and idio- or koinobiosis. All castes of ants and all developmental stages, excepting eggs, are possible targets. Some species parasitize adult worker ants while foraging or performing other activities outside the nest; however, in most cases, parasitoids attack ant larvae either inside or outside their nests. Based on their abundance and success in attacking ants, some parasitoid wasps like diapriids and eucharitids seem excellent potential models to explore how parasitoids impact ant colony demography, population biology, and ant community structure. Despite a significant increase in our knowledge of hymenopteran parasitoids of ants, most of them remain to be discovered.

## 1. Introduction

Ants are distributed all over the world, and their colonies provide both a stable food resource and numerous niches for thousands of other organisms, termed myrmecophiles, that exhibit a diverse array of relationships with their hosts [1–7]. Among myrmecophiles, numerous species of hymenopterans are associated with ants through predation, parasitism on the brood and/or adults, cleptoparasitism, parabiosis, mimetism, true symphily, or indirect parasitism through trophobionts and/or social parasites. However, in most cases, the precise nature of their relationship with their ant hosts remains obscure.

A review of the diversity of parasitoid wasps attacking ants has not been attempted since the work of Schmid-Hempel [7]. In his extensive review of the parasites of social insects, he pointed out the wide variety of hymenopteran parasitoids that attack these insects but, with the exception

of the family Eucharitidae (with 33 valid species really involved), his list provided very few other examples (only 10) of true parasitoidism, that is, cases where the attack of the wasp species on ants (adults or brood) has been reliably demonstrated.

Knowledge has increased greatly in the intervening years, and numerous cases of parasitic associations involving wasps and ants have been reported. Moreover, changes in nomenclature and phylogeny have been numerous in the last two decades (see, e.g., [8–15]), and many species names of both the parasitoids and their ant hosts required emendations.

In the present paper, we address only hymenopteran parasitoids and focus strictly on ant-parasitoid wasp associations in which parasitism has been established beyond any doubt, and where ants are proved to be the primary hosts. Therefore, no bethylid species are considered here even though various members of the genera *Pseudisobrachium* and

*Dissomphalus* are strongly suspected of being parasitoids of ant brood [16–18]. Neither are any species of ceraphronid, dryinid, figitid, platygastriid, proctotrupid, or pteromalid wasps considered although several species belonging to the genera *Ceraphron*, *Conostigmus*, *Gonatopus*, *Kleidotoma*, *Platygaster*, *Exallonyx*, and *Spalangia* are known to be associated with ants, most of them probably as parasitoids [2, 19–24]. All of these species were omitted from the present paper because wasps have not been reliably reared from ants or their brood. Moreover, according to the definition of “parasitoid” which implies the killing of a single host, associations such as those involving numerous sphecid species, particularly those of the genera *Aphilanthops*, *Clypeadon*, and *Tracheliodes*, which are known to specialize with preying on and storing numerous adult ants (of the genus *Formica*, *Pogonomyrmex*, or *Liometopum*, resp.) [25–27] are not dealt with. Likewise, the highly interesting associations of ants with some braconid species such as *Compsobraconoides* sp. [28] and *Trigastrotheca laikipiensis* Quicke [29], which are known to consume various stages of their ant hosts (*Azteca* spp. and *Crematogaster* spp., resp.) during their development, are not covered in the present paper.

In spite of such restrictions, the list of hymenopteran species reliably involved in parasitic associations with ants remains impressive and represents more than a quarter of all of the hymenopteran species known to be associated with ants [30]. Here, we attempt to provide an overview of both the diversity of the species of parasitoid wasps known to attack ants and the diversity of the interactions they have developed with their hosts. By so doing, we also call attention to this little known biodiversity.

## 2. Checklist of Hymenopteran Parasitoids of Ants

Records of associations of hymenopteran wasps with ants involve more than 500 wasp species [30], but only a fraction have unambiguously been reported as parasitoids. The term parasitoid applies to organisms whose juvenile stages are parasites of a single host individual, eventually sterilizing, killing, or even consuming their host, while the adult parasitoid is free living [31]. With few exceptions, female parasitoid wasps oviposit on or inside the body of their host, typically another arthropod, and all stages of development of the host are susceptible to attack. After hatching, the parasitoid larva feeds on the host’s tissues, gradually killing it. A survey of the literature since 1852 and some of our own unpublished results have allowed us to identify at least 138 species (see Table 1 and Supplementary Material available online at doi:10.1155/2012/134746) reported as primary endo- or ectoparasitoids of larvae, pupae, or adult ants. All of these species are included in 3 superfamilies: Chalcidoidea (with 6 families concerned), Ichneumonoidea (2 families), and Diaprioidea (only 1 family) (Table 1). In 2007, Sharkey [12] estimated that there were approximately 115,000 described species of Hymenoptera (perhaps up to 1,000,000 if undescribed species—especially species of parasitoid wasps—were included), and that Chalcidoidea and

Ichneumonoidea were the most species-rich superfamilies among the parasitoid hymenopterans. So, it is not surprising that most of the parasitoid wasps attacking ants belong to these two superfamilies, especially the Chalcidoidea which alone includes more than 70% of all of wasp species parasitizing ants registered until now.

In the following text, we follow Sharkey [12] for the higher-level phylogeny of the order Hymenoptera (see also [15]). The taxonomic validity of the scientific names is in accordance with Bolton [8, 9] for ants, and with different databases available on the web for other hymenopterans: Hymenoptera Name Server (version 1.5) ([http://osuc.biosci.ohio-state.edu/hymDB/nomenclator.home\\_page](http://osuc.biosci.ohio-state.edu/hymDB/nomenclator.home_page)), Global Name Index (version 0.9.34) ([http://gni.globalnames.org/name\\_strings](http://gni.globalnames.org/name_strings)), Universal Chalcidoidea Database [32] (<http://www.nhm.ac.uk/chalcidoids>), and Home of Ichneumonoidea (version 2011) (<http://www.ichneumonoidea.name/index.php>). Authors of all scientific names are given throughout the text only when they are not already reported in Table 1.

**2.1. Diaprioidea.** The superfamily Diaprioidea is a monophyletic group, with 4 recognized families [15], and accounts for more than 4000 species around the world in over 210 genera [8, 155–157], almost all in the family Diapriidae. Most diaprioids are primary endoparasitoids of dipterans (eggs, larvae, or pupae), but several species are known to attack Hymenoptera, Homoptera, or Coleoptera, and some are facultative or obligate hyperparasitoids. Some of the species attacking Diptera have been considered as potential biological control agents, but their efficiency has not been demonstrated [157, 158].

**2.1.1. Diapriidae.** Despite their number, the members of this large family are relatively unknown and less than half of the 4000 species estimated to occur worldwide have been described [8, 156, 159]. Three subfamilies are currently recognized: Ambositrinae, Belytinae, and Diapriinae [15]. Their biologies are diverse, but most species are primary parasitoids of puparia of Diptera [156–160].

Although some diapriids have only occasionally been found in ant nests, a number of species are closely associated with ants (all belonging to the Belytinae and Diapriinae subfamilies). However, there are few behavioral data on host-diapriid myrmecophile interactions (but see [36]). These symphyles are often highly adapted to their hosts, exhibiting morphological and behavioral adaptations to living with ants (extensive morphological mimicry of the host ants—coloration, ocellus regression, convergence in sculpture—, presence of appeasement substances in specialized structures and trichomes, trophallaxis, etc., [161–166]), which presumably aid them in avoiding detection and/or aggression by host ants [34]. The adaptations can include secondary apterism in which the wings of the wasps are assumed to have been bitten off by either the parasite itself or its host (e.g., *Mimopria*, *Bruchopria*, *Lepidopria*, and *Solenopsia*, [156, 161, 164, 167, 168]). Most often, the presence of a diapriid in an ant nest is suspected to be just circumstantial [160] and related to its

TABLE 1: List of parasitic wasps recorded as true primary parasitoids of ants (brood or adult). As all of the eucharitids are true parasitoids of ants, all known associations with ants have been included, but see \*\*. For further details, see text.

Hymenopterous parasitoids		Associated ant host		References
Species	Referred to as	Species	Referred to as	
Diaprioidea: Diapriidae (26)				
<i>Acanthopria</i> sp.	—	<i>Cyphomyrmex salvini</i> Forel	—	[33]
<i>Acanthopria</i> sp.	—	<i>Trachymyrmex</i> cf. <i>zeteki</i> Weber	—	[34]
<i>Acanthopria</i> sp. no. 1	—	<i>Cyphomyrmex transversus</i> Emery	—	[35]
<i>Acanthopria</i> sp. no. 2	—	<i>Cyphomyrmex transversus</i> Emery	—	[35]
<i>Acanthopria</i> sp. no. 3	—	<i>Cyphomyrmex transversus</i> Emery	—	[35]
<i>Acanthopria</i> sp. no. 4	—	<i>Cyphomyrmex transversus</i> Emery	—	[35]
<i>Acanthopria</i> sp. no. 5	—	<i>Cyphomyrmex transversus</i> Emery	—	[35]
<i>Acanthopria</i> sp. no. 6	—	<i>Cyphomyrmex transversus</i> Emery	—	[35]
<i>Acanthopria</i> sp. no. 7	—	<i>Cyphomyrmex transversus</i> Emery	—	[35]
<i>Acanthopria</i> sp. no. 8	—	<i>Cyphomyrmex transversus</i> Emery	—	[35]
<i>Acanthopria</i> sp. 1	—	<i>Cyphomyrmex minutus</i> Mayr	—	[36]
<i>Acanthopria</i> sp. no. 1'	—	<i>Cyphomyrmex rimosus</i> (Spinola)	—	[36]
<i>Acanthopria</i> sp. no. 2'	—	<i>Cyphomyrmex rimosus</i> (Spinola)	—	[36]
<i>Acanthopria</i> sp. no. 3'	—	<i>Cyphomyrmex rimosus</i> (Spinola)	—	[36]
<i>Acanthopria</i> sp. no. 4'	—	<i>Cyphomyrmex rimosus</i> (Spinola)	—	[36]
<i>Mimopriella</i> sp.	—	<i>Cyphomyrmex rimosus</i> (Spinola)	—	[36]
<i>Mimopriella</i> sp. 1	—	<i>Trachymyrmex</i> cf. <i>zeteki</i> Weber	—	[34]
<i>Mimopriella</i> sp. 2	—	<i>Trachymyrmex</i> cf. <i>zeteki</i> Weber	—	[34]
<i>Oxypria</i> sp.	—	<i>Trachymyrmex</i> cf. <i>zeteki</i> Weber	—	[34]
<i>Plagiopria passerai</i> Huggert and Masner	—	<i>Plagiolepis pygmaea</i> (Latr.)	—	[37]
<i>Szelenyiopria lucens</i> (Loiácono)	<i>Gymnopria lucens</i>	<i>Acromyrmex ambiguus</i> (Emery)	—	[38]
<i>Szelenyiopria pampeana</i> (Loiácono)	<i>Gymnopria pampeana</i>	<i>Acromyrmex lobicornis</i> (Emery)	—	[39]
<i>Szelenyiopria</i> sp. 1	—	<i>Trachymyrmex</i> cf. <i>zeteki</i> Weber	—	[34]
<i>Szelenyiopria</i> sp. 2	—	<i>Trachymyrmex</i> cf. <i>zeteki</i> Weber	—	[34]
<i>Trichopria formicans</i> Loiácono	—	<i>Acromyrmex lobicornis</i> (Emery)	—	[40]
<i>Trichopria</i> sp.	—	<i>Acromyrmex lobicornis</i> (Emery)	—	[40]
Chalcidoidea: Chalcididae (2 + 2*)				
<i>Smicromorpha doddi</i> Girault	—	<i>Oecophylla smaragdina</i> (Fabr.)	—	[41, 42]
<i>Smicromorpha keralensis</i> Narendran*	—	<i>Oecophylla smaragdina</i> (Fabr.)	—	[43]
<i>Smicromorpha masneri</i> Darling	—	<i>Oecophylla smaragdina</i> (Fabr.)	—	[44]
<i>Smicromorpha minera</i> Girault *	—	<i>Oecophylla smaragdina</i> (Fabr.)	—	[42]
Chalcidoidea: Encyrtidae (1)				
<i>Blanchardiscus</i> sp. ? <i>pollux</i> Noyes	—	<i>Pachycondyla goeldii</i> (Forel)	—	[45]
Chalcidoidea: Eucharitidae (86 + 7** + 1***)				
<i>Ancylotropus manipurensis</i>	—	<i>Camponotus</i> sp.***	—	[11, 46]
<i>Ancylotropus</i> sp.	—	<i>Odontomachus troglodytes</i> Santschi	—	[11]
<i>Athairocharis vanhoorti</i> Heraty	—	<i>Anoplolepis</i> sp.	<i>Anaplolepis</i> sp.	[11]
<i>Austeucharis fasciiventris</i> (Brues)	<i>Psilogaster fasciiventris</i>	<i>Myrmecia gulosa</i> (Fabr.)	—	[47]
<i>Austeucharis implexa</i> (Walker)	—	<i>Myrmecia pilosula</i> F. Smith	—	[11]

TABLE 1: Continued.

Hymenopterous parasitoids		Associated ant host		References
Species	Referred to as	Species	Referred to as	
<i>Austeucharis myrmeciae</i> (Forel)	<i>Eucharis myrmeciae</i> Cameron	<i>Myrmecia forficata</i> (Fabr.)	—	[48]
<i>Austeucharis</i> sp.	—	<i>Myrmecia pavidata</i> Clark	<i>M. atrata</i> Clark	[49, 50]
	—	<i>Myrmecia nigriceps</i> Mayr	<i>M. nigriceps</i> Smith	[49, 50]
	—	<i>Myrmecia pilosula</i> F. Smith	—	[50]
	<i>Epimetegea</i> sp.	<i>Myrmecia pyriformis</i> F. Smith	—	[51]
	—	<i>Myrmecia tarsata</i> F. Smith	—	[50]
	—	<i>Myrmecia vindex</i> F. Smith	<i>M. vindex</i> Forel	[50]
<i>Chalcura affinis</i> (Bingham)	<i>Rhipipallus affinis</i>	<i>Odontomachus ruficeps</i> F. Smith	<i>O. ruficeps</i> subsp. <i>coriarius</i> Mayr	[52]
	<i>Chalcuroides versicolor</i> Girault	<i>Odontomachus</i> sp.	<i>Myrmecia</i> sp.	[53, 54]
<i>Chalcura deprivata</i> (Walker)	—	<i>Odontomachus haematodus</i> (L.)	<i>O. haematodes</i>	[55]
<i>Chalcura nigricyanea</i> (Girault)	—	<i>Rhytidoponera metallica</i> (F. Smith)	<i>R. metallicum</i>	[11]
<i>Chalcura polita</i> (Girault)	—	<i>Rhytidoponera metallica</i> (F. Smith)	<i>R. metallicum</i>	[11]
<i>Chalcura</i> sp.	—	<i>Formica rufa</i> L.	—	[56]
<i>Chalcura</i> sp. nr. <i>polita</i> (Girault)	—	<i>Rhytidoponera chalybaea</i> Emery	—	[11]
<i>Dicoelothorax platycerus</i> Ashmead	—	<i>Ectatomma brunneum</i> F. Smith	—	[57]
<i>Dilocantha lachaudii</i> Heraty	—	<i>Ectatomma tuberculatum</i> (Olivier)	—	[58, 59]
<i>Eucharis adscendens</i> (Fabr.)	—	<i>Formica ?cunicularia</i> Latr.**	<i>F. glauca</i> Ruzsky	[60]
	—	<i>Formica rufa</i> L.	—	[61]
	—	<i>Messor barbarus</i> (L.)**	<i>Aphaenogaster</i> <i>barbara</i> L.	[62]
<i>Eucharis bedeli</i> (Cameron)	—	<i>Cataglyphis bicolor</i> (Fabr.)***	<i>C. viaticus</i>	[63]
	<i>Chalcura bedeli</i>	<i>Cataglyphis viaticus</i> (Fabr.)	<i>Myrmecocystus</i> <i>viaticus</i>	[64, 65]
	<i>Chalcura bedeli</i>	<i>Formica rufa</i> L.***	—	[61, 65]
<i>Eucharis esakii</i> Ishii	<i>E. scutellaris</i> Gahan	<i>Formica japonica</i> Motschoulski	<i>F. fusca fusca japonica</i> Mots.	[66]
	<i>E. scutellaris</i> Gahan	<i>Formica</i> sp.	—	[55]
<i>Eucharis microcephala</i> Bouček	—	<i>Cataglyphis nodus</i> (Brullé)	<i>C. bicolor</i> ssp. <i>nodus</i>	[67]
<i>Eucharis punctata</i> Förster	—	<i>Messor concolor</i> Santschi**	<i>M. barbarus</i> r. <i>semirufus</i> v. <i>concolor</i> Sm.	[68]
<i>Eucharis rugulosa</i> Gussakovskiy	—	<i>Cataglyphis</i> sp.**	—	[60]
<i>Eucharis shestakovi</i> Gussakovskiy	—	<i>Messor structor</i> (Latr.)**	—	[69]
<i>Eucharis</i> sp.	—	<i>Formica neorufibarbis</i> Emery**	<i>F. fusca neorufibarbis</i>	[70]
	—	<i>Myrmica incompleta</i> Provancher**	<i>M. brevinodis</i> Emery	[70]
<i>Galearia latreillei</i> (Guérin-Ménéville)	<i>Thoracantha bruchi</i>	<i>Pogonomyrmex cunicularius</i> Mayr**	<i>P. carnivora</i> Santschi	[11, 71]
<i>Gollumiella longipetiolata</i> Hedqvist	—	<i>Paratrechina</i> sp.	—	[72]
<i>Hydrorhoa</i> sp. <i>striaticeps</i> Kieffer complex	—	<i>Camponotus maculatus</i> (Fabr.)	<i>C. maculatus</i> Mayr	[11]
<i>Isomerala coronata</i> (Westwood)	<i>Isomaralia coronata</i>	<i>Ectatomma tuberculatum</i> (Olivier)	—	[73]
	—	<i>Ectatomma ruidum</i> Roger***	—	[11]
<i>Kapala atrata</i> (Walker)	<i>K. surgens</i>	<i>Pachycondyla harpax</i> (Fabr.)	—	[11]
<i>Kapala cuprea</i> Cameron	—	<i>Pachycondyla crassinoda</i> (Latr.)	—	[74]
<i>Kapala floridana</i> (Ashmead)	—	<i>Pogonomyrmex badius</i> (Latr.)**	—	[70]
<i>Kapala iridicolor</i> (Cameron)	<i>K. sulcifacies</i> (Cameron)	<i>Ectatomma ruidum</i> Roger	—	[75, 76]

TABLE 1: Continued.

Hymenopterous parasitoids		Associated ant host		References
Species	Referred to as	Species	Referred to as	
	—	<i>Gnamptogenys regularis</i> Mayr	—	[76]
	—	<i>Gnamptogenys striatula</i> Mayr	—	[76]
	—	<i>Gnamptogenys sulcata</i> (F. Smith)	—	[76]
	—	<i>Pachycondyla stigma</i> (Fabr.)	—	[76]
<i>Kapala izapa</i> Carmichael	—	<i>Ectatomma ruidum</i> Roger	—	[76]
<i>Kapala</i> sp.	—	<i>Dinoponera lucida</i> Emery	—	[77]
	—	<i>Ectatomma brunneum</i> F. Smith	—	[78]
	—	<i>Ectatomma tuberculatum</i> (Olivier)	—	[79]
	—	<i>Gnamptogenys sulcata</i> (F. Smith)	—	[80]
	—	<i>Gnamptogenys tortuolosa</i> (F. Smith)	—	[78]
	—	<i>Hypoponera nitidula</i> (Emery)	—	[81]
	—	<i>Odontomachus bauri</i> Emery	—	[11]
	—	<i>Odontomachus brunneus</i> (Patton)	—	[80]
	—	<i>Odontomachus haematodus</i> (L.)	—	[77]
	—	<i>Odontomachus hastatus</i> (Fabr.)	—	[11]
	—	<i>Odontomachus insularis</i> Guérin-Méneville	<i>O. haematodes</i> <i>insularis pallens</i> Wheeler	[66]
	—	<i>Odontomachus laticeps</i> Roger	—	[80]
	—	<i>Odontomachus mayi</i> Mann	—	[78]
	—	<i>Odontomachus meinerti</i> Forel	—	[81]
	—	<i>Odontomachus opaciventris</i> Forel	—	[80]
	—	<i>Pachycondyla apicalis</i> (Latr.)	—	[80]
	—	<i>Pachycondyla harpax</i> (Fabr.)	—	[81]
	—	<i>Pachycondyla stigma</i> (Fabr.)	—	[81]
	—	<i>Pachycondyla verena</i> (Forel)	—	[78]
	—	<i>Typhlomyrmex rogenhoferi</i> Mayr	—	[81]
<i>Kapala terminalis</i> Ashmead	—	<i>Odontomachus insularis</i> Guérin-Méneville	<i>O. haematodes</i> <i>insularis pallens</i> Wheeler	[66]
<i>Lophyrocera variabilis</i> Torrén, Heraty and Fidalgo	—	<i>Camponotus</i> sp.	—	[82]
<i>Mateucharis rugulosa</i> Heraty	—	<i>Camponotus</i> sp.	—	[11]
<i>Neolosbanus gemma</i> (Girault)	—	<i>Hypoconer</i> sp.	—	[83]
<i>Neolosbanus palgravei</i> (Girault)	—	<i>Hypoconer</i> sp.	—	[83]
<i>Obeza floridana</i> (Ashmead)	—	<i>Camponotus floridanus</i> (Buckley)	<i>C. abdominalis</i> <i>floridanus</i>	[84]
<i>Orasema aenea</i> Gahan	—	<i>Solenopsis quinquecupis</i> Forel	—	[85]
<i>Orasema argentina</i> Gemignani	—	<i>Pheidole nitidula</i> Santschi	<i>P. strobili misera</i> Snts.	[71]
<i>Orasema assectator</i> Kerrich	—	<i>Pheidole</i> sp.	—	[86, 87]
<i>Orasema coloradensis</i> Wheeler	<i>O. coloradensis</i> Ashmead	<i>Diplorhoptrum validiusculum</i> (Emery)	<i>Solenopsis molesta</i> <i>validiuscula</i>	[70]
	<i>O. coloradensis</i> Gahan	<i>Formica oreas comptula</i> Wheeler	—	[88]
	<i>O. coloradensis</i> Gahan	<i>Formica subnitens</i> Creighton	—	[88]
	<i>O. coloradensis</i> Ashmead	<i>Pheidole bicarinata</i> Mayr	<i>P. vinelandica</i> Forel	[70]
<i>Orasema costaricensis</i> Wheeler and Wheeler	—	<i>Pheidole flavens</i> Roger	—	[63]

TABLE 1: Continued.

Hymenopterous parasitoids		Associated ant host		References
Species	Referred to as	Species	Referred to as	
	—	<i>Pheidole vallifica</i> Forel	—	[89]
<i>Orasema fraudulenta</i> (Reichensperger)	<i>Psilogaster fraudulentus</i>	<i>Pheidole megacephala</i> (Fabr.)	—	[90]
<i>Orasema minuta</i> Ashmead	—	<i>Pheidole</i> nr. <i>tetra</i> Creighton	—	[11, 83]
	—	<i>Temnothorax allardycei</i> (Mann)	—	[11, 83]
<i>Orasema minutissima</i> Howard	—	<i>Wasmannia auropunctata</i> (Roger)	—	[91]
	—	<i>Wasmannia sigmoidea</i> (Mayr)	—	[92]
<i>Orasema monomoria</i> Heraty	—	<i>Monomorium</i> sp.	—	[93]
<i>Orasema occidentalis</i> Ashmead	—	<i>Pheidole pilifera</i> (Roger)	—	[94]
<i>Orasema pireta</i> Heraty	—	<i>Solenopsis</i> sp.	—	[85]
<i>Orasema rapo</i> (Walker)	—	<i>Eciton quadriglume</i> (Haliday)**	—	[83]
<i>Orasema robertsoni</i> Gahan	—	<i>Pheidole dentata</i> Mayr	—	[95]
<i>Orasema salebrosa</i> Heraty	—	<i>Solenopsis invicta</i> Buren	—	[85]
	—	<i>Solenopsis richteri</i> Forel	—	[96]
<i>Orasema simplex</i> Heraty	—	<i>Solenopsis invicta</i> Buren	—	[97]
	—	<i>Solenopsis macdonaghi</i> Santschi	—	[85]
	—	<i>Solenopsis quinquecuspis</i> Forel	—	[85]
	—	<i>Solenopsis richteri</i> Forel	—	[96]
<i>Orasema simulatrix</i> Gahan	—	<i>Pheidole desertorum</i> Wheeler	—	[98]
<i>Orasema sixaolae</i> Wheeler and Wheeler	—	<i>Solenopsis tenuis</i> Mayr	—	[63]
<i>Orasema</i> sp.	B1 nr. <i>bakeri</i>	<i>Solenopsis geminata</i> (Fabr.)	—	[83]
	B1 nr. <i>bakeri</i>	<i>Solenopsis xyloni</i> MacCook	—	[83]
<i>Orasema</i> sp.	B2 nr. <i>bakeri</i>	<i>Pheidole</i> nr. <i>californica</i> Mayr	—	[83]
	B2 nr. <i>bakeri</i>	<i>Pheidole</i> nr. <i>clementensis</i> Gregg	—	[83]
	B2 nr. <i>bakeri</i>	<i>Pheidole</i> sp.	—	[83]
	B2 nr. <i>bakeri</i>	<i>Tetramorium</i> sp.	—	[83]
<i>Orasema</i> sp.	C1 nr. <i>costaricensis</i>	<i>Pheidole dentata</i> Mayr	—	[83]
<i>Orasema</i> sp.	—	<i>Pheidole bilimeki</i> Mayr	<i>P. anastasioi</i> Emery	[99]
<i>Orasema</i> sp.	—	<i>Pheidole paiute</i> Gregg	—	[94]
<i>Orasema</i> sp. nr. <i>bouceki</i> Heraty	—	<i>Pheidole</i> sp.	—	[83]
<i>Orasema</i> sp. <i>uichancoi</i> -group	—	<i>Pheidole</i> sp.	—	[93]
<i>Orasema susanae</i> Gemignani	—	<i>Pheidole</i> nr. <i>tetra</i> Creighton	—	[83]
<i>Orasema tolteca</i> Mann	—	<i>Pheidole hirtula</i> Forel	<i>P. vasleti</i> var. <i>acohlma</i>	[100]
<i>Orasema valgius</i> (Walker)	<i>O. pheidolophaga</i> Girault	<i>Pheidole</i> sp.	—	[53]
<i>Orasema wheeleri</i> Wheeler	<i>O. wheeleri</i> Ashmead	<i>Pheidole ceres</i> Wheeler	—	[70]
	<i>O. viridis</i> Ashmead	<i>Pheidole dentata</i> Mayr	—	[55, 70]
	<i>O. viridis</i> Ashmead	<i>Pheidole sciophila</i> Wheeler	—	[55, 70]
	<i>O. viridis</i> Ashmead	<i>Pheidole tepicana</i> Pergande	<i>P. kingi</i> subsp. <i>instabilis</i> Emery	[55, 70]
	<i>O. viridis</i> Ashmead	<i>Pheidole tepicana</i> Pergande	<i>P. carbonaria</i> Pergande	[55, 70]
<i>Orasema worcesteri</i> (Girault)	<i>O. doello-juradoi</i> Gemignani	<i>Pheidole radoszkowskii</i> Mayr	<i>P. nitidula</i> Emery	[71, 96]
<i>Orasema xanthopus</i> (Cameron)	—	<i>Solenopsis invicta</i> Buren	—	[83, 96]
	—	<i>Solenopsis quinquecuspis</i> Forel	—	[85]
	—	<i>Solenopsis richteri</i> Forel	—	[101]
	—	<i>Solenopsis saevissima</i> (F. Smith)	—	[102]

TABLE 1: Continued.

Hymenopterous parasitoids		Associated ant host		References
Species	Referred to as	Species	Referred to as	
<i>Orasemorpha eribotes</i> (Walker)	—	<i>Pheidole</i> sp.	—	[54]
<i>Orasemorpha myrmicae</i> (Girault)	—	<i>Pheidole</i> sp.	—	[83]
<i>Orasemorpha tridentata</i> (Girault)	<i>Eucaromorpha wheeleri</i> Brues	<i>Pheidole proxima</i> Mayr	—	[103]
<i>Orasemorpha xeniades</i> (Walker)	—	<i>Pheidole tasmaniensis</i> Mayr	—	[83]
<i>Pogonocharis browni</i> Heraty	—	<i>Gnamptogenys menadensis</i> (Mayr)	—	[11]
<i>Pseudochalcura gibbosa</i> (Provancher)	—	<i>Camponotus herculeanus</i> (L.)	—	[46]
	—	<i>Camponotus laevigatus</i> (F. Smith)	—	[104]
	—	<i>Camponotus novaeboracensis</i> (Fitch)	<i>C. ligniperdus</i> var. <i>novaeboracensis</i>	[70]
	—	<i>Camponotus</i> sp. ? <i>vicinus</i> Mayr	—	[104]
<i>Pseudochalcura nigrocyanea</i> Ashmead	—	<i>Camponotus</i> sp.	—	[105]
<i>Pseudochalcura sculpturata</i> Heraty	—	<i>Camponotus planatus</i> Roger	—	[11]
<i>Pseudometagea schwarzii</i> (Ashmead)	—	<i>Lasius neoniger</i> Emery	—	[106]
<i>Rhipipalloidea madangensis</i> Maeyama, Machida, and Terayama	—	<i>Camponotus</i> ( <i>Tanaemyrmex</i> ) sp.	—	[107]
<i>Rhipipalloidea mira</i> Girault	—	<i>Polyrhachis femorata</i> F. Smith	—	[11]
<i>Schizaspidia convergens</i> (Walker)	—	<i>Odontomachus haematodus</i> (L.)	<i>O. haematodes</i>	[55]
<i>Schizaspidia nasua</i> (Walker)	—	<i>Odontomachus rixosus</i> F. Smith	—	[11]
<i>Stilbula arenae</i> Girault	—	<i>Polyrhachis</i> sp.	<i>Cyrtomyrma</i> sp.	[54]
<i>Stilbula cyniformis</i> (Rossi)	<i>S. cynipiformis</i>	<i>Camponotus aethiops</i> (Latr.)	<i>C. marginatus</i> Latr.	[68]
	<i>Schizaspidia tenuicornis</i>	<i>Camponotus japonicus</i> Mayr	<i>C. herculeanus</i> ssp. <i>japonicus</i>	[108]
	<i>Schizaspidia tenuicornis</i>	<i>Camponotus obscuripes</i> Mayr	<i>C. herculeanus</i> ssp. <i>ligniperdus</i> v. <i>obscuripes</i>	[66, 108]
	<i>S. cynipiformis</i>	<i>Camponotus sanctus</i> Forel	<i>C. maculatus</i> r. <i>sanctus</i>	[62]
<i>Stilbula polyrhachicida</i> (Wheeler and Wheeler)	<i>Schizaspidia polyrhachicida</i>	<i>Polyrhachis dives</i> F. Smith	<i>Polyrhachis</i> ( <i>Myrmhopla</i> ) <i>dives</i>	[109]
<i>Stilbuloida calomyrmecis</i> (Brues)	<i>Schizaspidia calomyrmecis</i>	<i>Calomyrmex purpureus</i> (Mayr)	—	[103]
<i>Stilbuloida doddi</i> (Bingham)	<i>Schizaspidia doddi</i>	<i>Camponotus</i> sp.	—	[52]
<i>Timioderus acuminatus</i> Heraty	—	<i>Pheidole capensis</i> Mayr	—	[93]
<i>Tricoryna chalcoponerae</i> Brues	—	<i>Rhytidoponera metallica</i> (F. Smith)	<i>Chalcoponera metallica</i> var. <i>critulata</i>	[103]
<i>Tricoryna ectatommae</i> Girault	—	<i>Rhytidoponera</i> sp.	<i>Ectatomma</i> sp.	[110]
<i>Tricoryna iello</i> (Walker)	—	<i>Rhytidoponera</i> sp.	—	[11]
<i>Tricoryna minor</i> (Girault)	—	<i>Rhytidoponera metallica</i> (F. Smith)	—	[11]
	—	<i>Rhytidoponera victoriae</i> (André)	—	[11]
<i>Tricoryna</i> sp. nr. <i>alcicornis</i> (Bouček)	—	<i>Rhytidoponera violacea</i> (Forel)	—	[11]
<i>Zulucharis campbelli</i> Heraty	—	<i>Camponotus</i> sp.	—	[11]
Chalcidoidea: Eulophidae (5)				
<i>Horismenus floridensis</i> (Schauff and Bouček)	<i>Alachua floridensis</i>	<i>Camponotus atriceps</i> (F. Smith)	<i>C. abdominalis</i> (Fabr.)	[111]
	<i>Alachua floridensis</i>	<i>Camponotus floridanus</i> (Buckley)	—	[111]
<i>Horismenus myrmecophagus</i> Hansson, Lachaud, and Pérez-Lachaud	—	<i>Camponotus</i> sp. ca. <i>textor</i> Forel	—	[112]

TABLE 1: Continued.

Hymenopterous parasitoids		Associated ant host		References
Species	Referred to as	Species	Referred to as	
<i>Myrmokata diparoides</i> Bouček	—	<i>Crematogaster</i> sp.	—	[113]
<i>Pediobius marjoriae</i> Kerrich	—	<i>Lepisotia</i> sp.	<i>Acantholepis</i> sp.	[114]
Unidentified sp. (? <i>Horismenus</i> )	nr. <i>Paracrias</i>	<i>Crematogaster acuta</i> (Fabr.)	—	[109, 112]
Chalcidoidea: Eurytomidae (4)				
<i>Aximopsis affinis</i> (Brues)	<i>Conoaxima affinis</i>	<i>Azteca</i> sp.	—	[115]
	<i>Conoaxima affinis</i>	<i>Azteca alfari</i> Emery	<i>Azteca alfari</i> subsp. <i>lucidula</i> var. <i>canalis</i>	[116]
	<i>Conoaxima affinis</i>	<i>Azteca pittieri</i> Forel	—	[117]
<i>Aximopsis aztecicida</i> (Brues)	<i>Conoaxima aztecicida</i>	<i>Azteca alfari</i> Emery	<i>Azteca alfaroi</i>	[115]
	<i>Conoaxima aztecicida</i>	<i>Azteca constructor</i> Emery	—	[115]
<i>Aximopsis</i> sp.	<i>Conoaxima</i> sp.	<i>Azteca salti</i> Wheeler	<i>Azteca xanthochroa</i> (Roger) subsp. <i>salti</i>	[116]
<i>Aximopsis</i> sp. (? <i>aztecicida</i> )	<i>Conoaxima</i> sp. (? <i>aztecicida</i> )	<i>Azteca alfari</i> Emery	—	[118]
	<i>Conoaxima</i> sp. (? <i>aztecicida</i> )	<i>Azteca australis</i> Wheeler	—	[118]
	<i>Conoaxima</i> sp. (? <i>aztecicida</i> )	<i>Azteca ovaticeps</i> Forel	—	[119]
	<i>Conoaxima</i> sp. (? <i>aztecicida</i> )	<i>Camponotus balzani</i> Emery	—	[118]
Chalcidoidea: Perilampidae (1)				
Unidentified sp.	—	<i>Pachycondyla luteola</i> (Roger)	—	[119]
Ichneumonoidea: Braconidae (11 + 4*)				
<i>Elasmosoma berlinense</i> Ruthe	—	<i>Camponotus</i> spp.	—	[120]
	—	<i>Camponotus vagus</i> (Scopoli)	—	[121]
	—	<i>Formica fusca</i> L.	—	[48]
	—	<i>Formica japonica</i> Motschoulsky	—	[122]
	—	<i>Formica pratensis</i> Retzius	—	[123]
	—	<i>Formica rufa</i> L.	—	[124–126]
	—	<i>Formica sanguinea</i> Latr.	—	[48]
	—	<i>Formica</i> spp.	—	[120]
	—	<i>Lasius niger</i> (L.)	—	[48, 56]
	—	<i>Polyergus</i> sp.	—	[127]
<i>Elasmosoma luxemburgense</i> Wasmann	—	<i>Formica rufibarbis</i> Fabr.	—	[128, 129]
<i>Elasmosoma michaeli</i> Shaw	—	<i>Formica obscuripes</i> Forel	—	[130]
	<i>E. sp. nr. pergandei</i> Ashmead	<i>Formica obscuriventris clivia</i> Creighton	—	[131]
<i>Elasmosoma pergandei</i> Ashmead*	—	<i>Camponotus castaneus</i> (Latr.)	<i>C. melleus</i> (Say)	[132]
	—	<i>Formica integra</i> Nylander	—	[126]
	—	<i>Formica subsericea</i> Say	—	[126]
<i>Elasmosoma petulans</i> Muesebeck*	—	<i>Formica integra</i> Nylander	—	[133]
	—	<i>Formica opaciventris</i> Emery	—	[127, 133, 134]
	—	<i>Formica pergandei</i> Emery	<i>F. rubicunda</i> Emery	[133, 134]
	—	<i>Formica rubicunda</i> Emery***	—	[127, 133, 134]
	—	<i>Formica subintegra</i> Wheeler	<i>F. subintegra</i> Emery	[133]
	—	<i>Formica subsericea</i> Say	—	[133]

TABLE 1: Continued.

Hymenopterous parasitoids		Associated ant host		References
Species	Referred to as	Species	Referred to as	
<i>Elasmosoma schwarzi</i> Ashmead*	—	<i>Formica schaufussi</i> Mayr	—	[127]
	—	<i>Polyergus lucidus</i> Mayr	—	[127]
<i>Elasmosoma vigilans</i> Cockerell	—	<i>Formica perpilosa</i> Wheeler	—	[94]
	—	<i>Formica subpolita</i> Mayr	—	[135]
<i>Elasmosomites primordialis</i> Brues	—	<i>Lasius</i> sp. (? <i>schiefferdeckeri</i> Mayr)	—	[136]
<i>Kollasmosoma marikovskii</i> (Tobias)	—	<i>Formica pratensis</i> Retzius	—	[137]
<i>Kollasmosoma platamonense</i> (Huddleston)	<i>Elasmosoma platamonense</i>	<i>Cataglyphis bicolor</i> (Fabr.)	—	[127]
	—	<i>Messor semirufus</i> (André)	—	[138]
<i>Kollasmosoma sentum</i> van Achterberg and Gómez	—	<i>Cataglyphis ibericus</i> (Emery)	—	[129]
<i>Neoneurus auctus</i> (Thomson)	<i>Euphorus bistigmaticus</i> Morley	<i>Formica pratensis</i> Retzius	—	[139, 140]
	<i>Euphorus bistigmaticus</i> Morley	<i>Formica rufa</i> L.	—	[139, 140]
<i>Neoneurus clypeatus</i> (Förster)*	<i>Elasmosoma viennense</i> Giraud	<i>Formica rufa</i> L.	—	[141]
<i>Neoneurus mantis</i> Shaw	—	<i>Formica podzolica</i> Francoeur	—	[142, 143]
<i>Neoneurus vesculus</i> van Achterberg and Gómez	—	<i>Formica cunicularia</i> Latr.	—	[129]
Ichneumonoidea: Ichneumonidae (3 + 2*)				
<i>Eurypterna cremieri</i> (de Romand)	<i>Pachylomma cremieri</i>	<i>Formica rufa</i> L.	—	[144]
	<i>Pachylomma cremieri</i>	<i>Lasius fuliginosus</i> (Latr.)	<i>Formica fuliginosa</i>	[145–148]
	—	<i>Lasius niger</i> (L.)	—	[123]
	<i>Pachylomma cremieri</i>	<i>Lasius nipponensis</i> Forel	—	[149]
<i>Ghilaromma fuliginosi</i> (Donisthorpe and Wilkinson)*	<i>Paxylomma fuliginosi</i>	<i>Lasius fuliginosus</i> (Latr.)	—	[150, 151]
<i>Hybrizon buccatus</i> (Brébisson)	<i>Pachylomma buccata</i>	<i>Formica rufa</i> L.	<i>F. rufa</i> var. <i>rufo-pratensis</i>	[152]
	<i>Pachylomma buccata</i>	<i>Formica rufibarbis</i> Fabr.	—	[152]
	<i>Pachylomma buccata</i>	<i>Formica sanguinea</i> Latr.	—	[152]
	<i>Pachylomma buccata</i> Nees	<i>Lasius alienus</i> (Förster)	<i>Donisthorpea aliena</i>	[24]
	<i>Pachylomma buccatum</i>	<i>Lasius brunneus</i> (Latr.)	—	[144]
	<i>Pachylomma buccata</i>	<i>Lasius flavus</i> (Fabr.)	—	[152]
	—	<i>Lasius grandis</i> Forel	—	[129]
	<i>Pachylomma buccata</i>	<i>Lasius niger</i> (L.)	—	[140]
	<i>Pachylomma buccata</i> Nees	<i>Myrmica lobicornis</i> Nylander	—	[24]
	<i>Pachylomma buccata</i> Nees	<i>Myrmica ruginodis</i> Nylander	—	[24]
	<i>Pachylomma buccata</i>	<i>Myrmica scabrinodis</i> Nylander	—	[153]
	<i>Pachylomma buccata</i>	<i>Tapinoma erraticum</i> (Latr.)	—	[152]
<i>Hybrizon rileyi</i> (Ashmead)*	—	<i>Lasius alienus</i> (Förster)	—	[154]
Unidentified Hybrizontinae (gen. nov. sp. nov.)	—	<i>Myrmica kotokui</i> Forel	—	[149]

\*: attack was not observed, but there is strong evidence that all of the species of this genus reported as associated with an ant species are true primary parasitoids of this host.

\*\*: uncertain report of association with the host (e.g., ants of the genera *Pogonomyrmex* and *Messor* do not have cocoons—contrary to what is reported in the original reference—and were probably misidentified), uncertain identification of the ant host (ambiguity between 2 or more species), or wasps not found directly within the nest of the presumed host (e.g., found near a nest—perhaps only by chance—or found on refuse deposit—perhaps as a prey—).

\*\*\*: erroneous report (misidentification of either the parasitoid or the ant host), or erroneous emendation of the host species.



FIGURE 1: Winged females of the diapiiid wasp, *Plagiopria passerai* (white pointer) in a nest of the formicine ant *Plagiolepis pygmaea*, just after emergence from queen pupae. Photos courtesy of L. Passera.

search for dipterous hosts, such as *Tetramopria aurocincta* Wasmann found in nests of *Tetramorium caespitum* (L.) [128]. This wasp is in fact a parasitoid of the puparia of *Compsilura concinnata* Meigen (Diptera: Tachinidae), a primary parasite of the lepidopteran *Hyphantria cunea* (Drury) [160]. Occasionally, diapiiids enter ant nests for temporary shelter since some species hibernate in the host nest as do *Solenopsis imitatrix* Wasmann and *Lepidopria pedestris* Kieffer in the nests of *Solenopsis fugax* (Latr.) [37, 164].

Only a few diapiiids are true parasitoids of ant brood. Ever since the pioneering work of Wasmann in 1899 [128], most diapiiids found in ant nests were assumed either to parasitize insect myrmecophiles (dipteran or coleopteran) inside the host nest or, less frequently, to be primary parasitoids of ant larvae. However, the first record of a diapiiid positively reared from ant brood was reported just in 1982 by Lachaud and Passera [37], who reared *Plagiopria passerai* from cocoons of queens of the formicine *Plagiolepis pygmaea* (Figures 1(a) and 1(b)). As far as known, diapiiid parasitoids attacking ants develop as solitary or gregarious, koinobiont endoparasitoids of the host larvae [34, 36, 38, 169], and worker and/or reproductive immature stages can be parasitized [37, 169, 170]. Ramos-Lacau et al. [35] observed oviposition of *Acanthopria* sp. in young ant larvae under laboratory conditions. Late parasitized larvae are easily recognized by their dark coloration, compared to nonparasitized larvae, due to the developing wasp visible through the cuticle [35, 36, 38]. Worker ants do not discriminate between parasitized and nonparasitized larvae

[35, 38, 169], but adult parasitoids are aggressively attacked by their hosts under laboratory conditions [35, 36].

From the 121 diapiiine species in 34 genera that have been collected in association with ants [30], development of immature stages as parasitoids of ant larvae has been demonstrated for only 26 species in 7 genera, most of which are only known at the level of morphospecies (Table 1): 15 species of *Acanthopria*, 3 of *Mimopriella*, 1 of *Oxypria*, 1 of *Plagiopria* (*P. passerai*), 4 of *Szelenyopria*, and 2 of *Trichopria* (*T. formicans* and *Trichopria* sp.) [34–38, 169, 170]. The ant hosts of these diapiiines belong to 8 species in only 4 genera: the myrmicine fungus-growing ants *Cyphomyrmex*, *Trachymyrmex*, and *Acromyrmex* and the formicine *Plagiolepis*. Fifteen species of Belytinae belonging to 11 genera have also been reported from ant nests [30, 171–173], but none has been reliably reared from the ants, and their actual relationship with their hosts remains unknown.

In some cases, the rate of parasitism can reach high levels. Two recent studies have provided important details of the biology of diapiiids and have also investigated their impact on ant-host populations. Fernández-Marín et al. [36] found that between 27 and 70% of the colonies of 2 species of *Cyphomyrmex* were parasitized by one species in Puerto Rico and by up to 4 concurrent morphospecies of diapiiids in Panama. Similarly, the work of Pérez-Ortega et al. [34] showed that another fungus-growing ant, *Trachymyrmex* cf. *zeteki*, was attacked by a diverse community of diapiiids in Panama, with a mean intensity of larval parasitism per ant colony of 33.9%, and a prevalence across all ant populations of 27.2% (global data for all 6 diapiiid morphospecies present at the study site).

**2.2. Chalcidoidea.** The superfamily Chalcidoidea is considered as one of the most abundant, species-rich, and biologically diverse groups of insects with 23,000 species described and a conservative estimation of about 400,000 to 500,000 species in over 2040 genera distributed in 19 families [32, 174–178]. Though some species are phytophagous, most Chalcidoidea are parasitoids of other insects, and numerous species are currently used as biological control agents against insect pests.

**2.2.1. Chalcididae.** Chalcididae is a moderate-sized family with more than 1450 species and over 85 genera. Chalcids are primary parasitoids of Lepidoptera or, to a much lesser extent, of Coleoptera, Diptera, Hymenoptera, and Neuroptera, and various species are hyperparasitoids of other hymenopterous parasitoids [179]. Most often they parasitize host larvae or pupae, but a few species can parasitize eggs.

Very few species, like *Epitranus chilkaensis* (Mani) (referred to as *Anacryptus chilkaensis*) found with the formicine *Camponotus compressus* (Fabr.) in the Barkuda Island (India) [180], are known to be associated with ants [179, 181], but true parasitoidism has rarely been documented. Only species of the genus *Smicromorpha* seem to be specialized as parasitoids of the larvae of the green ant, *Oecophylla smaragdina*. The only unquestionable (see [44]) record of parasitoidism is that of Dodd in the early 20th century, describing *Smicromorpha doddi* in North

Queensland (Australia) parasitizing larvae of this weaver ant, “depositing eggs upon them when the workers are using their silk-spinning larvae for the purpose of binding the leaves together when building a new nest” [41]. No other example of true parasitoidism has ever been quoted for the genus *Smicromorpha* but, more recently, adults of another species of this genus, *S. masneri*, were reported emerging from *O. smaragdina* nests collected in Vietnam and maintained in controlled green-house conditions in the USA, which strongly suggests that these wasps are also primary parasitoids of weaver ants [44]. Moreover, two other species, *S. keralensis* [43] and *S. minera* [42], have been observed hovering over nests of *O. smaragdina* in India and Australia, respectively, a behavior likely to be related to parasitism of ants (see below under Braconidae and Ichneumonidae). For such reasons, all these members of the genus *Smicromorpha* can reasonably be suspected of being true parasitoids of the larvae of this ant host and were included in our list (Table 1).

**2.2.2. Encyrtidae.** Encyrtidae is a large family of parasitic wasps, currently including more than 460 genera and 3700 species, and is one of the key chalcidoid families for the biological control of insect pests [178, 182, 183]. Most encyrtids are primary endoparasitoids of immatures or, less commonly, adults of Coccidae and Pseudococcidae; others are hyperparasitic through other hymenopterous parasitoids, and some can attack insects in other orders, mites, ticks, or spiders [184, 185]. Some species are polyembryonic, a single egg multiplying clonally in the host, producing large numbers of identical adult wasps.

At least 25 species of encyrtid wasps representing 16 genera are known to be indirectly associated with ants through primary parasitism of the trophobionts they exploit and protect [32]; for example, the species *Anagyrus ananatis* Gahan is indirectly associated with the ant *Pheidole megacephala* through the trophobiotic Pseudococcidae present in their nest [186]. However, very few encyrtids have been reported as directly associated with ants. Apart from *Taftia prodeniae* Ashmead, which was found to exhibit a phoretic association (wasps were found clinging to the ant’s antennae) with the dolichoderine ant *Dolichoderus thoracicus* (F. Smith) (referred to as *D. bituberculatus* (Mayr)) [187], and an unidentified species recently reported from a refuse deposit of the ecitonine ant *Eciton burchellii* [188], only *Holcencyrtus wheeleri* (Ashmead) (referred to as *Pheidoloxenus wheeleri*), found in nests of the myrmicine ants *Pheidole tepicana* Pergande (referred to as *P. instabilis*) [70] and *P. ceres* Wheeler (referred to as *P. ceres* var. *tepaneca* Wheeler) [100], has been suspected of being “probably also entoparasitic on these ants or their progeny during its larval stages” [1]. However, the parasitic relationship was never proved. Only very recently a Neotropical, gregarious endoparasitoid species, *Blanchardiscus* sp. (?*pollux*) (determination by J. S. Noyes), was recorded from French Guiana attacking pupae of the ponerine ant *Pachycondyla goeldii* [45] and thus constitutes the first true case of parasitism on ants for this family. However, no information has yet been published, and the exact identification of the species still needs to be confirmed.

**2.2.3. Eucharitidae.** This is a small family but the largest and most diverse group of hymenopteran parasitoids attacking ants since all of its members, where the host is known, parasitize ant brood [11, 66, 72, 78, 83, 189–191]. Fifty-three genera and more than 470 species are currently described and distributed in three subfamilies: Oraseminae, Eucharitinae, and Gollumiellinae.

All of the species have a highly modified life cycle [63, 66, 76, 83, 108]. Like the Perilampidae [191] and the ichneumonid species *Euceros frigidus* [192], but unlike most parasitic wasp species, eucharitid females deposit their eggs away from the host nest, in or on plant tissue (leaves and buds) [72, 189] (Figures 2(a) and 2(b)), and the very active, minute (less than 0.13 mm), strongly sclerotized first-instar larva is termed a “planidium” (Supplementary material 2 available online at doi:10.1155/2012/134746). It is responsible for gaining access to the host ant brood by using various phoretic behaviors including either attachment to an intermediate host (as in some orasemine species [11, 72, 83, 86, 88, 93] and, possibly, in *Gollumiella antennata* (Gahan) ([190] but see [72]) or, more generally, to foraging ant workers. On occasions (as is apparently the case for *Pseudochalcura gibbosa* and *Gollumiella longipetiolata*), attractive substances are suspected to be present in or on the eggs [46, 72]. Within the nest, the planidium attaches itself to an ant larva (Figures 2(c) and 2(d)): Eucharitine planidia attach externally to the host larva, whereas orasemine and gollumielline planidia partially burrow into the host larva, in the thoracic region just posterior to the head capsule [11, 70, 72]. All of the Eucharitidae develop as koinobiont, larval-pupal ectoparasitoids. At molting of the host larva, the planidium migrates to the ventral region, just under the legs (Figure 2(e)), of the newly formed ant pupa for further development which is only completed when the host pupates [76, 83, 93, 189] (Supplementary material 3 available online at doi:10.1155/2012/134746). In general, only one parasitoid develops per host but, occasionally, more than one adult eucharitid can develop in a single host (superparasitism) (Figure 2(f)) [72, 83], especially when larger brood (sexual brood) is parasitized [193, 194], and one exceptional case of multiparasitism involving two different species from two different eucharitid genera (*Dilocantha lachaudii* and *Isomeralla coronata*) has even been reported from a single pupa of the ectatommine ant *Ectatomma tuberculatum* [79]. In almost all of the cases, adults emerge among ant brood (but see [77]), and, even if in some cases they are well treated within the nest by their hosts (as is the case for *Orasema coloradensis* which is transported, cared for, and even fed by the workers of *Pheidole bicarinata* [70]), they have to leave the host nest to reproduce. Ants show only moderate aggression to newly emerged eucharitids [58, 70, 75, 106, 189, 195, 196], suggesting passive or active chemical mimicry of the host ants [58, 75, 195]. If the parasitoid wasps do not exit their host nest by themselves, ant workers transport them outside (Figure 2(g)) as if they were refuse [58, 77, 196], ultimately enhancing wasp dispersal. Parasitism is very variable and localized in time and space [106, 193, 194]. A very high local prevalence may lead to only a low impact at the regional scale, suggesting that these parasitoids do not

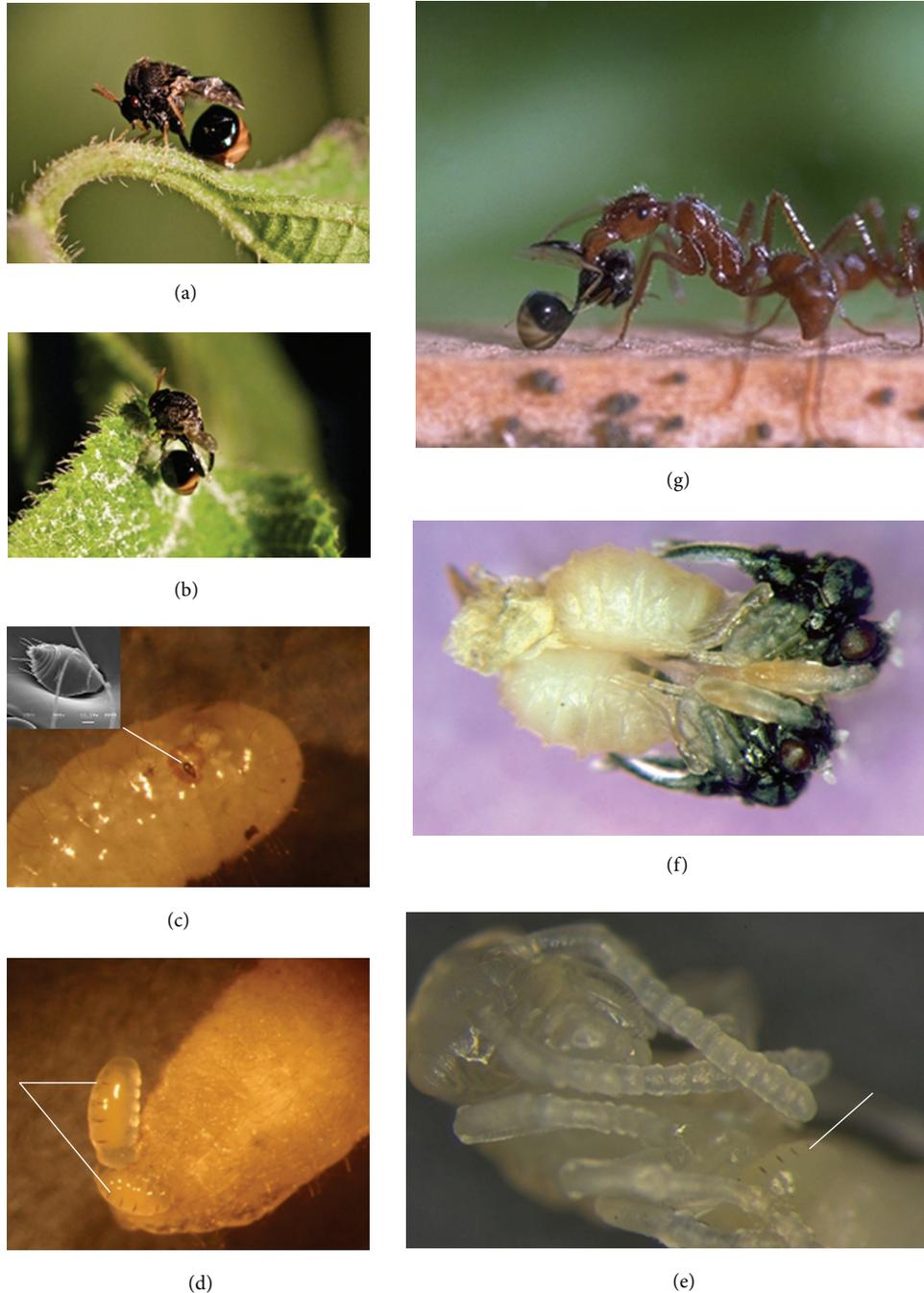


FIGURE 2: Life cycle of a typical eucharitid wasp. (a) Female *Dilocantha lachaudii* ovipositing on *Lantana camara* L. (Verbenaceae). (b) *D. lachaudii* female with eggs scattered on leaf surface. (c) Planidium (white pointer) attached upon an *Ectatomma tuberculatum* larva. Insert: SEM picture of a planidium. (d) Two *D. lachaudii* swollen planidia (white pointers) feeding upon an *E. tuberculatum* larva. (e) 2nd instar larva (white pointer) relocated after host pupation. (f) Two *D. lachaudii* pupae from a single host pupa. The host cocoon has been removed. (g) *E. tuberculatum* worker transporting a recently emerged *D. lachaudii* female. Photos: J.-P. Lachaud and G. Pérez-Lachaud.

have a major influence on the dynamics of their ant host population [194].

According to Heraty [11], the hypothesized phylogeny of Eucharitidae is highly correlated with the subfamilies of their ant hosts and responsible for differences in behavior related with egg placement, activity of the planidium, and

access to the ant host. Oraseminae (*Orasema*, *Orasemorpha*, and *Timioderus*) primarily attack myrmicine ants (numerous species of *Pheidole* and *Solenopsis*, and some species of *Diplorhoptrum*, *Monomorium*, *Temnothorax*, *Tetramorium*, and *Wasmannia*, see Table 1), and exceptionally formicines (*Formica subnitens* and *F. oreas comptula* in the case of

*O. coloradensis*, [88]) or ecitonines (*Eciton quadriglume* in the questionable case of *O. rapo*, [11, 83]). For Eucharitinae, the only two host records for the tribe Psilocharitini (*Neolosbanus*) concern the ponerine genus *Hypoponera* [83], while the numerous members of the tribe Eucharitini are essentially parasitic on medium to large ponerines (*Pachycondyla*, *Odontomachus*, and *Dinoponera*) and ectatommines (*Ectatomma*, *Gnamptogenys*, *Typhlomyrmex*, and *Rhytidoponera*), but also on myrmeciines (*Myrmecia*) and numerous formicines (*Anoplolepis*, *Calomyrmex*, *Camponotus*, *Cataglyphis*, *Formica*, *Lasius*, and *Polyrhachis*); without exception, all of the scarce records of associations of eucharitines with myrmicine ants (*Messor*, *Myrmica*, and *Pogonomyrmex*) are highly doubtful (Table 1). Finally, the only host record for the Gollumiellinae concerns a formicine (*Paratrechina*).

The hosts of most eucharitid genera seem to be restricted to only one or a few closely related ant genera and, for a long time, all species were considered as host-specific parasitoids, at least at the host genus level [83]. However, recent results [76, 78, 79] raised questions concerning the degree of host specificity in eucharitids and about the factors that determine the association of these parasitoids and their hosts. Results in the guild of eucharitid parasitoids associated with ponerine ant species in southeastern Mexico and French Guiana suggest that some eucharitid wasps tend to be oligophagous in their host choice: some eucharitid species can attack different hosts from different genera and different subfamilies such as *Kapala iridicolor*, which parasitizes one species of *Ectatomma*, two of *Gnamptogenys*, and one of *Pachycondyla* [76, 78]. Furthermore, concurrent parasitism has been reported for *Ectatomma tuberculatum*, which is simultaneously parasitized by *Dilocantha lachaudii*, *Isomeralla coronata*, and *Kapala* sp. [79], or for *E. ruidum* parasitized by two *Kapala* species, *K. iridicolor*, and *K. izapa* [76, 193].

**2.2.4. Eulophidae.** The family Eulophidae is the largest of the Chalcidoidea with up to 4470 species in 297 genera. The majority of the species are primary parasitoids attacking a large variety of insects (mainly Lepidoptera and Coleoptera, but also Diptera, Thysanoptera, and Hymenoptera), and occasionally mites or spiders. Many species are facultative or obligate hyperparasitoids of other Hymenoptera, and some are even phytophagous. Entomophagous larvae can develop as koino- or idiobionts, gregarious or solitary, and ecto- or endoparasitoids, and according to the species, eulophids can attack eggs, larvae, pupae, or even the adults of their hosts [197].

Despite the large number of species in this family, parasitization of ants is uncommon among Eulophidae, and only few associations involving eulophid wasps and ant hosts have been reported to date. Almost all are from genera belonging to the subfamily Entedoninae. Three concern species indirectly associated with ants as they parasitize insects living in ant nests: *Pediobius acraconae* Kerrich which has been reported [114] from a last instar larva of the pyralid lepidopteran *Acracona remipedalis* Karsch found in a nest of *Crematogaster depressa* (Latr.) or *C. africana* Mayr in Nigeria, and both *Microdonophagus woodleyi* Schauff in Panama



FIGURE 3: Larva of the neotropical weaver ant *Camponotus* sp. ca. *textor* parasitized by the gregarious endoparasitoid *Horismenus myrmecophagus* (Eulophidae). Several wasp larvae can be observed through the host cuticle. Photo: G. Pérez-Lachaud.

and *Horismenus microdonophagus* Hansson et al. in Mexico, which parasitize larvae of *Microdon* sp. syrphid flies living in nests of the dolichoderine *Techomyrmex fulvus* (Wheeler) (referred to as *Tapinoma fulvum*) [198] and of the formicine *Camponotus* sp. ca. *textor* [112], respectively. Three other species (two Entedoninae and a Tetrastichinae) have been reported associated with ant nests, but direct parasitism on the ant brood was not clearly established in any of these cases: *Myrmobomyia malayana* Gumovsky and Bouček with nests of an ant species of the genus *Dolichoderus* in Malaysia [199], an unidentified species of *Horismenus* from the bivouac and refuse deposits of the army ant *Eciton burchellii* [188], and an unidentified species of *Tetrastichus* from a nest of the formicine *Myrmecocystus mexicanus* Wesmael in Nevada [94].

In fact, only five species are known as true primary parasitoids of ants (Table 1). An unidentified gregarious parasitoid, apparently closely related to the genus *Paracrius* (according to Gahan in [109]), possibly *Horismenus* sp. [112], was recorded parasitizing larvae of the myrmicine *Crematogaster acuta* in Guyana, the prepupae of another unidentified species of *Crematogaster* were parasitized by *Myrmokata diparoides* [113] in Cameroon, *Pediobius marjorieae* was reared from cocoons of the formicine ant *Lepisiota* sp. in Uganda [114], and two species of *Horismenus*, *H. floridensis* and *H. myrmecophagus*, were found parasitizing the pupae of *Camponotus atriceps* and *C. floridanus* in Florida [111], and of the weaver ant *Camponotus* sp. ca. *textor* in Mexico [112], respectively. In the latter two cases, *Horismenus* larvae develop as gregarious endoparasitoids of the ant larvae (Figure 3), and large numbers of parasitoid individuals can develop from the same host: up to 21 for *H. floridensis* and between 4 and 12 for *H. myrmecophagus*. Finally, two other cases deserve to be added to this list since two other ant species have recently been found parasitized by eulophids: the ponerine ant *Pachycondyla crenata* (Roger) in Mexico and an unidentified species of *Camponotus* (*Dendromyrmex*) in French Guiana [112]; however, the identity of the parasitoids has not been confirmed yet.

2.2.5. *Eurytomidae*. Eurytomidae is a moderate-sized family with 90 genera and at least 1400 nominal species [13, 32, 200, 201]. Eurytomid wasps exhibit a wide range of biologies, but most of the larvae are endophytic either as seed or plant stem eaters or as parasitoids of gall formers or other phytophagous insects. Most species are primary or secondary parasitoids, attacking eggs, larvae, or pupae of various arthropods (Diptera, Coleoptera, Hymenoptera, Lepidoptera, Orthoptera, and Araneae).

A few species have been reported as indirectly associated with ants, like *Eurytoma rosae* Nees von Esenbeck found with *Lasius flavus* and *Eurytoma* sp. found with *Formica* (?) *rufibarbis* (misidentified as *Polyergus rufibarbis*) [20], but most probably these eurytomids only fed on the gall-forming cynipid larvae and/or on the gall tissue on *Rosa* spp. which are visited by these ant species, without any direct relationship with the ants. Recently, various adults of a new genus and species, *Camponotophilus delvarei* Gates, were found within nests of the weaver ant *Camponotus* sp. ca. *textor* [202], but the exact nature of their relationship with the ants remains unclear. As a matter of fact, only 3 or 4 species from the single genus *Aximopsis* (see Table 1) have been reported from Guatemala, Costa Rica, Guyana, Colombia, and Peru as parasitoids of queens of various species of dolichoderine ants (*Azteca alfari*, *A. australis*, *A. constructor*, *A. pitieri*, *A. ovaticeps*, and *A. salti*) and one formicine (*Camponotus balzani*), all of which colonize *Cecropia* spp. internode chambers by chewing a hole through a prostoma and entering the internode. The parasitoids attack only founding queens and feed on their host, while the internode chamber is sealed with parenchyma scraped from the internal stem walls [115, 116, 118]; there is never more than one wasp larva or pupa per foundress ant [117]. Queen parasitization was thought to occur before they entered their dwellings (Bailey, in [115]); however, as suggested by Davidson and Fisher [119], the location of the ant host may occur through searching for host plants since female *Aximopsis* were observed to visit various seedlings, where they inspected newly sealed prostoma. This fact has been confirmed recently. A picture of an *A. affinis* female ovipositing through a prostoma into an *Azteca* queen at La Selva Biological Station, Costa Rica, was provided by Weng et al. [203] (their Figure 16). In this site, among the internodes that harbored *Azteca* ants, 43% contained dead queens, of which 13% contained *A. affinis* [203].

2.2.6. *Perilampidae*. Perilampidae is a small family closely related to the Eucharitidae, composed of up to 270 species from 15 genera. A feature shared with Eucharitidae is that the first-instar larva, the “planidium”, is responsible for gaining access to the host, rather than the egg-laying female [191]. Most species are hyperparasitoids on ichneumonid wasps or tachinid flies which are primary parasitoids of Hymenoptera or Lepidoptera, or parasitoids of wood-boring platypodid and anobiid beetles, and some species can attack Orthoptera, Neuroptera, or Hymenoptera [190, 204].

Association of perilampids with ants seems extremely casual. The only report deals with an unidentified species from Peru found parasitizing cocoons of the ponerine ant

*Pachycondyla luteola*, inhabiting internode chambers of a *Cecropia*, with as many as nine perilampid wasps emerging from a single pupa of this ant [119]. However, no other details were ever published, and the species apparently remained undescribed.

2.3. *Ichneumonoidea*. The superfamily Ichneumonoidea, with only two extant families, accounts for more than 40,000 species around the world, and there are estimated to be approximately 100,000 species [205–207]. Most are primary ecto- or endoparasitoids, idio- or koinobionts, especially attacking immature stages of a wide variety of insects and arachnids, and more occasionally adults. Some members use many different insects as hosts, and others are very specific in host choice. Various ichneumonoids are successfully employed as biological control agents in controlling insect pests such as flies or beetles.

2.3.1. *Braconidae*. This is a very large family with 48 subfamilies, more than 1050 genera and about 17,600 described species worldwide and exhibiting a variety of biologies [207–209]. The total number of species is estimated to be 40–50,000. Many braconids parasitize nymphal stages of Hemiptera, Isoptera, and Psocoptera; a few genera also parasitize adult Coleoptera and Hymenoptera [209]. Two major lineages occur within the Braconidae: (a) the cyclostome braconids, most of which are idiobiont ectoparasitoids of concealed Lepidoptera and Coleoptera larvae although many are koinobiont endoparasitoids of Diptera and Hemiptera, and (b) the noncyclostome braconids which are all endoparasitoids, and most generally koinobionts, typically attacking an early instar of their hosts (see [210] for a comprehensive overview of their biology).

Numerous braconid species have been reported in association with ants. Some, such as *Compsobraconoides* sp. [28] and *Trigastrotheca laikipiensis* [29], are predatory on several developmental stages of ants. Others, such as *Aclitus sappaphis* Takada and Shiga found in nests of *Pheidole fervida* Smith [211, 212], *Paralipsis enervis* (Nees von Esenbeck) found with *Lasius niger* [213], or *P. eikoeae* (Yasumatsu) found with *L. japonicus* Santschi (referred to as *L. niger* (L.)) and *L. sakagami* Yamauchi and Hayashida [212, 214], are in fact primary parasitoids of root aphids and can only be considered as indirectly associated with the aphid-attending ants; however, they have developed highly sophisticated relationships with their hosts involving chemical mimicry and chemical and tactile communication to obtain regurgitated food (trophallaxis).

For several other species, the exact nature of the association with the ant host has not been clearly established, but at least 15 euphorine species can be considered as true parasitoids of adult ants even if direct evidence of oviposition has been obtained for only 11 of them (see Table 1). All of these parasitoids are grouped in three extant genera, *Elasmosoma*, *Kollasmosoma*, and *Neoneurus*, and one fossil genus, *Elasmosomites*, all belonging to the tribe Neoneurini. Evidence from Eocene Baltic amber, as demonstrated from an individual of *Elasmosomites primordialis* emerging from the abdomen of a *Lasius* worker (Figure 4(a)), indicates that

the parasitoid association between neoneurine braconids and ants has been in existence for at least 40 million years [136]. Although oviposition into the abdomen of adult worker ants has been reported on several occasions [56, 120, 121, 126, 127, 140], detailed descriptions were rare and, until recently, restricted to only two species. In the case of *N. mantis* attacking *Formica podzolica*, Shaw [142, 143] gave interesting information both on the “perching” behavior displayed by the parasitoid females in their ambush strategy to locate their hosts and on the attack sequence which is completed in less than 1 s and is characterized by a reduction of the usual braconid oviposition sequence, the first two steps (antennation of the host and ovipositor probing) being entirely lost in favor of speed. For *E. michaeli*, Poinar [131] not only described the attack behavior, exclusively focused on major workers of *Formica obscuriventris clivia* (Figure 4(b)), but also provided invaluable information on the altered behavior of parasitized ants, on the development of the immature stages, and on cocoon formation and adult emergence. Immature stages of Neoneurini parasitoids attacking adult ants develop as koinobiont endoparasitoids in the abdomen of workers, and fully developed larvae leave the host to pupate in the soil [131].

Very recently, slow motion video recordings were used to describe the oviposition behavior in adult ants for 3 other species [129], and we refer the reader to their excellent films, which show the variability in oviposition behavior within the tribe. Neoneurini wasps parasitize worker ants in the vicinity of the nest entrance(s), or while foraging. Females of *Elasmosoma luxemburgense* hover over the nest entrance of *Formica rufibarbis* and attack workers from behind, grasping the ant abdomen with the three pairs of legs involved, and probably ovipositing through the anus. The whole behavioral sequence (alighting, grasping, ovipositor insertion, and takeoff) lasted a mean of 0.73 s. The ants were aware of these attacks, turning around and chasing the wasps with open mandibles ([129] doi: 10.3897/zookeys.125.1754.app1). Females of *Kollasmosoma sentum* attack workers of *Cataglyphis iberica* in the vicinity of nest entrances, or when carrying prey and walking more slowly than usual. Attacks usually occurred during the brief stops characterizing *Cataglyphis* workers walks. The wasps were extremely fast and attacked the ants from behind. Oviposition took place in both the dorsal and ventral surfaces of the ant’s gaster, likely through intersegmental membranes. Wasps adjusted their alighting strategies according to the direction of their own approach to the targeted ant, and to the position of the ant’s gaster (horizontal or vertical position, distinctive for the genus *Cataglyphis*), and accomplished extraordinary pirouettes. The whole oviposition behavior lasted only 0.05 s on average. The ants were often aware of the presence of the parasitoids, aggressively turning around with open mandibles, or extending their hind or middle legs to hit them ([129] doi: 10.3897/zookeys.125.1754.app2). Finally, *N. vesculus* females alight and probably oviposit in the mesosoma of *Formica cunicularia* workers. As for *N. mantis* [142, 143], they were observed ambushing or hovering over the nest entrance. Females preferentially attacked ants while at a vertical



(a)



(b)

FIGURE 4: (a) *Elasmosomites primordialis* larva (white pointer) emerging from the abdomen of a *Lasius* worker in Baltic amber. Photo courtesy of G. Poinar Jr. (see [136]). (b) *Elasmosoma michaeli* larva leaving its *Formica obscuriventris clivia* host to pupate in the soil. Photo courtesy of G. Poinar Jr.

position (going up a tree trunk, e.g.). The wasps approached the ants from behind, alighted, held the ant’s thorax with their raptorial fore legs, bent their abdomen towards the postero-lower part of the ant’s thorax, and oviposited. The ovipositor is thought to be inserted near the posterior coxal cavities. The whole oviposition behavior lasted a mean of 2.02 s ([128] doi: 10.3897/zookeys.125.1754.app3).

With few exceptions, neoneurine wasps have been found in association with formicine ants [129, 207, 215, 216]. It is thought that formic acid used by these ants could serve also as a kairomonal stimulant to host-seeking hymenopterous parasitoids [120, 127, 129]. Far less is known about the fate of parasitized ants. According to Poinar [131], *Formica* ants parasitized by *E. michaeli* form an assembly along the edge of their superficial nest when the parasitoid larvae are about to leave the host to pupate. This behavioral modification is thought to increase the survival of adult wasps.

Several morphological and behavioral adaptations, apart from rapidity of attack, contribute to the success of these wasps in parasitizing aggressive adult ants: for example, the vestigial tarsal claws and enlarged pulvilli (suction like disks, [130, 131, 217]) of *Elasmosoma* spp., or the raptorial fore

legs of *Neoneurus* spp., enable wasps to grasp and hold the ant firmly while ovipositing. Likewise, the peculiar ventral spine of *K. sentum* females, located on the fifth sternite, could help to fix the wasp's position during oviposition, when the body of the wasp goes back tending to the vertical position, and fore legs detach from the ant's cuticle. Finally, the longitudinal disposition of *K. sentum* females's tarsi on the ant metasoma, one over the other, enables the necessary rotation of the body to adjust itself to the position of the ant's gaster, before oviposition. The wasp rotates counterclockwise if the right tarsus is placed over the left one; and if the left tarsus is placed over the right one, the rotation is clockwise.

**2.3.2. Ichneumonidae.** Ichneumonidae is the largest family in the Hymenoptera with about 23,330 described species worldwide in 46 subfamilies and 1207 genera; the total number of species is estimated to be more than 60,000 [207, 218, 219]. Most of the members of this large family are parasites of holometabolous insects, but a few species parasitize spiders (egg sacs, spiderlings, or adults) or egg sacs of pseudoscorpions. Many ichneumonids are hyperparasitoids of other ichneumonoids or of tachinid flies, and some species are egg-larval parasitoids, laying an egg in the host egg but consuming the host in its larval stage [218, 219].

Various species of the genus *Gelis* (all of them initially referred to as *Pezomachus*) and a few others of the genera *Agrothereutes*, *Aptesis*, *Pleolophus*, and *Thaumatogetelis* have been reported by various authors to be associated with ants of the genera *Lasius*, *Formica*, *Myrmica*, *Temnothorax*, and *Solenopsis* [24, 56, 220–222]. However, no information is available on the exact relationship with their ant host, except that in some cases (such as *Pleolopus micropterus* (Gravenhorst) (referred to as *Pezomachus micropterus*) and *T. vulpinus* (Gravenhorst) (referred to as *Pezomachus vulpinus*)), they were clearly reported as “found in the nest of *Formica rufa*, not reared from cocoons” [220]. Until now, true ichneumonid parasitism on ants has been demonstrated only for 3 species, all belonging to the subfamily Hybrizontinae and very likely to the same tribe Hybrizontini. The most ancient report dates back to 1852 [145] and concerns *Eurypterna cremieri* described as hovering over a nest of *Lasius fuliginosus* in Germany. This behavior, suspected to be related to the search of an appropriate host, was later confirmed by different authors not only for the same host species in France and Italy [146–148] but also for three other species of ants in the genera *Lasius* and *Formica* in France, England, and Japan [123, 144, 149]. In the early 20th century, Cobeli [148] described how four females of *E. cremieri* were hovering over trails of *L. fuliginosus*, while ants were moving their nest to another nest site, inspecting each ant worker that was transporting a larva. The female parasitoids quickly drew closer to the larva, and folding up the abdomen touched it, presumably depositing an egg. Such behavior was only observed with ants transporting a larva and did not trigger any reaction from the workers. In spite of the interesting information supplied, this report passed more or less unnoticed until 2010 when the parasitic nature of this behavior could be confirmed (and even photographed) concerning *Lasius nipponensis* transporting brood between

two nests [149]. Only workers carrying something in their mandibles were tracked by *E. cremieri* females hovering about 2 cm above them. And only those carrying a larva were attacked after a sudden dive of the wasp which gripped the targeted larva with the tarsi of its fore and middle legs, bent its abdomen down, exerted its ovipositor, and oviposited in the larva before flying away in search of a new host. The complete sequence lasted less than 1 s and elicited some brief excitement from the worker ant. Dissection of a stung ant larva showed that a wasp egg was present in the somatic cavity. Another undescribed Hybrizontinae species (gen. nov. sp. nov.) was similarly reported by the same authors as hovering over workers of the slow moving ant *Myrmica kotokui* which were holding something in their mandibles. As for *E. cremieri*, only those carrying a larva were more closely inspected and were attacked in a similar manner as previously described, but in that case, the complete attack sequence lasted longer (3–4 s), and oviposition itself took at least 1 s. A third case of ant larval parasitism has very recently been confirmed and involves *Hybrizon buccatus* females. This species had been frequently reported in association with (or hovering over) different ant species from various genera (*Myrmica*, *Lasius*, *Formica*, and *Tapinoma*, see Table 1) [24, 140, 144, 146, 152, 153] and was reared from nests of *Lasius alienus* where the ichneumonid naked pupae had been found among ant-host cocoons [150]. But it was not until 2011 that the oviposition into larvae transported by *Lasius grandis* workers could be observed and filmed during brood transfer between two nest entrances [129]. Only final instar larvae were attacked, in a very similar way to that previously described for *E. cremieri*, and the complete sequence lasted between 0.40 and 0.58 s. Chemical and/or visual cues are likely to be involved in the location of the ants' trail since *H. buccatus* females have been observed continuously hovering over the trail for a period of time, even in the absence of ants. Finally, considering both the hovering behavior as a reliable evidence of parasitism and the fact that all three ichneumonid parasitoids known until now to attack ants are restricted to the Hybrizontinae, two other cases are likely to be added to our list: *Ghilaromma fuliginosi* and *H. rileyi* which have been reported swarming and hovering over the nests of *Lasius fuliginosus* [150, 151] or attracted to a disturbed nest of *L. alienus* [154], respectively. However, in both cases, direct oviposition into ant larvae or adults needs to be confirmed.

### 3. Conclusions

Since the last paper on parasites of social insects by Schmid-Hempel [7], the number of reliable records of parasitoid wasps attacking ants and their brood has grown dramatically from about 43 species to at least 138 belonging to 9 hymenopteran families. Furthermore, the knowledge of the biology and behavior of those wasps and the nature of their interactions with ants has significantly progressed, though many gaps still remain. Most likely, hymenopterous parasitoids of ants are more abundant than suggested by our list of reliable records, and future studies focusing on the

immature stages of ants under close scrutiny would certainly increase this list substantially.

All castes of ants and all developmental stages, excepting eggs, are the target of parasitoid wasps. For example, neoneurine braconids parasitize adult worker ants while foraging or performing other activities outside the nest [129, 131, 143], while eurytomids of the genus *Aximopsis* attack adult queens at the very moment of nest foundation [115, 116, 118, 119]. However, in most cases, ant larvae are the target of parasitoid attacks, either inside or outside their nests. Larvae can be parasitized outside the protective walls of the nest during transportation when ants move from one nest to another as for some euphorine braconids and hybridine ichneumonids [129, 149], or while being employed to fix or build a new nest as occurred for the green weaver ant larvae attacked by the chalcidid *Smicromorpha* [41]. Most often, ant larvae are attacked inside the nest, notwithstanding the pugnacious character of ants. For eucharitid and perilampid wasps, planidia are transported by phoresis into the targeted nest where they actively search for a larval host. The extremely small size of the planidia is assumed to facilitate both entrance into the host colony and initial parasitization [195], but in most other parasitoid wasps (diapriids, encyrtids, entedonine eulophids, and some eurytomids), it has been assumed that it is the female that searches for a host nest, enters it, and oviposits on or in the larval host. So far, however, how the females gain access into the ant nest and complete the oviposition process has never been described, and the initial stages of development of these parasitoids are in most cases unknown (but see [35, 131]).

Hymenopterous parasitoids attacking ants exhibit a wide array of biologies and developmental strategies: ecto- or endoparasitism, solitary or gregarious, and idio- or koinobiosis. Besides, the behavioral strategies evolved to cope with ant aggression or to exploit the communication system of ants are also impressive. Most of these parasitoids belong to families with species using a wide range of insects or arthropods as primary hosts, and in many cases of recorded associations between parasitic wasps and ants [20, 23, 112, 114, 128, 160, 186, 198], the primary host of the parasitoids is not the ant but another insect species present in the ant nests. Such indirect association through parasitism of trophobionts or other myrmecophiles suggests that a possible path to the parasitization of ants by hymenopterous parasitoids could have evolved as a shift from the initial primary host (Diptera, Coleoptera, or other insect myrmecophiles) to the ant host larvae through a gradual process of association and integration with the ant hosts. Such a hypothesis proposed for diapriids by Huggert and Masner [160] and widened by Hanson et al. [223] to hymenopterous parasitoids in general might apply for numerous families, and a supporting example has recently been suggested among eulophids [112]. However, other evolutionary paths are likely to be involved in the case of eucharitids and perilampids and those species that attack adult ants and deserve further study.

Despite a significant increase in our knowledge of hymenopterous parasitoids of ants in the last 15 years, the remark of Schmid-Hempel [7] concerning parasitism in social insects in general: “the existing knowledge is bound to

be a massive underestimation, since the true abundance and distribution of parasites remain to be discovered” is still, more than ever, a topical subject. Most hymenopterous parasitoids attacking ants remain to be discovered. Moreover, despite the presumed importance of some of them as natural enemies of ants, few quantitative data are available on the impact of these natural enemies on their hosts (see [224]). Based on their abundance and success in attacking ant hosts [36, 83, 193, 194], some parasitoid wasps like, for example, diapriids and eucharitids, seem excellent potential models to explore how parasitoids impact ant colony demography, population biology, and ant community structure, and further studies focusing on these issues will certainly contribute to deepening our knowledge on this important group of parasites.

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## Research Article

# Predatory Behavior of *Canthon virens* (Coleoptera: Scarabaeidae): A Predator of Leafcutter Ants

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We present a detailed description of the predatory behavior of the beetle *Canthon virens* Mannerheim, 1829, on the leafcutter ant *Atta* sp. We observed 51 acts of predation, which were also recorded on film and subjected to behavioral analysis. *Canthon virens* exhibited 28 behaviors while predating upon *Atta* sp. queens. Adult beetles search for queens while flying in a zigzag pattern, 15 to 20 cm above the ground. After catching a queen, the predator stands on its back and starts cutting the queen cervix. Once the prey is decapitated, the predator rolls it until an insurmountable obstacle is reached. The distance from the site of predation to the obstacle can vary widely and is unpredictable. The beetle rolling the queen also buries it in a very peculiar way: first, it digs a small hole and pulls the queen inside, while another beetle is attached to the prey. The burial process takes many hours (up to 12) and may depend on the hardness of the soil and the presence of obstacles. In general, one or two beetles are found in a chamber with the queen after it is buried. They make the brood balls, which serve as food for the offspring. This study contributes to the knowledge of the predatory behavior of *Canthon virens*, a predator poorly studied in Brazil and widespread in the country.

## 1. Introduction

*Canthon dives* Harold, 1868, and *Canthon virens* Mannerheim, 1829, prey on queens of leafcutter ants, *Atta* spp., after the queen nuptial flight. For this reason, the beetles are considered natural biological control agents of these ants. In 1937, Lichti [1] was the first to report the predation of leafcutter ant queens by *Canthon dives* beetles. Later, Navajas [2] reported that not only queens but also winged forms of both sexes are preyed upon by *Canthon virens*.

*Atta* queens lose their wings after their nuptial flight and start looking for a place to dig. When searching for a suitable place to build their nests, and during nest digging, ant queens are most vulnerable to their beetle predators. They can be attacked by one to over six individuals simultaneously [3]. The predation rate of *Atta* by *Canthon virens* [4] alone is 7.6%, but when other species of *Canthon* are considered, this proportion can reach more than 50% [3].

Using the tissues of their prey, *Canthon* beetles build two or three brood balls for their larvae. Detailed observations of this behavior have been documented for *Canthon virens*: first, the beetle positions itself, with some difficulty, on the back of the queen. Then, using the clypeus as a lever and the jagged edges of the tibiae as a saw, the predator cuts the prey cervix or neck [5, 6]. Both male and female beetles prey on queens [7], but a relationship between the size of the prey (queen) and the predator (beetle) has not been found. However, there is a positive relationship between the densities of queens and beetles, suggesting that the relationship between *Atta* spp. and *Canthon virens* might be obligatory [3, 6].

The brood balls are buried  $7.7 \pm 2.3$  cm deep. Each female beetle lays one egg in each brood ball, and males and females remain in the burrow until the offspring has developed [3].

Little is known about the predation sequence of *Atta* queens by *Canthon virens*. In view of this gap, we have

investigated and provide a detailed description of the steps from searching for *Atta* queens to the confection of the brood balls.

## 2. Material and Methods

The behavior of the beetle predator *C. virens* was monitored for 5 years in a natural situation, in several commercial plantations of *Eucalyptus grandis* or natural vegetation (Cerrado) in the municipalities of Botucatu, Itatinga, and Anhembi, in the state of São Paulo, Brazil.

Fifty-one cases of queen predation by beetles were filmed on VHS, using two Panasonic M-8 cameras. Filming began when the queens came to the ground after their nuptial flight. The videos were labeled and stored for later analysis.

All behaviors were quantified by counting the number of times each occurred during 1 minute, at 2-minute intervals. We then used those results to calculate the frequencies of each behavior. Behaviors were then analyzed for a pattern.

## 3. Results

**3.1. Behaviors during Predation.** A total of 28 behaviors was recorded during the predation of *Atta* queens (Figure 1), as follows: (1) A single beetle flies over the queen in a zigzag pattern before capturing it. (2) A single beetle on the ground climbs onto the back of a walking queen. (3) Two beetles fly over a queen, but only one attacks and beheads it. (4) A single beetle flies over a queen and catches it. (5) A single beetle flies over a queen, is momentarily unable to catch it, but ends up succeeding. (6) A single beetle flies over a queen, lands on it, and then abandons it. (7) A beetle positions itself on the thorax of a queen, with its head pointing to the queen cervix. (8) A beetle attaches itself to the thorax of a queen, with its head turned to one side, that is, the time required for the beetle to turn in the direction of the queen head and start beheading it. (9) A beetle attaches itself to the queen thorax, with the head facing the queen gaster; the beetle then points its head towards the head of the walking queen, that is, the time required for the beetle to stand with its head toward the head of the queen and start cutting. (10) A beetle attaches itself to the queen thorax while the queen is digging and then changes position, standing up to kill it. (11) A beetle attaches itself to the queen thorax and then abandons it. (12) A beetle, on the ground, secures the queen abdomen, leaves its prey momentarily, and then comes back to catch it again. (13) A beetle, on the ground, secures the queen abdomen but does not turn its head toward the prey head. (14) A beetle cuts the queen cervix, the time needed to start and finish the process. (15) A beetle walks over a queen that is moving with difficulty. (16) A beetle attempts to cut the queen neck, but does not succeed. (17) After cutting the queen neck, the predatory beetle walks on the back of its prey, examining it. (18) After cutting the queen neck, the predatory beetle walks away. (19) After cutting the queen neck, the predatory beetle examines its prey, walks to the side, and comes back to roll it, that is, the time spent from cutting the queen cervix to starting to roll it. (20) After cutting the neck of the queen, the predator examines it, walks beside it, but does not continue with the

process of predation. (21) Numerous beetles show up and “dispute the prey” by rolling it together. When only two beetles are left, one is attached to the queen and the other one rolls it, that is, the time from the dispute to the burial. (22) Numerous beetles dispute the dead body by rolling it together. When only one beetle is left, it rolls the queen to the burial site. (23) More beetles show up and dispute a live queen (walking), which is being preyed upon by another beetle. When only three beetles are left, one goes away and two remain. (24) More beetles show up and dispute a live, yet immobile queen, which is being preyed upon by another beetle. One beetle goes away and the other stays with the original predator. (25) The beetle that killed the queen rolls it. From time to time it climbs up and down the queen, and then goes back to rolling it, that is, the time spent rolling and going up and down. (26) A beetle, after rolling its prey, begins to excavate the soil to bury it. It goes to the bottom of the hole and comes back to the surface. If two beetles are present, one digs and the other remains on the queen. (27) A beetle, after rolling the queen, digs a small hole in the ground, buries the queen only partially, and remains on the surface with the abdomen facing up, that is, the time spent to dig, to bury the queen superficially, and to position the legs up. (28) A beetle buries the queen very deep and then sits on the surface with the abdomen facing up.

**3.2. Descriptive Sequence of Behaviors.** Adult beetles showed up for the nuptial flight only. They were seen in greater numbers a little before and during the nuptial flight flying in a zigzag pattern, more or less 15 to 20 cm from the ground, searching for prey. They flew very fast, and periodically interrupted their activity to rest on the ground or on the vegetation.

A total of 92% of the queens (47 beetles) were captured by a beetle that was flying in zigzag and came upon the prey thorax (Figure 3). In 4% of the cases (2 beetles), however, the queen was not captured. One queen (2%) was chased by two beetles, but only one beetle finished the task.

An alternative, rarer capture method observed in 4% of the cases did not involve a zigzag flight. In that case, a beetle walking on the soil surface tried to climb on the back of a queen to kill it. In some instances, a beetle that failed to succeed using this method tried again, but seldom (2%) on the same individual.

An interesting result of our study is the observation that female beetles are those that capture and behead *Atta* queens. When a female beetle sits on the thorax of its prey, it often has the head turned to one side (40% of cases, 20 beetles) of the queen body. The predator then turns its head toward the head of the queen. Often, the female beetle sits in the correct position to cut the queen neck (Figures 1, 2(a), and 2(b)), but sometimes the female has its head turned toward the queen gaster (27% of cases, 14 beetles) (Figure 2(d)). Once the predator is positioned correctly (Figure 2(b)), it immediately begins to cut the queen neck. After a queen is killed, many beetles join the killer (Figure 2(e)), but only two are left to bury the prey (Figure 2(f)).

Division of labor according to sex in *C. virens* is well defined. The female catches, kills, rolls, and buries the prey.

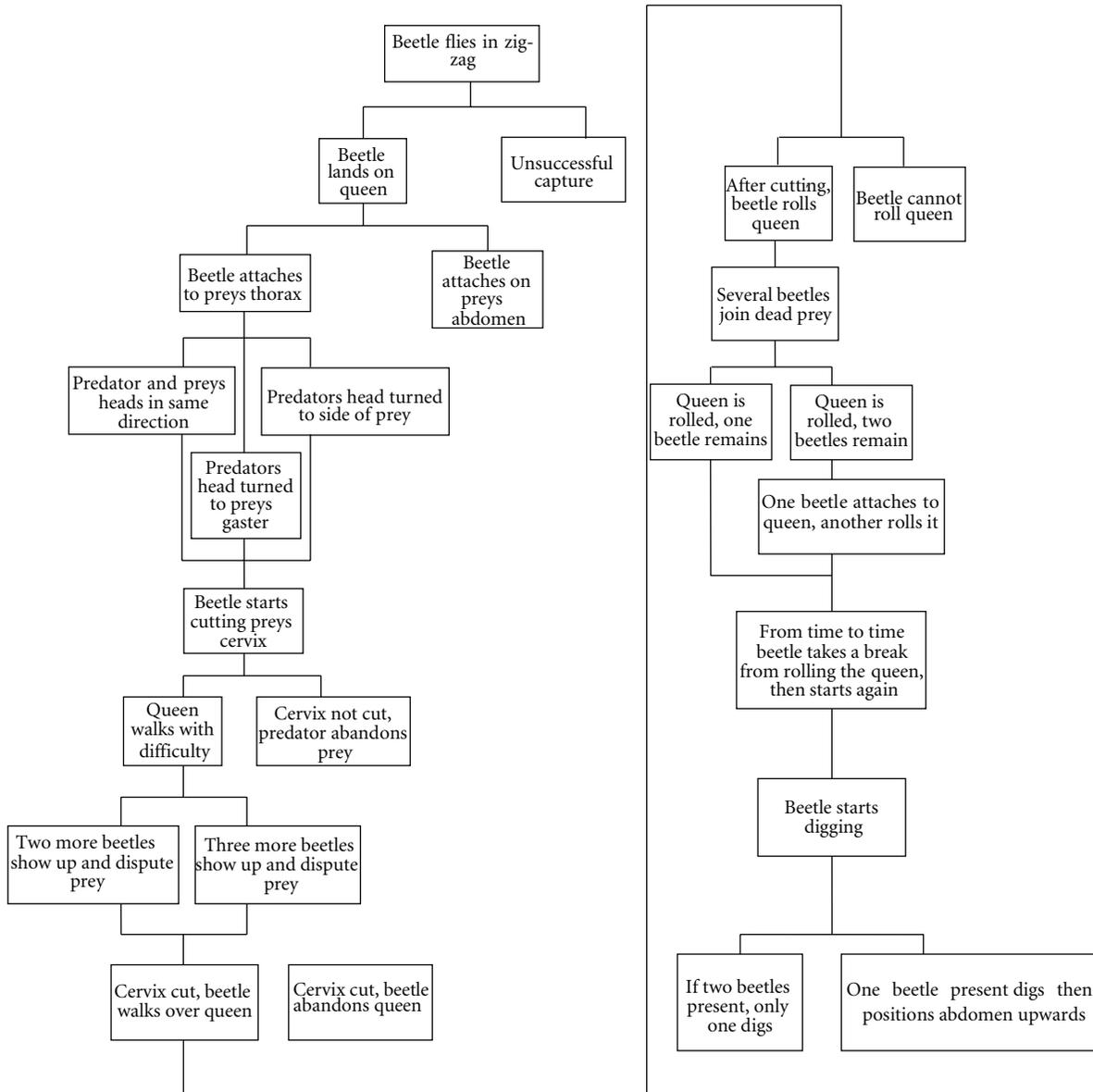


FIGURE 1: Fluxogram showing the behavior of *Canthon virens* preying on leafcutter ants (*Atta* spp.).

The male joins the female after the murder. In some cases, we observed females burying without a male, with her abdomen facing upward and her body partially buried in the tunnel under construction (Figure 2(g)).

A beetle can roll a queen for distances of 2 to 3 m. Some beetles that roll in push feces up to 9 m. *Canthon virens* individuals roll their prey until they find an insurmountable obstacle. The distance between the site of predation and the obstacle can vary widely and is unpredictable. Usually a beetle rolling a queen will stop from time to time, go away a few inches, then return, climb on the dead body, and start rolling it again.

The burial is always conducted by the beetle that pushed the queen: first, the beetle digs a small hole and pulls the queen into the pit, while the other beetle remains on the prey. The burial process takes up to 12 hours and may be

dependent on soil compaction and/or the presence of obstacles. As the queen is buried, the soil is deposited on the surface in the form of small pellets.

Up to three beetles were found buried with the queen during the first 24 hours, but the most common is to find one or two beetles per chamber with the queen (Figures 2(h) and 2(i)).

#### 4. Discussion

Predation is an unusual form of specialization in Scarabaeinae beetles because the majority of them are coprophagous. Some species of *Canthon* specialize in the predation of *Atta* queens. Other authors have described [2, 3, 5] the specialized predatory behavior of these beetles, beginning after the nuptial flight: first the beetle approaches the queen,

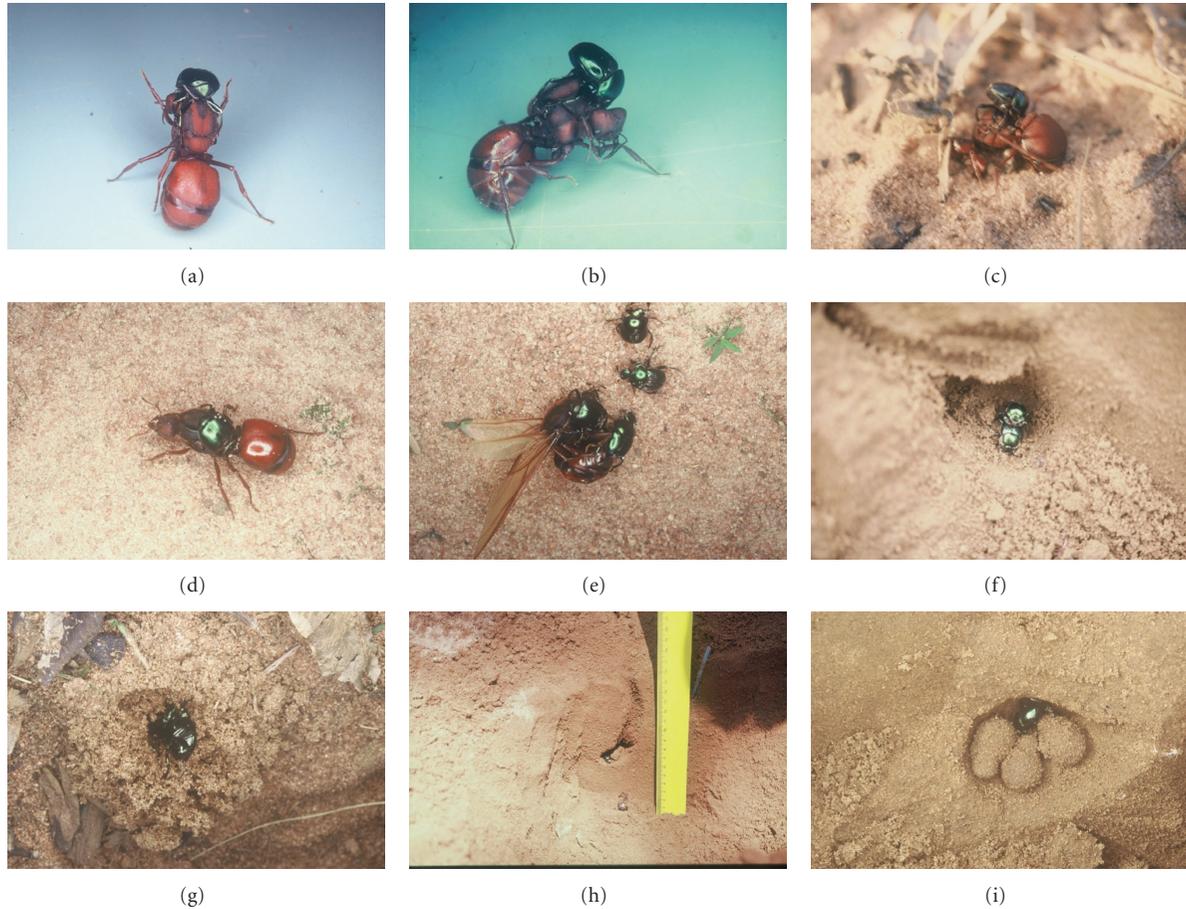


FIGURE 2: (a) A beetle flies zigzag over prey and lands on its back. (b) The beetle cuts the neck of the queen and is attached to it by its legs. (c) The queen has been killed: the beetle examines the prey, before rolling it. (d) Sometimes the beetle may attach itself to the thorax of the queen, having the head turned toward the queen abdomen. (e) When the queen is dead, many other beetles may join. (f) After a few minutes only two beetles remain to bury the queen. (g) The beetle is partially buried and has its abdomen and hind legs facing up and is partially buried; this phenomenon is observed when the beetle buries its prey by itself. (h) The nest of *Canthon virens*, with a channel about 1 cm in diameter and 15 cm deep. (i) Chamber at the end of the canal, with the brood balls.

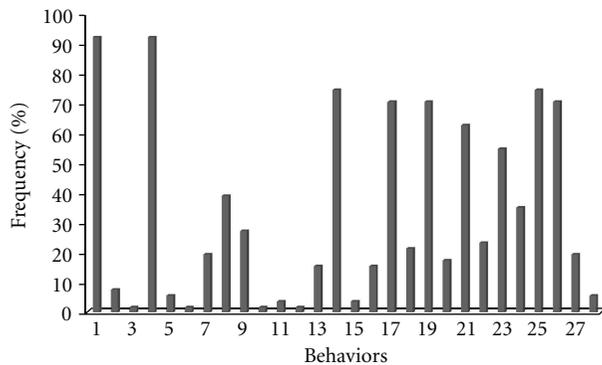


FIGURE 3: Frequency of predatory behaviors of *Canthon virens*.

lands on its back, and then beheads it. However, previous descriptions of the predation sequence were insufficient in view of the amount of information that can be obtained. In our study, we found that 28 behaviors are performed,

showing the complexity of the sequence of predation events (Figure 1).

First, *Canthon* beetles were observed flying at about 15 to 20 cm from the soil surface in search of prey. They fly extremely fast and periodically stop to rest on the ground or on the vegetation. Because queens are located by their beetle predators regardless of whether they remain still or move, we believe that individuals of *Canthon virens* use their sight to locate them. However, because vision is generally poorly developed in Scarabaeinae beetles, whereas their tactile sense is sharp [5], we cannot rule out the possibility that smell, such as that produced by allelochemicals, plays a role in prey location. It is well known that, when an odor current is detected by dung beetles, they take flight and perform a short-distance search [5]. The same could apply to *Canthon virens* beetles.

Once a queen is found walking on the ground, the predator flies in a zigzag fashion, lands on it, and later beheads it. In this study, we found that female beetles are responsible for catching the ant queens. This result contrasts with those

of Navajas [2] and Silveira et al. [7], who found that both males and females prey on the queen and that only the males attack it.

After landing on the queen, the female beetle proceeds to decapitate it, as shown in Figure 1. According to Halffter and Matthews [5], the beetle cuts the queen cervix with the aid of its front legs and clypeus, a fact we also observed. When a female beetle lands on the thorax of its prey, it most often positions itself with the head facing sideways. The predator then turns to face the queen cervix. Frequently a beetle will land on the queen thorax with its head pointing to the head of its prey. In rare cases, the predator stands on the queen thorax with its head pointing to the queen gaster.

The queen keeps walking for a while as the predator proceeds to behead it. At a certain moment, the queen stops and assumes a position in which her body is sideways, arched, and immobile. Infrequently, two or three other beetles may join before the queen has been beheaded and while it is still walking. Most often, however, beetles will group around a victim that is already dead. In some cases, we have observed up to 11 beetles surrounding a single queen. Apparently, some are there to compete for the female beetle. In the end, usually one or two beetles remain at the scene.

Forti and Rinaldi [3] reported that a queen may be attacked by up to six beetles when it is looking for an ideal place to start digging its nest. We believe that their reports actually describe what happens after the queen is already dead. Normally one beetle rolls the queen, while the other is attached to it.

Most Scarabaeini make balls from vertebrate feces and have developed specialized ways to roll them. The balls are rolled either by one or two beetles [5, 8]. When two beetles are involved, they usually belong to different sexes, and the roles taken by each member of the couple differ from one genus to another. In some species of *Canthon*, for instance, the male always rolls, while the female remains on the top of the ball. However, in *C. virens*, the female rolls the queen while the male is rolled along with it.

As soon as the beetle finds an insurmountable obstacle, it starts digging. Similarly, Silveira et al. [7] observed that beetles roll the headless queen until they find a dead leaf and, from that moment on, start digging.

In other coprophagous species of *Canthon*, for instance *C. lituratus*, either the male or the female, or both, prepares fecal balls. However, the female is the one to bury the ball, even when a male is present [9, 10]. In *C. virens*, the queen is buried by the beetle that was rolling it. First, the beetle digs a small hole and pulls the queen into the pit, while the other beetle remains stuck on the prey. The same behavior was observed in *C. lituratus*: the female buries the fecal ball while the male, when present, sits on it [9].

When a beetle excavates alone, it returns to the surface with the abdomen and hind legs facing up, remaining partially buried (Figure 2(g)). Silveira et al. [7] also noted that the beetle remains with the hind legs pointing up and suggested that this may be an indication that it is emitting sex pheromones to attract the opposite sex to the nesting site [11].

Finally, the soil is deposited on the surface at the same time that the queen is being buried. Within 24 hours, the beetles, usually one or two individuals, are buried in the chamber next to the brood ball (Figures 2(h) and 2(i)). This study contributes to the knowledge of the predatory behavior *C. virens*, a predator poorly studied in Brazil.

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## Review Article

# Cleptobiosis in Social Insects

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In this review of cleptobiosis, we not only focus on social insects, but also consider broader issues and concepts relating to the theft of food among animals. Cleptobiosis occurs when members of a species steal food, or sometimes nesting materials or other items of value, either from members of the same or a different species. This simple definition is not universally used, and there is some terminological confusion among cleptobiosis, cleptoparasitism, brood parasitism, and inquilinism. We first discuss the definitions of these terms and the confusion that arises from varying usage of the words. We consider that cleptobiosis usually is derived evolutionarily from established foraging behaviors. Cleptobionts can succeed by deception or by force, and we review the literature on cleptobiosis by deception or force in social insects. We focus on the best known examples of cleptobiosis, the ectatommine ant *Ectatomma ruidum*, the harvester ant *Messor capitatus*, and the stingless bee *Lestrimellita limão*. Cleptobiosis is facilitated either by deception or physical force, and we discuss both mechanisms. Part of this discussion is an analysis of the ecological implications (competition by interference) and the evolutionary effects of cleptobiosis. We conclude with a comment on how cleptobiosis can increase the risk of disease or parasite spread among colonies of social insects.

## 1. Introduction

For animals, foraging is often time consuming, risky and may not result in the discovery of food or other resources. A commonly observed alternative to searching for resources that may be dispersed in the environment is to take, either by force or deception, the resource from another animal. This theft of food or other resources from foragers or from larder caches is common enough to merit considerable interest in behavioral ecology. Many social insects act as thieves, and the focus of this paper is food theft that in some way involves a social insect species, but we also consider theft of other items, such as nesting material. We also consider how theft among social insects fits into the larger evolutionary picture of resource theft in animals. Social insects can be either the victim or the villain in food thefts. Some social insects frequently victimize other social insects, and these thieves may be members of the same species or of a different species. Social insects can also be victimized by commensals that are able to plug into social feeding mechanisms that normally direct food among colony members.

From this point forward, we use the term cleptobiosis to describe food theft, or theft of other items of value such as nesting material, by one animal from another. The evolutionary roots of cleptobiosis are simple; finding food and subduing prey items is key to animal survival, and cleptobiosis provides, for some species, an alternative to foraging costs in terms of time, energy, and exposure to the possible risk of the forager becoming, itself, prey. Most cleptobionts facultatively engage in theft, when profitable opportunities appear, but obligate cleptobiosis has evolved in at least one genus of stingless bee and in inquilines residing within social insect colonies. While the subject of this paper is cleptobiotic relationships involving social insects, the evolutionary principles apply broadly across many types of animals.

## 2. What Is Cleptobiosis?

This is an easy question with an unfortunately complicated answer. At the outset we gave a simple definition; in more formal terms we consider cleptobiosis to be “an ecological

relationship in which members of one species, as of ants, steal food from another” (<http://universalium.academic.ru/93503/cleptobiosis>), but we would add that the stolen items may also include nesting materials or other items of value. The term can also be spelled kleptobiosis, and, in studies of animals outside the social insects, kleptoparasitism (cleptoparasitism) is often used synonymously with cleptobiosis. However, serious confusion can arise from the different ways in which these terms have been used in the literature on insects and the literature on vertebrates, and in the remainder of this section we discuss how this terminology has been applied by investigators studying different types of organisms. Iyengar [1] presents a general review of cleptobiosis in animals.

In insect studies, cleptobiosis refers to food theft (or other valuable materials), and cleptoparasitism refers to brood parasitism (Table 1). Alternatively, the term cuckoo or cuckooism is used to describe brood parasites among birds and mammals, as well as bees. Cuckoo bees are brood parasites (strictly speaking, this is family Apidae, subfamily Nomadinae, but also refers to other bees with similar lifestyles) and are often termed cleptoparasites. These bee species lay their eggs in the nests of other bee species, rather than stealing food. Their young are then reared by adults of the host species [2]. Brood parasitism of this sort is also characteristic of birds such as cuckoos (family Cuculidae) [3] and cowbirds (Icteridae). Unfortunately, the term cleptoparasitism (or kleptoparasitism) is also frequently used to describe food theft in bird and mammals [4]. Adding to the terminological confusion is the occasional use of lestopobiosis to refer to more furtive (as opposed to forcible) forms of cleptobiosis.

We view cleptobiosis as theft of food or other valuable items and prefer other terms to describe brood parasitism. In some cases, as in gulls stealing from one another on a beach, the theft is direct interference with immediate consumption. In other cases, such as honeybees robbing honey from a hive, the target is stored food in a nest or cache. To avoid confusion, species that lay eggs in the nests of others (either conspecific or heterospecific) are probably better termed cuckoos or brood parasites, rather than cleptoparasites. This paper focuses specifically on cleptobiosis, defined as food thievery, in social insects.

Adding to the confusion, Crespi and Abbot [10] call thrips that steal galls induced by other thrip species cleptoparasites. Nest usurpation is also known in honeybees and in a broader context is similar to territorial contests in birds and mammals, in which an individual may lose a nesting site. We do not include nest usurpation in our review.

Theft of brood for the purpose of employing the stolen individual’s efforts in support of the thief is dulosis (in the older literature this is called slave-making, but the preferred terms is now dulosis). Dulosis is common in ants but is only incidentally observed in other types of animals. Inquilinism is social parasitism in which a reproductive enters a host colony, lays eggs, and relies on the host colony to rear its offspring. Unlike brood parasitism, the inquiline remains within the nest and typically its brood does not outnumber the host’s brood. Inquilines are typically evolutionarily close

to their hosts, as with ants living within the colonies of another ant species. Inquilines may, of course, take food from the host colony, potentially making this behavior a kind of cleptobiosis. Consumption of brood, as by corvids (family Corvidae: ravens, crows, magpies, and jays), which target eggs and nestlings of smaller birds [16], is predation rather than cleptobiosis.

Before moving on to our consideration of cleptobiosis, one other type of interspecific interaction that should be discussed is “guests” in social insect colonies. This type of symbiosis is related in some ways to cleptobiosis. Ant and termite colonies, in particular, often have guests, which are termed myrmecophiles and termitophiles, respectively. Guests living with ants include other ant species, beetles, flies, and Collembola. Larvae of lycaenid butterfly species display a variety of mechanisms—chemical, morphological, and behavioral—to induce care from ants [17, 18]. Like furtive cleptobionts, many of these symbionts “gain the keys to the kingdom” by mimicking the chemical recognition signatures of the host colony, a mechanism that we discuss in more detail below.

### 3. Nonsocial Insect Cleptobionts

Social insects are not the only members of cleptobiotic associations. Many animal species use cleptobiosis to exploit social information. This makes their search for food more efficient [19, 20]. By observing other animals’ foraging activities, an animal can take advantage of information that took another individual’s time and energy to discover. Birds in a flock may assemble, for example, to obtain social information about food [21]. In some cases such information theft leads to direct stealing of food. Heron gulls voraciously attempt to steal each other’s food discoveries [7]. Spiders steal food from each other in a similar manner [22, 23]. Interspecific theft of food items is common among carnivorous mammals, with thefts by hyenas in which wild dogs are victimized having been particularly well studied [24], although no mammalian or avian species makes their living solely by stealing food items from other species.

Taking this logic a step further, targeting cached food can be a highly efficient strategy [25]. Scatter caching is a form of food storage in which single food items are placed in caches within an animal’s home range. Sometimes, as in jays or squirrels, this can result in a high number of caches of food to be retrieved in a later season. Scatter caching is also subject to pilferage, but scatter caching animals typically do not defend individual caches. Ravens, for example, seek out each other’s food caches [25]. Raven defenses against cache pilferage largely rely on clever storage, rather than aggressive defense. However, when a mountain lion caches a deer carcass, it may then defend its food. In scatter caching, cache locations are associated with where prey was killed, rather than a central nest or den.

Among mammals and birds, caching seems responsive to immediate evolutionary pressures. Tree squirrels exemplify evolutionary flexibility in caching strategies, as some species are scatter cachers (e.g., the Eastern fox squirrel, *Sciurus*

TABLE 1: Terminology for food theft, brood theft, brood parasitism, and related phenomena. The term “cleptoparasite” has been used with such diverse meanings that it is probably best dropped from the lexicon for this area.

Term	Function	Example
Cleptobiosis	Theft of food or another item of value from another animal	Gulls [5], honeybees [6], <i>Ectatomma</i> ants [7, 8], bowerbirds (mating display materials) [9]
Lestobiosis	Cleptobiosis by furtive or deceptive means	<i>Ectatomma</i> ants [7, 8]
Nest usurpation	Theft of a nest structure, perhaps including brood or food cached within the nest	Honeybees [6], thrips [10]
Brood parasitism	Laying eggs in the nest of another animal, to be reared by that animal, functionally, this is theft of brood rearing	Cuckoos (birds) [3], cuckoo bees [2]
Dulosis	Theft of brood to rear as workers	“Slave-making” ants [11]
Inquilinism	Living within a social group as a social parasite, a conspecific or heterospecific reproductive that exploits the host colony by laying brood that are cared for by the host colony	<i>Psithyrus</i> bees in bumblebee ( <i>Bombus</i> ) colonies, <i>Dolichovespula arctica</i> and <i>D. adulterina</i> , (initially inquilines, often become usurpers) in other <i>Dolichovespula</i> colonies [12, 13], numerous ant species within other ant colonies [11]
Guests, myrmecophiles, and termitophiles	Live within a social insect colony, often adopting chemical recognition signature of host colony, may consume resources but do not represent a lethal drain on colony resources	Many species representing many insect orders, as well as noninsects. Specific examples are lycaenid Lepidoptera in ant colonies [14, 15] and wax moths, <i>Galleria mellonella</i> , in honeybee colonies [6]
Parasites	Live within a social insect colony but represent a potentially lethal presence	<i>Varroa</i> mites in honeybee colonies [6]
Brood predation	Eating eggs or brood from within a nest	Corvids (ravens, crows, magpies) consuming other bird’s eggs, army ants, many species of which target brood of other social insects [1]

*niger*), and others are larder cachers (e.g., the pine squirrel, *Tamiasciurus hudsonicus*). The difference between these species may in part reflect differing pressures from cache pilferage.

#### 4. Larder Caches and the Evolution of Cleptobiosis

A larder cache is food stored in a central place for future use. Food stored in larder caches is particularly attractive to potential thieves, and many larder caching social insect species, such as harvester ants, most stingless bees, and honeybees, have evolved impressive modes of colony defense. The evolution of larder caching is probably driven by the value of having food in a central place during unfavorable seasons, but the threat of loss of the cache, to animals of the same species or different species, is clearly a countervailing evolutionary force. Larder caching occurs in some ants and social bees, and to a lesser extent in social wasps, and is particularly important in our discussion of cleptobiosis in social insects. The long-term survival of social insect nests containing stored food, sometimes spanning many years, may help to make their colonies particularly susceptible to repeated raids.

For some individuals within a population, taking food from others can become the predominant mode of food

collection, but robbing conspecifics is not an evolutionarily stable strategy for all individuals within a population—it is clearly a dead-end if all animals in a population are robbers and none are foraging independently. Heterospecific food theft, on the other hand, can lead to obligate cleptobiotic specialization. An example of an obligate cleptobiont is the stingless bee, *Lestrimellita limão* [14, 15].

Social insect species may be particularly well equipped to engage in cleptobiosis, as the victim and thief share social mechanisms, and thief workers can evolve for specialized foraging roles without conflict with reproduction, which is the province of the queen in the colony [26]. Larder caching social insects are also victimized by birds and mammals. Perhaps most famously the western honeybee, *Apis mellifera*, is well armed to protect itself against vertebrate cache thieves attempting to access stored honey.

#### 5. From Foraging to Cleptobiosis

Cleptobiosis often appears to have arisen from foraging behavior that is redirected to stored foods. Stored food presents some of the same sensory profiles as the food in its original state; honey is both sweet and imbued with floral scents even though it has been concentrated from nectar and is found in honeycombs rather than flowers. Western honeybee, *A. mellifera*, colonies commonly rob honey from

other colonies. The western honey bee is a feeding generalist and forages on both floral and nonfloral sources of sugars, such as extrafloral nectaries. The evolutionary switch from foraging on nectar collected at flowers and extrafloral nectaries to foraging on stored honey in other bees' colonies is within the foraging flexibility of honeybees and may not have required any particular adaptations to allow bees to make this switch.

Other social insects, such as the ectatommine ant, *Ectatomma ruidum*, have made similar shifts, but have mechanisms for evading detection by workers in the colony being robbed. In more extreme cases, cleptobiosis involves specific adaptations for thievery from colonies of other social insects. Members of the stingless bee genus, *Lestrimellita*, are good examples of this. *Lestrimellita* species no longer forage on floral sources, instead they have evolved as an obligate robber of other stingless bee species. These examples, *E. ruidum* and *L. limão*, are discussed in more detail below.

Cleptobiosis can be intraspecific or interspecific. The focus on food collected by the victim, rather than the victim itself, differentiates this behavior from predation. The evolution of cleptobiosis appears to draw from foraging behaviors, but also may involve the evolution of specific mechanisms for overcoming defenses of the victimized colony. Guarding honey bees [27], for example, serve a primary function of preventing cleptobiosis. The number of guards present and the intensity of guarding behavior are responsive to the intensity of pressure from robbing bees from other colonies [28]. This is an interesting example of intraspecific coevolution, in which robbing behavior yields a fitness reward for their colony, but pressure from robbers results in the evolution of heightened defensive behavior [28].

A shift in foraging preferences is a reasonable hypothesis for the initial evolution of cleptobiosis. But how does obligate cleptobiosis evolve? The most likely evolutionary scenario seems to be first facultative cleptobiosis on other species, followed by the evolutionary loss of noncleptobiotic foraging preferences. Hölldobler and Wilson [11, Chapter 12], give a detailed discussion of how predation and territoriality might lead to dulosis and inquilinism. Nearly all of our examples of species exhibiting cleptobiosis are facultative cleptobionts. For individuals of these species, the ability to be a cleptobiont adds foraging opportunities and often allows these animals to take advantage of public information concerning food availability. Obligate cleptobiosis enables the evolutionary loss of specializations, such as pollen carrying structures, and the evolution of special abilities for overcoming nest entrance guards; both of these evolutionary outcomes are seen in the stingless bee genus *Lestrimellita*. However, obligate cleptobionts are uncommon, suggesting that perhaps this is a narrow niche which can only be filled under fairly conscribed circumstances.

## 6. Army Ants and Predation on Social Insect Brood

Army ants cross the line from cleptobiosis to predation, but they merit mention here because many army ant species are feeding specialists on the brood of other social insect species.

Army ants subdue their prey by force, rather than by deception, but their match in body size, social behavior, and their ability to recruit colony mates to food sources, makes their predatory behavior in some ways homologous to the brood capturing behavior of dulotic ants. The culminative act, predation or capture of labor, surely differs, but the ultimate outcome for the victimized colony is similar. Hölldobler and Wilson [11, Chapter 12] give a more detailed discussion of the possible intertwining of evolutionary pathways between predation and dulosis.

## 7. How Do Cleptobionts Succeed?

There are two basic strategies that cleptobionts can use to enter a target colony: deception and force. Deceptive entry usually involves furtive behavior, sometimes combined with manipulation of the chemical signals involved in nestmate recognition. We discuss these two strategies in more detail in the following two sections.

*7.1. Cleptobiosis by Deception: Evading Nestmate Recognition and Guards.* In some cases successful cleptobiosis is dependent on exploitation of social mechanisms. One common mechanism is evasion of a species' nestmate recognition system. For a colony to defend itself against potential cleptobionts, workers in the colony must be able to discriminate nestmates from nonnestmates. The presence of a nonnestmate within the colony, or at the colony entrance, is typically detected through differences in surface chemistry between colony residents and nonresidents [6, 29–31]. Residents defensively respond to perceived differences, biting and stinging (if a sting is present in the species) the intruders. Often the defenders are specialized guards, which are primed to respond to nonnestmates.

The chemical cues used in nestmate recognition are typically hydrocarbons that are probably coopted from their original role as cuticular waterproofing [32]. In *Polistes* wasps and in honeybees, nestmate recognition cues are acquired from nesting materials and all individuals in a colony present similar chemical signatures [6]. The mode of cue acquisition varies among ant species, but in at least some ants the postpharyngeal gland serves as a “gestalt” organ for a unifying colonial odor [33]. Potential cleptobionts can evade a chemical nestmate recognition system either by mimicking the chemical signature of the target colony or by not presenting a chemical signature of their own (figuratively speaking, they are a blank slate). The threat of cleptobiosis and social parasitism may be driving forces in the evolution of efficient nestmate recognition systems.

In *E. ruidum*, a neotropical ectatommine ant, cleptobiosis is common among colonies [7, 8, 34–36]. *Ectatomma ruidum* colonies are small, with typically fewer than 100 workers, and are abundant in many lowland dry, moist, and wet habitats. *Ectatomma ruidum* forages on small arthropods, seeds, and nectar. Where they occur they are abundant, with a mean distance of 1 to 2 meters between neighboring colonies. Foragers will repeatedly attempt to gain entrance into neighboring nests. These repeated attempts cooccur

with a reduction of concentration of cuticular hydrocarbons and with a convergence of cuticular hydrocarbons between the thief ant and the target colony. After repeated attempts, thieves can gain entry into target colonies without resistance and position themselves to receive food from foragers returning to the colony. They then depart and carry the food to their own colony. Ironically, colonies of equal strength, in terms of the number of workers, tend to equally rob each other, with no net effect on food flow into the colonies. Small colonies are at a distinct disadvantage and tend to lose considerably more food than they gain [37]. This is a clear example of cleptobiosis by deception.

**7.2. Cleptobiosis by Force.** The stingless bee, *L. limão*, is an obligate cleptoparasite of other stingless bees, and sometimes has been observed robbing honeybee colonies [38]. The highly sclerotized workers of *L. limão* [14] have a strong lemon odor due to the presence of citral. A common victim of *L. limão* is *Tetragonisca angustula*, a very common bee in the neotropics. One kind of guard in *T. angustula* hovers around the nest entrance [39–41]; hundreds of these hovering guards are present at any given time, making the presence of a nest of this species easily observable. When a *L. limão* worker enters the defensive perimeter of a *T. angustula* colony, the guards are alerted by the flight pattern, color, and odor of the intruder. *Tetragonisca angustula* guards cannot outfight the larger, more heavily armored *L. limão* workers, but can successfully disable *L. limão* by biting onto a leg or wing, once the hold on the intruder is secured, the *L. limão* worker ability to fly is compromised by the weight and imbalance created by the attached *T. angustula* worker [42]. If a *T. angustula* colony is effective against the first arriving *L. limão*, then the attack is not serious. If *L. limão* workers evade detection, then they recruit massive numbers of additional attackers, and the *T. angustula* colony will be overrun by cleptobionts. *Lestrimellita* may steal plant resins used in nest construction, as well as food [43].

In the western honey bee, *A. mellifera*, rich food resources, in the forms of pollen and honey, are stored within the nest. This food supports survival of the colony through cold temperate winters and provides reserves that allow rapid colony buildup in the spring and investment in reproduction (swarms and drones) early in the growing season. The presence of massive nutritional reserves provides a tempting target, and honey bee colonies can target each other for theft of food, particularly honey. This behavior fits well within our definition of cleptobiosis. Guard honeybees use chemical cues to discriminate nestmates from nonnestmates and act to exclude robbers from the nest [27, 44]. Weaker honeybee colonies are more susceptible to being robbed, and if guards are unable to exclude the first few robbers, massive recruitment of additional robbers may result in the targeted colony being overwhelmed [28].

*Ectatomma* ants, which were described above as deceptive cleptobionts, forcibly gain food from the ant *Pheidole radoszkowskii* [8]. *Ectatomma* ants have also been observed as victims of interspecific cleptobiosis. *Ectatomma tuberculatum* workers collect nectar and carry droplets externally, between

their mandibles, making them a potential target for cleptobiosis. Richard et al. [45] observed *Crematogaster limata parabiatica* workers robbing nectar from *E. tuberculatum* workers. *Crematogaster* workers were able to enter *E. tuberculatum* nests, but food theft was targeted at *E. tuberculatum* workers that were returning to their nest.

Espadaler et al. [46] observed ant, *Messor barbarus* and *Aphaenogaster senilis*, workers robbing Euphorbiaceae seeds from workers of other species, particularly *Tapinoma erraticum* (referred to as *T. nigerrimum*). Ants in the genus *Messor* are seed harvesters, so it is easy to see cleptobiosis of seeds by *Messor* workers as a fairly simple shift in foraging strategy. *Aphaenogaster* species show a wider range of food preferences, but *A. senilis* workers collect Euphorbiaceae seeds in the Mediterranean habitat in which this study was conducted. *Messor capitatus* workers engage in a variety of interference tactics that affect foraging by congeners, including cleptobiosis of seeds [47, 48]. Similarly, honey ant, *Myrmecocystus mimicus*, workers rob insect prey items from harvester ant, *Pogonomyrmex*, workers returning to their colonies [49].

Additional examples expand the range of species that can engage in forcible cleptobiosis. LaPierre et al. [50] observed *Polybioides tabida* F. (Ropalidiini) wasps robbing workers of the ant, *Tetraponera aethiops*. The wasp, *Charterginus* sp., (Epiponini) robs food normally collected by mutualistic ants on acacias. Corbara and Dejean [51] reported theft of paper nest material from ants by the social wasp *Agelaiia fulvofasciata*. In sum, cleptobiosis is exhibited by ants, bees, and wasps, giving support to the hypothesis that cleptobiosis is easily derived from preexisting features such foraging preferences, territorial behavior, and social mechanisms such as kin recognition.

## 8. Cleptobiosis and the Risk of Disease Spread

Cleptobionts, by coming into close contact with conspecifics, put themselves at risk for exposure to any disease their victim might carry. This becomes particularly important if the victim's weakness from disease makes them an easier target for cleptobiosis. Increased risk of disease transmission through cleptobiosis is best known in honeybees, *A. mellifera* [52]. Colonies weakened by the bacterial disease, American Foul Brood, *Paenibacillus larvae*, the intestinal parasite, *Nosema*, or by *Varroa* mites are much less well able than healthy colonies to defend themselves against robber bees from other colonies [52].

## 9. Discussion

Food theft—cleptobiosis—is an important form of inter-colonial interaction in social insects. In this paper we have defined cleptobiosis and discussed the difference between cleptobiosis and other types of interactions among social insect colonies, such as dulosis. We also suggest that the term “cleptoparasitism” has been used in so many different ways that it has lost its usefulness and should be avoided. We point out that brood parasitism, inquilinism, and “guests”

in social insect colonies all may involve mechanisms similar to cleptobiosis. Cleptobionts may be divided into deceptive and forceful types. Deceptive cleptobionts bear considerable resemblance to “guests” in the manner in which they gain entrance to target colonies.

The ecological implications of cleptobiosis are clear, and the reader should refer to Hölldobler’s [49] discussion of the significance of cleptobiotic ants in interference competition among ant colonies. Cleptobiosis can function as an extension of territorial behavior of a colony, weakening nearby colonies. In larder hoarding social insects, such as the honeybee, attacks on stored food can contribute to colony decline and failure.

The evolutionary effects of cleptobiosis are largely seen in well-developed nestmate recognition systems. The ability to discriminate and exclude nonnestmates can help to prevent entry by cleptobionts, brood parasites, and inquilines, as well as functioning in territorial interactions. Understanding the relative importance of these factors in shaping nestmate recognition systems remains an important goal for future research.

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## Research Article

# Behavior of *Paussus favieri* (Coleoptera, Carabidae, Paussini): A Myrmecophilous Beetle Associated with *Pheidole pallidula* (Hymenoptera, Formicidae)

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Several specimens of the myrmecophilous beetle *Paussus favieri* were reared in ant nests of *Pheidole pallidula*. Their interactions were recorded and all behaviors observed are described. Duration and frequency of five behaviors of *P. favieri* were analyzed with ANOVA and post hoc Tukey tests; these comprised rewarding, antennal shaking, antennation, escape, and “no contact”. Significant differences both in duration and in frequency among behaviors were detected. The main result is that the rewarding behavior, during which the beetle provides attractive substances to the host, is performed significantly more frequently than all others. This result strongly supports the hypothesis that the chemicals provided by the beetles and licked by the ants are of great importance for the acceptance and the full integration of *P. favieri* in the ant society. This result also suggests that, contrary to previous findings and interpretations, the myrmecophilous strategy of *P. favieri* is very similar to the symphilous strategy described for *P. turcicus*. The occasional interactions of some beetle specimens with the *P. pallidula* queen were recorded, illustrated, and discussed, indicating the possibility of a more complex strategy of *P. favieri* involving a chemical mimicry with the queen. In addition, the courtship performed by the beetle is described for the first time, together with a peculiar “cleaning” behavior, which we hypothesize functions to spread antennal chemicals over the body surfaces.

## 1. Introduction

Ant nests are very attractive for many organisms, because they represent well-protected and stable environments that are rich in various resources (ants, their brood, stored food, waste materials, etc.). In particular, a large number of insects establish relationships with ants for a considerable part of their life cycle [1–3] and are classified as true myrmecophiles [4]. Insect-ant interactions range from commensalism to specialized predation, parasitism, and mutualism [1]. The most specialized myrmecophiles are able to deceive the complex communication and recognition systems of the ants, infiltrating their societies and exploiting their resources

[1, 4, 5]. These ant parasites represent about 10% (~10,000 species) of known myrmecophilous insects and most are members of Coleoptera, Lepidoptera, Orthoptera, and Diptera [6]. They show several refined adaptations (e.g., chemical and morphological mimicry, specialized feeding behaviors, structural modifications) to avoid ant attacks, to be accepted by ants, and to develop and reproduce within ant nests [7].

All members of the ground beetle tribe Paussini (Coleoptera, Carabidae, Paussinae) are myrmecophiles and are considered to be ant parasites [8]. Like many other parasites of ants, they show striking adaptations, such as greatly modified antennae (flattened, enlarged, lenticular, globular,

concave, elongate, etc.), slender or compact bodies, elongate or flattened legs, and peculiar “myrmecophilous organs” composed of trichomes (tufts of hairs) connected to exocrine glands for the release of chemical secretions.

Paussini (known as “ant nest beetles”) are typically rare insects living in concealed environments which makes it difficult to observe their behavior in nature [7]. Therefore, while they have been extensively studied from a taxonomic point of view [8], information about their interactions with hosts and their life cycle is limited and largely indirect (i.e., inferred from their structural adaptations) with few ethological observations [9]. Although several attempts have been made to rear Paussini with their host ants, this has proven to be particularly difficult, and promising results have been achieved only for a few species (5 out of the currently recognised 572 Paussini species) [8].

The first observations of Paussini behaviors in captivity were reported by Péringuey [10, 11] for *Paussus lineatus* Thunberg, in 1781, and *P. linnaei* Westwood, in 1833, and, to a lesser extent, for *P. burmeisteri* Westwood, in 1838. Other early ethological notes were reported by Escherich [12] for *P. turcicus* Frivaldszky, in 1835, *P. favieri* Fairmaire, in 1851 [13], and *P. arabicus* Raffray, in 1885 [14]. These authors carefully reported their annotations mainly emphasizing the obligate association of these beetles with the ants (especially the ant genus *Pheidole*), their feeding strategy on larvae of the host ants, and some interactive behaviors between beetles and ants (e.g., dragging, grooming, aggressive behaviors). According to this first, though limited and speculative set of information gathered in captivity, and to previous anecdotal observations in nature reported by several authors (e.g., [15–19]), Escherich [14] tentatively categorized the strategies of the members of the genus *Paussus* in three main levels of interactions, referring to Wasmann’s [20, 21] myrmecophilous categories: synectrans (e.g., *P. linnaei*), synecoetes (e.g., *P. arabicus* and *P. lineatus*), and symphilous (e.g., *P. turcicus*). Later, Le Masne [22–24] successfully reared *P. favieri*, adding valuable and detailed information to the knowledge on the biology of this species which is a guest of the facultatively polygenic ant *Pheidole pallidula* (Nylander, 1849). Le Masne mainly focused his observations on the predatory strategy of *P. favieri* while feeding on adults and ant larvae [22, 24], and on the mechanisms of adoption of the beetle inside the nest [23]. More recently, Escherich’s [14] classification has been reviewed and updated by Geiselhardt et al. [8], and three different strategies have been identified, exemplified by three *Paussus* species: (1) the strategy of *P. arabicus* reported by Escherich [14] which is considered the most basal, since the initial contact with the ants triggers their aggression; however, the attacks cease after the contact with the ants [10, 12, 14, 25], and for this reason, the authors speculated that a chemical camouflage might occur in this species [8]; (2) the costly strategy of *P. turcicus*, which is actively groomed by its host ants, to which the beetle supplies an attractive and possibly rewarding antennal secretion [12, 13]; and (3) the strategy of *P. favieri*, which is considered the most derived, since it has no apparent costs. According to the observations by Escherich [13] and Le Masne [23], this beetle is readily accepted and fully integrated within the

colony without hostility. It is usually ignored by the ants, only rarely touched, quickly groomed, and dragged, and it moves undisturbed within the nest, free to feed on brood and adults. Probably, an advanced chemical mimicry mediates the mechanism of this association [8].

*Paussus favieri* was also the object of recent researches, being one of the most common species of Paussini in Northern Africa and one of the two species present in Europe. Cammaerts et al. [26–28] showed that *P. favieri* preferentially follows the pheromone trail produced by the poison glands of its host ant, discriminating this from pheromones of nonhost ant species. Lastly, Di Giulio et al. [7] reared and described the first instar larva of *P. favieri* that, like other *Paussus* larvae, shows remarkable adaptations to a myrmecophilous lifestyle (e.g., shortened and degenerated head capsule, reduced mouthparts, partial atrophy of legs, fused terminal disk), with specialized feeding behaviors that suggest that the larvae are fed by the ants through trophallaxis.

To clarify the mechanisms underlying host-parasite relationships between *P. favieri* and its host ant *P. pallidula*, we investigated the interspecific and intraspecific behaviors performed by the beetles inside the ant nests maintained in laboratory. In particular, our aims were (1) to describe the main behaviors performed by *P. favieri* and its host ant, (2) to analyze the duration and frequency of the behaviors performed by the beetles, and (3) to discuss the possible functional and adaptive significance of the observed behaviors.

## 2. Materials and Methods

**2.1. Material Examined and Rearing Conditions.** During an expedition to Morocco (High Atlas Mountains) in May 2010, adults of *P. favieri* were collected under stones, in nests of *P. pallidula* (Figure 1). Beetles and ants were then transported to the laboratory for behavioral experiments. Each beetle was reared with the ants from the nest in which it was found; when multiple specimens of *P. favieri* were found in the same nest, all specimens were reared together. Ants and beetles were housed in transparent glass boxes (32 × 22 × 15 cm) lined with a layer of plaster, and the walls were coated with fluon to prevent ants from escaping. Colonies were kept under controlled conditions (21–24°C; 12 h : 12 h light : dark; 60% humidity), following the procedures described by Detrain and Deneubourg [29], and maintained on a diet of sugar or honey, and fruit flies or moth caterpillars provided three times per week. The boxes were kept open to facilitate observations. After the ants and beetles were acclimated to these new conditions (about for 10 days), behavioral observations were made.

Ten colonies were established but we used only five, well-structured colonies with at least 100 nestmates (70% minors, 30% majors and queen) for behavioral observations.

**2.2. Descriptions of Behaviors.** Host-parasite interactions and intraspecific behaviors (cleaning and mating) were observed under natural light. Video was recorded with an NV GS120EG Panasonic video camera for a total of 20 hours. Because manipulation could have unpredictable effects on



FIGURE 1: *Paussus favieri* with minor and major worker of *Pheidole pallidula* (photo by P. Mazzei).

the host-parasite interactions, beetles and ants were not marked and beetles were not sexed. For the analysis of the host-parasite interactions, we selected 14 beetles for which recording sessions of at least 15 minutes were available.

All behaviors of both the beetles and the ants were described and classified into five categories (see Sections 3.2 and 3.3). The behaviors performed by *P. pallidula* during the interactions with the beetles were described following the behavioral repertoire suggested by Hölldobler and Wilson [1], Passera and Aron [30], and Sempo and Detrain [31]. Beetle cleaning and sexual behaviors were described after analyzing the videos in slow motion.

**2.3. Statistical Analyses of Behaviors.** We statistically analyzed five behaviors performed by the beetle while interacting with the host ant (see Section 3.2). Recording sessions were analyzed using the observation transcription tool EthoLog 2.2 [32] to continuously record the time that the beetle spent performing different behaviors. We tested whether different behaviors of beetles have significantly different durations, that is, if there are differences in the amount of time a beetle spends engaged in different behaviors when it interacts with ants. Differences between behavior duration were tested using a main effect ANOVA. A total of 1030 measurements of behavior duration (dependent variable) were analyzed. Because the beetles were not obtained by rearing but were collected from ant nests in the field, we have no information about possible interindividual variation due to genotypic differences, or previous experience with ants, age, days of fasting, and so forth. Thus, we combined all of these unknown factors into the concept of “individuality”. To control for this “individuality”, beetles were numbered from 1 to 14 and “beetle identity” was introduced as a second factor in the ANOVA. Therefore the identity of the beetle, which exhibited a behavior, and the type of behavior (classified into five categories, A–E, see Section 3.2) were used as categorical predictors (factors). Post hoc comparisons were performed using Tukey HSD tests. To determine whether different behaviors were performed more frequently than others, we executed analogous analyses on

the recorded frequency of the behaviors. Statistical analyses were performed with Statistica for Windows version 7.2 (StatSoft Inc., Tulsa, OK, USA).

**2.4. Scanning Electron Microscopy.** Morphological structures of *P. favieri* (Figure 2) involved in the interactions with host ants and with others conspecifics were studied using a Philips XL30 scanning electron microscope at L.I.M.E. (Interdepartmental Laboratory of Electron Microscopy, University “Roma Tre”, Rome). Specimens used for morphological study were kept overnight in a detergent water solution, cleaned by ultrasounds for 15 seconds, rinsed in water, dehydrated through a series of EtOH baths of increasing concentration (70, 80, 90, 95, and 100%), critical point dried (Bal-Tec CPD 030), mounted on a stub (by using self adhesive carbon disks), and sputter-coated with gold (Emitech K550 sputter coater).

### 3. Results

**3.1. General Morphology of *Paussus favieri*.** The beetle is small (length ~ 4 mm), much bigger than minor workers of *P. pallidula*, with intermediate dimensions between majors and queen (Figures 1 and 3). The body is slim with slender elongated legs and bulged modified antennae. The body color is light brown, similar to that of minor and major workers of the host ant, with shining, oily appearance. The head is subhexagonal with elongate palpi and dark eyes, bearing dorsally a long medial tuft of trichomes (Figures 2(a) and 2(b)). The antennae are particularly modified, composed by three joints: (1) a cylindrical and slightly elongated scape; (2) a globular, ring-like pedicel; and (3) a single segment “antennal club” (resulting from the fusion of 9 flagellomeres) that is wide, sub-triangular, swollen, and strongly asymmetrical (Figures 2(a) and 2(b)). The scape and the antennal club are covered by several modified trichomes and glandular pores (Figure 2(d)), while chemoreceptors are mainly distributed apically. The antennal club has a pointed basal spur with two tufts of trichomes (myrmecophilous organs, Figures 2(a) and 2(c)), and ventral pockets (Figure 2(d)) where glandular secretions are stored. The prothorax is elongated, of about the same width as that of the head, strongly constricted in the middle, without tufts of trichomes. Like the other *Paussus* species, a stridulatory organ is present on the ventral side, composed of finely ridged pars stridens on the hind femora and a plectrum (row of cuticular spines) on the basal part of the abdomen. The elytra are parallel and covered with elongate, branched trichomes. The pygidium is truncate with short-fringed trichomes. The ventral side of the body is smooth, without trichomes.

#### 3.2. Description of *Paussus favieri* Behaviors When Interacting with Host Ant

(a) *Rewarding.* The beetle remains still, while it is antennated and actively licked by ant minor and major workers (Figure 1; see Supplementary Material 1 available online

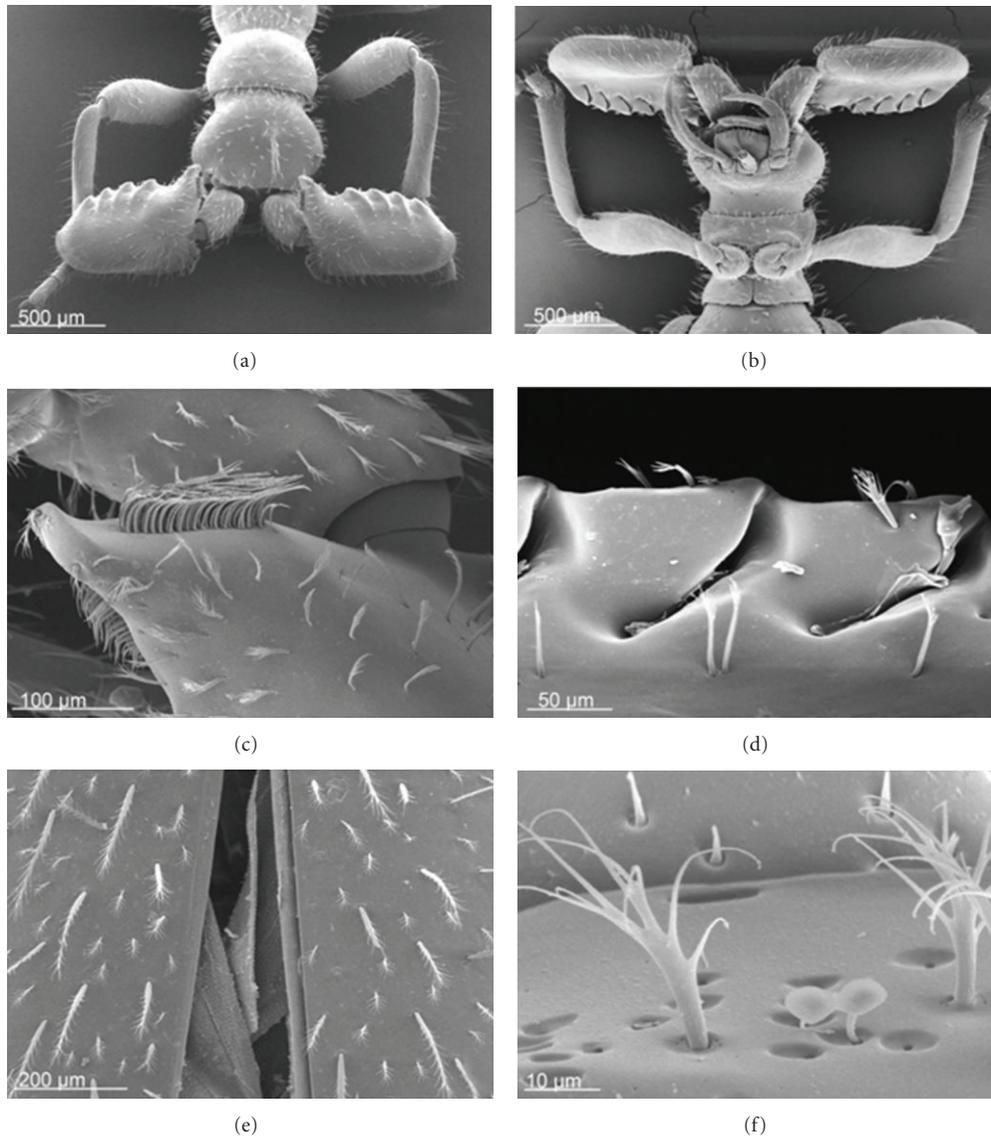


FIGURE 2: SEM micrographs of *Paussus favieri*: (a) anterodorsal view of head and thorax; (b) ventral view of head and thorax; (c) basal spur of the antennal club, dorsal view; (d) ventral antennal pockets with visible secretion; (e) elytra with modified sensilla chaetica; (f) modified sensilla chaetica on head with glandular pores.

at doi: 10.1155/2012/940315). This behavior is generally associated with movements of the beetle's hind legs, either singly or in combination.

(b) *Antennal Shaking*. The beetle vibrates the antennae, quickly shaking them forward and backward in the vicinity of the ants. This behavior mostly occurs after a long period of rewarding (see above).

(c) *Antennation*. The beetle moves its antennae in a slow, alternate, vertical way, oriented toward the object of interest. The beetle usually explores an ant's body with the apices of the antennae, which are particularly rich in sensorial structures.

(d) *Escape*. The beetle tries to elude the host ant in a temporary negative reaction. This behavior is not connected with aggression by the host, but rather in most cases it is a consequence of the presence of a high number of excited ants antennating and licking the beetle, or after an extended rewarding period.

(e) *No Contact*. The beetle does not interact with the ants. This state includes many different activities like exploring, resting, cleaning, interacting with partners, mating, and so forth.

Feeding and mating behaviors were observed rarely. The beetle feeds on ant larvae by piercing the integument with its mandibles and carrying around the victim while sucking blood and soft tissues from the abdomen. In these situations,



FIGURE 3: Interactions between *Paussus favieri* and queen of *Pheidole pallidula* (photo by P. Mazzei).

the ants do not react aggressively toward the beetle. These behaviors never occurred in the movies selected for analyses.

Beetles were observed directly interacting with the queen (Figures 3(a) and 3(b)). In a few cases, the beetles remained in the queen's chamber for some days, antennating and rubbing against the queen's body without any aggressive reaction from the queen or the workers.

### 3.3. Description of *Pheidole pallidula* Behaviors When Interacting with Beetles

(a) *Antennation*. The ants touch the beetles with their antennae on all exposed parts of the body, but especially on the beetle's antennae (Figures 2(a) and 2(b)).

(b) *Alarm*. The ants antennate frenetically and widely open their mandibles, similarly to alarm behaviors performed during dangerous situations [1, 31]. This behavior is rarely observed against beetles, but when it is, it is not followed by biting.

(c) *Licking*. The ants lick all exposed parts of the beetle's body that are rich in trichomes (antennae, head, legs, elytra and pygidium) (Figures 2(a), 2(e), and 2(f)). This licking behavior is very similar to the ants' allogrooming behavior ([31] and references therein). The ants spend most time licking the trichomes on the basal spur and the pockets of the antennae (Figures 2(c) and 2(d)). This activity can be performed simultaneously on one beetle by many ants (minor and major workers), and it is the reciprocal behavior to "rewarding" by *P. favieri* (see Section 3.2).

(d) *Dragging*. The ants, mostly minors, occasionally bite the antennal club of *P. favieri* and quickly drag the beetle around the nest. The beetles, though much bigger than ants, do not resist being dragged around. In two cases, we observed minor workers dragging a beetle inside the queen's chamber.

3.4. *Cleaning Behavior of Paussus favieri*. The cleaning behavior is characterized by the following phases (see Supplementary Material 2).

(1) *Antennal Cleaning*. The forelegs clean the antennae one at time, starting from ventral to dorsal side of antennal surface. In particular, the tarsus and the hairy apical part of tibia rub the apex and the posterior part of the antennal club, the ventral pockets (Figures 2(b) and 2(d)), and the posterolateral teeth (Figure 2(a)), with numerous quick movements. The tarsus also rubs against the whole antennal club, moving laterally from base to apex with slow movements. Additionally, the tibia cleans the dorsal side of antennal surface, with a single movement. During this phase, the antenna is highly movable and it is rotated according to the side to be cleaned.

(2) *Head Cleaning*. One of the forelegs moves over the head, rubbing the apical tuft of long sensilla (Figure 2(a)). This behavior has been rarely observed.

(3) *Leg Cleaning*. This cleaning is performed mutually by pairs of legs of the same side, the fore against the middle, and the middle against the hind legs. The tarsus and the tibia of one leg slowly rub the reciprocal leg from the base to the apex. In addition, the tarsi are rubbed together (fore-middle, middle-hind) repeatedly.

(4) *Elytral Cleaning*. The elytra are cleaned in the antero-posterior direction with slow repeated movements of the middle and hind tibiae and tarsi of the same side. The tarsi of the middle and posterior legs also rub the lateral surface of the abdomen.

3.5. *Mating Behavior of Paussus favieri*. The mating behavior of *P. favieri* is characterized by two distinct phases: courtship and copulation (see Supplementary Material 3).

*Courtship*. Males actively search for females, approaching them by antennal contact (antennal approach) in one of two different ways: (a) with a slow alternate vertical movements of his antennae touching the female's antennae (frontal approach), and (b) his antennae touching laterally the side of the female's elytron (lateral approach). After the lateral antennation, the male forelegs are moved up and down,

touching the female elytra and pronotum. The female replies by moving her antennae and the hind legs. After this preliminary antennal approach, the male climbs upon the female’s body, dorsally positioning himself in the opposite direction of the female, touching his antennae the apex of the female’s abdomen. This dorsal inverted phase lasted a few seconds; afterwards the male turns 180°, reaching the typical mating dorsal phase. During this phase, the partners reciprocally touch their antennae, and the female often moves her hind legs. In the 10 sequences analyzed, the dorsal phase lasts from 5 to 12 minutes and, in a few cases, it was followed by copulation attempts.

*Copulation.* From the dorsal phase, the male of *P. favieri* slides backwards, bends the abdominal apex downward, extrudes the aedeagus, and tries to insert it into the female’s genitalia. The antennae of the male are frenetically moved up and down. The copulation with complete insertion of genitalia was observed only once. In fact, the female often rejects the male and avoid copulation.

During mating, the ants frequently interact with the beetles, antennating them and/or actively licking their antennae and legs.

*3.6. Analyses of the Behaviors of Paussus favieri during Interactions with Its Ant Host.* The following behaviors of *P. favieri* were analyzed statistically: (A) rewarding, (B) antennal shaking, (C) antennation, (D) escape, and (E) no contact. We detected significant differences in the time a beetle spends performing different behaviors (Table 1). Post hoc Tukey tests showed significant differences between E versus A, B, C, and D ( $P < 0.0001$  in all pairwise comparisons). Individuality was not significant, which indicates that behavioral patterns do not vary significantly among individuals. Differences in the mean duration of different behaviors are shown in Figure 4.

We found that significant differences among the frequencies that different behaviors were performed (Table 2). Post hoc Tukey tests showed significant differences between A versus B, C, D and E ( $P < 0.0001$ ) and between C versus B and D ( $P < 0.05$ ). Differences in the mean values of frequencies of different behaviors are shown in Figure 5.

#### 4. Discussion

According to Wasmann [33, 34], two defensive structural types are generally recognized in myrmecophile morphology: the “protective” type, characterized by a compact body with hard and smooth surfaces, and retractable appendages; and the “symphilous” type, characterized by slim bodies with long slender appendages and many trichomes covering the body and/or crowded in myrmecophilous organs [8, 35]. These body forms suggest different strategies both for entering the host nests and for avoiding ant attacks. Both body types are present in the Paussini, sometimes with intermediate forms, with the symphilous type generally present in the most derived taxa that are considered to be the best integrated into ants’ colonies [1, 36]. *Paussus favieri* is

TABLE 1: Results of a main effect ANOVA for values of times spent performing different behaviors by beetles when interacting with ants. d.f.: degrees of freedom; SS: sum of squares; MS: mean sum of squares; *F*: Fisher; *P*: probability.

Effect	d.f.	SS	MS	<i>F</i>	<i>P</i>
Individuality	13	6183.000	475.620	0.848	0.6090
Behavior	4	27389.200	6847.310	12.201	<b>0.0001</b>
Error	1012	567925.800	561.190		

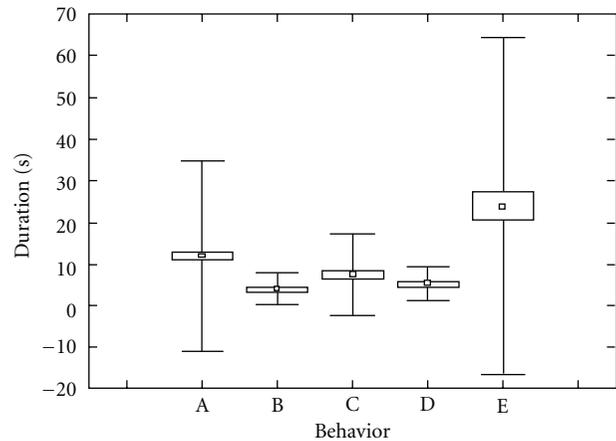


FIGURE 4: Differences in the duration of behaviors performed by beetles when interacting with ants. Mean values are shown by squares, standard errors as boxes, and standard deviations as whiskers. A: rewarding; B: antennal shaking; C: antennation; D: escape; E: no contact.

TABLE 2: Results of a main effect ANOVA for values of frequency of different behaviors performed by beetles when interacting with ants. d.f.: degrees of freedom; SS: sum of squares; MS: mean sum of squares; *F*: Fisher; *P*: probability.

Effect	d.f.	SS	MS	<i>F</i>	<i>P</i>
Individuality	13	643.238	49.480	1.027	0.4370
Behavior	4	4498.345	1124.586	23.336	<b>0.0001</b>
Error	66	3180.655	48.192		

clearly assignable to the latter type, showing all the distinctive characters noted previously. Our observations confirm that *P. favieri* is fully integrated in the host ant society since almost no aggressive behaviors against the beetles were observed. On the contrary, ants were strongly attracted by the beetle’s secretions.

The results of our statistical analyses show that beetles and ants spend a significantly longer amount of time not interacting (no contact, E) than the time they spend interacting with one another in a specific behavior. The state of no contact (E) can be the effect of a temporary withdrawal of the beetle, or the absence of caring by the ants. This is an expected result, since it is reasonable that the beetle spends most time in a number of activities that do not involve host interactions (i.e., exploring, mating, cleaning, resting, etc.).

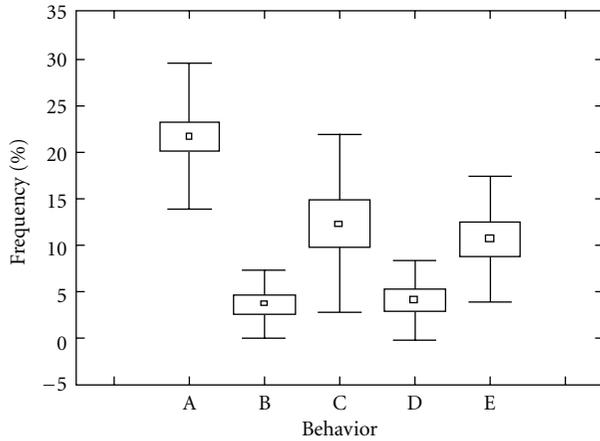


FIGURE 5: Differences in the frequencies of behaviors performed by beetles when interacting with ants. Mean values are shown by squares, standard errors as boxes, and standard deviations as whiskers. A: rewarding; B: antennal shaking; C: antennation; D: escape; E: no contact.

Concerning the behaviors performed by the beetle during the interactions with its host ants, the analysis of duration showed that rewarding (A), antennal shaking (B), antennation (C), and escape (D) are performed for similar amounts of time. However, it is notable that frequency of the rewarding behavior (A) is significantly greater than that of all other behaviors. During the rewarding behavior, *P. favieri* is antennated and actively licked by the ants, especially near the antennal symphilous organs (Figure 2(c)). This is consistent with the fact that the primary role of the highly modified antennae of *P. favieri* is glandular, producing substances that are highly attractive to the ants [37]. These substances are mostly stored inside the antennal pockets (Figure 2(d)). The chemical nature of this secretion is unknown, but it seems to be important for the acceptance and survival of the beetles within the ant nest [37] and for the success of the parasitic interaction. It has been speculated that, similar to other social parasites [1, 38], the chemicals secreted by Paussini beetles may have an appeasing function [8, 34]. Another hypothesis is that these substances provide a protective or rewarding food for the ants and their brood [8]. The rewarding behavior is generally associated with movements of the beetle's hind legs, an action possibly connected to the emission of stridulations. The high frequency of the rewarding behavior recorded in our experiments is quite in contrast with the previous observations by Escherich [13] and Le Masne [23], who reported that the ants only occasionally groom the beetle. According to our observations, the myrmecophilous strategy of *P. favieri* seems very similar to that of *P. turcicus* [12, 13], and the supposition that this strategy corresponds to a more derived (less costly) level of integration for *P. favieri* [8] seems unjustified.

The quick shaking of the antennae (antennal shaking (B)), not noted by Le Masne [22–24] and never recorded for any other coleopteran genera, has been occasionally observed in another species of *Paussus* [15]. Our observations suggest

that antennal shaking might be correlated with the glandular activity of the antennae, facilitating the spread of the viscous exudates from the antennal surface, or, most probably, with the spray of volatile allomones whose presence needs to be confirmed.

The antennation behavior (C) was described by Le Masne [22, 24], who interpreted it as a precursor to predation. Le Masne [24] observed that through antennation the beetle finds the ant's abdomen. Once found, the beetle pierces the abdomen with its sharp mandibles and feeds on the ant's hemolymph. However, in the videos analyzed for the present work, we never observed predation following the antennation behavior.

The occasional observation of some beetles interacting with the queen (Figures 3(a) and 3(b)), also for a prolonged time, is particularly interesting. We hypothesize that the physical interaction could supply a queen-specific chemical camouflage to the beetle and/or that the beetle could spread some of its attractive substances on the queen's body. In both cases, a chemical combination of beetle and queen odors could be reached, resulting in a deception of the hosts, allowing the beetle to achieve a higher social status inside the nest. The dragging of *P. favieri* inside the nest by *P. pallidula* minor and major workers is a behavior that this species (Maurizi and Di Giulio pers. obs.) and other *Pheidole* species [39] usually reserve for the queen [1, 8] and could be related to this possible mimicry. However, further research is required to confirm that this is a regular interaction, and that an exchange of cuticular hydrocarbons or other substances is involved.

The cleaning and mating behaviors performed by *P. favieri* inside the nest of *P. pallidula* have been observed and described in this work for the first time. Péringuey [10] mentioned a similar “brushing” behavior by fore and hind legs performed by males of *P. lineatus* after copulation. The complex cleaning behavior of *P. favieri* is quite different from the simple cleaning of other Carabidae [40] which mainly involves rubbing the comb organ of the forelegs (a row of spines positioned in an emargination of the inner edge of the fore tibiae) against antennae and mouthparts. In fact, the typical comb organ of ground beetles is vestigial or absent in Paussini [40, 41]. In *P. favieri*, the antennae have primarily a glandular function [37] and secrete a large amount of attracting substance. We interpret the rubbing of the forelegs against the antennae and then against middle and hind legs, head, elytra and abdomen, as a means of spreading antennal substances all over the body. This is also supported by the fact that the ants actively lick not only the antennae but also the head, legs, and elytra, suggesting that the attractants are present also on these body parts.

Little is known about the sexual behavior of Carabidae [42, 43], while no information is available for the Paussini except for a brief note of Péringuey [10] on *P. lineatus*. In this species, the male fixes his mandibles in the prothoracic excavation of the female and, with the hind legs, pulls the abdominal apex of the female towards him; in order to strengthen his position on the female's back, the male passes his antennae under the female's antennae, keeping this position for several hours. Serrano et al. [44] observed in

Portugal two specimens of *P. favieri* in copulation in an ant nest of *P. pallidula*, confirming that this beetle mates inside the colony, as is reported for other myrmecophilous beetles [45]. In captivity we observed the specimens of *P. favieri* mating in the ant nests several times and for a long duration. Precopulatory behavior includes exchanging tactile signals with antennae and legs, though it is possible that chemical signals are also involved. Unlike observations of *P. lineatus* [10], in both precopulatory and copulatory behaviors the mandibles are not used by *P. favieri*, while the dorsal position is maintained only by the male's legs. Of particular interest is the presence of an "inverted" dorsal phase (not noted in *P. lineatus*) that may be unique within the Carabidae.

Our experiments also suggest that acoustic signals are probably exchanged during the precopulatory behavior, since the female has been observed repeatedly moving the hind legs, a behavior possibly connected to the emission of stridulations (see Section 3.2(a)). However, the actual role of the acoustical communication in intra- and interspecific behaviors remains unknown.

In conclusion, the importance of the rewarding behavior confirms the primary role of the antennal secretions, possibly spread by a complex "cleaning" behavior, for the successful acceptance and integration of *P. favieri* inside the host colony. The identification of the secretions would be very important to verify their appeasing/rewarding properties, providing a more complete understanding of the myrmecophilous strategy of *P. favieri* and of other members of this tribe.

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## Review Article

# Specialized Fungal Parasites and Opportunistic Fungi in Gardens of Attine Ants

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Ants in the tribe Attini (Hymenoptera: Formicidae) comprise about 230 described species that share the same characteristic: all coevolved in an ancient mutualism with basidiomycetous fungi cultivated for food. In this paper we focused on fungi other than the mutualistic cultivar and their roles in the attine ant symbiosis. Specialized fungal parasites in the genus *Escovopsis* negatively impact the fungus gardens. Many fungal parasites may have small impacts on the ants' fungal colony when the colony is balanced, but then may opportunistically shift to having large impacts if the ants' colony becomes unbalanced.

## 1. Introduction

Restricted to the New World, the approximately 230 fungus-growing ant species in the tribe Attini cultivate basidiomycetous fungi on freshly harvested plant substrate [1–3]. A well-known subset of species in this tribe, the leaf-cutting ants, are considered the most important herbivores in the Neotropics [4, 5], due to the large amount of fresh leaves and flower parts that workers cut and use to nourish the mutualistic fungal cultivar.

The attine ant-fungal symbiosis is ancient and probably originated from ancestral ants occurring in the Amazon basin about 50 million years ago [6, 7]. Since then, the tribe Attini evolved five patterns of fungiculture that are currently recognized [6]. Thus, the lower and higher attine ant genera practice different types of fungiculture, which are classified according to the type of fungus and the type of substrates used to maintain the fungal partner [6]. Therefore, the various fungiculture can be defined as (i) lower attine agriculture performed by phylogenetically basal ant genera such as *Cyphomyrmex*, *Mycetagroicus*, and *Mycetophylax* which cultivate fungi in the tribe Leucocopriini [8, 9], (ii) a specific type of agriculture performed by some species of the lower attine genus *Apterostigma*, which cultivates fungi within the

family Pterulaceae (the “coral fungi” [10, 11]), (iii) a group of ants that cultivate Leucocopriini fungi in the yeast form (ants in the *Cyphomyrmex rimosus* group), (iv) the higher attine agriculture that encompass the derived genera *Trachymyrmex* and *Sericomyrmex* which cultivate phylogenetically derived fungi within the Leucocopriini, mostly on fallen vegetation or organic matter, and finally (v) a specific group of ants within the higher agriculture, the leaf-cutting ants, cultivate a recent clade of derived Leucocopriini fungi [12].

Evidence shows that attine ants domesticated their fungal cultivars during the evolution of the symbiosis [8]. Thus, the evolutionary history of fungus-growing ants was marked by several horizontal transfers (switches) of cultivars. Particularly, within the lower attine fungiculture these cultivar switches occurred multiple times [8]. Mikheyev et al. [13] demonstrated that leaf-cutting ants cultivate the same species of cultivar, *Leucoagaricus gongylophorus* in an association known as “many to one.” Interestingly, it was found that in colonies of leaf-cutting ant, just a single clone of the mutualistic fungus is cultivated by workers [14, 15].

Since the detailed study by Möller [16], it is known that attine fungiculture is continuously exposed to alien microorganisms. As fungus-growing ants rely on the mutualistic fungi as the main food source for the colony, fungiculture

requires from workers several mechanisms to keep their cultivars protected from alien microorganisms that would harm the symbiosis [17].

The most important strategies applied by attine ants in order to preserve their nests from harmful microbes consist of mechanical and chemical barriers including (i) careful cleaning of the leaf fragments used as substrate for the fungal cultivar in order to put away spores and microorganisms [18, 19]; (ii) massive inoculation of the mutualistic fungus mycelium onto the clean plant fragment increasing the colonization of this substrate by the cultivar [2]; (iii) the use of antimicrobial glandular secretions [20–24] and faecal droplets [25, 26]; (iv) weeding and grooming of infected parts of the garden when an undesired microorganism is detected [18]; (v) antagonistic activity of the mutualistic fungus against alien microorganisms [14, 19]; (vi) unspecific microbial interrelationship between microorganisms which benefit the whole nest [26]; (vii) control of humidity in disposal chambers [27]; (viii) association with antibiotic-producing bacteria [28–36]. Despite such mechanisms to suppress the development of alien microbes, a plethora of bacteria, filamentous fungi and yeasts are still found in ant gardens [35, 37–41].

Fungi on the genus *Escovopsis* are considered specialized parasites of attine gardens while others are consistently isolated in association with attine gardens and need further studies to understand their role as symbionts. Here, we focus on *Escovopsis* sp. and the additional filamentous fungi and yeasts found in attine gardens and address the few studies that have explored the role of such microorganisms in the attine ant-fungal symbiosis.

## 2. *Escovopsis* sp.: The Specialized Garden Parasite of Fungus-Growing Ants

The existence of the anamorphic fungus of the genus *Escovopsis* (Figure 1) was observed by various researchers [2, 42] and was uniquely discussed for the first time by Möller [16]. Recently, Currie et al. [37] reported that *Escovopsis* sp. is associated with several genera of attine ants and is considered a parasite of the fungus cultivated by these insects [43]. Except for fungus-growing ants in the *Cyphomyrmex rimosus* group, this parasite has been found in most attine ant genera with frequency of occurrence ranging from 11% to 75% [37, 38, 44, 45].

*Escovopsis* sp. can affect fungus gardens in various manners: in extreme cases, the parasite grows rapidly over the colony, resulting into its total collapse [45] (Figure 2). According to experiments conducted by Currie [45], *Escovopsis* can remain in the colony for an extended period of time, thereby, suppressing subsequent colony development. Such impacts on the ant colony are supposed to be due to the necrotrophic action of *Escovopsis* sp. towards the cultivar [43].

With respect to the occurrence of *Escovopsis*, so far, this parasite was not recorded from any other environmental source other than in association with attine ants, a pointer to a long history of coevolution with these ants and their

mutualistic fungi [46]. This ancient evolutionary pattern resulted in broad phylogenetic associations between the various types of fungiculture and specific phylogenetic lineages of *Escovopsis* [46–49] as it is the case of the relationship of this mycoparasite and ants in the genus *Apterostigma* that are naturally threatened by a specific lineage of *Escovopsis* sp. [44]. This lineage comprises four *Escovopsis* morphotypes defined on the basis of conidial colours ranging between white, yellow, pink, or brown [44].

Meanwhile, within a particular fungiculture group, the same *Escovopsis* sp. strain can be associated with many genera of ants and vice versa, demonstrating that the interrelationship is apparently nonspecific or weak at a finer phylogenetic level [50, 51]. Even in the same nest, different *Escovopsis* strains can be found as confirmed in the work of Taerum et al. [52], who verified that 67% of the colonies of *Atta* sp. and *Acromyrmex* sp. were infected by multiple strains of the parasite. Interestingly, such strains did not engage in interference competition for their hosts [52].

So far, only two species are formally recognized in this genus, namely, *Escovopsis weberi* [53] and *Escovopsis aspergilloides* [54]. These species were originally isolated from gardens of *Atta* sp. (in Brazil) and *Trachymyrmex ruthae* (in Trinidad and Tobago), respectively. Available data indicate that there exists a high variation in the morphology and genetic characteristics among strains of the two currently known *Escovopsis* species [44], suggesting that putative new species in this genus may be described in the near future [44, 55].

Several aspects of the biology of *Escovopsis* sp. still remain undiscovered. Nothing is known about their life cycle or whether there is a teleomorphic (sexual) state. Also, the mode of transmission between colonies is unknown. Regarding this aspect, Currie et al. [37] suggest that transmission may be through other arthropods that visit or inhabit the nests, such as mites. As a matter of fact, vertical transmission (from parental to offspring colonies) of this fungus has not been observed.

Considering the harmful effect and close relationship with the attine cultivar [49], it is not surprising to consider that this parasite could be used as a biocontrol agent. Accordingly, Folgarait et al. [56] studied the antagonistic effect of *Escovopsis* sp. towards three strains of the mutualistic fungus. The authors' findings indicate that, under *in vitro* conditions, *Escovopsis* sp. retarded the growth of the mutualistic fungus of *Acromyrmex lundii* and this effect is *Escovopsis* strain dependent. Similar results were previously reported by Silva et al. [57] in *in vitro* bioassays using one *Escovopsis* sp. strain and the mutualistic fungus of *A. sexdens rubropilosa*.

Despite these preliminary results about *Escovopsis* sp. as a potential agent of biological control, the ants' defensive mechanisms need to be considered. As a result of *Escovopsis* sp. infection ant colonies first mount a generalized response through a large mobilization of the individuals [58]. Second, workers physically remove and concentrate spores in the infrabuccal cavity [18, 59] (grooming); in addition, workers remove affected parts of the fungus gardens [18] (weeding). Regarding these two ant behaviours, there seems to exist caste specialization [60] and recruitment of workers to the site

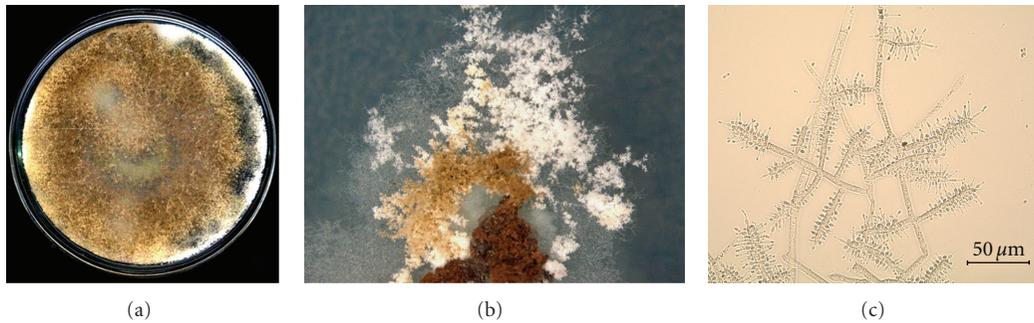


FIGURE 1: *Escovopsis* sp. parasites from fungus-growing ants. (a) General aspect of *Escovopsis* sp. isolated from the leaf-cutting ant *Atta sexdens rubropilosa* (Corumbataí, Brazil) cultured in potato dextrose agar (PDA) for 6 days at 25°C. (b) Close view of *Escovopsis* sp. isolated from *Acromyrmex lobicornis* (Santa Fé, Argentina) in PDA after 5 days at 25°C. (c) *Escovopsis* sp. conidiophores from (a). Note the cylindrical vesicles covered with ampulliform phialides.

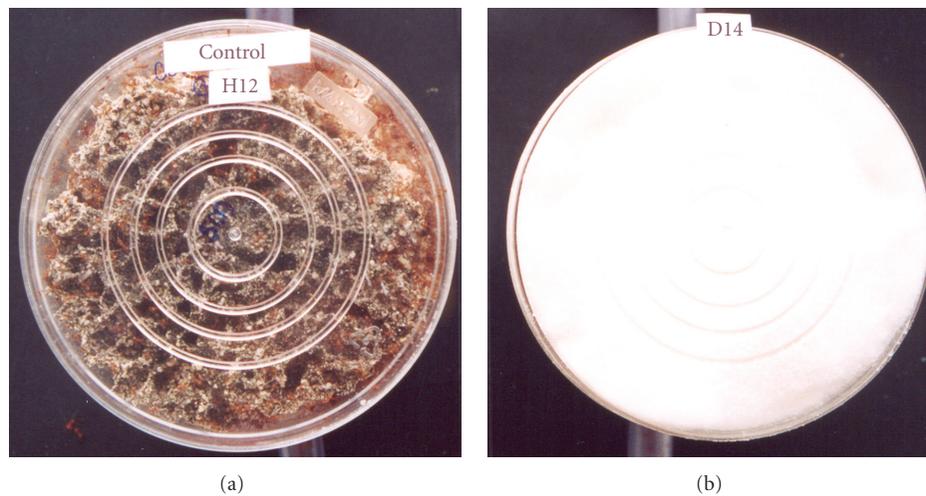


FIGURE 2: Laboratory colonies of *Atta sexdens rubropilosa* (Corumbataí, Brazil). Colonies were treated with baits without insecticide, control (a) and formulated baits containing the insecticide Hydramethylnon (b). *Escovopsis* sp. (white mycelia) emerged two days after treatment from fungus gardens of the infected colony (b), which depicts the aggressive effect of such parasite on attine ant colonies.

of infection [61]. An additional important factor that may impair the use of *Escovopsis* as agent of biological control is their low spore viability (about 3% viability [24]) and it should be considered on the development of biological control methods.

Perhaps the most effective defensive mechanism against *Escovopsis* sp. is the association of attine ants with microbial symbionts capable of producing antifungal substances. For example, ants are associated with *Pseudonocardia*, a group of bacteria found in the ant's exoskeleton that antagonize *Escovopsis* sp. and which is vertically transmitted during the foundation of new nests [28, 62]. Additionally, other microorganisms may antagonize *Escovopsis* sp. such as *Amycolatopsis* sp. [35], *Burkholderia* sp. [63], *Streptomyces* [32, 34, 35, 64], and yeasts [65]. Thus, rather than a one-to-one symbiosis between the ants and their fungi, recent work suggests that rather the ants rely on a consortium of microbes and their compounds to defend themselves against *Escovopsis* sp. parasites [26].

### 3. Occurrence of Additional Fungi on Gardens of Attine Ants and Their Possible Role as Symbionts

Evolutionary theory predicts that organisms with restricted genetic diversity are susceptible to exploitation by several parasites [66]. This can be the case of attine ants which cultivate a single strain of the mutualistic fungus [14, 15]. Since the fungus garden is an ideal environment for the growth of the fungal cultivar, it is expected that additional alien fungi would exploit this substrate.

As indicated earlier, Möller [16] was the first to record the existence of filamentous fungi in attine gardens. In addition to the fungus that was later named *Escovopsis* sp. and to which the author referred as the "strong state" of the mutualist cultivar, Möller [16] provided detailed information on the occurrence of *Aspergillus* sp., *Mucor* sp., *Penicillium*, and *Rhizopus* sp. on the fungus gardens of *Acromyrmex disciger* (collected in Blumenau, Brazil). This author reported

that such filamentous fungi covered the fungus gardens when left unattended by workers. Later, Spegazzini [67] found ascocarps of *Xylaria micrura* in abandoned nests of *Acromyrmex lundii* in Argentina and also provided detailed drawings of this structure. When working with laboratory nests of *Trachymyrmex septentrionalis*, Weber [68] observed that gardens were also overgrown by several filamentous fungi such as *Aspergillus* sp., *Mucor* sp., and *Penicillium* sp. Kreisel [42] also observed *Cunninghamella* sp., *Fusarium* sp., *Rhizopus* sp., and *Trichoderma* sp. when studying fungus gardens of *Atta insularis* (in Cuba) unattended by workers. Similarly, Bass and Cherrett [69] studying the roles that workers play on the fungus garden maintenance observed that *Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp., *Mucor* sp. and *Penicillium* sp. quickly overgrow gardens when left unattended by workers for several days. Luciano et al. [70] found *Aspergillus* sp., *Nigrospora* sp., and *Penicillium* sp. growing on the fungus garden of laboratory colonies of *Acromyrmex heyeri* in south Brazil.

Fisher et al. [39] demonstrated that *Atta cephalotes* colonies reared in the laboratory presented a shift in the fungal species composition when offered different plant substrates, thus providing the first experimental evidence that the fungal community on attine gardens may be influenced by the type of plant substrate used in the experiments. In addition, Currie et al. [37] demonstrated that gardens from diverse attine ant genera spanning all the phylogenetic diversity of the tribe Attini harbor alien fungi. These authors studied mostly attine ants from Central America and particular attention was drawn to the garden parasite *Escovopsis* sp. but several other fungi also occurred which were not identified [37]. Following this study, Ortiz et al. [71] reported the occurrence of *Fusarium* sp., *Rhizopus* sp., and *Trichoderma lignorum* when studying fungus garden fragments left unattended by workers of *A. cephalotes* (in Colombia). Barbosa et al. [72] and Barbosa [73] also reported a comprehensive list of species of filamentous fungi present in fungus gardens of *Atta laevigata* field nests in northeastern Brazil and concluded that the genus *Trichoderma* was prevalent in such gardens.

In addition to reports about filamentous fungi several authors also recorded a variety of yeasts on nests of attine ants. For example, Craven et al. [74] provided the first evidence that attine gardens contain yeasts using scanning electron microscopy. Pagnocca et al. [75] and Carreiro et al. [40] were the first to systematically study the yeast populations on laboratory colonies of *A. sexdens rubropilosa*. Such authors found variations in the abundance of yeasts populations in gardens and pointed out that *Candida*, *Cryptococcus*, *Pichia*, *Rhodotorula*, *Sporobolomyces*, *Tremella*, and *Trichosporon* were the prevalent genera. Carreiro et al. [76] showed that yeasts found on attine gardens produce the so-called killer toxins (or mycocins), which were proposed to be involved on the regulation of yeast populations on attine gardens. In this sense, Rodrigues et al. [65] proposed that yeasts may have a protective role in attine gardens against alien filamentous fungi. Up to date, the yeast survey on attine gardens rendered the description of three new species: *Cryptococcus haglerorum* [77], *Blastobotrys attinorum* (= *Sympodiomyces attinorum* [78]), and *Trichosporon*

*chiarellii* [79]; however, there is evidence that additional new yeast species associated with these insects await discovery. Recently, black yeasts in the genus *Phialophora* were reported to live on the exoskeleton of attine ants [80, 81] and the authors pointed out that they could antagonize the protective role of their symbiotic *Pseudonocardia*. Polysaccharidases secreted by yeasts and bacteria [82, 83] may also be important for the nest homeostasis and it is an open field for further investigation.

Despite the proposed roles that yeasts may play on attine gardens, few studies focused on the potential roles that filamentous fungi may perform on the attine ant-fungal interaction. Several filamentous fungi found in attine gardens are commonly found in soil or plant substrates, suggesting that these microorganisms are probably transported on the workers' integument or introduced into gardens via the plant material collected by the foraging workers [38, 41, 84]. Thus, it has been suggested that filamentous fungi are present in the fungus gardens as transient spores and may not play significant roles in the symbiosis [85]. In agreement with this hypothesis, Currie and Stuart [18] observed that when *Atta* sp. gardens are experimentally infected with *Trichoderma* sp. spores (a generalist fungus in comparison to *Escovopsis* sp.), the ants groom out spores efficiently that it is apparently removed from gardens. In contrast, gardens infected with *Escovopsis* sp. spores sustained long-term infections. Thus, this result suggests that general fungi like *Trichoderma* sp. may not play any role in the symbiosis [18].

On the other hand, recent studies address that filamentous fungi may play important roles in gardens of fungus-growing ants. Considering the studies reviewed here it is clear that a common trend arises: filamentous fungi (i) are found in association with diverse genera of attine ants, (ii) are found in attine nests from different localities, and, most important, (iii) quickly develop when the fungus gardens are unattended by workers (Figure 3).

In this sense, Rodrigues et al. [86] determined that the majority of microfungi found in gardens of *A. sexdens rubropilosa*, a leaf-cutting ant species spread all over Brazil, belong to genera commonly found in soil and plant substrate. Particularly, the fungus *Syncephalastrum racemosum* was found in 54% of gardens from laboratory nests treated with baits supplemented with the insecticide sulfluramid (commonly used in Brazil to control leaf-cutting ants). A variety of other fungi, including *Fusarium solani*, were found in such gardens but with fewer than 20% of prevalence. None of the laboratory nests used as control (either treated with *Eucalyptus* sp. leaves or baits without insecticide) had the fungus gardens overgrown by filamentous fungi. On the other hand, *Fusarium oxysporum* and *Trichoderma harzianum* were found in 23% and 38% of gardens from field nests treated with baits supplemented with sulfluramid, respectively. It is interesting to note that *S. racemosum* was not observed in gardens from nests treated with insecticides under field conditions [86]. In addition, *Escovopsis* sp. was isolated in 21% and 15% in gardens of laboratory and field nests treated with sulfluramid, respectively [86]. In another experiment, several microfungi species were observed to quickly overgrow the fungus garden of *A. sexdens rubropilosa*

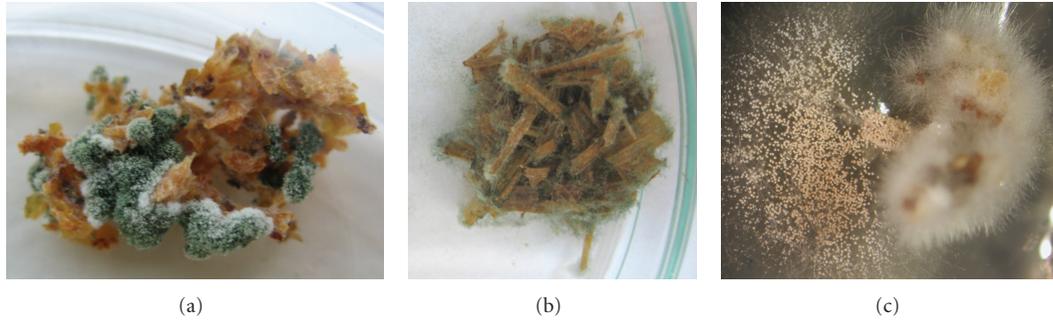


FIGURE 3: Fungus garden fragments of leaf-cutting ants overgrown by filamentous fungi. (a) *Atta texana* garden fragment (TX, USA) with green tufts of *Trichoderma* sp. (b) *Atta bisphaerica* garden fragment (Botucatu, Brazil) with green conidiation of *Trichoderma* sp. Workers from (a) and (b) were manually removed and garden fragments were kept in wet chambers for 5 days at 25° C. (c) Isolation plate showing fungus garden fragment on potato dextrose agar medium supplemented with 150  $\mu\text{g}\cdot\text{mL}^{-1}$  of chloramphenicol. On the right note the white mycelia of the mutualistic fungus of attine ants and on the left the microfungus *Aspergillus* sp. Both fungi emerged from the garden fragment.

( $n = 12$ ) when workers were experimentally removed [87]. The observed species included *Acremonium kiliense* (42%), *E. weberi* (42%), *Trichoderma* sp. (50%), and a fungus previously identified as *Moniliella suaveolens* (50%), which now is known to be a genus not yet described (Harry Evans, personal communication). Similarly, Carlos et al. [88] found several fungal species including *Penicillium* spp. and *S. racemosum* on *A. sexdens rubropilosa* gardens when treated with a variety of formulated insecticides.

In another systematic study, filamentous fungi were also reported from field nests of several species of *Acromyrmex* from south Brazil [38]. The authors observed a high diversity of fungi and noted that *F. oxysporum*, *Escovopsis* sp., and *Cunninghamella binariae* were present in 40.5%, 27%, and 19%, respectively, out of 37 nests. In contrast with previous studies, *S. racemosum* was found in 5.4% of the nests. Recently, Rodrigues et al. [41] showed that *Cyphomyrmex wheeleri* ( $n = 16$  nests), *Trachymyrmex septentrionalis* ( $n = 16$ ), and *Atta texana* ( $n = 4$ ) sampled in Texas (USA) harbor a diverse community of microfungi which varies across seasons and are structured, in part, by location where nests were collected, reflecting a spatial component on the structuring of fungal communities. Interestingly, both *Escovopsis* sp. and *S. racemosum* were not found in the studied nests.

Moreover, many filamentous fungi are carried by the female alates (gynes) of leaf-cutting ants during the foundation of a new nest [89–91]. These microorganisms were more prevalent on the integument than in pellets found in the infrabuccal pocket [37, 91]. Although such fungi may be accidentally transported by gynes, they compose the initial microbiota associated with the ant nests and might be involved in the success on the establishment of a new nest. In fact, Autuori [92, 93] reported that several incipient nests do not thrive the period following the nuptial flight. In addition to flooding and birds, this author argued that fungi were also responsible for the mortality of incipient nests [93].

An additional observation sheds light on the possible role of filamentous fungi as opportunistic antagonists. When laboratory subcolonies of *A. sexdens rubropilosa* were artificially infected with spores suspensions of *Fusarium solani*, *Trichoderma* cf. *harzianum*, *S. racemosum*, and *E.*

*weberi*, Rodrigues et al. [94] observed that only nests treated with *E. weberi* provided a persistent infection (detected up to 300 hours after infection). However, about twelve hours after treatment with *S. racemosum* spores, workers removed fragments of fungus gardens and dumped away from gardens. This observation parallels the weeding behavior originally described as a specific adaptation for removing germinated spores of *Escovopsis* sp. [18]. Dumped fragments were collected and after plating quickly revealed the presence of *S. racemosum* [94].

In addition to *Escovopsis* sp. other filamentous fungi were thought to be used as biological control agents [95]. Thus, attempts to use fungal spores on bait formulations demonstrated the effectiveness of this approach. Formulated baits with a combination of spores of *Metarhizium anisopliae* (an insect pathogenic fungi) and *Trichoderma viride* (opportunistic antagonist of the ant cultivar) controlled 100% of laboratory colonies of *Atta cephalotes* compared to the control (nests treated with baits without spores) [95]. Field experiments showed that baits with *M. anisopliae* and *T. viride* spores achieved 100% of nest mortality when compared to the insecticide Pirimiphos-methyl, which caused 60% of nest mortality. However, the time necessary to achieve 100% of nest mortality using the formulated baits was more than 60 days [95], which is considered ineffective for controlling leaf-cutting ants in large areas. Despite the failure of such attempts these initiatives are desirable and perhaps will guide the development of alternative techniques to control these pest ants.

#### 4. Conclusions and Future Directions

The evidence gathered so far suggests that filamentous fungi act as opportunistic antagonists on the attine ant-fungal interaction. In comparison to the specialized fungus *Escovopsis* sp., filamentous fungi are considered nonspecific antagonists of the ant cultivar. The antagonistic effect of these fungi is evident in disturbed gardens (either caused by insecticides or other unknown factor), when gardens are unattended by workers and on incipient nests. Future

experimental studies should systematically address whether filamentous fungi also influence healthy colonies. The results of such studies will ultimately help in the development of new strategies for controlling leaf-cutting ants.

Finally, despite the arguments in favour of the antagonistic nature of filamentous fungi, we do not rule out that some may have other unknown functions in the attine ant symbiosis. For instance, Freinkman et al. [96] demonstrated that fungi may be a potential source of new compounds as it is the case of bionectriol A, isolated from *Bionectria* sp. derived from the fungus gardens of *Apterostigma dentigerum*. Perhaps, future reports will unravel the existence of filamentous fungi that are beneficial to the ant colony. This aspect is totally unexplored and should also be considered when studying such microorganisms.

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## Research Article

# Exploitative Competition and Risk of Parasitism in Two Host Ant Species: The Roles of Habitat Complexity, Body Size, and Behavioral Dominance

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Habitat structural complexity can slow resource discovery by ants but can also lower the risk of parasitism during foraging. The relative importance of these two ecological facets of habitat complexity may differ in a species-specific manner and thus may be important in the outcome of exploitative competition over food resources. For the host ant species *Pheidole diversipilosa* and *P. bicarinata*, we used in situ experimental manipulations to explore whether the effects of habitat complexity on exploitative competition depended on host body size and behavioral dominance, two characteristics likely to affect mobility and utilization of refuge from specialist Dipteran parasitoids (*Apocephalus orthocladius* and *A. pugilist*, resp.). We found that habitat complexity affected the resource discovery and harvest components of exploitative competition in an opposing fashion for each species and discuss these results in light of the differences in body size and behavioral dominance between the two hosts.

## 1. Introduction

Characteristics of habitats in which animals forage influence their mobility, ability to compete, and the likelihood of encountering predators, among other things. The structural complexity of a habitat can act on several aspects of animal foraging simultaneously. In particular, exploitative competition or the consumption of a common resource without direct competitive interaction may be directly affected by habitat complexity because it constrains animal movement in a species-specific manner [1]. Architecturally complex substrates often take more energy and time to traverse [2–5], which can reduce a species' ability to find and efficiently harvest a resource. A given degree of habitat complexity will be more difficult for relatively small species to traverse because they must move around or through the substrate, instead of over it [1]. As a result, habitat complexity can mediate exploitative competition for a common resource because species of different sizes are differentially affected.

Although habitat complexity may have negative effects on exploitative competitive ability by constraining animal movement, it may also have positive indirect effects on

competitive ability by providing refuge from predators or parasitoids during resource acquisition. Numerous studies on a wide range of taxa have noted the importance of habitat complexity in providing refuge from predators ([6–13], but see [14, 15], e.g., of habitat complexity increasing predation). By impeding movement and providing refuge, habitat complexity may have opposing effects on resource discovery and acquisition, but the degree to which this is true may depend on species-specific characteristics such as body size or use of refuge from predators.

Ant communities are well suited for studying the role of habitat complexity in exploitative resource competition and escape from predators. Evidence suggests that exploitative competition between species has fitness consequences and is important in determining community composition [16–19]. Ants exhibit a wide range of body sizes [1], with larger ants being able to navigate complexity in the microhabitat more easily than small ants [20–23]. Increased habitat complexity does not appear to have an energetic cost to foragers [24], but does increase the time required to harvest resources and necessarily decreases harvest rate [25].

Ant communities are not traditionally considered to be structured by top-down forces from predators. However, community composition can be influenced by specialist Dipteran parasitoids (Apocephalus: Phoridae) that attack host ant species, induce behavioral responses in their hosts, and alter the outcome of interspecific competition in the community [26–32]. Habitat complexity has been shown to benefit the host ant species *Pheidole diversipilosa* and *P. bicarinata* during interference competition with nonhost ant species by providing refuge from parasitoids. Refuge allows hosts to maintain similar numbers of soldiers during head-to-head competition as in competitive bouts without parasitoids [13]. These two host ants cooccur in the same habitat and are dominant to most other ants in the community, but *P. bicarinata* is behaviorally subordinate to *P. diversipilosa* [30]. This difference in dominance has the potential to impact benefits derived from habitat complexity during exploitative competition.

Previous research indicates that *P. diversipilosa* wins a majority of contests, has access to the majority of resources, and experiences a resource environment that is not restricted by competition [29]. If such a host is attacked by its specialist parasitoid while exploiting an uncontested resource, it can simply abandon the resource, wait for parasitoids to leave, and return to the resource at a later time [33]. As a result, any refuge provided by habitat complexity would have marginal benefit to the colony during exploitative competition.

Predictions are different for the more subordinate species, *P. bicarinata*. Subordinate species only have access to a limited proportion of total available resources because they often lose resources to dominants [29]. Previous work has shown that subordinate hosts simply cannot afford to leave a resource when parasitoids arrive because successful foraging bouts are too rare [33]. For subordinates, a higher mortality risk must be accepted in order to satisfy energy requirements. Work on damselfly, passerine bird and ant communities has demonstrated that solutions to this ecological trade-off have evolutionary repercussions: subordinate competitors or species with higher resource requirements display little predator avoidance regardless of any pressure from dominant competitors [33–35]. Such observations have led to the hypothesis that subordinate species, who typically experience a more limited resource environment than dominants, will sacrifice predator avoidance to a greater extent than dominants in order to meet energy requirements [33, 36, 37]. While harvesting uncontested resources, subordinate hosts are likely to benefit from refuge provided by habitat complexity to a greater extent than dominant hosts because subordinates under attack by parasitoids must continue to forage even when resources are not contested by competitors, whereas dominants can avoid parasitism by returning to the nest.

The ecological and evolutionary consequences of host dominance discussed above suggest that benefits derived from habitat complexity may depend on whether foraging is occurring in an interference or exploitative competitive context. The benefits derived from habitat complexity during interference competition (head-to-head competition for resources) are investigated in a previous study [13]. In con-

trast, this study focuses on whether habitat complexity affects the exploitative component of competition (depression of the resource base in the absence of competitors). We explored the benefits derived from habitat complexity separately in interference and exploitative contexts because parasitoids have a greater impact on hosts engaged in interference competition (versus exploitative harvest of uncontested resources), due to a positive feedback between recruitment pheromones used during defense of resources and parasitoid behavior [29].

In addition, this study expands upon a previous study [13] by exploring whether habitat complexity affects the “discovery” and “harvest” components of exploitative competition separately. The effects of habitat complexity on each component of exploitative competition are interpreted in light of the body size and behavioral dominance of two host ant species. Of the two focal species, *P. diversipilosa* is approximately twice as large as *P. bicarinata* (workers: 0.12 versus 0.05 mg, resp.; soldiers: 0.44 versus 0.26 mg, resp.), and wins 15% more of its interactions with all other species in the local assemblage [30]. First, we determine whether habitat complexity influences the time it takes each host species to find resources (the “discovery” component of exploitative competition, [38]). Second, we ask whether the benefits hosts receive from refuge during harvest of uncontested resources (the “harvest” component of exploitative competition) depends on their dominance within the community. Benefits provided by habitat complexity during harvest of uncontested resources are measured in terms of the number of soldier ants because (1) soldier ants are crucial for the defense and harvest of large resources and (2) only soldiers are attacked by parasitoids. We then interpret our findings in the context of variation in habitat complexity.

## 2. Materials and Methods

**2.1. Study Site and System.** This study was conducted in oak, pine, and juniper woodlands in the Chiricahua Mountains of Southeast Arizona. The two focal ant species *P. diversipilosa* and *P. bicarinata* coexist in this habitat and are hosts to species-specific parasitoids (*Apocephalus orthocladius* and *A. pugilist*, resp., [39]). In July–August of 2003, *P. diversipilosa* was studied on National Forest land surrounding the Southwestern Research Station (31°52′ N 109°14′ W). In August–September of 2004, *P. bicarinata* was studied nearby on land owned by the Southwestern Research Station (31°53′ N 109°12′ W). Colonies of *P. diversipilosa*, *P. bicarinata*, and their respective parasitoids are found at both of these sites within meters of each other, but their relative abundances at each site differ (see Section 4).

**2.2. Experimental Design.** To investigate how habitat complexity affects exploitative competition for resources and host-parasitoid interactions, we forced field colonies to forage up into plastic bins and recorded their behavior under different levels of habitat complexity and parasitism. Cookie baits measuring 2 × 2 cm were placed 50 cm away from the nest entrance, and the number of soldiers harvesting and

defending these baits was recorded every 10 minutes for 2.5 hours in all treatments. Cookie baits are examples of large resources that require soldiers to break them into small pieces for efficient transport by workers. Placing baits 50 cm away from colony entrances ensured that baits were discovered and that colonies traversed a distance during which they were susceptible to parasitoid attack.

Foraging bins were 30 × 60 cm Sterilite storage containers, and had a 6 cm diameter hole at one end that could be placed directly over colony nest entrances. Using foraging bins allowed us to (1) minimize disturbance around nest sites and control exactly which resources hosts were harvesting and (2) introduce or exclude parasitoids from treatments using bridal veil to cover the foraging bin. We used soldier number as a response variable because soldiers (1) are able to carve up large resources for transport to the nest by workers, and thus are critical to harvesting resources, (2) can defend resources against competitors, and (3) are the only caste attacked by parasitoids in this system. We also recorded the time it took colonies to discover cookie baits.

We used a multifactor design with two levels of habitat complexity (complex or simple) and parasitoid exposure (parasitoids present or absent). Complex habitat treatments contained 5000 cm<sup>3</sup> of leaf litter that had been oven-dried for 72 h, while simple habitat treatments occurred in empty foraging bins. The addition of leaf litter closely approximated average leaf litter depth found in habitat where both species coexisted. Parasitoids were captured by aspiration during recruitment events instigated at unused host colonies nearby. In parasitoid-present treatments, two parasitoids were introduced after soldiers had recruited to resources. Foraging bins were covered tightly with bridal veil in all treatments to ensure that parasitoids could not escape from parasitoid-present treatments and that parasitoids could not gain access to parasitoid-absent treatments.

Treatments were replicated on seven colonies of *P. diversipilosa* and eight colonies of *P. bicarinata*. The experiment was performed in areas where *P. diversipilosa* and its specialist parasitoid *A. orthocladus* cooccurred with *P. bicarinata* and its specialist parasitoid *A. pugilist* in order to control for the surrounding competitive environment. Colonies were randomly assigned the order in which they received treatments such that all colonies on a given trial day received different treatments. This allowed us to control for the effects of environmental variation and cumulative treatment effects. In addition, we rested colonies for two days between treatments to control for energetic state after foraging on cookies. It was not possible to monitor all colonies at once due to time constraints and distance between colonies, so all replicates were divided roughly into two groups, and groups experienced treatments within 24 h of each other to control for environmental conditions. All treatments were shaded to control for temperature and humidity differences between colony locations.

**2.3. Analysis.** Exploitative competition can generally be divided into two components: discovery and harvest of resources. To determine the impact of habitat complexity on

resource discovery for each host, we conducted paired *t*-tests on the time it took hosts to discover cookie baits (TTD) in complex and simple habitat treatments. This experiment resembles a randomized block or repeated measures design, in which colonies are blocks and treatments are implemented within blocks. Since parasitoid treatments were not implemented until after hosts discovered cookie baits, TTD values were averaged across both levels of parasitism (e.g., for each complexity treatment, TTD values for each colony were averages of TTD in parasitoid absent and parasitoid present levels). Paired *t*-tests were then performed to compare each colony's average values for complex and simple habitats. To compare discovery speed between hosts within either simple or complex habitat treatments, we used two-sample *t*-tests because hosts were not intrinsically paired. To control for the multiple comparisons made within habitat complexity treatments and maintain an experiment-wide  $\alpha$  of 0.05, we used Bonferroni adjustments.

To determine whether the refuge benefits provided by habitat complexity during harvest of resources depend on host dominance level, we constructed a randomized block/repeated measures General Linear Model to test for differences among treatments. For each host, post hoc comparisons among means were conducted using Tukey's HSD method with degrees of freedom appropriate for randomized block/repeated measures designs and 0.05 experiment-wide  $\alpha$  levels. Replicate means were calculated by averaging recorded values of soldiers at cookie baits over the 2.5 h foraging period. Recorded values were averaged from the time colonies discovered the cookie bait for treatments without parasitoids, and from the point of parasitoid introduction for treatments with parasitoids. Means were transformed [ $\log(\text{mean} + 1)$ ] to meet homogeneity of variance and normality assumptions.

### 3. Results

The time it took *P. diversipilosa* to discover cookie baits did not differ significantly between complex and simple habitat treatments ( $t_{1,6} = -0.870$ ,  $P > 0.05$ ; Figure 1), although *P. diversipilosa* discovered resources slightly faster in complex habitat treatments. In contrast, *P. bicarinata* discovered resources in simple habitats much more quickly than in complex habitats ( $t_{1,7} = 5.276$ ,  $P < 0.005$ ; Figure 1). Within complex habitats, *P. diversipilosa* discovered resources more quickly than *P. bicarinata* ( $t_{1,13} = 2.538$ ,  $P < 0.05$ ; Figure 1), but *P. bicarinata* discovered resources more quickly than *P. diversipilosa* in simple habitats ( $t_{1,13} = -2.923$ ,  $P < 0.005$ ).

For both *P. diversipilosa* and *P. bicarinata*, general linear models indicated that significant differences in the number of soldiers harvesting resources existed between at least two treatments ( $F_{3,17} = 5.070$ ,  $P < 0.05$ ;  $F_{3,21} = 4.139$ ,  $P < 0.05$  resp.). *P. diversipilosa* maintained significantly more soldiers at resources in complex habitats without parasitoids than either complex or simple habitats with parasitoids (closed circle compared to closed and open triangles in Figure 2(a):  $Q_t = 4.244$ ,  $P < 0.05$ ;  $Q_t = 4.683$ ,  $P < 0.05$ ). There was a little

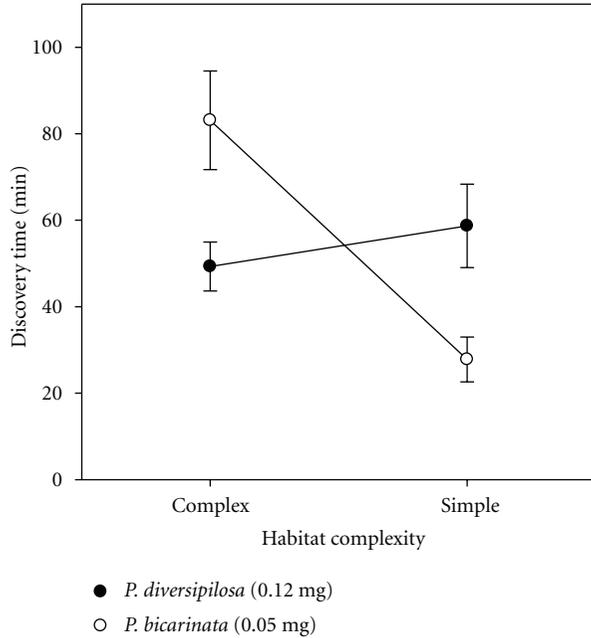


FIGURE 1: Differences in resource discovery time between *P. diversipilosa* and *P. bicarinata* in complex and simple habitats. Means and standard errors are presented.

difference in soldier number between simple and complex habitats without parasitoids (open and closed circles:  $Q_t = 1.358$ ,  $P > 0.05$ ). There was also no difference in soldier number between simple habitats without parasitoids and both simple and complex habitats with parasitoids (open circles compared to open and closed triangles:  $Q_t = 3.379$ ,  $P > 0.05$ ;  $Q_t = 2.886$ ,  $P > 0.05$ ).

*P. bicarinata* maintained significantly more soldiers at resources in complex and simple habitats without parasitoids than simple habitats with parasitoids (open and closed circles compared to open triangle in Figure 2(b):  $Q_t = 4.199$ ,  $P < 0.05$ ;  $Q_t = 4.191$ ,  $P < 0.05$ ). In contrast, no difference in soldier number existed between complex and simple habitats without parasitoids and complex habitats with parasitoids (open and closed circles compared to closed triangles:  $Q_t = 2.355$ ,  $P > 0.05$ ;  $Q_t = 2.347$ ,  $P > 0.05$ ). Soldier number was also statistically indistinguishable between complexity levels in both parasitoid and no parasitoid treatments (open compared to closed triangles and open compared to closed circles:  $Q_t = 1.844$ ,  $P > 0.05$ ;  $Q_t = 0.008$ ,  $P > 0.05$ ).

## 4. Discussion

**4.1. Exploitative Competition.** For a given habitat complexity level, such as the leaf litter used in this study, smaller species perceive their environment as more rugose than larger species. This theory, known as the size-grain hypothesis, predicts that larger species will traverse a moderately rugose habitat with greater ease than smaller species [1]. Results on resource discovery time show that smaller *P. bicarinata* take longer to discover resources in complex habitats than do larger *P. diversipilosa*, which is consistent with the size-grain

hypothesis. However, the observation that smaller *P. bicarinata* find resources in simple habitats more quickly than larger *P. diversipilosa* runs somewhat contrary to the predictions of the size-grain hypothesis. This observation suggests that, in addition to the limitations on movement predicted by the size-grain hypothesis, these two species either (1) differ in the degree to which they tolerate desiccation, (2) have different exploratory or recruitment strategies, or (3) exhibit differential sensory bias toward habitat complexity. First, differences in the degree to which species tolerate desiccation is not a plausible explanation for *P. bicarinata* discovering resources more quickly than *P. diversipilosa* in simple habitats because smaller ants such as *P. bicarinata* are more sensitive to desiccation stress than larger ants, and soil temperatures are much higher in more open, simplified environments [40–44]. Physiological limitations are also not a plausible explanation in the context of our experimental setup because physiological conditions between treatments were controlled (see Section 2). Second, *P. diversipilosa* and *P. bicarinata* may differ in their exploratory [45] or recruitment behaviors [46]. Unfortunately, the small scale of our experimental arena caused a rapid attenuation of recruitment curves, making insight into exploratory and recruitment behavior difficult in this study. Further work should be conducted to determine whether differences in exploratory or recruitment behavior can explain *P. bicarinata* discovering resources more quickly than *P. diversipilosa* in simple habitats. Finally, *P. diversipilosa* and *P. bicarinata* may exhibit differential sensory bias towards habitat complexity, a possibility that is discussed in detail below. Regardless of the mechanism behind these results, the ultimate consequence is that smaller *P. bicarinata* can discover resources faster in simple habitats, while larger *P. diversipilosa* can discover resources faster in complex habitats. Thus, habitat complexity has important but contrasting effects on the resource discovery component of exploitative competition for both species.

During initial attempts to find host colonies for this study, 44% of *P. bicarinata* and 64% of *P. diversipilosa* foraging bouts to cookie baits went unchallenged (data not shown). Thus refuge from parasitoids during uncontested harvest of resources may have important fitness consequences. *P. diversipilosa* and *P. bicarinata* harvesting resources in the absence of direct competition respond differently to habitat complexity, and this difference is best explained by the parasitoid avoidance behavior and dominance of each host. We predicted that *P. diversipilosa*, being behaviorally more dominant and having access to the majority of resources, would abandon uncontested resources when under attack by parasitoids regardless of the presence of refuge in complex habitats. This prediction follows from the resource loss-predation trade-off suggested to exist in a variety of systems [33, 36, 37]. We found that *P. diversipilosa* under attack by parasitoids do abandon uncontested resources regardless of whether refuge from habitat complexity is present. However, we also found that the number of soldiers *P. diversipilosa* maintains at resources in simple habitats without parasitoids is not statistically distinguishable from the number of soldiers maintained in simple habitats with parasitoids.

A lower physiological threshold for open habitats is one explanation for this pattern but is unlikely for reasons explained above. In addition, if desiccation tolerance were solely responsible for the observed foraging patterns of *P. diversipilosa* during exploitative competition, significant differences between complex habitat treatments in the presence and absence parasitoids should not exist. However, we cannot rule out the role of desiccation tolerance in *P. diversipilosa* foraging behavior. A more plausible explanation is that *P. diversipilosa* exhibits a sensory bias towards habitat complexity and is less willing to forage in any habitat that does not offer refuge from parasitoids. Numerous studies on a wide range of taxa suggest that animals make patch choices based on perceived predation risk ([47] and references therein, [48–50]). Work on vole, deer mouse and passerine bird populations suggests that competitive dominants may choose to forage in habitats with less predation risk, thereby forcing subordinates to forage in habitats with greater predation risk [50–53]. These patch choices take place in ecological time and are considered solutions to the problem of maximizing energy intake while minimizing mortality risk.

As predicted by the resource loss-predation trade-off, subordinates must accept a higher mortality in order to satisfy energy requirements. Therefore, we predicted that *P. bicarinata* under attack by parasitoids would benefit from refuge even while foraging on uncontested resources. The number of *P. bicarinata* soldiers at resources in complex habitats was similar regardless of parasitoid presence, but soldier number in simple habitats with parasitoids was much lower than without parasitoids. These observations support the predictions of the resource loss-predation trade off and suggest that refuge benefits associated with habitat complexity depend on host dominance during exploitative competition. Subordinate hosts harvesting uncontested resources benefit from habitat complexity because their need for resources does not allow them to avoid parasitism by ceasing foraging. In contrast, dominant hosts harvesting uncontested resources receive no benefit from habitat complexity because they can afford to cease foraging in the presence of parasitoids. The potential for resource loss increases when resources are directly contested by competitors (interference competition). As the potential for resource loss increases during interference competition, dominant hosts should become more willing to accept the risk of parasitism in order to retain resources, and refuge provided by habitat complexity may allow hosts to strike a balance between retaining resources and risking mortality. Prior work in this system has shown that *P. diversipilosa* under attack by parasitoids will not abandon resources if they are directly contested by competitors, as long as habitat complexity provides some refuge from attacking parasitoids [13]. This study expands upon previous work [13] by demonstrating that behavioral dominance and refuge provided by habitat complexity interact to influence how species balance the resource loss-predation trade-off in different competitive contexts. The acts of discovering resources and harvesting resources in the absence of competitors are two important components of exploitative competition between the focal species of this study. Habitat complexity provides

an advantage to *P. diversipilosa* during the discovery phase of exploitative competition because *P. diversipilosa* is larger and can traverse complex habitats more easily than *P. bicarinata*. The opposite is true while harvesting resources: habitat complexity provides an important refuge benefit to *P. bicarinata*, but no refuge benefit to *P. diversipilosa*. During exploitative competition, habitat complexity plays a dual role in impeding movement and providing refuge. These mechanisms work in opposing manners in this system because the focal species differ in body size and behavioral dominance. The degree to which the discovery and harvest components of exploitative competition are opposing will depend on the relative strength with which habitat complexity impedes movement and offers refuge for *P. diversipilosa* and *P. bicarinata*.

**4.2. Impact of Natural Heterogeneity on Movement and Benefits from Refuge.** For ants, the degree to which movement is impeded by habitat complexity depends largely on the abundance and quality of litter on the ground surface. Natural heterogeneity in habitat complexity could lead to local pockets in which movement was strongly impeded by habitat complexity, favoring *P. diversipilosa*'s resource discovery abilities, and other pockets where movement was unimpeded, favoring *P. bicarinata*'s discovery abilities. In extremely heterogeneous environments, the relative discovery abilities of both species may, therefore, be similar when summed across the community. Further work is needed to determine whether natural heterogeneity in habitat complexity could facilitate coexistence between these host species.

The degree to which habitat complexity provides refuge depends both on variation in litter and on the abundance of parasitoids. While under attack by a constant number of parasitoids, *P. bicarinata* benefits more from refuge than *P. diversipilosa* (Figure 2). If there is reliable parasitoid pressure on both hosts, *P. bicarinata* would experience a greater relative benefit from refuge. Working in the same system, LeBrun and Feener [29] found that parasitoids discovered *P. diversipilosa* exploiting resources in the absence of competitors ~50% of the time. Parasitoid discovery of *P. bicarinata* is less predictable, as *A. pugilist* exhibits wide fluctuations in abundance through space and time, but is rarely more than 50% [33]. Based on observed parasitoid abundance for each host, it appears that the potential to benefit from refuge is greater for *P. bicarinata*. Pockets of low habitat complexity will not counteract benefits that *P. bicarinata* receives from areas nearby with higher habitat complexity because, unlike *P. diversipilosa*, *P. bicarinata* forages willingly in simplified habitats, and also unlike *P. diversipilosa*, *P. bicarinata* maintains some foraging presence at resources regardless of whether refuge from parasitoids is available (see Figure 2).

**4.3. Conclusions.** While *P. diversipilosa* should have greater relative discovery abilities in complex habitats, natural heterogeneity in structural complexity will minimize this advantage by favoring *P. bicarinata* in simpler habitats. *P. bicarinata* is also likely to benefit from refuge from parasitoids during harvest of uncontested resources to a greater degree than

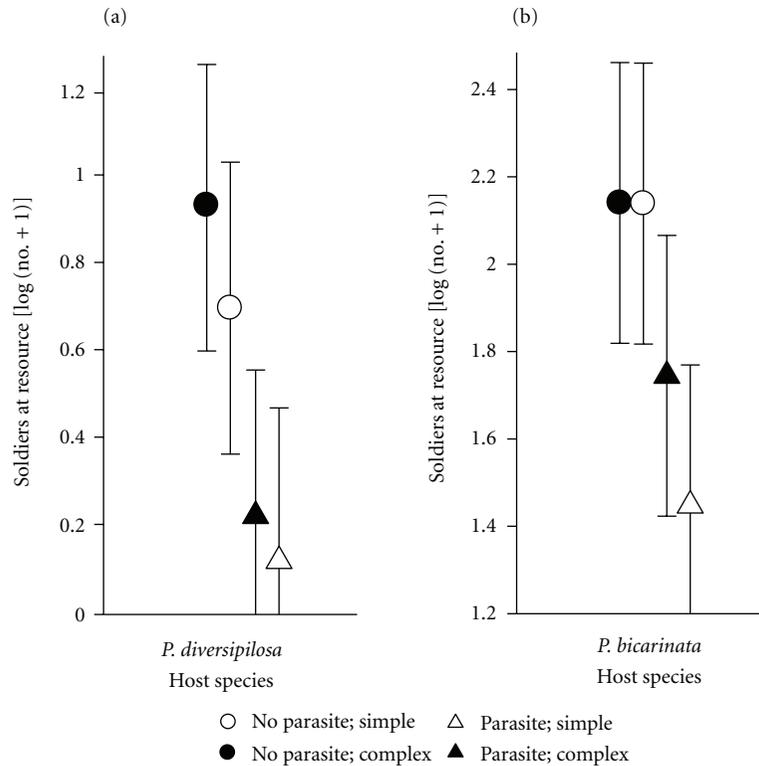


FIGURE 2: Number of (a) *P. diversipilosa* and (b) *P. bicarinata* soldiers harvesting resources in the absence of head-to-head competition when parasitoids are absent (circles) or present (triangles) in complex habitat (filled symbols) or simple habitat (empty symbols). Means and Tukey's minimum significant difference (MSD) comparison intervals are presented. Means whose comparison intervals overlap are not significantly different. Means whose comparison intervals do not overlap are significantly different at an experiment-wide  $\alpha$  of 0.05.

*P. diversipilosa*, and this advantage will not be affected by natural heterogeneity in structural complexity. These advantages in exploitative competitive ability experienced by *P. bicarinata* may partially explain why it is able to coexist along with *P. diversipilosa*, who is a superior interference competitor [29].

This study demonstrates how the dual roles of habitat complexity in impeding movement and providing refuge from parasitoids impact the exploitative competitive abilities of two host ant species. These two mechanisms by which habitat complexity mediates competition may function in an opposing manner because of differences in host body size and behavioral dominance. However, further work should be conducted to determine whether differences in exploratory or recruitment strategies offer additional insight into the effects of habitat complexity on each host [45, 46]. Natural variation in habitat complexity or variation caused by disturbance such as fire [13] may impact the relative importance of these mechanisms for each host, the degree to which they are opposing, and therefore the potential for coexistence between these species. Knowledge of the prevalence of complex versus simple substrates within and between habitats is important for predicting the degree to which these mechanisms oppose each other, but is currently lacking.

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## Review Article

# On the Use of Adaptive Resemblance Terms in Chemical Ecology

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Many organisms (mimics) show adaptive resemblance to an element of their environment (model) in order to dupe another organism (operator) for their own benefit. We noted that the terms for adaptive resemblance are used inconsistently within chemical ecology and with respect to the usage in general biology. Here we first describe how resemblance terms are used in general biology and then comparatively examine the use in chemical ecology. As a result we suggest the following consistent terminology: “chemical crypsis” occurs when the operator does not detect the mimic as a discrete entity (background matching). “Chemical masquerade” occurs when the operator detects the mimic but misidentifies it as an uninteresting entity, as opposed to “chemical mimicry” in which an organism is detected as an interesting entity by the operator. The additional terms “acquired” and “innate” may be used to specify the origins of mimetic cues.

## 1. Introduction

Social insects, especially ants and termites, dominate many terrestrial habitats in terms of abundance, biomass, and energy turnover [1, 2]. They accumulate considerable amounts of resources that can be of potential use for other organisms, in the form of living biomass, infrastructures (e.g., nest sites), or stored products [3]. The ecological success of social insects comes with the cost that predators and parasites may exploit their societies [4–6]. Since Wasmann’s [7] extensive study on organisms that developed close relationships with ants, a multitude of so-called myrmecophiles has been found to exploit ant colonies and their social resources in a variety of ways [5, 8]. Parasitic relationships may escalate in an evolutionary arms race where the hosts adapt towards protecting themselves from exploitation, while parasites adapt towards avoiding expulsion from the host [9].

In this context it is crucial that members of a society can be recognized reliably and distinguished from aliens, which can thus be aggressively expelled [10]. An efficient social recognition system is essential for a colony to function as a closed unit. The better such recognition works, the more effectively social exploitation can be prevented. Complex profiles of cuticular hydrocarbons (CHCs) are known to carry

information necessary for recognition of colony members in ants, bees, and wasps [10].

Macroparasites of ants have evolved a variety of strategies to cope with their hosts’ elaborate recognition system [5]. Potential strategies for avoiding or resisting the hosts’ defense behavior include the use of morphological, acoustical, and behavioral adaptations or the use of chemical repellents or attractants [1, 5, 11–13]. Particularly widespread and important are chemical strategies for avoiding recognition, either by not expressing relevant recognition cues or by matching host recognition cues [11, 14, 15]. For simplicity, we use the term “cue” referring to any chemical information that is potentially perceivable, irrespective of whether the information transfer is “intentional” or “unintentional” *sensu* Steiger et al. [16].

Chemical resemblances work analogously to other biological resemblances, such as acoustic or visual mimicry [17]. Unfortunately, different definitions exist in chemical ecology (see below), and thus different authors may describe different forms of chemical resemblances with identical terms or the same type of resemblance with different terms.

The aim of this paper is threefold. First, we identify how definitions of resemblances are generally used in biology. Second, we analyze the terminology that is used in chemical

TABLE 1: Summarized table of adaptive resemblance terms in general biology as used in important reviews. Systems can either be considered according to what a mimic pretends to be or according to what an operator perceives. We adopted the latter view.

By an operator, the mimic is . . .			
not detected as a discrete entity (causing no reaction)	detected as an uninteresting entity (causing no reaction)	detected as an interesting entity (causing a reaction beneficial to the mimic)	Reference(s)
Crypsis	Masquerade	Mimicry	Endler 1981 [21], 1988 [22]
Eucrypsis	Mimesis	Homotypy	Pasteur 1982 <sup>a</sup> [23]
Eucrypsis	Plant-part mimicry	Mimicry	Robinson 1981 [24]
Crypsis	Masquerade	Mimicry	Ruxton et al. 2004 [25], Ruxton 2009 [17]
Cryptic resemblance	Cryptic resemblance	Sematic resemblance	Starrett 1993 [18]
Crypsis	Masquerade	—	Stevens and Merilaita 2009 <sup>b</sup> [26]
Crypsis	Crypsis	Mimicry <sup>c</sup>	Vane-Wright 1976 [27], 1980 [20]
Camouflage or mimesis	Camouflage or mimesis	Mimicry	Wickler 1968 [19]

—: not considered.

<sup>a</sup>Pasteur [23] uses the term “camouflage” as generic term for both eucrypsis and mimesis.

<sup>b</sup>The term “camouflage” is used by Stevens and Merilaita [26] to describe all forms of concealment, including crypsis and masquerade.

<sup>c</sup>For the imitation of inanimate objects, Vane-Wright [27] uses the expressions “decoys” or “deflective marks”.

ecology. Finally, we attempt a synthesis and suggest a terminology that agrees best with the general biological definitions and with the chemical strategies observed in nature.

## 2. General Definitions of Biological Resemblances

Since the resemblance of organisms to elements of their environment (e.g., other organisms or background) is often not coincidental, but rather evolved for the benefit of the mimic, the term adaptive resemblance was coined [18]. In adaptive resemblance one organism (the mimic) modifies its appearance, pretending to be something different (the model), in order to dupe another organism (the operator) [19, 20]. Many different terms have been used to describe adaptive resemblance, including mimicry, camouflage, crypsis, masquerade, and mimesis. These terms have been debated intensively and defined repeatedly according to different criteria (see Table 1).

For the purpose of this paper, we adopted an operator’s view to narrow down the existing definitions of adaptive resemblance into a unified system. This means that we distinguish the cues of a mimic with respect to whether and how they are perceived by the operator. The resulting categories are only valid within a given perceptive channel between mimic and operator, and they can differ in other channels or if other organisms are considered. The first column of Table 1 defines resemblances in which a mimic is not perceived as a discrete entity by the operator and consequently causes no reaction in the operator. In such cases the mimic frequently blends with the background. We adopt the term “*crypsis*” for this phenomenon according to Endler [21], who first distinguished this type of resemblance from “*masquerade*”. In the latter a mimic is perceived by an operator as a discrete entity, which is however misidentified as uninteresting so that the operator also shows no reaction to the mimic. Accordingly, crypsis relies on the relationship

between the organism and the background, whereas the benefit of masquerade is thought to be independent of the background [28]. A stick insect, for example, is likely to be recognized as a stick by a potential predator independent of its surroundings (e.g., when lying on grass). A cryptic organism, however, depends strongly on the background. This fact allows testable predictions to be made. For example, a mimic performing masquerade should be treated similarly by the operator independent of its background. On the other hand a mimic that performs crypsis should be treated differently (e.g., recognized and attacked) by the operator when the background changes.

The third column of Table 1 defines adaptive resemblances in which a mimic is perceived by the operator as an entity of interest. This category was first described in a biological context by Bates [29] as “*mimicry*”, and this term is currently most frequently used, hence we adopt it here.

Finally, another mechanism exists to avoid detection by an operator, which is however not based on resemblance. The term “*hiding*” has been applied to cases in which the absence of informative cues is achieved by behavioral adaptations, making detection by an operator impossible [17]. In visual systems, for example, a rabbit is hiding if it stays in its burrow in the presence of a predator (operator), thereby avoiding detection [17]. If a hiding organism would be removed from the environment, the perceptive input of the operator will not change in the concerning channel. Hiding is not included in Table 1 because it does not fall into categories of resemblance; nevertheless this term will be of importance in our discussion on chemical interactions below.

## 3. The Use of Adaptive Resemblance Terms in Chemical Ecology

Compared to visual adaptive resemblances, chemical adaptive resemblances had initially been paid less attention to in scientific literature, despite the fact that chemical communication is the most widespread form of communication

among organisms [16, 30, 31]. However, more recent reviews on this topic show that understanding of chemical adaptive resemblance has increased markedly [11, 15, 32, 33].

According to this special issue on ants and their parasites, we focus here particularly on important reviews about parasites of social insects and on reviews about adaptive chemical resemblance. Reviews are suitable for analyzing how the terminology is used, since they provide overviews about specific fields, summarize the literature, and therefore mirror common practices.

We used the same categorization as in Table 1, adopting an operator's point of view. Note that two resemblance types were combined, that is, resemblances in which a mimic is not detected as discrete entity and resemblances in which a mimic is detected as an uninteresting entity (Table 2). We combined these two types of resemblances because none of the reviews distinguished them. Additionally, we included the origins of mimetic compounds in the table, since this is an interesting point regarding chemical resemblances and several authors based their terminology upon it.

Table 2 shows that the terms chemical mimicry and chemical camouflage are not used consistently. Some authors used the terms according to criteria similar to those used in general biology (see Table 1). They distinguished between chemical mimicry as the imitation of an interesting entity and chemical camouflage either as the imitation of an uninteresting entity or as the resemblance of background cues (*sensu* Dettner and Liepert [15]). This use of terms did not include the origins of mimetic compounds. In contrast, other authors focused primarily on the origin of mimetic cues. According to their terminology, chemical mimicry implies that mimetic cues are biosynthesized by the mimic, while chemical camouflage implies that the mimic acquires mimetic cues from the model (first defined by Howard et al. [38]). Additional definitions specifically focused on a mimic's avoidance of being detected as a discrete entity (Table 2). Chemical resemblances that allow mimics to avoid detection by background matching were defined as chemical mimesis by Akino [14] or as chemical crypsis by Stowe [31].

In addition to adaptive resemblances, another mechanism exists among parasites to prevent detection by an operator. This mechanism was called "chemical insignificance" [39]. However, chemical insignificance was originally brought up to describe the status of freshly hatched ant workers (callows), which typically carry very low quantities of cuticular hydrocarbons [39]. The term insignificance referred to these weak chemical cues, which are frequently not colony or even species specific, allowing the transfer and acceptance of callows into alien colonies [11]. The term chemical insignificance was also adopted to describe a status of ant parasites, which may benefit from displaying no or only small quantities of recognition cues to sneak unnoticed into host colonies [3, 11, 39, 40]. We discuss this point in more detail at the end of the following chapter.

Furthermore, chemical transparency was recently described as a chemical strategy in a wasp social parasite [41]. This strategy is somewhat similar to chemical insignificance, except that it refers particularly to a subset of cuticular compounds that are presumably responsible for recognition. We

discuss both strategies, chemical insignificance and transparency, in more detail at the end of the following section.

#### 4. Suggestions for a Consistent Terminology

As described above, adaptive resemblance terminology is used inconsistently in important reviews of chemical ecology, likely mirroring inconsistent use in this field generally. Most importantly, the terms chemical camouflage and chemical mimicry are inconsistently used by different approaches. While some authors distinguish them according to different models that are mimicked, others distinguish them according to the origin of mimetic cues (Table 2). To avoid confusion, we suggest a consistent terminology that is in line with the definitions used in general biology (Table 1). Consequently, adaptive resemblance of an entity interesting for the operator should be referred to as "*chemical mimicry*", irrespective of the origin of mimetic cues. Nevertheless, an additional distinction between biosynthesis and acquisition of mimetic cues might often be useful. Hence, we suggest using additional terms to distinguish the origins of mimetic cues; "*acquired* chemical mimicry" indicates that mimetic cues are acquired from the model, while "*innate* chemical mimicry" (as first mentioned by Lenoir et al. [11]) indicates that a mimic has an inherited ability to biosynthesize mimetic compounds. The two different mechanisms may affect coevolutionary dynamics in different ways. For example, a consequence of the acquisition of recognition cues by a parasite from its host is that the mimetic cues of model and mimic are of identical origin [3]. Coevolutionary arms races select in such cases for effective ways of acquiring chemical host cues by the mimic, for example, through specific behaviors such as intensive physical contact to the host. In the host, selection favors counterdefenses which prevent the acquisition of chemical cues. Selection pressures are somewhat different when a parasite biosynthesizes the mimetic cues [3]. In this case, the origins of the chemical cues of mimic and model are different, which allows coevolutionary arms races to shape on the one hand the accuracy of chemical mimicry of the mimic and on the other hand the discrimination abilities of the operator.

Mimics that are not detected as discrete entities or that are detected but misidentified as uninteresting entities by an operator have rarely been addressed in chemical ecological reviews, although they are common in general biology (first two columns of Table 1). Since the term camouflage is not used in general biology to distinguish these two forms of resemblances (Table 1) and since the term chemical camouflage is used inconsistently in chemical ecology (Table 2), we suggest abandoning this term so as to avoid confusion. Instead, we suggest using terms consistent to general biology. Accordingly, "*chemical crypsis*" describes cases in which an operator is not able to detect a mimic as a discrete entity, while "*chemical masquerade*" describes cases in which an operator detects a mimic as an uninteresting entity. In both cases, the operator shows no reaction. The terms "acquired" and "innate" can be applied to these categories as well to add further information on the origin of the disguising cues. Note that it is challenging but logically possible to

TABLE 2: Summarized table of the main terms used for chemical adaptive resemblances in reviews about parasites of social insects and in reviews about adaptive chemical resemblance. Systems can either be considered according to what a mimic pretends to be or according to what an operator perceives. We adopted the latter view. Furthermore, the terminology based on the origins of mimetic compounds is shown.

By an operator, the mimic is... not detected as discrete entity or detected as uninteresting entity <sup>a</sup>	detected as an interesting entity	Origin of mimetic compounds in cases where the mimic is detected as interesting entity by the operator		Reference
		Innate biosynthesis	Acquisition from host	
Chemical mimesis <sup>b</sup>	Chemical mimicry or camouflage	Chemical mimicry	Chemical camouflage	Akino 2008 <sup>c</sup> [14]
—	Chemical mimicry	No distinction		Bagnères and Lorenzi 2010 <sup>d</sup> [34]
Chemical camouflage	Chemical mimicry	No distinction		Dettner and Liepert 1994 [15]
Chemical camouflage	Chemical mimicry	No distinction		Geiselhardt et al. 2007 <sup>e</sup> [34]
—	Chemical mimicry	No distinction		Howard and Blomquist 2005 [32]
—	Chemical mimicry	No distinction		Keeling et al. 2004 [35]
—	Chemical mimicry	Chemical mimicry by biosynthesis	Chemical mimicry by camouflage	Lenoir et al. 2001 [11]
—	Chemical mimicry or camouflage	Chemical mimicry	Chemical camouflage	Nash and Boomsma 2008 <sup>c</sup> [3]
—	Chemical mimicry	—		Pierce et al. 2002 [36]
—	Chemical mimicry	Not specified	Chemical mimicry	Singer 1998 <sup>f</sup> [37]
Chemical crypsis <sup>g</sup>	Chemical mimicry	No distinction		Stowe 1988 [31]
—	Chemical mimicry	Not specified	Chemical camouflage <sup>h</sup>	Thomas et al. 2005 <sup>c</sup> [8]

—: not considered in the article. No distinction: the term chemical mimicry was used irrespective of the origin of mimetic cues. <sup>a</sup>According to the first two columns in Table 1. <sup>b</sup>Defined as being invisible through background matching. <sup>c</sup>Authors follow the definition of Howard et al. [38]. <sup>d</sup>Authors use the term mimicry irrespective of the origin of mimetic compounds but point out that different definitions exist depending on their origin. <sup>e</sup>Authors follow Dettner and Liepert [15]. <sup>f</sup>The term camouflage was used once to describe invading predators that biosynthesize CHCs of social insects. <sup>g</sup>Defined as resemblance of the background or of an entity in the background. <sup>h</sup>Inconsistent to the definitions of Dettner and Liepert [15].

TABLE 3: Proposed terminology for chemical adaptive resemblances. Chemical cues of a mimic can either be “*acquired*” from the environment (including the host), or they can be “*innate*”, that is, biosynthesized. In all cases of chemical adaptive resemblance, the operator is deceived by the mimic so that the mimic benefits.

Suggested term	By an operator, the mimic is. . .
<i>Chemical crypsis</i>	. . . not detected as a discrete entity due to the expression of cues that blend with the environment (causing no reaction in the operator).
<i>Chemical masquerade</i>	. . . detected but misidentified as an uninteresting entity (causing no reaction in the operator).
<i>Chemical mimicry</i>	. . . detected as an entity of interest (causing a reaction in the operator).

empirically separate cases of masquerade and crypsis [28], but this has yet to be done in a nonvisual context. Table 3 gives an overview on our proposed terminology for chemical adaptive resemblances. Please note that in our terminology it is only important whether and how mimics are perceived by an operator. Similarities in the chemical profiles of parasites and hosts may be important diagnostic tools, but they are not part of the definitions.

Finally, we want to stress the special case of organisms that suppress the expression of chemical cues which can potentially be detected by the operator. Following our aim of applying a consistent biological terminology, “*chemical hiding*” is the most appropriate definition. This definition includes two slightly different scenarios, the total absence of relevant cues and the presence of cues below the operator’s perceptive threshold. In both cases chemical perception of the organism is impossible. A host’s inability to detect any chemical cues of a parasite was also referred to as “chemical insignificance” [3]. However, the term chemical insignificance is unfortunately used ambiguously regarding the important point whether there are no detectable cues [3] or small yet detectable amounts of cues are present [39]. Clearly, it should be distinguished whether an operator is able to detect an organism or not. If resemblance cues are present and perceived (irrespective of the quantitative level), the phenomenon will fall per definition into one of the categories chemical crypsis, chemical masquerade, or chemical mimicry (Table 3). For example, if a callows’ weak chemical signature was expressed by a parasite and adult host ants misidentified this parasite as a callow, we would follow Ruxton [17] by assigning this to chemical mimicry (since callows are certainly interesting entities). Empirical evidence for a chemical mimicry of callows could result in practice from a combination of chemical data (callow resemblance) and behavioral data (hosts treat parasite as callows). However, an exhaustive discussion about methods is beyond the scope of this conceptual paper. Consequently, the original definition of chemical insignificance as a “weak signal” [39] appears not applicable to parasites without the risk of confusing it with chemical mimicry. If chemical cues are below an operator’s perceptive threshold, the definition of chemical

hiding will apply. However, the term chemical insignificance may be used as a functional term describing the lack of chemical information in a certain context. For example, callows are chemically insignificant in terms of nestmate recognition due to a lack of chemical information in that context. Nevertheless, callows carry apparently sufficient information in the context of caste identity since workers show characteristic behaviors towards them; for example, they receive assistance during hatching and are transported to new nest sites in migratory ants.

The above discussion on chemical insignificance applies also to the phenomenon of chemical transparency. If no cues are expressed that are perceivable by the operator, the focal organism would show chemical hiding, regardless of the presence of any other compounds. In contrast, if perceivable cues are present, chemical crypsis, chemical masquerade, or chemical mimicry applies. In the described case of chemical transparency [41], the parasite is most likely recognized and misidentified as an interesting entity (e.g., as brood), since social parasites usually exploit the brood care behavior of their hosts.

Notably, a parasite may alternatively avoid chemical detection through behavioral mechanisms by “hiding” according to the definition in general biology (see above) rather than “chemical hiding.” For example, if it avoids detection by staying in a cavity so that its chemical cues do not reach the operator, it is hiding. A parasite that performs “hiding” could potentially be detected if it was somehow confronted with the operator. In contrast, a parasite that shows “chemical hiding” cannot be detected by chemical senses of the operator at all.

## 5. Examples for the Use of Adaptive Resemblance Terms

In this section we want to discuss examples to clarify the use of terms regarding adaptive resemblances. The mimicking of CHC profiles of the host is widespread among ant parasites, and this is generally assumed to facilitate integration into the host colonies. Parasites are indeed frequently not recognized as alien species [11, 33]. This strategy of avoiding recognition as an alien species by expression of host CHCs could potentially be referred to as chemical crypsis (if the colony odor is regarded as the background) or as chemical masquerade (if a nestmate worker is regarded as an uninteresting entity). However, we argue that the strategy is best described by chemical mimicry for the following reasons. First, workers are certainly able to detect other workers, and hence parasites that mimic them are discrete entities, excluding the term chemical crypsis. Second, workers are certainly interesting entities to other workers because social actions are shared, such as grooming or trophallaxis. Consequently, a mimic that uses a worker as model resembles an entity of potential interest to ant workers, so that chemical mimicry rather than chemical masquerade applies.

It becomes more complicated when a parasite mimics the nest odor of its host. Lenoir et al. [42] demonstrated that the inner nest walls of the ant species *Lasius niger* are coated with the same CHCs as those that occur on the cuticle of workers. However, the CHCs on the walls occurred in different

proportions and showed no colony specificity. If a mimic resembles such a chemical profile, chemical crypsis will be the most appropriate term, because the mimic represents no discrete entity and rather blends with the uniform nest odor. To our knowledge, no clear evidence exists for this case.

Another example is worth highlighting in this context which was already pointed out by Ruxton [17]. The CHCs of *Biston robustum* caterpillars resemble the surface chemicals of twigs from its host plant [43]. *Formica japonica* and *Lasius japonicus* workers do not recognize the caterpillars on their native host plant, but when caterpillars were transferred to a different plant, the ants noticed and attacked them. In this case it depends on the operator's perception whether the example should be considered as chemical crypsis or chemical masquerade. If the ants did not detect a twig (and hence a caterpillar) as a discrete entity, but as background, chemical crypsis would apply. If the ants detected the caterpillar as a discrete but uninteresting entity, for example, as a twig, then chemical masquerade would apply. As Ruxton [17] emphasized, twigs are of huge dimension compared to the size of ants. Hence, it is more likely that ants do not detect caterpillars as discrete (uninteresting) entities, but rather perceive them as (uninteresting) background. Accordingly, chemical crypsis appears to be the most appropriate term for this example.

These examples may demonstrate that it can be rather difficult to assign appropriate terms to particular adaptive resemblance systems. Nevertheless, the definitions we proposed are generally straightforward, and they can be applied unambiguously if the necessary information about a system is available. We hope that this paper contributes to a careful and consistent use of adaptive resemblance terminology in chemical ecology.

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## Review Article

# Fire Ants (*Solenopsis* spp.) and Their Natural Enemies in Southern South America

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We review the fire ant research conducted by the ARS-South American Biological Control Laboratory (SABCL) since 1987 to find a complex of natural enemies in southern South America and evaluate their specificity and suitability for field release as self-sustaining biological control agents. We also include those studies conducted by the ARS-Center for Medical, Agriculture, and Veterinary Entomology in the United States with the SABCL collaboration. Ecological and biological information is reported on local fire ants and their microsporidia, nematodes, viruses, phorid flies, eucharitid wasps, strepsiptera, and parasitic ants. Their biology, abundance, distribution, detrimental effect, field persistence, specificity, and phenology are discussed. We conclude that the objectives of the ARS program in South America are being achieved and that the pioneering studies have served to encourage further investigations in the United States and other countries and advanced the implementation of biological control programs to decrease imported fire ant densities and damage. Still, several promising organisms should be further investigated for eventual field release in the near future.

## 1. Introduction

The ant genus *Solenopsis* Westwood (Hymenoptera: Formicidae: Myrmicinae) is represented in South America by 16 native species known as “fire ants” [1]. While, in general, these ants cause occasional local problems in their homeland, two species accidentally introduced into the southern United States in the early 1900s are considered pests with a high negative impact in rural and urban areas [2]. These pest species are the red imported fire ant, *Solenopsis invicta* Buren, and the black imported fire ant, *S. richteri* Forel, both included in a revision of the *S. saevissima* complex [3].

During the last decade, *S. invicta* has been considered one of the 100 worst invasive exotic species [4]; this fire ant became a more global problem when it invaded ecosystems in the Caribbean Islands [5], Australia [6], New Zealand [7], Hong Kong, Taiwan [8], and mainland China [9]. Its eradication has been accomplished only in New Zealand [10].

In the United States, the imported fire ants cause many problems in the southeast and in some patches in California. They are a major public health concern because of their

aggressive stinging behavior [11]. Although, for most individuals, this is just an irritating nuisance, for several hundred thousand people in the United States, sensitive to fire ants or highly allergic, the sting might cause severe reactions and eventually death [12]. Fire ants also injure domestic animals, livestock, affect wildlife [13, 14], native ants, and other arthropods [15, 16]. Structures, electrical devices, and agricultural crops can also be damaged [17, 18].

In disturbed North American environments, imported fire ants are dominant terrestrial arthropods [19]. *Solenopsis invicta* has displaced Nearctic species of fire ants in the United States and adversely affected the diversity of the ant assemblages [15, 16]; however, the nature of the impact on native ant species has been controversial [20]. Some beneficial effects of fire ants such as predation on several agricultural and livestock pests have been also reported [20, 21].

In the United States, chlorine insecticides were used to control fire ants in the 1950s and 1960s, but they brought negative consequences to the environment [21]. A new bait with the insecticide mirex was believed to make fire ant eradication possible. However, in 1971, its use was highly restricted because of many environmental concerns and

mirex registration was cancelled in 1977 [11, 21]. Since the 1980s, more environmentally friendly products have been used in the United States [11] and in other invaded countries. Still, the chemical approach is expensive, only provides temporary control, is detrimental to several nontarget organisms, and is not appropriate for large and/or sensitive environments. Consequently, the need of implementing control methods with less negative environmental impacts became a priority.

The first serious interest in biological control of fire ants was shown by the United States in the late 1960s. Scientists from the University of Florida and the ARS-Insects Affecting Man and Animals Research Laboratory (IAMARL, now the Center for Medical, Agricultural, and Veterinary Entomology, CMAVE), both in Gainesville, FL, conducted the first surveys for natural enemies in Brazil, Uruguay, and Argentina and provided information on several potential candidates [22–24].

In late 1987, after three years of cooperative work with Brazilian researchers in Mato Grosso and Mato Grosso do Sul, Brazil, scientists from the IAMARL formally established the fire ant biological control program at the ARS-South American Biological Control Laboratory (SABCL) in Hurlingham, Buenos Aires province, Argentina [25]. Since then, the main objective of the program has been to find a complex of natural enemies of fire ants in their homeland, evaluate their specificity, and determine their suitability for eventual use in the United States against the red and black imported fire ants.

In this paper, we review the fire ant research conducted by SABCL researchers in southern South America since 1987. Several studies carried on in the United States by CMAVE scientists in collaboration with SABCL researchers are also included. We cover not only the occurrence of fire ant natural enemies and aspects of their biology and ecology, but also ecological studies on other South American fire ants.

## 2. Fire Ants in Southern South America

South American fire ants occur in almost all habitats from the Amazon Basin of Brazil to 42° S in Río Negro province, Argentina [1, 26–28], and up to more than 3,200 m of altitude in the Puna region in the Andes [29]. *Solenopsis invicta* occurs along most of the Río de la Plata basin from the vicinity of Rosario, Santa Fe province, Argentina, to Paraguay, southern Brazil and eastern Bolivia [1, 28] (Figure 1). Recent surveys revealed that mitochondrial DNA haplotypes of *S. invicta* are distributed in Argentina up to 33°41' S in Mercedes, San Luis province, and 64°52' W and 1,100 m of altitude in the Calilegua National Park, Jujuy province [29–31]. The occurrence of *S. invicta* in the Amazon basin has been controversial since it has been previously recorded in Porto Velho, Rondonia state, Brazil [28], but it was virtually absent in more recent studies [30, 31]. *Solenopsis richteri* is native to central Argentina, southern Uruguay, and Brazil. In Argentina, it occurs mainly in the pampas surrounding Buenos Aires and along the lower reaches of the Río de la Plata basin, up north to Rosario area. Other common fire ant species in southern South

America are *S. quinquecupis* Forel, mostly cooccurring with *S. richteri*; *S. magdonaghi* Santschi, mostly cooccurring with *S. invicta*; *S. interrupta* Santschi, mostly cooccurring with *S. electra* Forel in northwestern Argentina and Bolivia; *S. weyrauchi* Trager, mostly occurring presumably alone above 2,000 m of altitude throughout the Andes from Perú to Argentina [1, 30].

Hundreds of studies on introduced populations of the red and black imported fire ants have been published since the 1970s, several of which have attributed their invasion success to the adaptation to disturbed habitats, the escape from natural enemies, or the competitive superiority [20, 32]. Despite their widespread impact in invaded habitats, little was known about these species in their homeland. The first studies in their native range focused on the occurrence and detrimental effects of natural enemies such as pathogens, social parasites, and parasitoids [20, 24, 33–36].

Several ecological studies on ant assemblages were conducted during the last decade in Argentina and Brazil [30, 37–42]. These studies were oriented (1) to know the position of *S. invicta* in the hierarchy of dominance of the ant assemblages, cooccurring not only with many competitor ants but also with their natural enemies, and (2) to investigate if its success in the introduced range is the consequence of a low-competitive environment more than the relative absence of their natural enemies [38–40]. These works revealed that, in several ant assemblages in Argentina and Brazil, overall, *S. invicta* occupied the top in the ecological dominance hierarchy, being the ant most frequently captured (64–82% of the samples) and numerically abundant (23–27% of total individuals captured) without showing the highest biomass. Most assemblages included at least 8–10 ant species that were also very common [38–42].

The ecological studies also showed that *S. invicta* was frequently a slow discoverer but almost always a good dominator of the food resources, allowing other cooccurring species of ants to be abundant [38–40]. This would indicate that its success was not necessarily based on the break of the discovery-dominance tradeoff, as it has been found in other invasive species, such as the Argentine ant, *Linepithema humile* (Mayr). Despite not being a good discoverer, *S. invicta* won, on average, 75% of the interactions in five ant assemblages in northern Argentina [38, 39] and Brazil [40]. In Argentina, its main competitor was *Pheidole obscurithorax* Naves (also exotic in the United States). Its ecological dominance was based on (1) the large numbers of individuals, (2) the well-developed recruitment system, (3) the aggressive behavior, and (4) the uninterrupted-foraging activity [30, 38–40].

The situation in southern South America strongly contrasts with that observed in North America, where *S. invicta* is the unique dominant ant representing most ant biomass [17, 20, 32]. At least in Argentina, the strong competitive environment and the indirect effect of natural enemies were suggested to be the most important factors limiting the success of *S. invicta*. Competitors and natural enemies would likely be locally adapted to the genetically divergent *S. invicta* populations inhabiting different parts of South America. An assessment of its genetic variation using 2,144 colonies from



FIGURE 1: Red dots showing localities in Argentina, Brazil, Uruguay, and Chile mentioned in the paper.

75 sites worldwide revealed that around 97% of all known mt DNA haplotypes of *S. invicta* only occur in the native range [31]. The dominant haplotypes in the United States and other newly invaded areas occur only at low frequencies (<5%) in eight populations in Formosa province (Figure 1) in northeastern Argentina [31], indicating that this area is more likely to be the source of *S. invicta* in the United States [43].

### 3. Natural Enemies

**3.1. Pathogens.** Preliminary explorations for fire ant diseases in Argentina were conducted by researchers from IAMARL and SABCL in 1987 in the provinces of Buenos Aires, Entre Ríos, and Santa Fe [25]. The vial sampling of 425 fire ant colonies in 47 sites and the subsequent microscopic examination revealed the presence of the following pathogens: (1) *Kneallhazia* (= *Thelohania*) *solenopsae* Knell, Allen, and Hazard (Microsporidia: Thelohaniidae) at 41% of the sites and 11% of the colonies; (2) *Vairimorpha invicta* Jovenaz and Ellis (Microsporidia: Burenellidae) at 11% of the sites

and 2% of the colonies; (3) *Myrmecomycetes annellisae* Jovenaz and Kimbrough (Deuteromycotina: Hyphomycetes) at 15% of the sites and 2% of the colonies; (4) *Mattesia* sp. (Neogregarinida) at 7% of the sites and 1% of the colonies; (5) a mermithid nematode at 7% of the sites and 0.5% of the colonies. This preliminary overall occurrence of *K. solenopsae* and *V. invicta* in 13% of the colonies of *S. richteri* and *S. quinquecuspis* almost doubled the prevalence (7.6%) of the same infections on *S. invicta* in the area previously surveyed of southwestern Brazil [25].

**3.1.1. Microsporidia.** *Kneallhazia solenopsae* and *V. invicta* are obligate intracellular pathogens first discovered infecting mainly the fat body of fire ants collected in the area of Cuiabá, Mato Grosso, Brazil [22, 44–46] (Figure 1). Both microsporidia show immature vegetative stages and reproductive stages represented by spore dimorphism with basically eight meiospores (octospores) bound by a membrane and nonbounded, or free, binucleate spores. More recent ultrastructural studies on *K. solenopsae* showed the presence of several other spore morphotypes [47–50].

*Field Surveys.* Subsequent surveys conducted in 1988 were mostly concentrated on microsporidia of *S. richteri* and *S. quinquecupis* in Buenos Aires province to select a field site for long-term ecological studies [51, 52]. The microscopic (phase-contrast) examination of 1,836 samples of fire ant colonies from 185 roadside sites revealed the presence of *K. solenopsae* (Figure 2) at 25% of the sites and 8% of the colonies and *V. invicta* (Figure 3) at 5% of the sites and 1% of the colonies. In some sites, *K. solenopsae* showed epizootic levels infecting 40–80% and *V. invictae* infecting 60% of the colonies.

This prevalence was the highest reported for South America. Simultaneous, or dual, infections of *K. solenopsae* and *V. invictae* in the same colony were not detected. The area of Saladillo, 180 km SW of Buenos Aires (Figure 1), was selected for long-term studies on *S. richteri* populations infected with *K. solenopsae* (see Detrimental Effect). At this stage, the fungus *M. anellisae* was found in 6% of the sites and 1% of the colonies; *Mattesia* sp. and the mermithid nematode were not found.

From 1991 to 1999, explorations were extended to northern Argentina in the search for *K. solenopsae* and, mainly, *V. invictae* infecting *S. invicta* [53]. The sampling of 2,528 fire ant colonies in 154 sites revealed the presence of *K. solenopsae* at almost 43% of the sites and in 12% of the colonies and *V. invictae* at 13% of the sites and in 2.3% of the colonies. Again, some sites in northcentral Santa Fe province showed more epizootic levels of *V. invictae* with up to 50% of the colonies infected; some of these sites were selected for long-term studies on *S. invicta* populations infected with *V. invictae* (see Detrimental Effect).

Both microsporidia were sympatric in 12 sites, in three of which 7 dual infected colonies (*S. richteri* and *S. macdonaghi* in Entre Ríos and *S. invicta* in Santa Fe) were found. This very low overall prevalence of dual infections ( $7/2,528 = 0.0028 = 0.3\%$ ) was identical to the combined probability of finding at random *K. solenopsae* (12%) and *V. invictae* (2.3%) simultaneously in the same colony ( $0.12 \times 0.023 = 0.0028 = 0.3\%$ ).

In 1993, a brief and opportunistic sampling of 61 *S. invicta* colonies at 18 sites in the area of Cuiabá (type locality for *K. solenopsae* and *V. invictae*) revealed the presence of 21% of the colonies infected with *K. solenopsae* and 6.6% with *V. invictae* (Briano and Patterson, unpublished data).

At least in Argentina and Paraguay, *K. solenopsae* and *V. invictae* showed the ability to infect both monogyne and polygyne colonies of *S. invicta* and *S. richteri*. In a sampling of 20 *S. invicta* colonies infected with *K. solenopsae*, 45% were polygyne and 55% were monogyne colonies; from 15 *S. invicta* colonies infected with *V. invictae*, 46% were polygyne and 54% were monogyne colonies [54]. Similarly, it was found that in a population of 41 colonies of *S. richteri* infected with *K. solenopsae*, 42% of the colonies were polygyne and 58% were monogyne [55].

It is important to remark that, during the course of the investigations in Argentina on *K. solenopsae*, this microsporidium was suddenly discovered in the United States [56] and subsequently found in most southern states. This fact redirected some of the studies on this candidate,

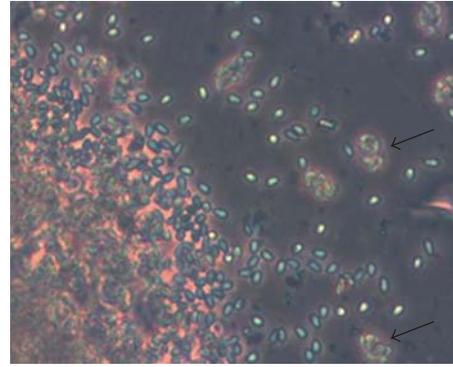


FIGURE 2: Phase contrast view (400x) of meiospores of *Kneallhazia solenopsae* in workers of *Solenopsis richteri*. Because of the grinding process, the oocyst membrane usually breaks and most spores are released in the aqueous extract. A few intact oocysts (arrows) are shown with meiospores inside.

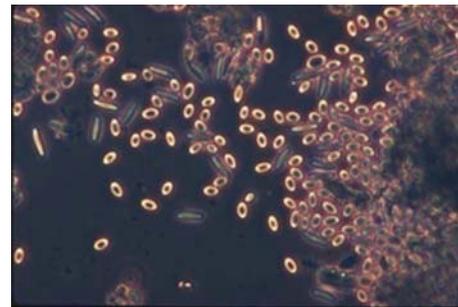


FIGURE 3: Phase contrast view (400x) of ovoid meiospores (octospores) and bacilliform binucleate free spores of *Vairimorpha invictae* in workers of *Solenopsis invicta*. Again, most oocysts with meiospores break during the grinding process.

since its presence in the US represented a change in the biological control approach for *S. invicta*.

New surveys for *V. invictae* and *K. solenopsae* were conducted from 2001 to 2005 in several central and Northern provinces (temperate and subtropical) of Argentina and in limited areas of Paraguay, Brazil, Chile, and Bolivia [57], including large western regions previously unexplored. A total of 2,064 colonies were sampled in 262 sites in roadsides, pastures, and recreational areas. *Vairimorpha invictae* was found at 12% of the sites and 10% of the colonies in Argentina, Brazil, Paraguay, and Bolivia. The provinces of Santa Fe and Entre Ríos showed the highest prevalence of infected colonies (20 and 7%, resp.); in certain *S. invicta* sites in Santa Fe (San Javier and vicinities; Figure 1), the prevalence was 50–54%, and, in *S. richteri* sites in Entre Ríos (Médanos), the prevalence was 60%. The prevalence of *V. invictae* in Paraguay and Bolivia was very low. *Kneallhazia solenopsae* showed a wider distribution occurring at 25% of the sites and 13% of the colonies and was reported for the first time in western and northwestern Argentina and Bolivia, at altitudes of almost 2,300 m and colder weather. It was also first reported infecting *S. interrupta* [57]. The province of Buenos Aires showed the highest prevalence with 68% of

infected sites and 34% of infected colonies. This time, both microsporidia cooccurred in 11 sites, 10 of which showed 46 dual infected colonies in several provinces. This prevalence of dual infections ( $46/2,064 = 0.0223 = 2.2\%$ ) was higher than the combined probability of finding *K. solenopsae* (13%) and *V. invictae* (10%) simultaneously in the same colony ( $0.13 \times 0.10 = 0.013 = 1.3\%$ ) and was the consequence of repeated and planned samplings in sites with high prevalence of dual infections. The highest prevalence of dual infections was found in Santa Fe with 3.9% of *S. invicta* infected colonies and in Entre Ríos with 2.7% of *S. richteri* infected colonies.

**Intracolony Prevalence.** The intracolony prevalence of *K. solenopsae* in fire ant colonies (mainly *S. richteri*) was very high. Vegetative stages (Figure 4) infected 28% (range 20–45%) of the immature fire ants including eggs and only 1.2% of the queens, while mature stages (spores; Figure 2) infected 42.3% (range 34–95%) of the workers, sexual adults, and queens and 0.8% of the pupae [58]. The presence of infected eggs revealed transovarial (vertical) transmission of *K. solenopsae*. The mean number of meiospores per worker ranged from  $9 \times 10^5$  to  $6.7 \times 10^6$ . Free spores were extremely rare.

The intracolony prevalence of *V. invictae* in fire ant colonies (mainly *S. invicta*) was also very high in most ant castes and stages [53]. Vegetative stages infected 30% (range 17–52%) of the fire ant larvae and 4.8% of the queens. Low prevalence of vegetative stages was also detected in a few eggs, providing evidence for transovarial transmission. However, the importance of the vertical transmission in the life cycle of *V. invictae* remained uncertain. Meiospores and binucleate spores of *V. invictae* (Figure 3) were found in all fire ant castes except eggs. Meiospores infected 33% (range 5–56%) of mature ants, and binucleate spores infected 39% (range 9.5–63%) of immature and mature ants. The occurrence of *V. invictae* was much more common in sexual males than in females. The mean number of meiospores per worker ranged from  $1.2 \times 10^4$  to  $6.4 \times 10^4$ . Free binucleate spores of *V. invicta* in *S. invicta* were much more common than those of *K. solenopsae* in *S. richteri*, ranging from  $3.2 \times 10^3$  to  $1.6 \times 10^4$ .

Dual infections showed lower intracolony prevalence. In *S. invicta*, it ranged from 4.5 to 22% of the individual pupae, workers, and sexual females. In *S. richteri* and *S. macdonaghi*, dual infections were found only in 2.7% of the workers [53]. Dual infections were suggested to represent an important mortality factor for fire ant colonies, but it was never confirmed with appropriate laboratory tests.

**Detrimental Effect.** The long-term field effect of *K. solenopsae* on *S. richteri* was studied in 6 plots established in natural pastures in the area of Saladillo, Buenos Aires province, and monitored 4–10 times per year from October 1988 to January 1993 for the density of colonies and the infection rates [51, 59, 60]. The study included the identification, sampling, and mapping of 1,348 active colonies. Although the fire ant densities showed cyclic variations unrelated to seasons, the overall density decreased from 162 to 28 colonies per hectare by the end of the study. The proportion of infected

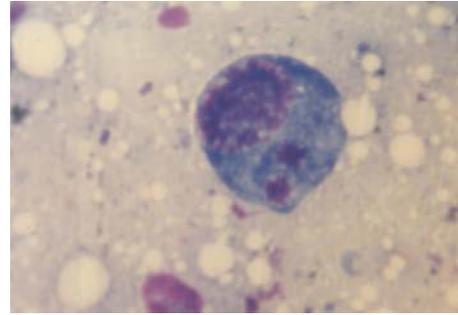


FIGURE 4: Cell of a *Solenopsis richteri* larva infected with a binucleate vegetative stage of *Kneallhazia solenopsae*. Giemsa's stain, 1000x.

colonies was very variable during the study and was positively related to rainfall. A weak negative association was found between the density of colonies and the rate of infection. The reduction of fire ant densities was attributed to the presence of *K. solenopsae*, although the loss of control plots because of natural dissemination of the infection obscured conclusive results.

During 9 months in 1992, the mound volumes of 84 *K. solenopsae*-infected colonies were compared with 88 healthy colonies from two different areas [55]. The presence of polygyny, number of queens per colony, and presence and abundance of brood and myrmecophiles were also compared between infected and healthy colonies. There was a strong negative association between mound size and infection with *K. solenopsae*. The mounds of infected colonies (mean  $\pm$  SD:  $4.9 \pm 1.0$  liters) were substantially smaller than those of healthy ones of the two different areas ( $14.7 \pm 1.8$  and  $18.7 \pm 1.7$  liters). No difference was found in the volumes of infected monogyne and polygyne colonies. The presence of multiple queens was common in both infected and healthy colonies, and the number of queens per colony did not differ significantly. The infection of *K. solenopsae* had no effect on worker brood presence, but there was less sexual brood in infected colonies. All the myrmecophiles found, *Neoblissus parasigaster* Bergroth (Hemiptera: Lygaeidae), *Martinezia* sp. (*Myrmecaphodius*) (Coleoptera: Scarabeidae), *Myrmecosaurus* sp. (Coleoptera: Staphylinidae), and the social parasite *Solenopsis daguerrei* (Santschi) were as common in infected as well as in healthy colonies, but *N. parasigaster* was more numerous in infected colonies. Within the Saladillo plots, no effect of *K. solenopsae* was reported on the fire ant colony movement [61].

The lethal effect of *K. solenopsae* was suggested for polygyne colonies of *S. richteri* originally collected in the field and later fragmented in the laboratory to one queen and 100 workers. The fragmented colonies were reared in small plastic containers for their residual longevity [62]. After 3 months of rearing, the mortality was 92% for infected colonies ( $n = 14$ ) and only 49% for healthy colonies ( $n = 22$ ).

In 1995 and 1996, the survival of 224 starved individual minor and major workers and 13 sexual females selected at random from infected and healthy colonies of *S. richteri* was compared [62]. At 27°C, the mortality rate of infected

workers was higher than that of healthy workers. Although the final mortality rate of infected and healthy sexual females was similar, the mortality rates occurred much sooner for infected sexuals. No differences were detected in the live weight of infected and healthy workers (minor or major). In a similar survival test with workers of *S. invicta* infected with *V. invictae*, mortality rates of infected workers were much higher than those of healthy ones [53].

The long-term field effect of *K. solenopsae* and *V. invictae* on *S. invicta* was studied in 8 roadside plots established in Santa Fe province and monitored 3–5 times per year from May 2000 to March 2004 for the density of colonies, the USDA population index (PI), and the infection rates [63]. As in the study of *S. richteri*, control plots were lost since they suffered the natural spread of the infections, making difficult the analysis of the results. Although the mean PI per plot showed abrupt reductions followed by reinfestations, important reductions of 53–100% were observed at the end of the test in 7 of the 8 plots, resulting in an overall PI reduction of 69%. From the total 394 colonies sampled, 82.5% were healthy and 17.5% were infected. The percentage of infection with both microsporidia also showed fluctuations and an overall reduction from 26 to 5% of infection rates. Only 3 colonies were found with dual infections in 2 plots. The proportion of infected and healthy colonies in the PI categories was significantly different for medium and large colonies with worker brood. More than 97% of the large colonies were healthy, suggesting that infected colonies did not produce large colonies.

**Field Persistence.** Several observational studies on field persistence were conducted to check the occurrence of the microsporidia over time. These repeated samplings were important to recognize field sites with high prevalence of infections to be used as source sites for eventual shipping of infected colonies to the US. One *S. quinquecupis* site in the area of Pergamino, 240 km N of Buenos Aires (Figure 1), infected with *K. solenopsae* was monitored every 1–2 months from October 1988 to July 1990 (Briano, unpublished data). The mean number of fire ant colonies sampled per monitoring date was 23 (range: 12–36), and *K. solenopsae* was always found infecting the colonies with ranges of 17–64%.

Another site in Saladillo with *S. richteri* infected with *K. solenopsae* was monitored 9 times from Oct 1992 to Jun 1998 (Briano, unpublished data). The mean number of fire ant colonies sampled per monitoring date was 14.2 (range: 6–50). In seven (78%) of the monitoring dates, *K. solenopsae* was found infecting from 22 to 67% of the fire ant colonies examined. In the other two sampling dates, no infected colonies were found.

Two *S. richteri* sites in Entre Ríos and one *S. invicta* site in Santa Fe infected with *K. solenopsae* and *V. invictae* were sampled 5–10 times from July 2001 to March 2005 [57]. On average, 18 colonies (range 7–70) were sampled per monitoring date and a high prevalence of both infections was detected, reaching epizootic levels in most occasions. The total prevalence of both microsporidia ranged from 46 to 78% of the colonies; in two occasions, 100% of the colonies were infected. Each microsporidium exhibited a

characteristic enzootic/epizootic wave; *V. invictae* occurred more sporadically, with sudden fluctuations in prevalence, while *K. solenopsae* showed a more sustained prevalence with fewer fluctuations. High peaks in prevalence of *K. solenopsae* coincided with low peaks of prevalence of *V. invictae* and vice versa. The mutual interference of both microsporidia was never confirmed with laboratory tests; but it was suggested that the successive high levels of both infections, one at a time, might represent a more constant pressure against fire ant host populations. However, this assumption was never checked with appropriate field tests.

**Transmission.** Many tests for the artificial horizontal transmission of *K. solenopsae* to individuals or colonies of *S. invicta* and *S. richteri* were conducted in the laboratory from 1992 to 2000 (Briano, unpublished data). Several approaches were used, such as (1) inoculation of healthy laboratory colonies with spore suspensions obtained from infected workers; (2) transference of infected fire ant larvae to healthy receptor colonies; (3) transference of infected fire ant adult workers to healthy receptor colonies; (4) transference of the myrmecophile *N. parasigaster* to healthy receptor colonies; (5) inoculation of *N. parasigaster* with spore suspensions obtained as above; (6) mixing of queenless infected colonies with healthy polygyne colonies; (7) inoculation of healthy field colonies with spore suspensions using various methods. After several days or weeks (depending on the approach used), the microscopic examination of the inoculated individuals, colonies, or myrmecophiles did not reveal infections.

However, horizontal transmission of *K. solenopsae* was achieved by scientists at CMAVE by transferring *S. invicta* infected brood to healthy colonies (approach number 2 above) and by mixing colonies (approach number 6 above) [64–66].

Tests were conducted to obtain artificial dual infections in *S. richteri* with *K. solenopsae* and *V. invictae* (Briano, unpublished data). Colonies infected with *V. invictae* were used as inocula with the following approaches: (1) transference of *Vairimorpha*-infected larvae to *Kneallhazia*-infected colonies; (2) transference of *Vairimorpha*-infected workers to *Kneallhazia*-infected colonies; (3) inoculation of *Kneallhazia*-infected colonies with *Vairimorpha* spore suspensions obtained from infected workers. Again, infections were not detected in the subsequent microscopic examination of the inoculated colonies.

In 2003, colonies of *S. invicta* infected with *V. invictae* were collected in Santa Fe, Argentina, and transported to quarantine at CMAVE for additional transmission tests. The following approaches were considered [67]: (1) inoculations of incipient *S. invicta* colonies reared from newly mated queens with larvae from the infected field colonies; (2) inoculations of incipient *S. invicta* colonies reared from newly mated queens with nonmelanized pupae from the infected field colonies; (3) inoculations of incipient *S. invicta* colonies reared from newly mated queens with larvae or melanized pupae from infected laboratory colonies; (4) inoculation of incipient *S. invicta* colonies with dead adults from the infected field colonies. The subsequent microscopic examination revealed, for the first time, positive transmission

in 40% (2/5) of the inoculated colonies in approaches number 1 and number 2, 100% (3/3) in approach number 3, and 33% (2/6) in approach number 4. Due to the limited number of colonies inoculated in each approach, the statistical analysis was not conducted. Also, the colony growths and brood volumes were significantly lower in infected than in healthy colonies.

**Specificity.** The field host range of *K. solenopsae* and *V. invictae* was first studied from 1993 to 2000 in eastcentral Argentina and southern Brazil by sampling terrestrial ants cooccurring with infected fire ants. Ants were sampled using 520 bait traps (glass vial with pieces of canned “Vienna sausage”) in 52 preselected infected roadside sites and by hand sampling of 585 colonies (*S. invicta*, *S. richteri*, and other ants species) in 90 sites [68]. *Kneallhazia solenopsae* and *V. invictae* were found infecting only *S. invicta*, *S. richteri*, *S. macdonaghi*, and *Solenopsis* sp. (unidentified fire ant species), while the other ants baited/sampled in the genera *Pheidole*, *Camponotus*, *Crematogaster*, *Linepithema*, *Brachymyrmex*, *Nylanderia* (= *Paratrechina*), *Acromyrmex*, and *Wasmannia* were not infected. A preference of *V. invictae* for *S. invicta* was suggested [68]. The infection in *S. macdonaghi* was a new host record. A few meiospores of *K. solenopsae* also had been found in some individuals of the myrmecophile *N. parasigaster* and the parasitic ant *S. daguerrei* [51, 59], but infections in host tissue were not confirmed. More recently, empirical evidence of *K. solenopsae* infections on *S. geminata* and *S. geminata* × *S. xyloni* hybrid was reported from Texas and Mexico [69].

In 2004, additional studies were conducted in 5 polygyne sites in Corrientes and Santa Fe to confirm the specificity of *V. invictae* for *Solenopsis* ants [70]. All sites had high levels of *V. invictae* infections in fire ant colonies. As above, baits and hand samplings were used to collect ants and other arthropods in the immediate areas of infected fire ants. *Vairimorpha invictae* infections were detected only in fire ants by microscopy and PCR. The other ants tested were in the genera *Ectatomma*, *Pachycondyla*, *Acromyrmex*, *Crematogaster*, *Pheidole*, *Wasmannia*, *Cephalotes* (= *Zacryptocerus*), *Dorymyrmex*, *Linepithema*, *Camponotus*, *Brachymyrmex*, and *Nylanderia*. The other tested arthropods were in the orders Araneae, Odonata, Orthoptera, Homoptera, Hemiptera, Psocoptera, Coleoptera, Diptera, Lepidoptera, and Hymenoptera.

The host specificity of *V. invictae* was studied in the laboratory at CMAVE evaluating the tropical fire ant, *S. geminata*, the southern fire ant, *S. xyloni*, and the Argentine ant, *Linepithema humile* [71]. Inoculations of *S. invicta* brood infected with *V. invictae* into lab colonies of the three recipient ant species resulted in infections only in the control *S. invicta* in 60% of the colonies. However, the adoption of congeneric brood was not consistent, and, within the first two days, all the *S. geminata* and most of the *S. xyloni* colonies had moved the inoculated brood in the trash pile. In the case of the Argentine ant, the inoculated *S. invicta* brood was initially tended in 2 of the 6 nests. However, inocula appeared to be finally discarded from all nests. Since alien brood survival seemed to have been temporary, whether the lack

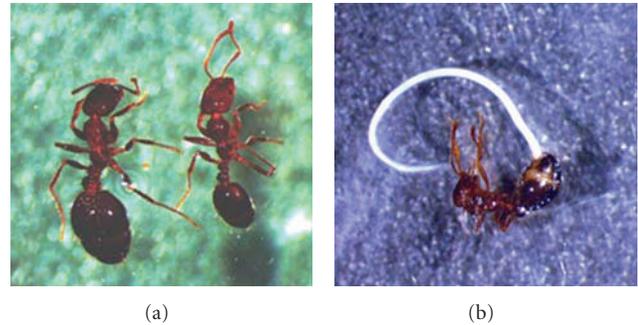


FIGURE 5: (a) Parasitized (left) and nonparasitized *Solenopsis* worker. (b) Juvenile *Allomeris solenopsi* emerging from worker.

of infection in the test ants was due to limited interspecific cross-fostering of brood or lack of physiological susceptibility was not determined [71].

**3.1.2. Nematodes.** Few records of parasitic nematodes exist for fire ants [72]. The diagnostic character for the occurrence of mermithid nematodes in ants is the enlarged gasters of the workers (Figure 5(a)). In the late 1980s, three species were reported for southern South America: (1) *Tetradonema solenopsis* Nickle and Jouvenaz from central-western Brazil in 2.9% of the 2,250 fire ant colonies examined; (2) a mermithid nematode from central-eastern Argentina in 0.5% of the 425 colonies examined; (3) an unidentified nematode from central-eastern Argentina, Uruguay, and southern Brazil in 4.3% of the 600 colonies sampled [73, 74].

In 2006, while researching for decapitating phorid flies in Corrientes province, Argentina, CMAVE scientist Sanford Porter redetected the presence of mermithids in *S. invicta*. The nematode was recently described and named as *Allomeris solenopsi* [72].

**Surveys and Parasitism Rates.** From 2006 to 2008, examination of 489 fire ant colonies in Buenos Aires and northeastern Argentina revealed infections in 17.3% of the 29 sites, where an average of 52.3% of *S. invicta* or *S. richteri* colonies was infected. The mean number of parasitized workers per colony was 52 (range 1–500). Also pupae were found with enlarged gasters and juvenile nematodes inside. Several positive sites were further revisited for the persistence of the infections; although the infection persisted over more than one year, the parasitism rates showed great variations (Varone, unpublished data).

**Life Cycle.** The life cycles of terrestrial or semiterrestrial mermithid nematodes are completely known only in a few cases [75–77]. In general, adults are free-living organisms, and developing stages are parasitic. Females usually lay eggs during periods of high moisture. Juveniles undergo one molt in the egg and emerge as a second-stage juvenile. The emerged juvenile enters the insect host through the anus, spiracles, or direct penetration through the cuticle to reach the body cavity [78]. Some species of mermithids develop little until the host reaches the adult stage and then migrate to the abdomen, mature, and kill the host on emergence [79].

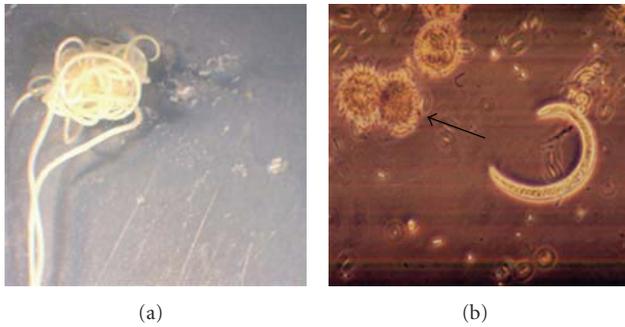


FIGURE 6: (a) Adults of *Allomeris solenopsi* forming a mating cluster and laying eggs (10x). (b) Eggs (arrow) and juvenile *A. solenopsi* (400x).

The *A. solenopsi* parasitism mechanism on fire ants remains unknown. However, in the laboratory, it seems that water is needed for reproduction and oviposition. It was observed that juvenile nematodes came out from dead parasitized workers only when placed in water (Porter, unpublished data). The emergence took several minutes to three days (Figure 5(b)) but failed in 65.8% of the cases. Once in the water, juvenile nematodes molted to adults and formed “mating clusters” (Figure 6(a)) with the subsequent egg laying. Juveniles were observed developing inside the eggs, and some emerged when the eggs were crushed (Figure 6(b)).

Rearing mermithid parasites of insects have been especially difficult. Since mermithids are relatively common parasites of aquatic insects, laboratory cultures have been established for a few mosquito parasites [77, 80, 81]. Creighton and Fassuliotis [82] cultured a mermithid parasite for the control of a terrestrial insect, *Diabrotica balteata* (Coleoptera: Chrysomelidae).

Several tests were conducted to spread the infection artificially under laboratory conditions by transferring eggs or newly emerged juveniles of *A. solenopsi* to uninfected host colonies. The juveniles were introduced to receptor colonies using hard-boiled egg, tenebrionid larvae, and crickets, or located by hand in the ant brood pile using a humid and folded wiper (Kimwipes). Parasitism was obtained only using crickets and the wiper but at very low rates.

**Detrimental Effect.** An important change in the behavior of infected workers was detected in the laboratory by voluntarily exposing for 10-minute 5 SABCL staff members to the fire ant sting (Varone, unpublished data). All infected workers tested ( $n = 10$ ) lost their stinging behavior. Infected ants did not show the typical stinging position of a curved gaster, probably due to the enlarged gaster. Linear relationships were found between the sizes of the ant heads and the venom glands in 20 healthy and 20 infected workers, indicating no atrophy of the venom gland in infected workers (Varone, unpublished data).

An effect was also observed with aggression tests among workers from different colonies. Contests involving two ants, one being infected and the other not, usually only resulted in

antennation, while interactions between two healthy workers mostly ended in aggression and/or fighting.

The infection with *A. solenopsi* also affected the residual survivorship of the worker ants. On average, parasitized workers ( $n = 872$ ) survived  $17.5 \pm 12.6$  days and nonparasitized ones ( $n = 1,078$ ) survived  $35.8 \pm 26.1$  days after the colony was removed from the field. *Allomeris solenopsi* adult survivorship as a free form in water was  $10.7 \pm 9.2$  days, showing great variability among individuals with a range of 2–72 days (Varone, unpublished data).

**3.1.3. Viruses.** Until recently, little effort has been invested on virus infections in fire ants [36]. Virus-like particles were reported from *Solenopsis* ants in Brazil [83]. More recently, three positive-strand RNA viruses were discovered infecting *S. invicta* in the United States: SINV-1, SINV-2, and SINV-3 [84–90]. These viruses were successfully transmitted to uninfected workers by feeding food. The viruses replicated within *S. invicta* [88] and were associated with significant mortality among workers and larvae once infected [89].

Surveys for these viruses in Argentina were conducted from 2005 to 2008 mainly on *S. invicta* and some *S. richteri* and *S. quinquecupis*. Almost 400 colonies were sampled at 32 sites in the provinces of Corrientes, Chaco, Formosa Santa Fe, and Entre Ríos. In addition, 6 colonies of *S. weyrauchi* and *S. interrupta* were sampled in two sites in Bolivia along with 17 *S. macdonaghi* at one site in Uruguay. In all cases, workers were preserved in ethanol 96%. Reverse transcription polymerase chain reactions (RT-PCR) and multiplex PCR were used [91].

In Argentina, the viruses occurred in 22 (73.3%) sites with a mean colony infection of 26%. SINV-1 was present in 12 (40%) sites and in 24 (12.8%) colonies; SINV-2 in 8 (26.7%) sites and 11 (5.8%) colonies and SINV-3 in 2 (6.7%) sites and 5 (3.2%) colonies. Although SINV-1 was the most common, it frequently occurred in combination with the other two; only 29.2% of the infected colonies were exclusively infected with SINV-1, 37.5% had double infections (SINV-1 plus SINV-2 or SINV-3), and the remaining 33.3% were positive for all three viruses [91]. Only one *S. invicta* colony was exclusively infected with SINV-2. In Bolivia and Uruguay, infected colonies were not detected (Varone and Calcaterra, unpublished data).

### 3.2. Parasitoids

**3.2.1. Phorid Flies.** At least 30 *Pseudacteon* species (Diptera: Phoridae) are parasitoids of *Solenopsis* fire ants in the New World and 23 attack South American fire ants in the *Solenopsis saevissima* species group [92, 93]. Fire ant decapitating flies are parasitoids of individual workers [94, 95].

Female flies chase live worker ants (Figure 7) and, in a rapid aerial attack, insert their eggs into the thorax. After hatching, the larva migrates into the ant’s head, consumes all the tissue, and ultimately decapitates the host ant. A single adult fly emerges from the oral cavity 2–6 wk after the egg was laid [96]. Once the adult fly emerges, it has only a few days to look for a mate to repeat the cycle.



FIGURE 7: *Pseudacteon* fly chasing a *Solenopsis invicta* worker for oviposition (photo by S. D. Porter).

**Field Surveys.** Before the mid 1990s, the biology, geographical distribution, and abundance of most of these South American flies on fire ants were scarcely known. Borgmeier described most of the species [97], and extensive collections were conducted in the early 1970's [98, 99].

Large-scale surveys were conducted by SABCL and CMAVE researchers from 1995 to 2002 documenting the occurrence of *P. tricuspis* Borgmeier, *P. curvatus* Borgmeier, *P. borgmeieri* Schmitz, *P. litoralis* Borgmeier, *P. obtusus*, *P. nocens* Borgmeier, and *P. affinis* Borgmeier at several sites in the provinces of Buenos Aires, Santa Fe, Chaco, Formosa, Corrientes, and Entre Ríos (Briano, unpublished data). Several sites with high densities of flies were used later to run field host preference tests. Five species of flies (*P. tricuspis*, *P. curvatus*, *P. litoralis*, *P. obtusus*, and *P. nocens*) were shipped to the quarantine facilities at CMAVE for mass rearing, host-specificity tests, and eventual field releases.

Later, surveys were extended to western Argentina, southern Bolivia, southern Paraguay, Uruguay, and central Chile [29, 30, 100]. Fourteen *Pseudacteon* species were collected from 52% of the 720 fire ant mounds examined at 146 sites: *P. curvatus*, *P. litoralis*, *P. tricuspis*, *P. nocens*, *P. obtusus*, *P. cultellatus* Borgmeier, *P. nudicornis* Borgmeier, *P. borgmeieri*, *P. solenopsidis* Schmitz, and *Pseudacteon* near *obtusus* (small biotype) were associated with *S. invicta*. Nine species were sympatric at one site in Corrientes.

*Pseudacteon obtusus* showed the southernmost geographical distribution in Corralito, Río Negro, Argentina (Figure 1; 40°44' S), and the westernmost in Bulnes, Bio Bio, Chile (Figure 1; 72°20' W), where it was recorded attacking *Solenopsis gayi* (Spinola) and *Solenopsis weyrauchi* in Santa Cruz (Figure 1; 2,280 m), Tucumán province, Prepuna bioregion. *Pseudacteon curvatus* was one of the most abundant and widely distributed species. Its density was negatively correlated with the densities of *P. obtusus* and *P. tricuspis* [30, 101], suggesting differential habitat/host preferences and/or competitive replacement. *Pseudacteon cultellatus* was found attacking *S. invicta* in a gallery forest gap next to the Río Paraná, in Corrientes province [100]. A total of 356 *P. tricuspis* and 204 *P. obtusus* males were collected from disturbed fire ant mounds while chasing females for mating, showing sex ratios female : male of 2 : 1 and 1 : 1, respectively [30].

These ratios were similar to those observed in the laboratory at CMAVE (S. Porter, pers. comm.). However, the primary field sex ratio remained unknown.

A new species, *P. calderensis* Calcaterra, was discovered attacking the fire ant *S. interrupta* in Salta and Jujuy provinces [100, 102], a region scarcely surveyed after the last fire ant decapitating fly was discovered in South America [103].

**Phenology and Phylogeny.** Seasonal activity of phorid flies was studied at two *S. invicta* sites in Corrientes [104]. Species showed different annual and/or daily activity patterns. The highest abundance was recorded in spring and the lowest in summer. Abundance was higher close to dusk, and species diversity was highest at midday. Weather conditions affected the presence and abundance of most species except *P. litoralis* and *P. nocens*, which represented 71–79% of all female flies captured at these two sites in Corrientes. These flies were genetically very similar and showed similar patterns, suggesting a shared derived trait from a recent common ancestor. In contrast, *P. cultellatus* and *P. nudicornis* were genetically quite similar but showed different activity patterns [104].

**Natural Parasitism and Detrimental Effect.** Studies on natural parasitism conducted at multiple sites in northeastern Argentina revealed a very low overall rate of 0.2–0.5% worker parasitism. The highest rates per site (1.2%) and per colony (2.8%) were reported for a gallery forest in spring [104].

The presence of phorids affected the foraging capacity of *S. invicta*. A 50% decrease in the numbers of workers baited after the arrival of the flies suggests a potential shift in the use of food resources in favor of other ant species. However, an effect on the hierarchy of dominance of the ant assemblage was not observed [39].

**Vectors.** Using PCR techniques, *Pseudacteon* flies from Argentina were tested in the United States for their potential as vectors of the bacterium *Wolbachia* [105]. Seven of ten species tested were positive for four *Wolbachia* strains. Multiple infections were detected only in *P. curvatus*. Strains infecting the flies were not closely related to the sequences obtained from strains infecting *S. invicta* and *S. richteri*, indicating that these flies were not vectoring *Wolbachia* into these fire ant species. *Pseudacteon* decapitating flies do not appear to vector fire ant viruses [106].

More recently, *Pseudacteon* flies were tested as potential vectors of the microsporidia *K. solenopsae* and *V. invictae* [107]. Several species of flies that were reared from *S. invicta*-infected workers were confirmed as carriers of *K. solenopsae*. Detrimental effects on the development of fly pupae and on emergence of adult flies were not observed. These results indicated that *Pseudacteon* flies might vector *K. solenopsae* but actual vectoring remains to be confirmed. In contrast, *V. invictae* did not infect phorids reared from infected fire ants [107].

**Field Releases in the United States.** In 1995, the first releases of *P. tricuspis* from South America (without the participation/collaboration of SABCL) were conducted in Texas by

scientists of the University of Texas at Austin. Later, other releases of *P. curvatus* and *P. obtusus* were conducted by the Texas Cooperative Extension Program in collaboration with APHIS and ARS [108]. The establishment and expansion of the flies have been systematically monitored [109–111].

Since 1997, five *Pseudacteon* species have been released by CMAVE researchers. These species and biotypes are those originally found in Brazil and Argentina and later tested in the US and/or in South America by CMAVE scientists with close collaboration of Brazilian and SABCL researchers. The species released are (1) *P. tricuspis*, biotype São Paulo, Brazil, released from 1997 to 2000 [112]; (2) *P. curvatus*, biotype Las Flores, Argentina, released in 2000 [113]; (3) *P. curvatus*, biotype Formosa, Argentina, released in 2003 [114]; (4) *P. litoralis*, biotype Formosa, released in 2005 [115]; (5) *P. obtusus*, biotype Formosa, released in 2008 (Porter, unpublished data); (6) *P. cultellatus*, biotype Corrientes, released in 2010 (Porter, in progress).

Several postrelease studies were conducted in the United States mainly on *P. tricuspis* and *P. curvatus*. These studies (1) confirmed predictions of the high host specificity of the pre-release studies [116, 117], (2) documented their establishment and spread [108, 109, 118, 119], (3) documented seasonal abundance and rates of parasitism [120], and (4) indicated little detrimental effect of *P. tricuspis* on *S. invicta* population densities [121]. Other studies on distribution and efficacy are in progress.

### 3.2.2. Eucharitid Wasps

**Biology.** Almost all species of the small wasps, *Orasema* spp. (Hymenoptera: Eucharitidae), are brood parasites of myrmicine ants in the genera *Pheidole*, *Solenopsis*, *Tetramorium*, and *Wasmannia* [24, 122–124]. Adult females lay their eggs into host plants, and the emerging larvae (planidia) attach themselves to foraging ant hosts and are carried into the nest [123, 125]. Adult wasps of *O. simplex* Heraty showed a short life span in the laboratory, estimated in  $3.6 \pm 1.5$  days, and a single female had more than 600 mature oocytes in the ovarioles indicating a high fertility at emergence [126]. The short female survivorship and high fertility strongly suggested that *O. simplex* is a proovigenic species, representing a way to counteract the low probability of the phoretic transport provided by foraging worker ants to reach the nest.

Occasional observations reported several types of plant tissues as oviposition sites [122, 127–130]. Recent laboratory nonchoice oviposition tests with plants of economic and ornamental importance such as corn, soybeans, lemon, red pepper, and *Vinca rosea* confirmed that all plants tested resulted appropriate substrates for oviposition [126].

Similarly, field observations in the surroundings of parasitized fire ant colonies located at three sites in the provinces of Corrientes and Entre Ríos revealed that 87% of the shrubs and grasses present in the genera *Smilax*, *Paspalum*, *Grindelia*, *Eupatorium*, *Sesbania*, *Asclepias*, *Verbena*, *Sida*, and *Stemodia* showed oviposition marks [126].

While associated with the brood, immature *Orasema* species produce or assimilate compounds that mimic the

cuticular hydrocarbon profile of the ant host, thus avoiding detection [131]. Pupation occurs in the brood, followed by adult emergence within the ant nest. The adults exit the nest for mating and oviposition [125, 132] (Figure 8).

**Surveys.** Fifty-five species of *Orasema* have been described worldwide [124, 133], and more than 200 species have been estimated for the Neotropic (Heraty, pers. comm.). *Orasema* parasitoids were first reported on fire ants of the *S. saevissima* complex in Uruguay [134]; *O. xanthopus* (Cameron) was later found parasitizing up to 40% of the colonies of *S. invicta* and other fire ant species of the same complex in Brazil [24, 25, 99, 135, 136]. In Argentina, 11 species were reported, three of which were parasitoids of fire ants in Buenos Aires, La Pampa, and some of the northwestern provinces [123, 137].

Between 2005 and 2007, the distribution of *Orasema* species and their ant hosts were intensively studied in Argentina and neighboring countries by excavating *Solenopsis* colonies in 73 sites in roadsides, pastures, and recreational areas [138]. A total of 731 colonies with brood were transported to the laboratory, separated from the soil by flotation [139] and the brood isolated [140] for *Orasema* individuals. *Orasema* was found in 29 sites parasitizing 13.5% of the 443 colonies in Argentina and 4.2% of the 288 colonies in Paraguay, Uruguay, and Bolivia. Five species were identified: (1) *O. simplex* was the most abundant, occurring at 17 sites and in 63.7% of the 72 parasitized colonies; (2) *O. xanthopus* and (3) *O. salebrosa* Heraty were found only at two sites; (4) *O. aenea* Gahan was found parasitizing fire ants for the first time at one site in Argentina; (5) *O. pireta* Heraty was found at one site parasitizing an unidentified *Solenopsis* species in Bolivia. In Paraguay and Uruguay, only *O. simplex* was present [138].

Two new host species of *Orasema* within the *S. saevissima* complex were discovered: *S. quinquecuspis* in Argentina and *S. macdonaghi* in Uruguay.

The wide variety of habitats and geographic distribution suggested that *Orasema* is a common parasitoid of fire ants in their native land. However, a second sampling of the Argentine sites conducted 6 to 18 months later revealed a field persistence in only 36.4% of the sites [138].

**Laboratory Rearing.** After several attempts and different approaches, the laboratory rearing and artificial transfer of this parasitoid to nonparasitized fire ants was achieved by placing planidia together with plant tissue in fragmented receptor colonies with abundant healthy brood [126]. However, this method had a very low success rate of 3.1% (only 12 adult wasps obtained from 385 planidia transferred). As previously observed by Vander Meer et al. [131], immature *Orasema* individuals were tended by ant workers as their own brood, with no aggression. However, several *Orasema* adults were found partially preyed, suggesting the loss of host-specific compounds soon after emergence.

### 3.2.3. Myrmecolacid Strepsipteran

**Life Cycle.** The ant parasitoid, *Caenocholax fenyesi* Pierce (Strepsiptera: Myrmecolacidae), has an unusual life cycle in

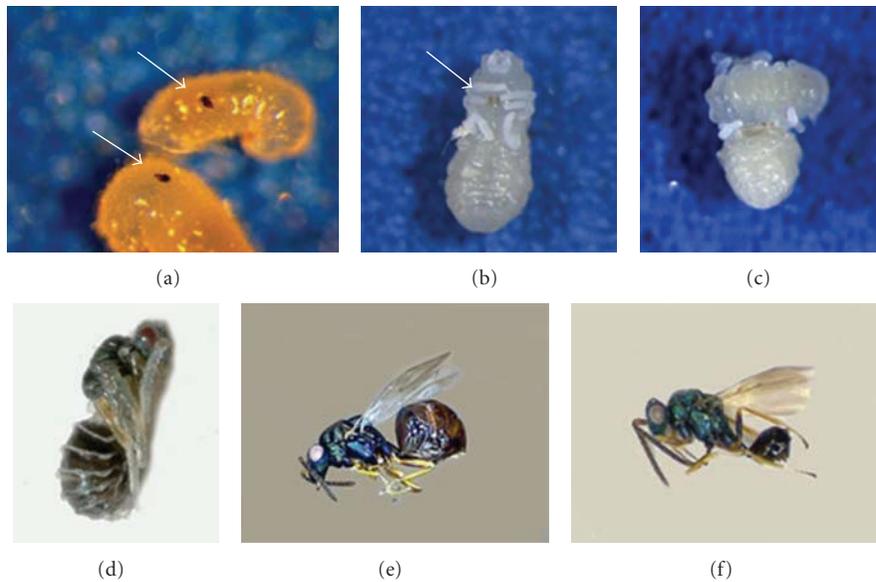


FIGURE 8: Life cycle of *Orasema*. (a) Planidia (arrows) attached to ant larvae. (b) First-instar larva (arrow). (c) Developed larva (ant larva behind). (d) Pupa. (e) Adult female. (f) Adult male.

which males parasitize ants while females parasitize crickets [141, 142]; it is currently the only extant species in its genus. The male of this species has the smallest genome (108 Mb) studied so far [143]. The lack of information on its host associations has led to several speculations.

**Distribution and Hosts.** *Caenocholax fenyessi* has a wide geographical distribution occurring from southern United States to Chile and Argentina and infects seven ant species from three subfamilies with discontinuous distributions [144–146]. In Mexico, Central America, or Ecuador where *S. invicta* did not occur, *C. fenyessi* parasitized other ant species [147]. Males of *C. fenyessi* had been previously collected in Salta province in northwestern Argentina where mtDNA haplotypes of *S. invicta* occur [31, 145].

**Parasitoid-Host Association.** In 2003–2004, 15 *C. fenyessi* males were isolated in the laboratory from four *S. invicta* colonies originally collected in Corrientes and Formosa provinces. This was the first report of *C. fenyessi* parasitizing *S. invicta* in South America [144]. However, the parasitism rate was less than 0.2%. In 2003 and 2005, additional surveys for *C. fenyessi* females using light traps, pitfall traps, and sweep nets were conducted in areas of Corrientes and Formosa where parasitized *S. invicta* colonies have been found previously. None of the 456 orthopterans, 9 dyctiopterans (Mantodea), and 6 phasmodeans (Proscopidae) collected were parasitized by *C. fenyessi* [144].

**Cryptic Diversity.** Recent molecular analysis revealed that *C. fenyessi* contained at least 10 cryptic lineages consistent with separate species and that the genetic diversity was strongly structured by geography and host association of the female [147]. Further studies revealed slight variation in key

morphological characters, so several species might not be strictly cryptic (J. Kathirithamby, pers. comm.).

### 3.3. Parasites

#### 3.3.1. Social Parasitic Ant

**Life History.** Only one social parasite, the parasitic ant *Solenopsis* (= *Labauchena*) *daguerrei*, has been effectively reported for fire ants [148]. Like most other socialinquilines, *S. daguerrei* has lost the worker caste and produces only reproductive queens and males [149] with a numerical sex ratio female : male of 3 : 1 [149]. The parasite commandeers the host's workers to care for its own brood and provide them with food [150]. Mature parasite queens that have shed their wings (dealates) are only one-tenth the weight of fire ant host queens [149]; they attach or “yoke” themselves to the neck of the host queen with their mandibles and ride around on her back or sides (Figure 9).

A host queen may have two or three parasite queens attached to her neck and another half a dozen to other parts of her body, apparently intercepting food intended for the host queens and inhibiting host queen egg production [149, 150]. Parasitic queens attached to hosts survived longer than those not attached [151].

Since the parasites are treated like nestmates by host workers, *S. daguerrei* apparently avoids the chemical recognition system of its hosts by mimicking or assimilating the cuticular hydrocarbons responsible for the host colony odor [150–152]. The ability of *S. daguerrei* to match host colony odor is likely to be sensitive to the strong patterns of genetic differentiation of the hosts, being consistent with the hypothesis that these parasites are locally adapted to their hosts, and thus specific to their associated host ecotype.



FIGURE 9: Two queens of *Solenopsis daguerrei* yoking a queen of *Solenopsis richteri*.

However, many laboratory and field attempts to artificially propagate this parasitic ant using sympatric colonies have failed [153]. Parasitized and nonparasitized colonies were used as source and receptor colonies, respectively. Several approaches were used, such as transference of parasitic queens, sexuals and/or pupae, contact of entire colonies, transplanting of entire field colonies, and the transfer of newly mated parasitic queens.

**Field Surveys.** Surveys for *S. daguerrei* in fire ant populations were conducted in Argentina, Brazil, Bolivia, Paraguay, and Uruguay by ARS scientists from 1974 to 1996 [154]. The examination of 12,180 fire ant colonies revealed occurrence of this parasitic ant in a variety of habitats in northeastern Argentina, Uruguay, and southern Brazil. Parasitization occurred in *S. richteri*, *S. quinquecupis*, *S. invicta*, *S. macdonaghi*, and *S. saevissima*, all members of the *S. saevissima* species group. The overall parasitism rates ranged from 1 to 7% of the colonies. The sites with the highest parasitism rates were San Eladio, Buenos Aires, Argentina, with 7% and Dourados, Mato Grosso do Sul, Brazil with 6,2% (Figure 1). However, surveys in the 1970s revealed that in some localities *S. daguerrei* was found in 24–70% of the colonies [59, 150].

**Phenology and Mating.** Most of the available information on *S. daguerrei* phenology and breeding biology was reported from *S. richteri* host populations in Buenos Aires province, Argentina. Adults of *S. daguerrei* were more common in fall-early winter, contrasting with the low seasonality showed by parasitic populations in northern Argentina and southern Brazil [148]. Mating flights were not observed. In a laboratory study, a total of 756 individuals of *S. daguerrei* were captured flying out from *S. richteri* host colonies; of those, 738 (98%) were females (87% were inseminated) and only 18 (2%) were males [151]. A later examination of the host colonies showed that no parasitic males were found inside and that 40% of the parasitic females were inseminated. As previously suggested [150], these observations confirmed that copulation occurred mainly inside the nests with nest-mates, resulting in a high level of inbreeding [155]. However, cryptic dispersal of males and mating with noninseminated females might occur, thereby reducing inbreeding. Also, the apparent poor dispersal ability of this parasite suggested a

strong genetic differentiation on both a micro- and macro-geographic scale (Bouwma, unpublished data).

**Detrimental Effect.** Field studies conducted in the area of San Eladio in Argentina on *S. richteri* colonies [149, 156] showed that, compared to nonparasitized colonies, parasitized colonies (1) had less worker brood, (2) produced the sexual caste later in the season, and (3) had fewer queens (2.9 versus 5.5). This suggested that the parasite might drive the host toward monogyny. Also, in field populations of *S. richteri*, lower mound densities were found in areas with presence of the parasite compared to parasite-free sites, suggesting some potential for the biological control of fire ants. In a few laboratory studies, *S. daguerrei* was reported to kill the host queens by decapitation [148] and to cause the colony to collapse [150].

**Evolutionary Traits.** Like other social parasites, it is believed that *S. daguerrei* must be highly specialized and has evolved the ability to exploit the social system of their hosts [155]. Recent studies on the evolutionary history of members of the *S. saevissima* species group were based on morphological characters [1] and mtDNA sequences [157]. These studies showed that *S. daguerrei* occupied a basal position in the group and that it was a close relative of its several hosts. It belonged to a larger clade, sister of the host clade, following the loose version of Emery's rule [158] and indicating that *S. daguerrei* would not have evolved directly from their hosts within the *S. saevissima* group (strict version of Emery's rule) [159–162]. This is supported by the single origin of social parasitism suggested for *S. daguerrei* collected from *S. invicta* and *S. richteri* host colonies over a vast geographic area [157].

In 2007, molecular studies to determine the genetic structure of *S. daguerrei* in Argentina, southern Brazil, and Paraguay revealed a high genetic variability and the probable presence of a complex with new species (Bouwma, unpublished data).

**Vectors.** To test if *S. daguerrei* was a vector of the bacterium *Wolbachia*, three individuals of the parasitic ant were found to be infected representing a new host record for *Wolbachia* [105]. Sequence analyses revealed that each individual contained the unusual number of eight *Wolbachia* variants. In total, nine unique sequences or strains were found, two of which were identical to the sequences obtained from their fire ant hosts *S. invicta* and *S. richteri*. This suggested horizontal transmission of *Wolbachia* between *S. daguerrei* and its hosts.

#### 4. Conclusions

Native fire ants in southern South America, mainly *S. invicta*, were dominant ants in several regions, but these ant communities usually included several abundant competitors. This strong competitive environment in their homeland contrasts with the situation in invaded communities in North America. The release from interspecific competition in the new habitats and the escape from coevolved natural enemies seem to strongly contribute to *S. invicta*'s successful invasions in North America.

Since 1987, the field surveys and the examination of approximately 14,000 fire ant colonies in almost 1,000 collecting sites in Argentina and neighboring countries have documented the presence of the microsporidia *Kneallhazia solenopsae* and *Vairimorpha invictae*, the nematode *Allomermis solenopsi*, three *S. invicta* viruses, 14 species of *Pseudacteon* decapitating flies, 5 species of the parasitoid wasp *Orasema*, the strepsipteran *Caenocholax fenyesei*, and the parasitic ant *Solenopsis daguerrei*.

*Kneallhazia solenopsae* was the most common pathogen of native fire ants, showing a wide distribution and high field persistence, mainly infecting *S. richteri*. On the other hand, *V. invictae* showed a narrower distribution, a lower and disjunct overall occurrence, and higher prevalence in *S. invicta*. Both diseases showed the ability to infect monogyne and polygyne populations, and, at times and in certain areas, they reached epizootic levels, representing the highest infection rates ever reported for South America. Despite this, the natural occurrence of dual infections in the field was very low and similar to probability predictions (combined probability of finding at random *K. solenopsae* and *V. invictae* simultaneously in the same colony).

Their high intracolony prevalence indicated that these microsporidia were important chronic diseases of fire ants. Both diseases showed several deleterious effects on individual colonies and field populations, and their ecological and physiological host ranges were restricted to closely related ants in the genus *Solenopsis*. These facts suggested that they might be good self-sustaining organisms for the classical biological control of the imported fire ants in the United States, with little or no risk to native ants and other arthropods.

The horizontal transmission of both pathogens accomplished by CMAVE scientists in the United States has allowed specificity and efficacy trials under laboratory and field conditions. Once field release of *V. invictae* is approved for the United States, the ability to transmit this disease into *S. invicta* colonies will accelerate the artificial field infection, its dispersal, and the eventual faster decline in imported fire ant population densities.

The finding and identification of the nematode *A. solenopsi* represented a new species discovery. The overall occurrence was low, and the parasitism rates in the field were highly variable. Laboratory rearing was difficult, and many aspects of its life cycle remain unknown. However, infected fire ants showed shorter longevity and interesting changes in their behavior. Further efforts with this organism are recommended.

The use of molecular techniques facilitated the screening for fire ant viruses. Of the three viruses found, SINV-1 was the most common and abundant and was frequently found in combination with the other two. Although no detrimental effect was observed in field infected colonies in the United States, under certain stress conditions, colonies might collapse. Consequently, a combination of these viruses and their genetic manipulation represent a potential alternative to traditional insecticides for controlling imported fire ants.

Among the parasitoids, by far, *Pseudacteon* flies were the most frequent and abundant, with 14 species collected, many of which were sympatric. *Pseudacteon curvatus* was the

most abundant in many areas followed by *P. obtusus*, one of the most widely distributed. However, the overall natural parasitism rate was very low, indicating low direct effect on worker mortality. As expected, the presence of phorids decreased the foraging capacity of the workers. A new species was discovered, and several new fire ant hosts were documented. Many of the species exhibited different annual and daily activity patterns, and some of them showed the possibility of vectoring *K. solenopsae*.

Five *Pseudacteon* species were released by ARS in the United States. Several postrelease studies documented their establishment, spread, seasonal abundance, and parasitism rates and confirmed their high specificity for the imported fire ants. Unfortunately, significant reductions of *S. invicta* population densities have not been observed yet.

A considerable amount of new information on the biology of *Orasema* wasps was obtained. Their overall occurrence was fairly high, and they were found in many habitats over a wide geographic range. In addition, five species were identified and two new *Solenopsis* hosts were discovered. The laboratory rearing and artificial transmission was obtained for the first time but at very low rates. A wide variety of economically important plants were confirmed as oviposition substrates. Cosmetic damage to many plants during the oviposition process probably precludes this organism from further testing for fire ant biological control.

Similarly, useful biological information was gathered for *Caenocholax fenyesei* mainly on its host associations. The finding of parasitism on *S. invicta* represented the first record in South America. However, parasitism rates were extremely low. In addition, preliminary attempts to elucidate its complicated life cycle by finding the females in the field have failed. At this point, the use of this organism as a biological control agent of fire ants seems very unlikely.

The parasitic ant *S. daguerrei* parasitizes several fire ant species within the *S. saevissima* group. The overall occurrence was low, but, in certain areas, it reached high parasitism rates, mainly on *S. invicta* in Brazil and *S. richteri* in Argentina. New biological observations were reported on phenology and mating, and several detrimental effects were documented for field populations of *S. richteri*. In addition, molecular studies on its evolutionary history revealed close host relatedness and wide genetic variability, suggesting the potential presence of a complex of species. Future studies on parasite-host matching are needed to identify the most suitable species or biotype for biological control of *S. invicta* in the US. Unfortunately, unsolved rearing problems and the inability to transfer this parasite to nonparasitized fire ant colonies have discouraged further testing.

In summary, after 23 years of intensive field work and laboratory research, the main objective of the program to find a complex of fire ant natural enemies and to evaluate their specificity and suitability for field release has, indeed, been accomplished. Many natural enemies were found, investigated, and developed in close collaboration with ARS scientists in the United States. Several of these organisms were field released, and their ecology and efficacy in the new habitats continue to be evaluated. Many of these biological

control agents could be available for use in other countries or regions invaded by fire ants.

The pioneering studies conducted in South America on native fire ants and their natural enemies have served to greatly encourage further investigations by many scientists and institutions in the United States and other countries. These efforts have advanced the implementation of area-wide biological control programs. Still, promising organisms such as *V. invictae*, *S. daguerrei*, *A. solenopsi*, viruses, and, maybe, more species or biotypes of *Pseudacteon* flies should be further investigated for eventual field release in the near future. It is expected that the final outcome of current and future programs will be the decrease of the imported fire ant population densities and their damage.

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## Research Article

# Diversity of Fungi Associated with *Atta bisphaerica* (Hymenoptera: Formicidae): The Activity of *Aspergillus ochraceus* and *Beauveria bassiana*

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The grass-cutting ant *Atta bisphaerica* is one of the most serious pests in several pastures and crops in Brazil. Fungal diseases are a constant threat to these large societies composed of millions of closely related individuals. We investigated the occurrence of filamentous fungi associated with the ant *A. bisphaerica* in a pasture area of Viçosa, Minas Gerais State, Brazil. Several fungi species were isolated from forager ants, and two of them, known as entomopathogenic, *Beauveria bassiana* and *Aspergillus ochraceus*, were tested against worker ants in the laboratory. The two species were highly virulent, achieving 50 percent worker mortality within 4-5 days. It is the first time *A. ochraceus*, a commonly found fungal species, is reported to infect *Atta* species at a high prevalence. Possible uses for the fungus within biological control are discussed.

## 1. Introduction

Leaf-cutting ants (*Atta* and *Acromyrmex* genera) are considered dominant herbivores in the Neotropics [1] and also a severe pest when attacking crops. The leaf-cutting ants include a number of species which cut predominantly monocotyledonous plants and are known as grass-cutting ants. Knowledge of the morphological, biological, behavioural, and breeding characteristics of these species is still lacking, and are important by the difficulties in controlling their colonies. Among the nine *Atta* species occurring in Brazil, *Atta bisphaerica* Forel, *Atta capiguara* Gonçalves, and *Atta vollenweideri* Forel cut mainly monocots, and one, *Atta laevigata* (Smith), cuts both monocots and dicots.

*Atta bisphaerica*, or “saúva mata-pasto” as it is commonly known in Brazil, stands out as a major pest of pastures and sugar cane [2], currently occupying a leading position as pest species in São Paulo sugar cane plantations and pastures of southeastern Brazil. The control of this species through

granular baits is not appropriate as the baits, which are available in the market, attract with the citrus pulp, which is unattractive to grass-cutting ants. This fact has stimulated the search for new materials for a bait production which is more attractive to these grass cutters [3, 4] and also the demand for natural control agents for leaf-cutting ants in general, including fungi [5] and parasitoids [6, 7].

The main advantage of biological control would be the reduction of the use of insecticides that pollute the environment and may constitute a barrier in the export of Brazilian products. Furthermore, in November 2009, the Forest Stewardship Council (FSC) approved the use of the sulfluramid, fipronil, and deltamethrin active ingredients of insecticide baits for another five years in the Brazilian eucalyptus plantations [8], which provides some time for the forestry companies to find alternatives for pest control.

The potential of using fungi to control leaf-cutting ants is large, considering that several entomopathogenic species have been found in worker ants, nearby or within the nests of

the ants [9–13] and may cause the death of the worker ants or of the fungus garden. While leaf-cutting ants are constantly in contact with a diversity of entomopathogenic fungi, they do not appear to be normally infected by them. This supports the view that they are effectively protected against these parasites by behavioural mechanisms such as intensive self-grooming or allogrooming [14, 15] and utilization of antibiotics derived from glands or symbiotic bacteria. Knowing the diversity of these fungi and the ants' resistance mechanisms is essential if one wants to implement safe and efficient microbial control methods for the pest control.

The purpose of this study was first to isolate and identify the filamentous fungal species associated with *A. bisphaerica*. Then, we tested the entomopathogenic activity of two species found in the first part of our study presented here: an *Aspergillus ochraceus* isolate, which was the most observed, and an isolate of *Beauveria bassiana*, a species widely used in microbial control of insects and also found in worker ants of *A. bisphaerica*, were tested in the laboratory.

## 2. Material and Methods

**2.1. Collection of Ants in the Field and Research of Pathogenic Fungi.** The ants were collected in a pasture in Viçosa, MG, Brazil (20° 44'S, 42° 50'W) on May 27, 2010, between 2:00 and 4:00 PM during their foraging time. We collected 100 foraging worker ants, which were on their return to the nest with grass fragments, each of three colonies (I, II, and III), which demonstrated foraging activity, totaling 300 workers ants. The ants were taken to the laboratory and isolated in Petri dishes supplied with a honey-water solution (1 : 1) and sterile distilled water, being monitored daily until death. The dead worker ants were washed in series with a solution of 70% ethanol, 4% sodium hypochlorite, and distilled water. After washing, they were transferred to sterile Eppendorf tubes, containing moist cotton and kept in the incubator at a temperature of  $25 \pm 1^\circ\text{C}$ , a relative humidity of  $70 \pm 10\%$ , and a 12-hour photoperiod, until the external appearance of fungal hyphae. Then the isolation of the fungi was performed in a vertical laminar flux hood. The fungi were inoculated in Petri dishes (9 cm diameter) with a potato dextrose agar (PDA) culture containing antibiotics (penicillin, 0.5 g/L, streptomycin, 0.5 g/L). After obtaining pure cultures, they were subjected to microscopic analysis by performing the identification and classification of the fungi with the aid of taxonomic keys [16–18] and then confirmed at the generic and species level by Dr. Harry Evans.

**2.2. Infection of the Ants in the Laboratory.** To assess the potential pathogenic fungi *B. bassiana* and *A. ochraceus*, isolated from the workers collected in the field as described in Section 2.1, we tested the mortality of the *A. bisphaerica* worker ants taken from colonies maintained in the laboratory and inoculated with a spore suspension. The colonies were collected 10 days before at Viçosa, Minas Gerais State, Brazil, and maintained in the laboratory according to the methodology developed by Della Lucia [19]. They were maintained at  $25 \pm 5^\circ\text{C}$ , relative humidity of  $75 \pm 5\%$ ,

and a 12 : 12 light : darkness regime. On a daily basis, they received cut leaves of *Hyparrhenia rufa* (Poales: Poaceae) and *Zea mays* (Poales: Poaceae), in addition to clean water. Five hundred and fifty workers (head width  $\sim 3.2$  mm) were collected in roughly equal proportions from the three colonies (A, B, and C, maintained under laboratory conditions) in the fungus garden, which probably were less likely to be contaminated. Conidial suspensions of the two fungi species were prepared from a Tween 80 solution of 0.05% at concentrations of  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$ , and  $10^9$  conidia/mL. For each concentration, 50 worker ants received 1  $\mu\text{L}$  of the suspensions on the pronotum. One  $\mu\text{L}$  of a 0.05% Tween 80 solution was applied to the control ants. This control group was used in the comparison with the two isolates since the experiments were conducted simultaneously. After receiving the treatments, the insects were held individually in Petri dishes supplied with a honey-water solution (1 : 1) and sterile distilled water renewed every two days. The mortality of the worker ants was daily monitored. The dead worker ants were washed as in the previous experiment and transferred to sterile Eppendorf tubes, containing moist cotton and kept in the incubator at temperature of  $25 \pm 1^\circ\text{C}$ , a relative humidity of  $70 \pm 10\%$ , and a 12-hour photoperiod, until the external appearance of fungal hyphae. Then we proceeded with the isolation of the fungi, which were randomly selected in all the used concentrations with 15 isolate plates for each individual concentration. This was done to confirm whether the death was due to the applied fungi.

**2.3. Survival Analysis.** Survival curves were generated as a function of the observation time through the Kaplan-Meier method. Firstly, we performed a comparison of multiple groups, and thereafter we compared the survival of two groups applying the nonparametric Log-rank test at 5% significance level with the aid of the Statistica software, version 7.0.

## 3. Results

The survival of the *A. bisphaerica* worker ants, which were brought from the field, did not vary among the three analyzed colonies ( $\chi^2 = 3.06$ ,  $P = 0.21$ ), and the maximum survival was 25 days (Figure 1). It was possible to isolate and identify 10 fungi species that may have deleterious effects on the worker ants. Among them, three *Aspergillus*, three *Penicillium*, one *Cladosporium*, two *Mucor*, and one *Beauveria* species were identified (Table 1). The most frequent fungus found in the worker ants was *A. ochraceus*, with 44 occurrences. It was found in individuals who had died about 5 days after the beginning of the analyses. The fungi that had not sporulated were classified as *Mycelia sterilia* (Table 1). The percentage of worker ants, which were infected with known entomopathogenic fungi, was 15.33%, namely, *A. ochraceus* (14.67%), *A. niger* (0.33%), and *B. bassiana* (0.33%).

During experimental infection, we found a significant difference in survival time of the worker ants subjected to different concentrations of both suspensions *A. ochraceus*

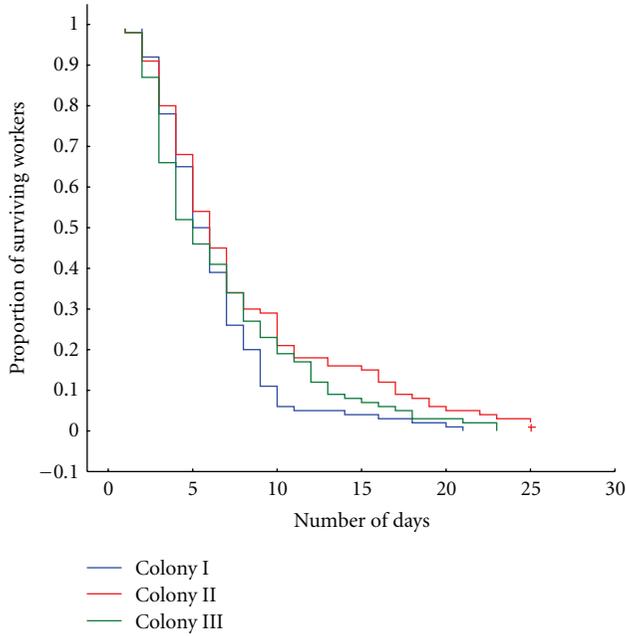


FIGURE 1: Cumulative time-related survival proportion (Kaplan-Meier curves) of the *Atta bisphaerica* forage worker ants from three different colonies.

TABLE 1: Species and frequencies of the fungi isolated from 300 foragers of *Atta bisphaerica*.

Fungus species	Frequency of occurrence (in 300 workers)
<i>Aspergillus niger</i>	1
<i>Aspergillus ochraceus</i>	44
<i>Aspergillus sclerotiorum</i>	5
<i>Beauveria bassiana</i>	1
<i>Cladosporium</i> sp.	15
<i>Mucor hiemalis</i>	4
<i>Mucor racemosus</i>	10
<i>Penicillium lilacinum</i>	2
<i>Penicillium</i> sp. 1	2
<i>Penicillium</i> sp. 2	1
<i>Mycelia sterilia</i>	5

( $\chi^2 = 42.27$ ,  $P < 0.001$ ; see Table 2 for comparing two survival curves with a Log-rank test) (Figure 2) and *B. bassiana* ( $\chi^2 = 94.03$ ,  $P < 0.001$ ; see Table 3 for comparing two survival curves with a Log-rank test) (Figure 3). The shortest survival time was observed at a concentration of  $10^9$  conidia/mL in both treatments, with an  $LT_{50}$  of four days at a  $10^9$  concentration of *A. ochraceus* (Figure 2) and an  $LT_{50}$  of four days for concentrations of  $10^8$  and  $10^9$  of *B. bassiana* (Figure 3). The  $LT_{50}$  of the control group resulted in 10.5 days.

#### 4. Discussion

Since certain entomopathogenic fungi present a high potential to control the ants, pathogenicity tests have been

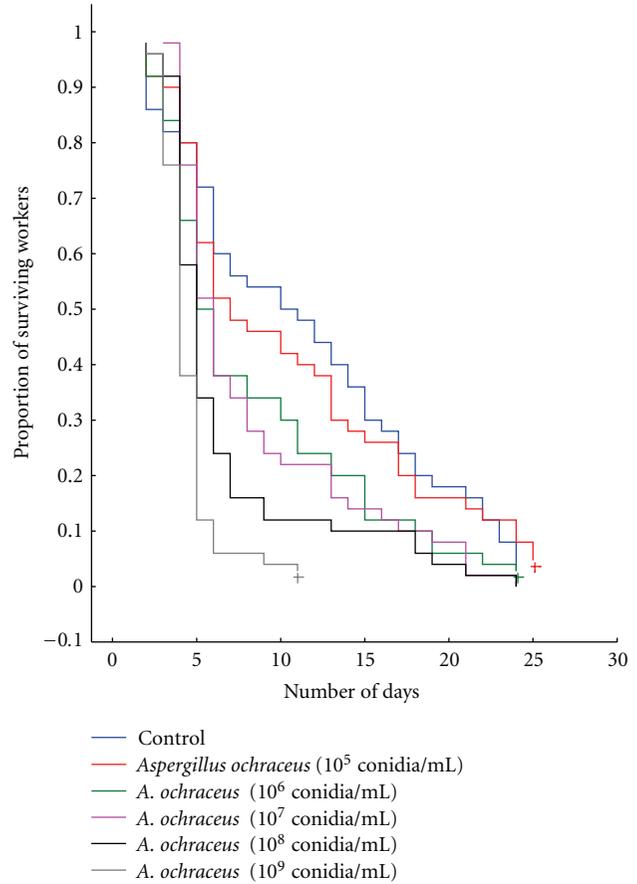


FIGURE 2: Cumulative time-related survival proportion (Kaplan-Meier curves) of the *Atta bisphaerica* worker ants after being treated with different suspension concentrations of the *Aspergillus ochraceus* fungus.

performed with fungi widely used within the microbial control of insects, such as *Metarhizium anisopliae* (Metsch.) and *Beauveria bassiana* (Bals.). They have proved to be highly virulent in the laboratory, although field tests do not reproduce the same results [20–22]. In our study, *M. anisopliae* was not found and *B. bassiana* was obtained only once. The *Aspergillus ochraceus* was the most frequent and, for the first time, reported to infect ants frequently. The mortality test results of ants infected with *A. ochraceus* and *B. bassiana* are similar to those obtained in other pathogenicity tests of *B. bassiana* or *M. anisopliae* against leaf-cutting ants [22–24]. Although the high control mortality indicates that the ants were stressed in some way (e.g., social isolation), and thus probably more susceptible to diseases, the parasite treatments caused significantly increased mortality. This allows us to say that *A. ochraceus* could be also a promising biological control agent of ants. Surely, more research should be undertaken before significant field use of the pathogen. Even though that *Aspergillus* are unusual pathogens of most insects, these fungi produce a diverse range of compounds that can be potent insect toxins and potentially useful as pesticidal [25]. However, the safety of these compounds is a major concern and more studies are

TABLE 2:  $P$  values of Log-rank test to compare two Kaplan-Meier survival curves for *Atta bisphaerica* workers treated with different conidial concentrations of *Aspergillus ochraceus*.  $P$  values less than 0.05 were considered significant.

Conidial concentrations	0	$10^5$	$10^6$	$10^7$	$10^8$	$10^9$
0	—					
$10^5$	$P = 0.90$	—				
$10^6$	$P = 0.06$	$P = 0.07$	—			
$10^7$	$P = 0.02$	$P = 0.04$	$P = 0.93$	—		
$10^8$	$P < 0.01$	$P < 0.01$	$P = 0.15$	$P = 0.10$	—	
$10^9$	$P < 0.0001$	$P < 0.0001$	$P < 0.001$	$P < 0.0001$	$P < 0.01$	—

TABLE 3:  $P$  values of Log-rank test to compare two Kaplan-Meier survival curves for *Atta bisphaerica* workers treated with different conidial concentrations of *Beauveria bassiana*.  $P$  values less than 0.05 were considered significant.

Conidial concentrations	0	$10^5$	$10^6$	$10^7$	$10^8$	$10^9$
0	—					
$10^5$	$P = 0.80$	—				
$10^6$	$P = 0.03$	$P = 0.02$	—			
$10^7$	$P < 0.0001$	$P < 0.0001$	$P < 0.01$	—		
$10^8$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	—	
$10^9$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.77$	—

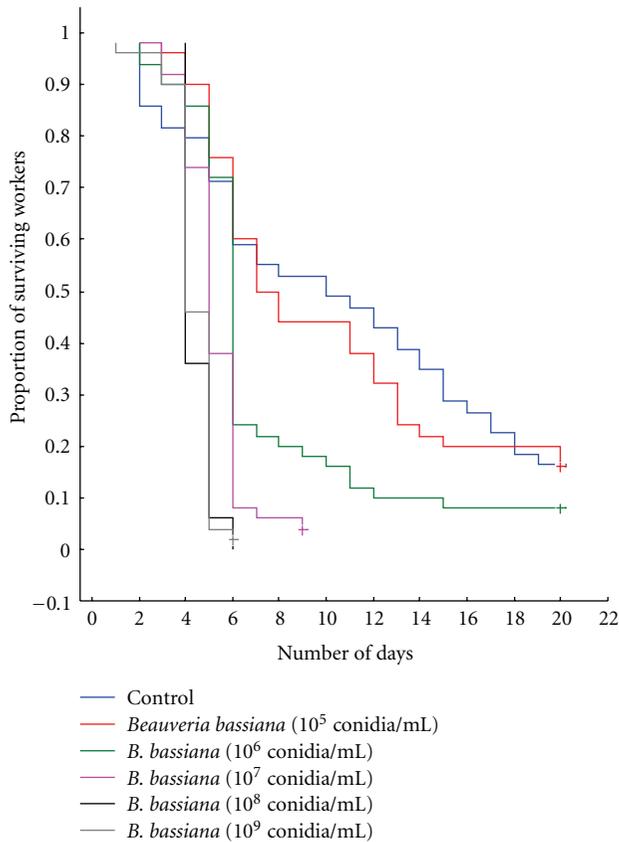


FIGURE 3: Cumulative time-related survival proportion (Kaplan-Meier curves) of the *Atta bisphaerica* worker ants after being treated with different suspension concentrations of the *Beauveria bassiana* fungus.

needed to evaluate potential risks to humans and nontarget species.

*Cladosporium* fungi are phytopathogens [26] and may be transported by various insects causing their death. In a study of leaf-cutting queen ants, the *Cladosporium* fungus was prevalent in *Atta laevigata* and in *A. capiguara* [27]. It is believed that these fungi are frequently found due to their cosmopolitan distribution, being acquired from the environment and dispersed during the founding of a colony by queens or forage worker ants carrying leaves. The role of the *Cladosporium* species inside the ant colonies is not known for sure, but they have been considered potential antagonists of the fungus garden [28]. The same kind of reasoning can be adopted for the *Mucor* fungi species, which have also been found in the nests of leaf-cutting ants, however, without knowing exactly how they interact with these ants or the fungus garden. The fungi of this genus are considered invertebrate pathogens. For example, *Mucor hiemalis* can cause the mortality of *Cupiennius* and *Ischnothele* spiders [29]. Toxic metabolites of these organisms present an insecticidal activity against the adults of *Bactrocera oleae* and *Ceratitis capitata* (Diptera: Tephritidae) [30].

Considering the diversity of fungi found in this study, the importance of new surveys and tests of entomopathogenic fungi isolates with the potential of being used in microbial control of these ants becomes evident. *Beauveria* and *Metarhizium* are the species which have been most studied and which have been evaluated on tests on their pathogenic potential. It is evident now that *A. ochraceus* should be further investigated for its pathogenic potential for leaf-cutting ants as well as other fungi genera found here.

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## Research Article

# Effect of Habitat Type on Parasitism of *Ectatomma ruidum* by Eucharitid Wasps

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Eucharitidae are parasitoids that use immature stages of ants for their development. *Kapala* Cameron is the genus most frequently collected in the Neotropics, but little is known about the biology and behavior of any of the species of this genus. We aimed to evaluate the effect of habitat type on eucharitid parasitism and to contribute to the knowledge of the host-parasite relationship between *Kapala* sp. and the poneromorph ant *Ectatomma ruidum* (Roger) in Colombia. Twenty *E. ruidum* colonies were extracted from two different habitat types (woodland and grassland), and larvae and cocoons (pupae) were examined in search for parasitoids in different stages of development. Globally, 60% of the colonies were parasitized, with 1.3% of larvae and 4% of pupae parasitized. Planidia (first-instar larvae), pupae, and adults of the parasitoid were observed. All of the pupae and adult parasitoids belonged to *Kapala iridicolor* Cameron. All the colonies collected in the woodlands were parasitized and contained more parasitized larvae (2%) and parasitized cocoons (8%) than those collected in grasslands (4/12 parasitized colonies, 0.5% parasitized larvae, 0.8% parasitized cocoons). The relationship observed between habitat type and parasitism prevalence is a novel aspect of the study of eucharitid impact on ant host populations.

## 1. Introduction

Several dipteran, strepsipteran, and hymenopteran parasitoids are natural enemies of ants [1–9]. Among the hymenopterans, the Eucharitidae *sensu stricto* is the only monophyletic group, at the family level, where all of its members are parasitoids of ants. They are also one of the largest and most diverse groups attacking social insects [8].

Eucharitidae have a specialized life cycle that includes oviposition away from the host, on or into a host-plant [2]. Although there are more than 400 species of Eucharitidae already described [8], the hosts and host-plants of only a few species are known [10], and knowledge on the life history and ecology of these wasps is even scarcer. In the New World, detailed studies on selected species have only been carried out in a few localities in Mexico, Argentina and North America (e.g., [1, 11–17]). For Colombia, there is no detailed report on the biology of any species of this family.

The impact of eucharitids on their host populations has recently been explored in detail for some Mexican and South American ant populations [12, 17–19]. These, and earlier reports (e.g., [2, 11, 20]), signaled the aggregated nature of eucharitid populations. In fact, prevalence of parasitism by eucharitids varies greatly in time and space [2], with 100% of colonies parasitized at some sites, and other colonies escaping from parasitism (e.g., [18, 19]). Differences in local parasitism, in general, can be attributable to several different factors such as the presence of resources, other than hosts, necessary for maintaining high parasitoid populations locally (e.g., floral and extrafloral nectar, and refuge sites for adults), suitable host-plants, microclimatic differences, and/or dispersal capacity of adult parasitoids [21, 22]. In some cases, for example, parasitoids may be less effective at parasitizing hosts in sites with simpler vegetation [23]. In the case of eucharitids, an aspect not yet studied in detail is the effect of the habitat on the impact of these parasitoids on

their ant-host populations, though preliminary results of a recent study suggest that differences in management in coffee agroecosystems (i.e., shade, pruning, weed management) might affect parasitism by eucharitids [24].

*Ectatomma ruidum* (Roger) (Hymenoptera: Formicidae: Ectatomminae) is a diurnal, earth-dwelling, Neotropical ant that nests in the soil. This ant is found from southern Mexico to Brazil, from sea level to an altitude of 1500–1600 m [25–27], and is dominant in several ecosystems such as forests [28], or economically important cultivated areas [29, 30]. Two species of *Kapala* (Eucharitidae) have been reported to parasitize this ant in Mexico [14, 31], and parasitism of *E. ruidum* by *Kapala* sp. is also known from Colombia (C. Santamaría and J. Herrera, unpub. data). The purpose of this study is to report observations of the host-parasite relationship between *Kapala* sp. and *E. ruidum* in Colombia and to compare the impact of this eucharitid on its ant host population in two different habitat types.

## 2. Materials and Methods

This study was carried out on the grounds of the Melendez Campus at the Universidad del Valle (3° 22' N, 76° 32' W), located at the south of the city of Cali, Department of Valle del Cauca, Colombia. The Campus has an area of approximately 100 ha, 8 ha of which are occupied by buildings, 44 ha by woodlands, 46 ha by grasslands, and 1 ha by two ponds. The average elevation is 970 m; mean annual temperature is 24.1°C and average relative humidity 73% [32]. Average annual rainfall is around 1500 mm, with two rainfall peaks, from March to May and from September to November (Instituto de Hidrobiología, Meteorología y Estudios Ambientales-IDEAM, unpublished data, cited by [32]). According to the Holdridge system, the study site is located in an area classified as Tropical Dry Forest (bs-T) [33].

Five sites on the campus were examined: 2 sites in grasslands dominated by Poaceae and other creeping plants and with no trees, and 3 sites located in woodlands. The sites in the latter habitat had a lesser amount of Poaceae among the creeping vegetation and had, in some cases, abundant litter. Common tree species in these sites were *Pithecellobium dulce* (Roxb.) Benth., *Samanea saman* (Jacq.) Merr., and *Calliandra pittieri* Standl. (Fabaceae); *Mangifera indica* L. (Anacardiaceae); *Ceiba pentandra* (L.) Gaertn. (Bombacaceae); *Ficus elastica* Roxb. (Moraceae); and *Tabebuia chrysantha* G. Nicholson, *T. rosea* (Bertol.) A. DC., and *Spathodea campanulata* P. Beauv. (Bignoniaceae) [32]. In each of the 5 sites chosen, we determined the number of *E. ruidum* nests in a plot of 8 × 8 m. One additional plot, placed 50 m from the closest grassland plot and comparable to the others, was censused for nest density evaluation in the grassland area, to get an even sample size. During April, May, and November 2009, 20 nests chosen at random were excavated (8 in woodlands and 12 in grasslands) and transported to the laboratory for examination.

Ant larvae were inspected for planidia (eucharitid first-instar larvae) attached to their cuticle by means of a stereoscopic-microscope (Nikon SZ645). Cocoons were kept

in petri dishes at room conditions for 5 days or more and were examined once daily to record emergence of adult eucharitids. At the end of the observation period, all of the cocoons were dissected to look for adults and pupae of dead, or not yet emerged, parasitoids, and to register the caste and sex of ants attacked by the parasitoids. Adult wasps were individually placed in vials covered with cloth mesh, and their survival time was evaluated. No food or water was provided. Pupae and adult eucharitids were identified with available keys [8, 34, 35], and their sex was determined, when possible, based on the dimorphism present in the antennae [8]. The material collected was measured using a stereomicroscope equipped with an ocular micrometer and preserved in 96% alcohol. Voucher specimens of both the ants and the parasitoids have been deposited in the Grupo de Investigación en Ecología de Agroecosistemas y Hábitats Naturales (GEAHNA) collection, at the Museo de Entomología of the Universidad del Valle, Colombia (MEUV), and at the Arthropod Collection of El Colegio de la Frontera Sur, Unidad Chetumal, Mexico (ECO-CH-AR).

A Fisher's exact test was carried out to establish whether there were significant differences between the proportions of parasitized colonies found in woodlands and in grasslands, and Z tests were used to search for differences in the number of parasitized larvae and parasitized pupae according to habitat. Nest density and colony size according to habitat (woodlands or grasslands), and colony size according to the presence or absence of parasitoids (both habitats), were compared using a Mann-Whitney test. Spearman nonparametric correlation was used to explore the relationship between the size of the colony (adults + brood) and total parasitized brood, between the number of larvae per colony and total parasitized larvae, and between the number of cocoons per colony and total parasitized cocoons. All statistics were calculated using STATISTICA 8.0 (StatSoft, Inc.) and R 2.13.1 (The Foundation for Statistical Computing) programs.

## 3. Results

Of the 20 *E. ruidum* colonies examined, 12 (60%) were parasitized (Table 1). The global rate of parasitism in the study area was 2.3% (parasitized brood per total ant brood, 27/1162), with 1.3% (9/714) of the larvae and 4.0% (18/448) of the pupae parasitized. In total, 29 eucharitid individuals or their remains were observed, with  $2.4 \pm 2.6$  (mean  $\pm$  standard deviation;  $n = 12$  colonies; range: 1–10) parasitoids per parasitized colony. Parasitoids in 3 different stages of development were found: planidia in 7 colonies ( $1.6 \pm 0.8$  parasitized larvae per parasitized colony; range: 1–3), pupae in 3 colonies ( $3.3 \pm 3.2$  individuals; range: 1–7), and adults in 5 colonies ( $1.6 \pm 0.9$  individuals; range: 1–3). Pupae and adults were identified as belonging to *Kapala iridicolor* (Cameron).

All of the colonies collected in the woodlands were parasitized ( $n = 8$ ), while in the grasslands only 33.3% (4/12) contained eucharitids (Table 1). Prevalence of parasitism and type of habitat were not independent (Fisher's two-tailed exact test:  $P = 0.0047$ ), and there was a greater frequency of parasitized nests in the woodlands than in

TABLE 1: Composition of *Ectatomma ruidum* colonies in two different habitat types, percent parasitized brood, and number and stage of development of *Kapala iridicolor* individuals.

Nest Number	Habitat	Queen Number	<i>Ectatomma ruidum</i>						<i>Kapala iridicolor</i>				
			Gynes	Males	Workers	Larvae	Pupae	Total	Parasitized larvae (%)	Parasitized pupae (%)	Planidia/scar	Pupae	Adults
1	Woodland	0	0	5	34	17	23	79	2 (11.8)	1 (4.4)	3	0	1(♂)
2	Woodland	0	0	0	9	8	3	20	1 (12.5)	0 (0)	1	0	0
3	Woodland	0	0	3	27	24	18	72	1 (4.2)	0 (0)	1	0	0
4	Woodland	0	0	0	5	20	58	83	0 (0)	10 (17.2)	0	7(5♂, 1♀, 1?)	3(1♂, 2♀)
5	Woodland	0	1	5	28	36	48	118	0 (0)	4 (8.3)	0	2(1♂, 1♀)	2(♀)
6	Woodland	0	0	2	47	121	46	216	0 (0)	1 (2.2)	0	0	1(♂)
7	Woodland	0	0	1	17	27	5	50	1 (3.7)	0 (0)	1	0	0
8	Woodland	0	0	14	18	91	0	123	2 (2.2)	—	2	0	0
9	Grassland	0	0	0	14	63	6	83	0 (0)	0 (0)	0	0	0
10	Grassland	0	0	0	9	18	22	49	0 (0)	0 (0)	0	0	0
11	Grassland	0	0	1	3	25	21	50	0 (0)	0 (0)	0	0	0
12	Grassland	0	0	0	15	25	32	72	0 (0)	0 (0)	0	0	0
13	Grassland	0	1	1	27	26	16	71	0 (0)	1 (6.3)	0	0	1(♂)
14	Grassland	0	0	1	38	31	23	93	0 (0)	0 (0)	0	0	0
15	Grassland	0	0	2	23	39	34	98	0 (0)	1 (2.9)	0	1(♂)	0
16	Grassland	0	0	2	20	1	19	42	0 (0)	0 (0)	0	0	0
17	Grassland	0	0	0	34	33	31	98	1 (3.0)	0 (0)	1	0	0
18	Grassland	1	0	0	39	42	20	102	0 (0)	0 (0)	0	0	0
19	Grassland	0	0	0	40	42	13	95	1 (2.4)	0 (0)	2	0	0
20	Grassland	0	0	0	3	25	10	38	0 (0)	0 (0)	0	0	0
Total		1	2	37	450	714	<b>448</b>	1652	9 (1.3)	18 (4.0)	11	10	8

the grasslands (Fisher's one-tailed exact test:  $P = 0.039$ ). Furthermore, the proportion of parasitized pupae differed between both habitats ( $Z$ -test,  $Z = 2.9$ ;  $P = 0.003$ ), with a greater number of parasitized pupae in the woodlands than in the grasslands (Figure 1). Although a greater proportion of parasitized larvae was also observed in the woodlands (Figure 1), there was no statistical difference according to habitat ( $Z$ -test,  $Z = 1.79$ ,  $P = 0.07$ ). The global rates of parasitism for the woodlands and the grasslands were 4.2% and 0.65%, respectively. The average number of parasitoids per parasitized colony was greater in the woodlands ( $3 \pm 3.1$  parasitoids; range: 1–10) than in the grasslands ( $1.25 \pm 0.5$  parasitoids; range: 1–2).

There was a significant, positive correlation between the number of parasitized pupae and the number of available pupae (Spearman correlation test,  $r = 0.63$ ,  $P = 0.004$ ,  $n = 19$  colonies). However, no correlation was found between the following variables: (1) total parasitized brood and colony size (Spearman,  $r = 0.39$ ,  $P = 0.08$ ,  $n = 20$  colonies), (2) total parasitized larvae and number of available host larvae (Spearman,  $r = -0.04$ ,  $P = 0.86$ ,  $n = 20$  colonies), and (3) total parasitized brood and number of workers (Spearman,  $r = 0.22$ ,  $P = 0.36$ ,  $n = 20$  colonies).

Workers and ant larvae were present in all of the *E. ruidum* colonies but there were no cocoons in one of them (Table 1). The global mean size of the colonies (queen, gynes, workers, males, pupae, and larvae) was  $82.6 \pm 41.7$

individuals. Colony size was greater in colonies from the woodlands and in those parasitized ( $95.1 \pm 59.2$  and  $93.8 \pm 47.8$  individuals, respectively) than in those from the grasslands ( $74.3 \pm 24.0$ ), or from nonparasitized colonies ( $66.1 \pm 24.7$ ). Nevertheless, there were no significant differences in colony size between parasitized and non-parasitized colonies (Mann-Whitney test,  $U = 29$ ,  $P = 0.14$ ,  $n_1 = 12$ ,  $n_2 = 8$ ), nor according to the habitat from which the colonies came (Mann-Whitney test,  $U = 40$ ,  $P = 0.62$ ,  $n_1 = 12$ ,  $n_2 = 8$ ). A significantly greater density of *E. ruidum* colonies was estimated for the grasslands (3281 colonies/ha), compared to that for the woodlands (1563 colonies/ha) (Mann-Whitney test,  $U = 0$ ,  $P = 0.049$ ,  $n_1 = n_2 = 3$ ).

Six planidia attached to the cuticle of *E. ruidum* larvae (Figure 2(a)) were observed. Five were in the interior of a sclerotized ring (Figures 2(b) and 2(c)) while one was not (Figure 2(e)). In 5 cases, sclerotized rings with no planidia were observed (Figure 2(d)). In 2 host larvae from different colonies, 2 planidia (or empty scars) were observed (representing 22% of the parasitized larvae, 2/9). Very small host larvae were found parasitized. The length of parasitized larvae ranged from 2.77 to 10.10 mm ( $n = 8$ ). Planidia were on average  $0.086 \pm 0.006$  mm in length (range: 0.08–0.09 mm,  $n = 3$ ), and the sclerotized rings had a diameter of  $0.165 \pm 0.072$  mm (range: 0.07–0.27 mm,  $n = 9$ ) and a thickness of  $0.043 \pm 0.026$  mm (range: 0.08–0.09 mm,  $n = 9$ ). More male (64.7%, 11/17) than female (35.3%, 6/17)

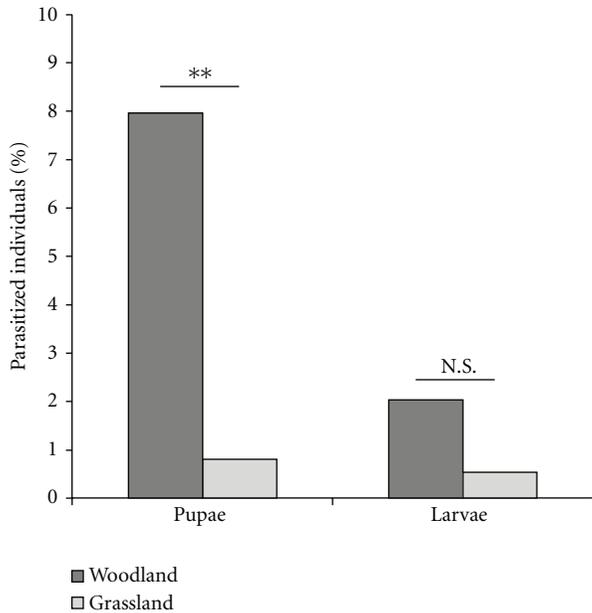


FIGURE 1: Global percentage of *Ectatomma ruidum* immature stages parasitized by *Kapala iridicolor* in two different habitat types (woodland versus grassland). \*\* $P < 0.01$ ; N.S.: nonsignificant.

eucharitids were observed in six colonies with wasp pupae and adults. Adult *K. iridicolor* males, on the average, lived longer ( $5.3 \pm 2.2$  days, range: 2–7 days,  $n = 4$ ) than females ( $4 \pm 0.8$  days, range: 3–5 days,  $n = 4$ ) although no significant differences were found (Mann-Whitney test,  $U = 4$ ,  $P = 0.25$ ).

No cases of superparasitism were observed during dissection of cocoons. Empty cocoons from which eucharitid adults emerged had an operculum at the anterior end, opposite to the one with the ant's meconium, forming a regular circular cut (Figure 3(a)) which is made by the adult wasps with their falcate jaws. From the remains of the host ants found during dissection of the cocoons, it can be stated that male, queen, and worker pupae are attacked (Figure 3(b)). Queen pupae were not completely consumed by the developing wasp.

#### 4. Discussion

In this study, a comparison of eucharitid parasitism was made between two contrasting habitats differing in tree cover and associated understory vegetation. This perspective had not previously been considered in detail in studies of the impact of eucharitids on their ant host populations. Although early works (e.g., [2, 11]) indicated variation of parasitism in space, no comparison of parasitism by eucharitids in different habitats involving grasslands is available. However, a recent study also found a greater parasitism of poneromorph ants in forest fragments, compared to the more disturbed areas of Mexican coffee plantations [24].

Because the plots in our study (woodlands and grasslands) were interspersed, a site effect is less possible than a “habitat” effect. Our results showed that parasitoids

were more prevalent in woodlands than in grasslands with respect to the number of parasitized colonies and parasitized host pupae, although the percentage of parasitized larvae did not differ statistically between both habitats. These findings suggest that the probability of an encounter between eucharitids and their host ant colonies is higher in more complex habitats such as those of the Valle del Cauca dry forest. Alternatively, the survival of eucharitids may be increased in shaded areas. The two habitats are different in vegetation (composition and structure) and environmental characteristics such as temperature and humidity [36], which may affect host and host-plant availability/distribution, and ant foraging strategies.

This study also represents the first detailed record of the interaction between *K. iridicolor* and *E. ruidum* in Colombia, which is the second locality for these species where aspects of the impact of parasitism and other information on the natural history of this eucharitid are known. *Kapala iridicolor* is known to parasitize several species of poneromorph ants in Mexico (*E. ruidum*, *Gnamptogenys regularis* Mayr, *G. sulcata* (Fr. Smith), *G. striatula* Mayr, and *Pachycondyla stigma* (F.) [14]), and it might probably interact with other ants in Colombia. On the Melendez Campus and in the City of Cali, *K. iridicolor* had been reported earlier [37], but its ant host was unknown. In the locality studied, other species of poneromorph ants have also been reported including *Odontomachus bauri* Emery, *O. erythrocephalus* Emery, and *Pachycondyla* sp. [37, 38].

The percentage of *E. ruidum* colonies with *K. iridicolor* parasitoids is very close to that observed for the interaction of *E. ruidum* with *K. iridicolor* and *K. izapa* Carmichael in Mexico [18]. The percentage of immature stages attacked was, however, low compared with the results of some studies that indicate over 16% of the brood parasitized (e.g., [11, 13, 20]). Nevertheless, these figures are within the range observed by Lachaud and Pérez-Lachaud [18] in their year-long study on *E. ruidum* in Mexico.

It is worth noting that a lower density of *E. ruidum* nests was found in the woodlands compared to the grasslands. These observations are consistent with those for the Departments of Valle del Cauca and Cauca [39], but not for Guajira, a drier region in the extreme north of Colombia, where a high abundance of *E. ruidum* nests was found in areas with higher presence of trees [40]. Furthermore, and although not studied in detail, we did note differences in the foraging hours of *E. ruidum* workers in the two habitats sampled. Foragers of this ant species displayed very low activity in the grasslands during the warmest hours (10:00 am to 16:00 pm), while ants were observed foraging during these hours in the woodlands. Nest distribution and nest density, as well as foraging times of ants, are factors that could also contribute to differences in parasitism by eucharitids. *Kapala iridicolor* is known to use a wide range of host plants for oviposition, including species of several plant families (Malvaceae, Boraginaceae, Asteraceae) [14, 34]. The plant(s) used by this eucharitid in our study site remain(s) unknown; however it is likely that differences in understory vegetation between the woodland and the grassland contribute also to the observed differences in

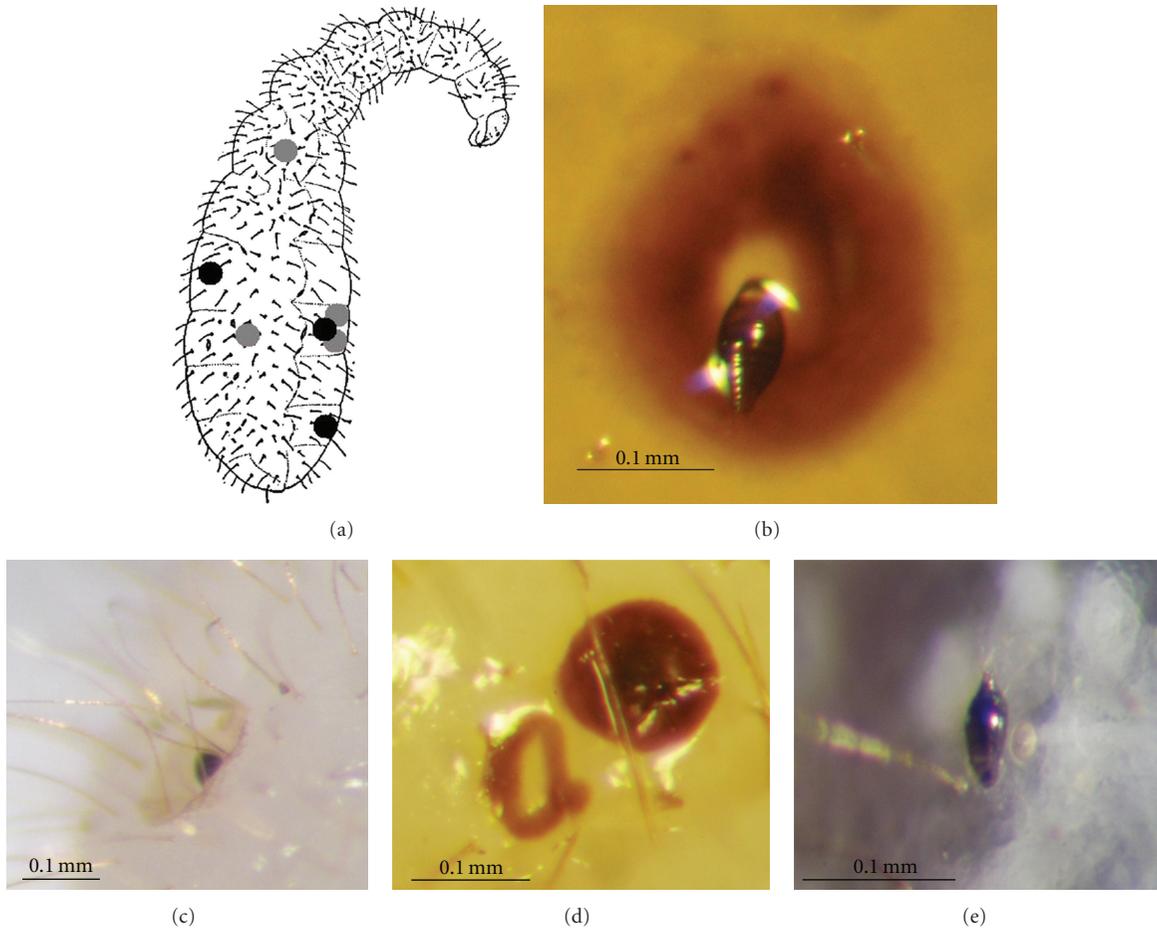


FIGURE 2: First larval stage (planidium) of *Kapala iridicolor* on its *Ectatomma ruidum* larval host. (a) Position of planidia (black dots) and scars (gray dots) on the host larva (modified from [43]). (b) Planidium with scar (sclerotized ring) around it. (c) Extreme back side view of a planidium on the interior of a partially sclerotized ring. (d) Scar without planidium. (e) Planidium joined to the host without formation of a sclerotized ring.

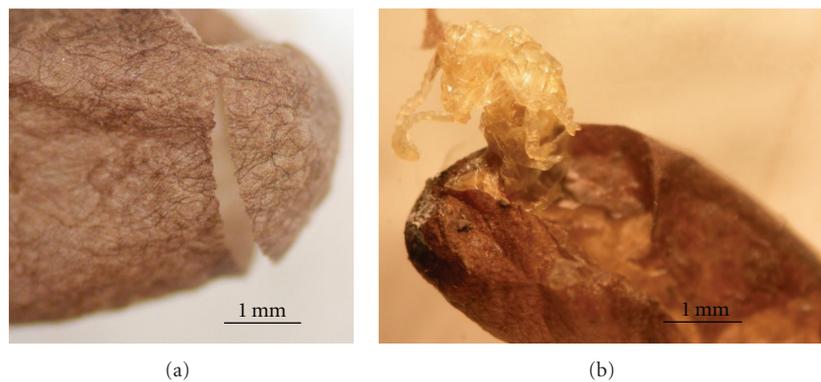


FIGURE 3: Operculum made by *Kapala iridicolor* wasp on emergence from the host cocoon. (a) View of the cocoon with operculum. (b) Host remains (only the ant cuticle is left).

parasitism of *E. ruidum* by *K. iridicolor*, as has been suggested for differences in eucharitid parasitism between coffee agroecosystems and the forest [24]. This issue deserves further study.

A positive correlation was established between the number of parasitized pupae and the number of pupae available in the colonies. This pattern was similar to that reported elsewhere [18, 19]. It was also found that parasitized ant

larvae were quite variable in size and covered almost all sizes of larvae present in the colonies. This observation contrasts with previous reports [11–14, 41] where, in general, only late larval instars were found attacked by planidia, but very young host larvae with planidia have been reported in some cases [19, 42].

In summary, the results of this study showed an effect of habitat type (woodlands versus grasslands) on parasitism of *E. ruidum*, a widely distributed, dominant poneromorph ant, by *K. iridicolor*. It also records some aspects of the natural history of this parasitoid in Colombia.

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## Review Article

# Evolutionary Perspectives on Myrmecophily in Ladybirds

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Myrmecophiles are species that usually have developed specialized traits to cope with the aggressiveness of ants enabling them to live in their vicinity. Many coccinellid species are predators of Hemiptera; the latter is also often protected by ants. Therefore these ladybirds frequently interact with ants, and some species have become myrmecophilous. In this paper, we aim to provide an overview of the evolution of myrmecophilous traits in ladybirds. We then discuss the costs and benefits of myrmecophily and the dietary shift to myrmecophagy observed in a few species.

## 1. Introduction

Ants represent a highly ecologically successful and most often dominant group of insects. Their predominance in almost all terrestrial ecosystems leads them to interact with many other organisms. One of the best-known examples of such interaction is their mutualism with Hemiptera. Ants protect the sap-feeding insects, and in return they benefit from the honeydew provided by the Hemiptera [1–3]. Honeydew is rich in carbohydrates and in some amino acids, which are attractive and nutritionally valuable for ants [4–7]. Additionally, the ants sometimes use aphids as a source of protein by consuming them [8–11]. Aside from protecting the Hemiptera, ants may also reduce their risk of getting fungal infections via hygienic behaviors [12–14], reduce indirect competition with untended Hemiptera [15], and they can even transport the Hemiptera to suitable feeding sites when a host plant's quality deteriorates [16]. Nevertheless, the main benefit for the Hemiptera when tended by ants is the protection the ants provide from natural enemies [1, 3, 13, 17–21]. Untended colonies experience higher predation and parasitism rates. The colonies of the aphid *Tuberculatus quercicola*

(Matsumura), for example, had lower survival rates when ants were excluded [17], and the black cherry aphid *Myzus cerasi* (Fabricius) reached higher densities of individuals on trees with ants than on those without ants [18]. As a result of the protection they provide to Hemiptera, ants are in competition with predators such as ladybirds and syrphid larvae as well as parasitoids.

Ladybird species are well known for their aphido- and coccidophagy, which have popularized them as biocontrol agents in agricultural systems and private gardens. They exhibit, however, a large trophic diversity from mycophagous and phytophagous to predatory species. The latter species mainly eat coccids or aphids and, also to some extent, aleyrodids, psyllids, chrysomelids, and mites, although coccidophagy is considered more primitive than aphidophagy [22, 23]. The fact that many ladybird species prey on Hemiptera brings them into frequent contact with ants. Some of them use behavioral, physical, and chemical characteristics to cope with these aggressive competitors. Species found living regularly or only with ants are called “myrmecophiles” (from the Greek words for ants, “myrmex”, and loving, “philos”). Their interactions span from facultative and diffuse relationships,

which in ladybirds stems from their general defensive traits, to more obligate and integrated interactions which rely on specific adaptations. In this paper, we aim to provide an overview of the evolution of myrmecophilous traits in ladybirds [22–24]. We then discuss the costs and benefits of such specialization on ant-tended Hemiptera and the dietary shift to myrmecophagy demonstrated in a few species.

## 2. Diversity and Biology of Myrmecophilous Species

The Coccinellidae family consists of seven subfamilies [25] among which five include myrmecophilous species: Scymninae, Ortaliinae, Chilocorinae, Coccinellinae, and Coccidulinae [26–31] (Table 1). Interestingly, only one myrmecophilous species has been recorded to date in each of the three subfamilies Ortaliinae, Chilocorinae, and Coccinellinae and two in the subfamily Coccidulinae whereas there are eight species from the Scymninae subfamily belonging to four different tribes: one species from the Diomini tribe, two species from the Hyperaspidini and Brachiacanthadini tribes, and three species from the Scymnini tribe. Therefore, myrmecophilous species seem to be more strongly represented at the base of the phylogenetic tree [22–24]. Some myrmecophilous species have evolved more obligate relationships with ants and use chemical mimicry (i.e., the passive or active acquisition of a chemical signature by the myrmecophile allowing acceptance by the host) to evade ant aggressiveness. This adaptive trait has appeared in two different subfamilies with mimicry of the ant brood in the Scymninae [28, 32] and mimicry of the aphid prey in the Chilocorinae [33]. All of these characteristics and their distribution in the phylogeny show that myrmecophily appeared independently several times during the evolution of ladybirds [22–24], even in the case of the dietary shift to myrmecophagy which appeared both in the Scymninae [27, 28] and the Ortaliinae [34].

### 2.1. General Defensive Traits

**2.1.1. Physical Traits.** Many ladybird species, though not strictly myrmecophilous, encounter ants and show variation in their sensitivity to ant aggressiveness or to their venom [35]. Adults can, for example, hold the body tightly pressed against the plant surface when attacked by ants. Some species can completely conceal their legs under the body when cowering, such as individuals from the Chilocorinae subfamily, so that ants cannot seize any appendages which usually causes ant aggression to quickly cease [33, 35]. Moreover, ladybirds use reflex bleeding as a general defense mechanism against their natural enemies. It has a mechanical protective effect since, as the haemolymph coagulates, it becomes more viscous and sticky, impeding the ants' movements [36].

Adult ladybirds are rather well protected by their sclerotized elytrae, while their larvae and pupae have soft bodies that are more sensitive to ant bites. The pupae are often protected by the larval skin shielding them and sometimes by their ability to use reflex bleeding [36] or by a dense covering of hair [33]. In some species, the pupae are also able to move

up and down in response to a tactile stimulus [37], which could deter ant attacks. Many ladybird larvae have a waxy covering protecting them from their natural enemies, including ants [38]. When ants try to attack the larvae, their mandibles become covered by the covering's sticky filaments, and this usually causes them to stop and start grooming themselves. The two myrmecophilous species, *Scymnus nigrinus* (Kugelann) and *S. interruptus* (Goeze), are able to prey on ant-tended aphid colonies and to better survive predator attacks thanks to their waxy covering [30]. Myrmecophilous *Brachiacantha quadripunctata* (Melsheimer) and *B. ursina* (Fabricius) even get inside the ants' nest where they feed on ant-tended coccids and adelgids (Hemiptera, Aldegidae) [39, 40]. The waxy filaments also allow the myrmecophilous ladybird *Azya orbiger*a (Mulsant) to feed on coccids tended by *Azyca instabilis* (F. Smith) [29], and the larvae of the ladybird *Ortalia pallens* (Mulsant) to feed on *Pheidole punctulata* (Mayr) workers [34]. However, this waxy coating does not always provide an efficient protection as *Pheidole megacephala* (Fabricius) ants prey on *Cryptolaemus montrouzieri* (Mulsant) and *A. orbiger*a ladybird larvae since they are able to remove their protective coating [19].

Some ladybird larvae that are devoid of a waxy covering, such as *Diomus thoracicus* (Fabricius) [28], *Platynaspis luteorubra* (Goeze) [33], and *Scymnodes bellus* (Blackburn) [27], show a convergent adaptation to ants in their general morphology: they are all ovate and flat with expanded marginal setae and short, stout legs. This body shape, with few exposed extremities, could be considered a protective type as has been found in other myrmecophilous Coleoptera [41].

**2.1.2. Behavioural Traits.** In myrmecophilous species, the larvae tend to move slowly and inconspicuously, as has been observed for *Coccinella magnifica* (Redtenbacher), *P. luteorubra*, and *B. quadripunctata* [33, 40, 42]. The comparison between the myrmecophilous species *C. magnifica* and its close nonmyrmecophilous relative *C. septempunctata* (Linnaeus) has shown that *C. magnifica* uses physical, behavioral, and chemical defenses adapted from the general defenses observed in Coccinellidae [42, 43]. No novel behavior or development of specific traits have been observed in *C. magnifica* [43]. One way for *C. magnifica* larvae to limit ant aggressiveness is to minimize the time spent on an aphid colony and thus the chance of encountering ants; for example, the larvae frequently pick up and carry their prey away from the colony before consuming it [42]. The fact that the nonmyrmecophilous *C. septempunctata* is also sometimes observed near ant-tended aphid colonies, especially at the end of the colonies' cycle when aphid colonies become scarce, and that reciprocally *C. magnifica* has been observed preying on untended colonies, suggests that the scarcity of prey may have been a selective pressure in the evolution of myrmecophily [43, 44]. Indeed, during prey shortages, the limited availability of untended colonies might have forced ladybirds to prey on ant-tended colonies, opening the path to developing a tolerance towards ant aggressiveness. However, the myrmecophily of *C. magnifica* ladybirds has not been observed throughout its European habitat, suggesting that

TABLE 1: Taxonomy and some biological characteristics of myrmecophilous ladybirds. Facultatively myrmecophilous species and species for which no reliable information is available are not included.

Taxon	Ant associate	Larval diet	Myrmecophilous traits	References
Chilocorinae				
<i>Platynaspis luteorubra</i>	<i>Lasius niger</i> , <i>Myrmica rugulosa</i> , <i>Tetramorium caespicum</i>	Ant-tended aphids	Behavior, prey odor mimicry, flat body, marginal setae, short and stout legs	[33]
Coccinellinae				
<i>Coccinella magnifica</i>	<i>Formica rufa</i>	Ant-tended aphids	Behavior, chemical deterrent	[42, 43]
Coccidulinae				
<i>Azya orbigera</i>	<i>Azteca instabilis</i>	Ant-tended coccids	Waxy covering	[29]
<i>Bucolus fourneti</i>	Unknown	Ant workers	Waxy covering	[31]
Ortalinae				
<i>Ortalia pallens</i>	<i>Pheidole punctulata</i>	Ant workers	Waxy covering	[34]
Scymninae				
<i>Brachiacantha quadripunctata</i>	<i>Lasius umbratus</i> , <i>Formica subpolita</i>	Ant-tended aphids and adelgids	Behavior, and Waxy covering	[40]
<i>Brachiacantha ursina</i>	<i>Lasius</i> sp.	Ant-tended aphids and adelgids	Waxy covering	[39]
<i>Hyperaspis reppensis</i>	<i>Tapinoma nigerrimum</i>	Apparently ant-tended fulgorids	Body oval, and waxy covering	[60]
<i>Scymnodes bellus</i>	<i>Iridomyrmex</i> sp.	Ant workers	Flat body, marginal setae, short and stout legs	[27]
<i>Scymnus interruptus</i>	<i>Lasius niger</i>	Ant-tended aphids	Waxy covering	[30]
<i>Scymnus nigrinus</i>	<i>Formica polyctena</i>	Ant-tended aphids	Waxy covering	[30]
<i>Diomus thoracicus</i>	<i>Wasmannia auropunctata</i>	Ant brood	Prey odor mimicry, flat body, marginal setae, short and stout legs	[28]
<i>Thalassa saginata</i>	<i>Dolichoderus bidens</i>	Unknown	Ant mimicry	[32]

myrmecophily might be facultative or limited to some populations [45]. Concerning oviposition, two strategies have been observed. The eggs can be laid close to untended aphid colonies on which the emerging larvae can feed such as in *C. magnifica* [42], or females may try to oviposit directly in the Hemiptera colony despite possible ant aggressiveness. In the case of *A. orbigera*, females oviposit in the coccid colonies and lay the eggs under scale exuvia or carcasses to protect them from predation [46].

**2.1.3. Chemical Traits.** In addition to its mechanical impediment of ant movement, the haemolymph released during a reflex bleeding event often has a repellent effect due to the presence of alkaloids [47]. The alkaloids are synthesized by the ladybirds and seem to originate from fatty acids, as has been shown for the biosynthesis of coccinelline in *C. septempunctata* fat bodies [48]. Furthermore, their presence in eggs also provides them with a chemical protection that deters predators [47]. They could also act as an ant repellent but this remains to be demonstrated. The extremely repellent effect of the myrmecophilous ladybird *C. magnifica* has been suggested, but it has not been demonstrated yet [42, 43]. Finally the waxy coating might also possess chemical properties helping to attenuate ant aggressiveness as it does for *Scymnus louisianae* (Chapin) [49].

**2.2. Chemical Adaptation.** Some myrmecophilous species employ a chemical strategy using a specific chemical signature on the cuticle [28, 32, 33]. They rely on a specific cuticular profile which can be obtained through passive or active acquisition [50] and results in the chemical mimicry of its prey odour, such as in *P. luteorubra* [33], or of the ant's brood in *Thalassa saginata* (Mulsant) [32] and *D. thoracicus* [28]. Thus, chemical mimicry helps some ladybirds to decrease ant aggressiveness, as in the case of *P. luteorubra* larvae, and even to disguise themselves as nestmates as in the case of *T. saginata* and *D. thoracicus*. Interestingly, chemical mimicry probably results from an adaptation as opposed to a preexisting trait, and it has only been observed in larvae thus far. Indeed, we would expect a preexisting trait to be observed in adults too, as it would help them to get the same benefits as their larvae, in particular avoiding ant aggressiveness at emergence. This adaptation might not be necessary in adults since they are protected by their hard elytrae and can readily fly away from the aphid colony in the case of *P. luteorubra* or even occupy a different niche as in the two nest-integrated ladybird larvae.

### 3. Why Specialize on Ant-Tended Hemipteran Colonies?

Since myrmecophilous interactions vary from facultative to obligate, the extent of the associated costs and benefits varies

accordingly with the most integrated species having the highest costs but also the highest benefits.

**3.1. Benefits.** The first and most obvious benefit of being able to prey on ant-tended Hemiptera is gaining access to better food sources [19, 20, 26, 44]. Indeed, ant-tended hemipteran colonies are usually larger, have a longer lifespan, and thus persist longer in late summer in temperate regions than untended colonies. For *P. luteorubra*, better foraging success was measured in ant-tended colonies and resulted in a higher adult weight, which is likely to positively influence adult fitness and survival [33]. This better foraging success has been explained by a decrease in the defensive behavior of aphids and a shorter searching distance in ant-tended colonies [33].

A second and important benefit of myrmecophily may arise from the access it provides to an enemy-free space. Ants limit interspecific and intraguild competition as well as access to the parasitoids and predators of ladybirds [29, 30, 33, 51, 52]. The competitors of *S. interruptus* and *S. nigrinus*, for example, have been noted as being less present on ant-tended colonies [30]. Moreover, the presence of ants reduced larval parasitism by *Homalotylus platynaspidis* (Hoffer) (Hymenoptera, Chalcidoidea) in *P. luteorubra* [33], and the aggressive behaviour of *A. instabilis* disturbed the oviposition behavior of *H. shuvakhinae* (Trjapitzin), the most common parasitoid of *A. orbiger*a [29]. Nonetheless, the observed decreases in parasitism rates do not always directly result from ant protection. *C. magnifica* larvae are less parasitized by the parasitoid *Dinocampus coccinellae* (Schrank) than the larvae of its close nonmyrmecophilous relative *C. septempunctata* found in the same area. Laboratory studies have shown that this is linked to the unsuccessful parasitism of *C. magnifica* and not to the presence of ants [53].

Ant parasitoids might also influence the interaction between ants and ladybirds. Phorid flies affect ant worker behavior by decreasing their activity [54–57]. During periods of low ant activity induced by the disturbance generated by phorid flies, *A. orbiger*a adults can prey on the coccids at the same rate as in untended colonies and oviposit in the colony [46].

**3.2. Costs.** As discussed above, the specialization in preying on ant-tended Hemiptera depends mostly on the ability of ladybirds at all stages to be protected from ants. Ants aggressively protect the Hemiptera colonies, which disturbs ladybird foraging and can cause them to leave and stop exploiting a patch, and most importantly they can be injured or killed.

Another possible cost is that adaptation to ants would render the respective coccinellid species difficult to live without them. Myrmecophilous ladybirds could be poor competitors or poorly defended against predators and parasites as suggested by Majerus et al. [26]. The production of chemical defenses might be at the cost of other traits such as immunity or strong defenses against predators or parasites [26]. Furthermore, the association with ants is likely to decrease the habitat range available for the ladybird, especially for the most specific parasites specialized on one ant species.

Consequently, any reduction in the host habitat or abundance would directly affect the ladybirds' fitness and survival.

## 4. Dietary Shift to Myrmecophagy

Only four ladybird species larvae are currently known to feed on ants: *Bucolus founeti* (Mulsant) [31], *O. pallens* that eats *P. punctulata* ants [34], *S. bellus* feeding on *Iridomyrmex* sp. [27], and *D. thoracicus* feeding on *Wasmannia auropunctata* (Roger) [28]. The first three species feed on ant workers outside the ant nest, relying on ant's foraging habits to get close to them. Thus, these species can stay relatively immobile and wait for prey to approach. In the case of *D. thoracicus*, the larvae are parasites that live inside the ant nest. The larvae are usually found in or near the brood pile where they have access to a constant food source. The integration of this species into the ant colonies relies on the chemical mimicry of its cuticular profile with the one of the ants' brood [28]. Only the adults leave the colonies early after emergence to avoid being attacked, as they do not share the same cuticular profile as the ants.

Such a dietary shift to myrmecophagy provides several important benefits. First, the ladybirds gain access to a food source available all year round and for many years since ant colonies are usually long-lived. Second, in the case of *D. thoracicus*, the larvae might be better protected from predators, parasitoids, and competitors lacking the adaptations needed to enter the ant nest. They may also benefit from a rather homeostatic environment in which temperature and humidity are rather constant and individuals are protected from climatic events.

The shift by ladybirds to myrmecophagy most probably followed the development of myrmecophily and as such bears the same costs, such as a more restricted niche due to specialization on ants. Nevertheless, these costs are largely balanced out by the access to a constant food resource both in time and quantity. This removes the constraints of resource limitation which are important in the evolution of habitat preferences and diet in predatory ladybirds [58]. Hemipteran colonies, and especially aphids, are a transient resource, and even if adult ladybirds can track them down by moving between patches, ladybird larvae are less mobile and limited to the colonies surrounding them. Therefore, the females of aphidophagous species tend to lay eggs early in the development of an aphid colony, known as the "egg window" [59], to ensure that their larvae have sufficient food. By feeding on ants, such limitation does not occur, which probably compensates for the costs of a myrmecophilous life style.

## 5. Perspectives

Despite being a highly species-rich group with around 6000 species described, the biology of most ladybird species, especially those found in the tropics, remains largely or even completely unknown. Only a few myrmecophilous species have been identified to date, but because most coccinellids encounter ants very frequently and often supplement their

essential food sources with other food items (which might help in their being able to shift to new diet and habitat), it would not be surprising that many more myrmecophilous species still remain to be discovered. Among the seven subfamilies of Coccinellidae, myrmecophilous species have been identified in only five of them with, moreover, most of them concentrated in the Scymninae subfamily [26–30]. The predominance of myrmecophily in the Scymninae raises the question of the evolution of traits that promoted such interactions with ants. Gaining more knowledge on the biology of myrmecophilous ladybirds, especially the ones having shifted to myrmecophagy, would provide insights on the evolution of myrmecophily and myrmecophagy in this family.

The ladybird diet is usually similar in larvae and adults; nonetheless, ants attack adult *D. thoracicus* suggesting that they rely on a different food source than do the larvae. The biologies of adult *O. pallens* and *S. bellus* ladybirds are unknown as well. Unraveling the diet of all stages in these three species would shed light on the origin of the dietary shift to myrmecophagy. The adults might be adapted to preying on ant-tended Hemiptera colonies which would have favored a dietary shift in the larvae. It has also been hypothesized that the limited availability of prey at some point in time might have been a selective pressure in the evolution of myrmecophily in the case of *C. magnifica* [43, 44]. A similar constraint could apply to the tropical and subtropical myrmecophagous species with the advantage of a dietary shift to ants associated with a food source available all year round since there is no dormancy period. In temperate areas, ladybirds overwinter at the adult stage. Thus, one can hypothesize that the lifecycles of ladybirds and ants might not be synchronized enough to have permitted myrmecophagy to appear in these regions. Finally, another hypothesis might be that since both ladybird species from subfamilies rooted at the base of the phylogenetic tree and coccids are more abundant and diversified in the Southern hemisphere, there might be more opportunities for myrmecophily to arise in these areas, both from a larger number of possible interactions and a longer common evolutionary history.

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## Review Article

# Nematode Parasites and Associates of Ants: Past and Present

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Ants can serve as developmental, definitive, intermediate, or carrier hosts of a variety of nematodes. Parasitic ant nematodes include members of the families Mermithidae, Tetradonematidae, Allantonematidae, Seuratidae, Physalopteridae, Steinernematidae, and Heterorhabditidae. Those nematodes that are phoretically associated with ants, internally or externally, are represented by the Rhabditidae, Diplogastridae, and Panagrolaimidae. Fossils of mermithids, tetradonematids, allantonematids, and diplogastrids associated with ants show the evolutionary history of these relationships, some of which date back to the Eocene (40 mya).

## 1. Introduction

Nematodes are one of the most abundant groups of animals known. Studies on their evolutionary history suggest that they probably arose in the Precambrian, which explains their wide abundance today in the terrestrial and marine environments. While only some 20,000 have been described, their species diversity has been estimated to be as high as 10 million [1].

One would assume that with their strict housekeeping habits, ants would not tolerate nematodes in or around their nests and would quickly dispose of any nest mates that might have become infected. However nematodes have been able to use some astonishingly sophisticated tactics to successfully parasitize these social insects. The present work covers the systematics, life history, pathology, and records of all described extant and fossil nematodes associated with formicids. This includes representatives of the nematode families Mermithidae, Tetradonematidae, Allantonematidae, Seuratidae, Physalopteridae, Steinernematidae, Heterorhabditidae, Rhabditidae, Diplogastridae, and Panagrolaimidae. Fossil records of mermithids, tetradonematids, allantonematids, and diplogastrids associated with ants reveal the evolutionary history of these associations, some of which date back 40 million years.

## 2. Mermithidae

The family Mermithidae includes parasites of invertebrates, especially insects. Because of their large size, mermithids are

easily detected in ants upon dissection (Figure 1) or as they leave their hosts (Figure 2). Most mermithid species, including those that attack ants, parasitize only a specific host species, genus, or family while others can infect representatives of several insect orders. Mermithids that attack aquatic insects, such as midges (Chironomidae, Ceratopogonidae) and mosquitoes (Culicidae), have a direct life cycle. Direct life cycles occur when, after growth and development is completed in the host, the mermithid emerges, molts to the adult stage, mates, and oviposits in the host's environment. The infective stage mermithid emerges from the egg, actively locates and enters a host, and initiates development in the hemocoel.

Some mermithids have an indirect life cycle, which is more complicated but allows hosts to be parasitized in environments hostile to nematodes. In an indirect cycle, the mermithid emerges from the host, molts, mates, and oviposits in the environment. But instead of emerging from the egg to search for a developmental host, the infective stage remains in the egg, waiting to be ingested by an invertebrate that serves as a paratenic host. When mermithid eggs are ingested by a paratenic host, the hatching infective stage penetrates the gut wall and enters the body cavity. But instead of developing, the mermithid encysts and enters a diapause. The encysted nematode can be carried through the different stages of host metamorphosis, but for its cycle to be completed, the paratenic host must be captured and fed to the brood of the developmental host. At the completion of the mermithids growth phase in the development host (like an ant), the latter is attracted to an aquatic or semiaquatic habitat favorable



FIGURE 1: Mermithid exposed in the gaster of *Camponotus* sp. from the Sierra Nevada, California.

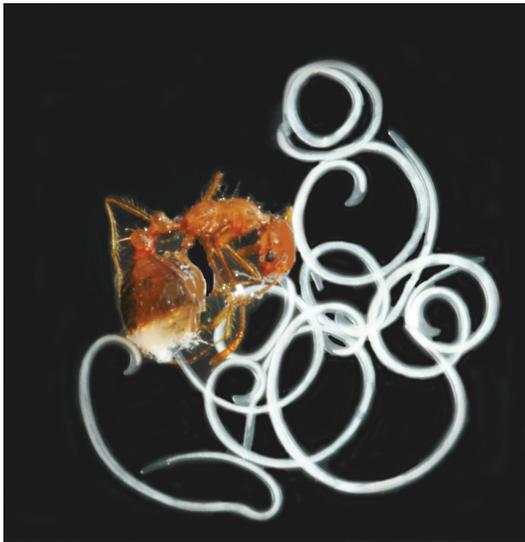


FIGURE 2: Parasitic juveniles of *Allomeris solenopsi* removed from the gaster of a fire ant worker. Photo courtesy of S. D. Porter, USDA-ARS.

to the nematode. This is when the mermithid exits, leaving the dying host behind. The developmental host is usually not only larger, but usually in a completely different taxonomic category and environment from the paratenic host. While the developmental host can live in a relatively dry habitat, the paratenic host usually inhabits an aquatic, semiaquatic, or damp biome. Also, both hosts can be widely separated taxonomically and may not even belong to the same phylum.

The first written account of a nematode parasite of ants was made by the Reverend William Gould in his 1747 book *An account of English Ants* (Table 1) [2].

The “white and long kind of worm, which is often met within their bodies” certainly refers to mermithid nematodes. For a number of years, mermithids were listed under “*Filaria*,” “*Gordius*,” or “*Mermis*,” and that is why mermithid systematics can be confusing and why early names for Gould’s ant mermithid included *Gordius formicarum* Diesing [3] and *Filaria formicarum* von Siebold [4].

The first described ant mermithid was *Pheromermis myrmecophila* from *Lasius* spp. [5]. However it was originally described in the genus “*Mermis*,” then assigned to the genus

TABLE 1: Section from Gould [2] referring to the first reported instance of mermithid parasitism of ants.

Amongst other Incidents that tend to lessen and destroy Ant-Flies, it is observable that abundance of them are demolished by a white and long Kind of Worm, which is often met with in their Bodies. You may frequently take three from the Infides of the large, but seldom more than one from a small Ant-Fly. These Worms lie in a spiral Form, and some of them may be extended Half an Inch.

TABLE 2: Mermithid nematodes described from ants.

Mermithid	Host	Reference
<i>Agamomermis cephaloti</i>	<i>Cephalotes minutus</i>	[11]
<i>Agamomermis costaricensis</i>	<i>Odontomachus hastatus</i>	[11]
<i>Agamomermis ecitoni</i>	<i>Eciton burchellii</i>	[11]
<i>Allomeris solenopsi</i>	<i>Solenopsis invicta</i>	[12]
<i>Camponotimermis bifidus</i>	<i>Camponotus aethiops</i>	[13]
<i>Comanimermis clujensis</i>	<i>Formica fusca</i>	[14]
	<i>Camponotus aethiops</i>	
* <i>Heydenius formicinus</i>	<i>Prenolepis henschei</i>	[15]
* <i>Heydenius myrmecophila</i>	<i>Linepithema</i> sp.	[11]
<i>Meximermis ectatommi</i>	<i>Ectatomma ruidum</i>	[11]
<i>Pheromermis lasiusi</i>	<i>Lasius niger</i>	[16]
<i>Pheromermis myrmecophila</i>	<i>Lasius</i> spp.	[5]
<i>Pheromermis villosa</i>	<i>Lasius flavus</i> , <i>L. niger</i>	[17]

\* fossil.

*Pheromermis* [6], then moved to the genus *Allomeris* [7] and lastly to the genus *Camponotimermis* [8]. Its position in the genus *Pheromermis* was recently confirmed by Kaiser, who showed its similarity with the European ant mermithid, *Pheromermis villosa* [9]. Over the years, a large number of ant species have been reported parasitized by mermithids. A list of Holarctic parasitized ants was presented by Passera [10] and Neotropical parasitized ants by Poinar et al. [11]. A compilation of all described mermithids from ants is presented in Table 2.

Fossils, such as the postparasitic juvenile of *Heydenius formicinus* emerging from a male *Prenolepis henschei* (Figure 3) [15], as well as from a worker ant (Figure 4) in Baltic amber [1] show that ants have been parasitized by mermithids for at least 40 million years and probably much longer. The fossil record of Neotropical mermithid parasites of ants is represented by a parasitic juvenile of *Heydenius myrmecophila* adjacent to its ant host, *Linepithema* sp. in 20–30-million-year-old Dominican amber (Figure 5) [11]. It is assumed that the traumatic events of the ant host entering the resin caused the mermithid to emerge prematurely from an opening in the gaster of the ant.



FIGURE 3: The fossil nematode, *Heydenius formicinus*, emerging from a male *Prenolepis henschei* in Baltic amber.



FIGURE 4: *Heydenius formicinus* adjacent to its worker ant host in Baltic amber.

Depending on the caste and length of time the mermithid is associated with its host, various degrees of host intercastes and abnormalities appear. Wheeler [18] was the first to provide an explanation for these phenomena by correlating the unusual morphological conditions with mermithid infections (Figure 6). Parasitized queen ants (mermithogynes) are shorter, have a smaller thorax (stenothoraxy), reduced wings (brachyptery), enlarged abdomen (physogastry), and smaller head (microcephaly) than their uninfected counterparts. Parasitized worker ants (mermithergates or macroergates) often develop morphological features characteristic of queens and soldiers. Attacked male ants (mermithaners) have shorter wings but enlarged heads, eyes, and gasters. Infected soldiers (mermithostratiotes) have reduced heads, an ocellus, and changes in pilosity (Figure 7) [19–24].

The life cycle of most ant mermithids remains a mystery. Crawley and Baylis [5] assumed that *P. myrmecophila* has a direct cycle, where infection is brought about by the eclosing



FIGURE 5: *Heydenius myrmecophila* adjacent to its *Linepithema* ant host in Dominican amber.

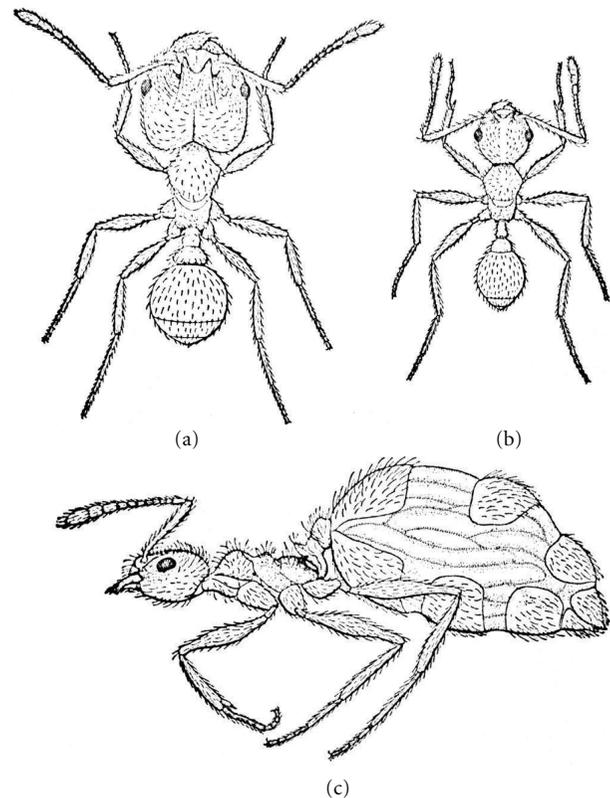


FIGURE 6: Plate (modified) of *Pheidole dentata* (referred to as *P. commutata*) from [18] showing the first evidence that mermithid nematodes could cause intercastes of ants. (a) Normal soldier; (b) normal worker; (c) parasitized worker (mermithergate).

preparasitic mermithid entering the ant host. When development is completed, the postparasitic juvenile emerges, molts to the adult stage in the ant's habitat, mates, oviposits and the cycle continues. However, no one has demonstrated a direct cycle for any mermithid parasite of ants. In 1934, Vandel [25] studied a mermithid parasite of *Pheidole pallidula* and realized that the infection must be initiated in the ant larva. He assumed that the nematodes were in the soil surrounding the ant colony so the infective stages could penetrate directly into



FIGURE 7: Two mermithid-infected soldiers (mermithostratiotes) (arrows) of *Pheidole pallidula* adjacent to smaller workers and an uninfected soldier (with large head). Note smaller heads on infected soldiers. Photo courtesy of Luc Passera.



FIGURE 8: *Pheromermis villosa* in a carpenter ant from Holland.

the ant larva; however he was unable to confirm the infection process.

The first life cycle of an ant mermithid was achieved by Kaiser with the European *Pheromermis villosa* [17, 26] (Figure 8). Kaiser showed that *P. villosa* had an indirect cycle involving oligochaetes as paratenic hosts. Workers of *Lasius* spp. collecting protein for the brood capture oligochaetes containing the infective stages of *P. villosa* and, unknowingly, feed them to the developing larvae. At this point, the nematode becomes active, penetrates into the ant larva's hemocoel, and initiates development. It was a significant discovery and raises the question whether all mermithid infections of ants have indirect life cycles. Other possible paratenic hosts for *Pheromermis* could be small aquatic insects that ingest mermithid eggs from the bottom debris of seepage areas or the edges of other water sources. The wasp parasite, *Pheromermis pachysoma* [6], also has an indirect cycle and uses caddis flies as paratenic hosts, which the eusocial wasps (Vespidae) feed to their brood [27].

Thus far, seven genera of mermithids are known to infect ants, namely, *Agamomermis*, *Allomermis*, *Camponotimermis*, *Comanimermis*, *Heydenius*, *Meximermis*, and *Pheromermis* (Table 2). All of the ant hosts of these mermithids feed their brood animal protein (in contrast to other genera, such as

the leaf cutting ants), and this behavior suggests they have an indirect life cycle involving a paratenic host. The two genera, *Agamomermis* and *Heydenius*, are collective group genera for immature extant and fossil mermithids, respectively [1].

There are some morphological and behavioral patterns that characterize mermithids with indirect cycles. They normally have smaller eggs with thicker shells than the eggs of direct development soil or freshwater mermithids. Also their eggs are completely embryonated when laid. Finally, the deposited eggs will not hatch in the environment even though the enclosed parasitic juvenile is fully developed. Hatching only occurs when a potential invertebrate paratenic host ingests the eggs. The eggs of *Pheromermis* spp. are small, numerous, fully embryonated when laid and do not hatch in the environment. Fully embryonated eggs ensure that the infective stages are ready to enter paratenic hosts as soon as they are ingested [6, 17].

The ant mermithid, *Allomermis solenopsi* [12], possess an unusual morphological feature on the mature eggs that could play a crucial role in its life cycle. The surface of the eggs is covered with elongate, erect, spiny adhesive processes. How these function in the life cycle is unknown, but the related species, *A. trichotopson*, possesses similar structures [28]. Since *A. solenopsi* parasitizes the fire ant, *Solenopsis invicta* in Brazil (Figure 2), the related *A. trichotopson*, whose host is unknown, may infect *Solenopsis geminata* in Jamaica. Could these egg processes somehow be connected with parasitism of *Solenopsis* spp.?

Can mermithids be manipulated to control ants? Aside from killing the ant host upon emergence, mermithids drain the host of food, reduce the flight muscles and fat body, and cause morphological modifications as mentioned above [9, 17, 24, 26]. Since mermithid-infected *Solenopsis* has reduced reproductive organs and die shortly after the nematodes emerge [11, 12, 29], it has potential as a biological control agent. However, if the cycle is always indirect as shown for *P. villosa*, it would be very difficult to artificially infect the ant brood. It would be necessary to first infect the paratenic host and then supply large numbers of these infected invertebrates to worker ants for transport back to the nest. Working with a mermithid that has a direct cycle would be easier; however there is still the problem of raising and disseminating the nematodes.

### 3. Tetradonematidae

The tetradonematids are a diverse group of nematodes that have traditionally been aligned with the Mermithidae. However, aside from some distinctive morphological characters, female tetradonematids normally mature, mate, and produce eggs within the host, which does not occur with mermithids. Two tetradonematids have been described from extant ants. *Tetradonema solenopsi* is a parasite of the red imported fire ant, *Solenopsis invicta*, in Brazil [30, 31]. Very little is known about this nematode aside from the scant description showing that females contained eggs and worker infection levels reached 12.5%. Parasitized ants that succumbed to the infections could be recognized by their slightly enlarged gaster with scallop-appearing dorsal sclerites.

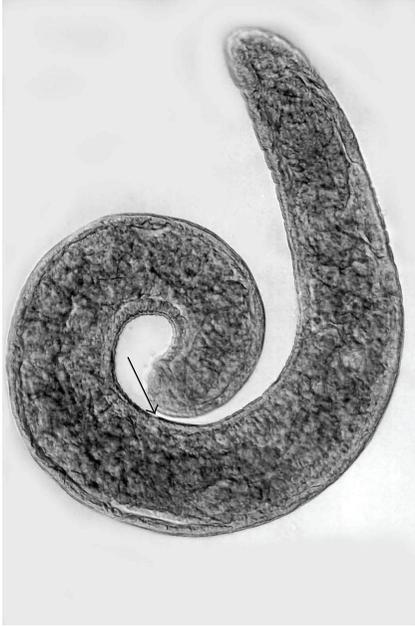


FIGURE 9: Mature female of *Myrmeconema neotropicum* in the early stages of egg production removed from a pupa of *Cephalotes atratus*. Arrow shows position of vulva.



FIGURE 10: Mature females of *Myrmeconema neotropicum* packed with eggs in the gaster of a *Cephalotes atratus* worker in Peru.

The second tetradonematid from extant ants is *Myrmeconema neotropicum* from *Cephalotes atratus* in Peru and Panama [32]. *Myrmeconema* is the only nematode that causes its ant host to radically change color (from black to red), which is crucial for completion of its life cycle [33]. This color change was a mystery for early taxonomists and the variety *Cephalotes atratus* var. *rufiventris* was erected solely on the basis of its red abdomen, which was later shown to be the result of *Myrmeconema* infections [32].

Developing females of *M. neotropicum* occur in ant pupae (Figure 9) but do not produce masses of eggs until they are carried into the adult ant (Figure 10). As the females deteriorate, eggs are released into the ant's hemocoel (Figure 11). At this stage of development, the gasters of the infected worker ants turn from black to red and are held high in the air (Figure 12) [33]. Birds mistake the red gasters for fruits and the nematode eggs are passed through the birds' digestive

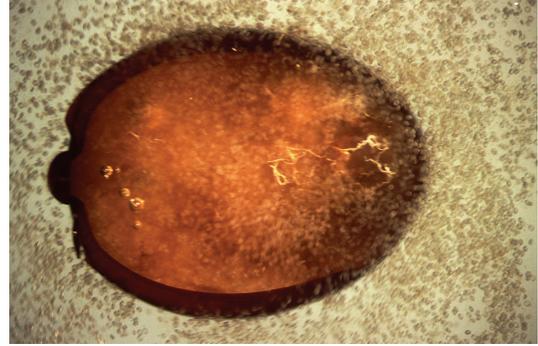


FIGURE 11: Eggs of *Myrmeconema neotropicum* released from the gaster of an infected *Cephalotes atratus* worker in Peru.



FIGURE 12: Worker of *Cephalotes atratus* infected with *Myrmeconema neotropicum*. The raised, red abdomen occurs when the nematode eggs are infective and ready for transport by birds. Photo courtesy of Stephen P. Yanoviak.

system and end up in the droppings, which are deposited on leaves and branches. *Cephalotes* workers collect and feed the infested excreta to their brood, which is how the larvae become infected [33].

Aside from their red gasters, parasitized ants are smaller with reduced head widths. They are sluggish, clumsy, generally less aggressive, and about 40% heavier than nonparasitized workers. They do not bite when handled, and their alarm/defense pheromone supply is significantly reduced or absent.

*Myrmeconema* is probably widely distributed throughout the Neotropics since this association has been in existence for some 20–30 million years. The fossil worker ant, *Cephalotes serratus* in Dominican amber, is surrounded by the eggs of *Myrmeconema antiqua* (Figure 13) [1]. The ant has a hole in its abdomen that quite possibly was made by a bird. Many of the eggs, which closely resemble those of *M. neotropicum* in size and shape, contain fully developed juveniles (Figure 14). All indications suggest that *M. antiqua* had a similar life history to the extant *M. neotropicum* and involved bird carriers.

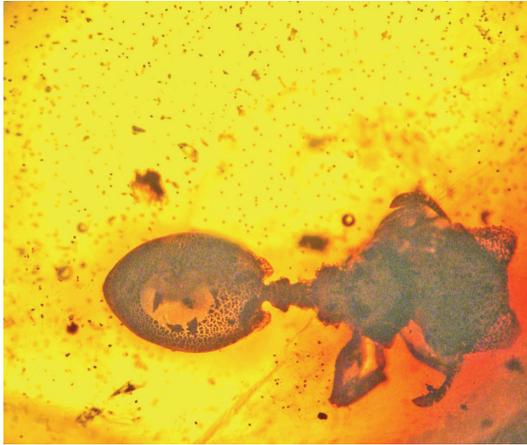


FIGURE 13: Worker of *Cephalotes serratus* infected with *Myrmeconema antiqua* in Dominican amber. Note microscopic eggs widely distributed in the amber that were released from a hole in the ant's gaster.

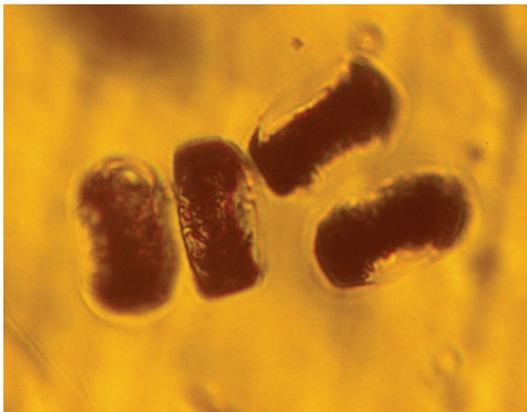


FIGURE 14: Detail of eggs of *Myrmeconema antiqua* in various stages of development in Dominican amber.

#### 4. Allantonematidae

It is curious why so few cases of allantonematid infections have been reported in ants. Since ants are probably one of the most investigated insect groups, is the absence of tylenchid parasitism due to a lack of observations or its rarity? The first and only described allantonematid parasite of extant ants is *Formicitylenchus oregonensis* that was parasitizing a queen *Camponotus* ant in Western Oregon, USA [34]. The queen had already chewed off her wings and appeared to be searching for a nesting site. There was a single large parasitic female (Figure 15) and 120 third-stage juvenile nematodes in the ant's gaster. The third-stage juveniles exited through the ant's reproductive and digestive tracts and molted twice to reach the adult stage. The enlarged pharyngeal glands in the free-living females suggest that they penetrate the cuticle to enter the body cavity of the host, probably ant larvae. Although the complete life cycle is unknown, the nematodes are clearly distributed by infected queen ants. The gonads of the infected ant were greatly reduced, and her eggs were



FIGURE 15: Female of the allantonematid *Formicitylenchus oregonensis* removed from the body cavity of *Camponotus vicinus* in Oregon, USA.

abnormal. Since carpenter ants can be damaging to structures, *F. oregonensis* can be considered as a potential biological control agent.

Since the original report of this parasite, the present author recovered a worker carpenter ant also infected with *F. oregonensis*, thus indicating that *Formicitylenchus* is probably restricted to ant hosts, especially members of the genus *Camponotus*. *Formicitylenchus* shows a close relationship with the allantonematid beetle parasite, *Metaparasitylenchus* [34]. It is possible that their last common ancestor parasitized beetles and the host shift from arboreal beetles to arboreal ants occurred during the anagenesis of *Formicitylenchus*. The close physical association between wood-boring beetles and *Camponotus* ants may be significant. Rogers [35] commented that "...the potential parasite would be expected to find its hosts in organisms which occupy the same niche largely independent of their phylogenetic position. In fact the specificity of many parasites is based on the ecological relationship of the hosts, especially in groups which have only recently become parasitic."

Another reason that allantonematid parasitism of ants may be more widespread than presumed is the discovery of juveniles of a fossil allantonematid, *Palaeoallantonema cephalotae*, in the ant, *Cephalotes serratus*, in Dominican amber [1] (Figure 16). Just before this fossil was discovered, Steven Yanoviak submitted an extant worker of *Cephalotes christophersenii* from Peru that was also infected with an allantonematid. The parasitic female (Figure 17) of this still undescribed species and the developing juveniles inside her body (Figure 18) show features typical of the family.

#### 5. Seuratidae

The discovery of adults of *Rabbiium paradoxus* [36] inside the gaster of worker *Camponotus castaneus* in Florida (Figure 19) was a surprise since all known nematodes of the Seuratidae are heteroxenous and develop to the adult stage in the digestive tract of vertebrates [37]. However, in *R. paradoxus*, the

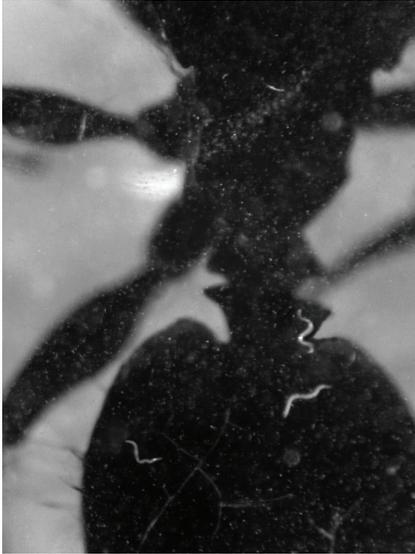


FIGURE 16: Three juveniles of the allantonematid, *Palaeoallantonema cephalotae*, that emerged from a worker *Cephalotes* ant in Dominican amber.



FIGURE 17: Parasitic female of an undescribed allantonematid from workers of *Cephalotes christopherseni* in Peru.

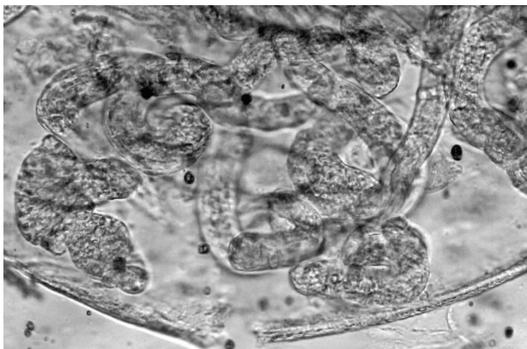


FIGURE 18: Juveniles developing inside the body of the female allantonematid shown in Figure 17.



FIGURE 19: Adults of *Rabbium paradoxus* adjacent to their ant host, *Camponotus castaneus*, in Florida.

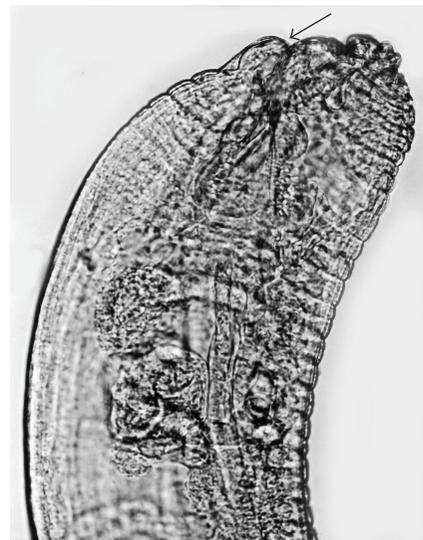


FIGURE 20: Head of a female of *Rabbium paradoxus*. Arrow shows anteriorly located vulva.

vertebrate host is obviously not required for adult development. The females of *R. paradoxus* have an anteriorly placed vulva (Figure 20), and the eggs embryonate inside the uterus (Figure 21). Since the other member of the genus, *R. caballeroi*, occurs in the gut of lizards in the Bahamas [38], it is likely that *R. paradoxus* originally had (or still has) a lizard definitive host. If the complete life cycle occurs just in ants, then *C. castaneus* would serve as both intermediate and definitive hosts. *C. castaneus* is a generalist feeder and will ingest vertebrate feces so it could acquire nematode eggs from lizard droppings. Parasitized worker ants had swollen gasters and showed unusual behavior by foraging during the day instead at night. This would make them easily captured by vertebrate predators.



FIGURE 21: Embryonated eggs in the uterus of *Rabbium paradoxus*.

The original life cycle of *R. paradoxus* may have been similar to that of the seuratoid *Skrjabinelazia galliardi*, a parasite of sphaerodactyline lizards in Brazil [37]. The female nematodes living in the gut of the lizard produce eggs that are passed out and ingested by insects. These eggs hatch in the insect gut and the juveniles enter the body cavity without further development. Growth is resumed when the insect intermediate hosts are eaten by lizards [38]. Unfortunately, the complete life cycle of *R. paradoxus* remains a mystery, but its precocious development is quite interesting.

## 6. Physalopteridae

There are few reports of heteroxenous nematodes utilizing ants as intermediate hosts, that is, hosts where the nematodes develop only to the third-stage infective juveniles. Maturity to the adult stages occurs when the intermediate host is eaten by a vertebrate definitive host. One such nematode is the physalopterid, *Skrjabinoptera phrynosoma* that lives in the stomach of the Texas horned lizard, *Phrynosoma cornutum*, and uses the harvester ant, *Pogonomyrmex barbatus*, as an intermediate hosts [39]. However instead of depositing isolated eggs that would pass from the lizard, the gravid nematodes die with the retained eggs enclosed in thick walled capsules. The females with their enclosed eggs pass out of the lizard and are collected by worker ants that feed them to their brood. The nematode eggs hatch in the gut of the ant larvae and the juveniles enter the fat body, where they develop only to the third stage. These juveniles are carried through the pupal and into the adult stage of the ant, where they eventually reside in membranous capsules. The nematodes complete their development to the adult stage when infected ants are eaten by the lizards. Worker ants with more than 10 nematodes were still active but had enlarged, lighter colored gasters. The interesting, pivotal stage in this life cycle is the attractiveness of the dead, egg-laden female nematodes to worker ants.

## 7. Rhabditidae, Diplogastridae, and Panagrolaimidae

This category includes juvenile nematodes living in the postpharyngeal glands of ants (internal phoresis) or being carried on the outside surface of ants (external phoresis) (Table 3). While these might not be considered parasites, in some instances where the association has been examined critically [40] damage has been inflicted on the ant's postpharyngeal glands and some of the nematodes increased in size during their stay in this location. Thus at most, they could be considered weak parasites. If they break through the glands and introduce microbes into the body cavity of the ant, they could even be regarded as pathogenic. However the latter scenario has not been documented.

Most of the nematodes in the postpharyngeal glands are dauer juveniles of free-living microbotrophs living in the ant's environment. The dauers enter the glands when external conditions become unsuitable (low humidity or diminished food supply). These resistant dauer juveniles can survive for relatively long periods. The nematodes may leave the glands when the environment is more suitable (moist with associated microbes), if the ant dies and the dauer initiates development within the decomposing ant, or when the nematodes are transferred from ant to ant during trophallaxis.

Janet [43] was the first to discover postpharyngeal rhabditids (*Oscheius dolichurus*) in *Lasius flavus* and *Formica rufa* in France. Wahab [42] was the first to systematically study these associations in the ant genera *Lasius*, *Formica*, *Tetramorium*, and *Myrmica* in Germany (Table 3). More recently Köhler [41] examined nematodes in the heads of ants collected from sap fluxes and rotten wood on trees in Germany. The most common ant that visited these fluxes was *Lasius brunneus* and, from a total of 262 workers collected, 43.5% carried nematodes, with *Koerneria histophora* being the most common associate. While most ants carried a single nematode, numbers occasionally reached up to 85 dauers per ant. Köhler [41] also found diplogastrid dauers in 4 males and a queen of *L. brunneus*. The infection rate of ants associated with *L. brunneus* workers varied depending on the weather cycle. There were more nematodes in ants during the dry period in August than during the rainy months of April and May. Also important in determining the rate of nematodes being carried by the ants was the location of the nests. Rates of infestation by nematodes in *L. brunneus* were much higher when the ants were collected from sap fluxes and rotten wood [41], than were collected from under stones and leaf litter [42].

Köhler [41] was able to infest ants by placing them in a Petri dish with rotten wood containing waving dauer stages. Both Wahab [42] and Köhler [41] provided evidence that the dauers can be transmitted from ant to ant via trophallaxis, which was supported in part by the experiments of Naarmann [53] showing that *Formica* ants mix food with secretions from the postpharyngeal gland before regurgitating it to nest mates. These ant-dauer associations probably occur worldwide since Markin and McCoy [40] reported *Diploscapter lycostoma* in the postpharyngeal glands of the Argentine Ant, *Linepithema humile* in California and Nickle & Ayre

TABLE 3: Juvenile nematodes of Rhabditida and Tylenchina associated with ants.

Nematode	Family	Host	Reference
<i>Diplogasteroides spengelii</i>	Diplogastridae	<i>Lasius brunneus</i>	[41]
<i>Diploscapter lycostoma</i>	Rhabditidae	<i>Formica</i> spp., <i>Lasius</i> spp.	[42]
“	“	<i>Myrmica rugulosa</i>	[42]
“	“	<i>Linepithema humile</i>	[40]
* <i>Formicodiplogaster myrmenema</i>	Diplogastridae	<i>Azteca alpha</i>	[1]
<i>Halicephalobus similigaster</i>	Panagrolaimidae	<i>Lasius brunneus</i>	[41]
<i>Koerneria histophora</i>	Diplogastridae	<i>Lasius</i> spp.	[42]
“	“	<i>Lasius brunneus</i>	[41]
<i>Oscheius dolichurus</i>	Rhabditidae	<i>Formica rufa</i> ,	[42, 43]
“	“	<i>Lasius flavus</i>	[42, 43]
“	“	<i>Tetramorium caespitum</i>	[42]
“	“	<i>Camponotus herculeanus</i>	[44]
“	“	<i>Lasius claviger</i>	[44]
“	“	<i>Lasius brunneus</i>	[41]
<i>Pristionchus lheritieri</i>	Diplogastridae	<i>Formica rufa</i> , <i>Lasius</i> spp.	[42]
*Unknown	“	<i>Azteca</i> spp.	[1]
Unknown	“	<i>Formica obscuriventris</i>	Present work (Figure 22)

\* Fossil.

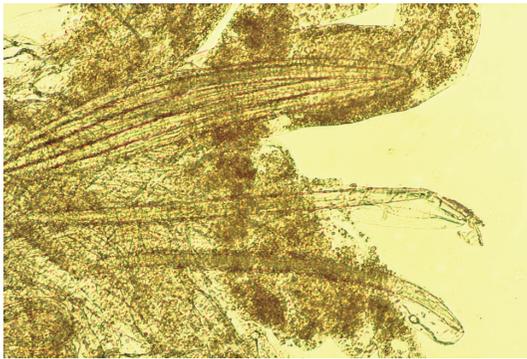


FIGURE 22: Dauer juveniles of a diplogastrid in the postpharyngeal glands of *Formica obscuriventris clivia* from Oregon.

[44] found *Oscheius dolichurus* in the head glands of *Camponotus herculeanus* and *Lasius claviger* in Ontario, Canada. The author has also found dauer diplogastrids in the postpharyngeal glands of workers of *Formica obscuriventris* in Oregon (Figure 22).

The association between dauer nematodes and ants is at least 20–30 million years old. Evidence for this is the discovery of dauer juveniles of the fossil diplogastrid, *Formicodiplogaster myrmenema*, carried by *Azteca alpha* workers in Dominican amber [1] (Figures 23 and 24). The dauer stages appear to be associated with the abdomen of the ants, suggesting that they were being carried in the segmental membranes of the gaster (external phoresis). None of the fossil stages occurred around the mouthparts of the ants. Also, developing stages of *F. myrmenema* were associated with nest material adjacent to worker *Azteca* ants in Dominican amber



FIGURE 23: Three dauer juveniles of *Formicodiplogaster myrmenema* adjacent to a worker of *Azteca alpha* in Dominican amber.

[1]. This indicates that *F. myrmenema* was developing in the nests of *A. alpha*, which is probably the case with extant nematodes in the head glands of ants. Whether the dauers of *F. myrmenema* were also in the postpharyngeal glands of the fossil ants is unknown.

TABLE 4: Ants infected by entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) under laboratory and/or field conditions.

Ant	Nematode	System	Reference
<i>Acromyrmex octospinosus</i>	<i>S. carpocapsae</i>	Aqueous	[45]
<i>Camponotus</i> sp.	<i>S. carpocapsae</i>	Sucrose	[46]
<i>Camponotus</i> sp.	<i>S. carpocapsae</i>	Aqueous	[47]
<i>Myrmica</i> sp.	<i>S. carpocapsae</i>	Aqueous	[47]
<i>Pogonomyrmex</i> sp.	<i>S. carpocapsae</i>	Sucrose	[48]
<i>Solenopsis</i> spp.	<i>S. carpocapsae</i>	Alginate capsules	[46]
<i>Solenopsis geminata</i>	<i>S. carpocapsae</i>	Aqueous	[49]
<i>Solenopsis invicta</i>	<i>S. carpocapsae</i>	Aqueous	[50, 51]
<i>Solenopsis invicta</i>	<i>H. bacteriophora</i>	Aqueous	[52]
<i>Solenopsis richteri</i>	<i>H. bacteriophora</i>	Aqueous	[52]
<i>Solenopsis richteri</i>	<i>S. carpocapsae</i>	Aqueous	[50]



FIGURE 24: Detail of a dauer juvenile of *Formicodiplogaster myrmecoma* adjacent to a worker of *Azteca alpha* in Dominican amber.



FIGURE 25: Developing stages of *Steinernema carpocapsae* removed from the body of an infected queen of *Solenopsis invicta*.

## 8. Steinernematidae and Heterorhabditidae

Included in this section are the so-called entomopathogenic nematodes belonging to the genera *Steinernema* and *Heterorhabditis*. It is quite likely that entomopathogenic nematodes infect ants under natural conditions, but no reports are known. Infection is initiated by a third-stage infective juvenile that enters the host's body cavity, apparently per os [50]. After reaching the hemocoel, the infective stage initiates development and, in so doing, releases a symbiotic bacterium (*Xenorhabdus* spp. in *Steinernema* nematodes and *Photorhabdus* spp. in *Heterorhabditis* nematodes) that is carried in the infective stage's gut lumen. The bacterium kills the insect soon after it is released in the body cavity. The nematodes feed on the mixture of bacteria and insect hemolymph and develop to the adult stage in the body cavity. With adequate nourishment, the nematodes undergo a second generation but when nourishment is limited, the juveniles form third-stage infective juveniles. By introducing the bacteria that quickly kill the hosts, these nematodes avoid specific defense responses and have a wide host range, attacking representatives of many insect orders and even other arthropods [54].

Laboratory experiments have shown that these nematodes can infect a number of ant species (Table 4) and they also have been used in the field against pest ants [50, 52, 54–56]. Poole [50] attempted to control field populations of ants (*Solenopsis richteri* and *S. invicta*) with *Steinernema carpocapsae*. Using a dose of 1 million infective stages per mound for *S. invicta*, the nematodes caused 35% mortality in the fall and 80% mortality in the spring. With *S. richteri*, the death rate was 80% in the spring and 36% in the fall. Poole [50] noted that workers were infected less than other stages, possibly because of their greater activity and grooming behavior. However, workers regurgitated infective stages to the alates and larvae. Queen ants were more susceptible and up to 3,000 infective stage juveniles could be produced in some infections (Figure 25).

Further field trials of *S. carpocapsae* and *Heterorhabditis bacteriophora* against the red imported fire ant, *S. invicta*,

TABLE 5

(1)	Nematodes represented as dauer or postdauer juveniles in the pharyngeal glands of ants Nematodes developing in the body cavity of ants	Rhabditidae, Diplogastridae and Panagrolaimidae
(2)	Only juvenile nematodes present Adult nematodes with or without juveniles	(2) (3) (4)
(3)	Elongate nematodes normally over 15 mm in length at completion of development; not enclosed in membranous capsules Nematodes under 10 mm in length; enclosed in membranous capsules	Mermithidae Physalopteridae
(4)	Nematodes reproducing in dead ants; infective juveniles produced Nematode adults, eggs and/or juveniles in living ants; infective juveniles absent	(5) (6)
(5)	Males with a bursa; females with a pointed tail Males without a bursa; females with a bluntly rounded tail, often bearing a small point at tip	Heterorhabditidae Steinernematidae
(6)	Eggs and juveniles present Eggs, but no juveniles present	Allantonematidae (7)
(7)	Vulva positioned at middle or lower half of body Vulva positioned in upper fourth of body	Tetradonematidae Seuratidae

gave control rates of 37.5% with *S. carpocapsae* but less with *H. bacteriophora* [52]. In field trials comparing applications of *Steinernema carpocapsae* and amidinohydrazone against *S. invicta*, Morris et al. [55] estimated that nematode applications at a rate of 2 million per gallon per mound resulted in 47% mortality.

Controlling fire ants in the field is difficult because of the small mound opening through which the nematodes are introduced. Also, it is desirable to have recycling of the nematodes in the nests, but healthy ants appear to remove infected individuals before the cycle is completed. Since the number of nematodes needed to overwhelm a colony of ants is quite high using inundative methods, consideration was given to the development of baits or other more efficient delivery systems [46, 48, 52, 55, 56]. These other methods are still under investigation.

## 9. Unknown Nematodes

Gösswald [57] reported the presence of several encysted nematodes in the flight muscles of a queen *Teleutomyrmex schneideri* in Germany. The cysts were quite small, being only 25  $\mu\text{m}$  in diameter. Except for their small size, the cysts are similar in appearance to those of the vespid mermithid, *Pheromermis pachysoma*, formed in the body wall of Trichoptera paratenic hosts [27] and the ant parasite, *P. villosa*, in the body of oligochete paratenic hosts [26]. However, the *Pheromermis* cysts are 60–100  $\mu\text{m}$  and 80  $\mu\text{m}$  in diameter, respectively. It is possible that juvenile nematodes of a mermithid parasite were acquired after the queen was fully formed and the nematodes preferred to encyst rather than initiate development. The other likelihood is that the nematodes were the infective stages of a heteroxenous nematode parasite and were waiting for transfer to a vertebrate definitive host. However, the only cysts of heteroxenous nematodes known from ants are those of the physalopterid, *S. phryn-*

*osoma*, the smallest of which measures 633  $\mu\text{m}$  in diameter [39].

In 1907, Janet [58] found nematodes 7–8 mm in length developing in the head cavities and emerging from the labial region of workers of *Formica fusca*. Just before the nematodes emerged, the infected ants began trembling and eventually died. The head cavities of infected ants were empty upon nematode exit. This behavior of developing in the head of ants is known for some phorid flies but not for nematodes. Whether this was a mermithid with an unusual developmental location or a heteroxenous nematode using the ant as an intermediate host is unknown.

## 10. Identification Key to Nematode Families Associated with Ants

See Table 5.

## 11. Conclusions

Representatives of most invertebrate parasitic nematode families attack ants, with the exception of sphaerulariids, entaphelenchids, and oxyurids. While mermithids are the most commonly encountered nematode parasites of ants, the complete life cycle of only a single species is known. The life cycle of ant mermithids can be quite complicated when it involves paratenic hosts living in completely different habitats. Even less is known about the life cycles of other ant parasitic nematodes, certainly not enough to consider using them as biological control agents. While the inundative application of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) can control ants in isolated colonies, establishing nematodes for the sustained control of ant populations has not been achieved.

There are probably many additional nematode parasites of vertebrates utilizing ants as intermediate hosts. Reptiles,

mammals, and amphibians eat ants, and it follows that nematodes other than *Skrijabinoptera phrynosoma* would have devised methods of cycling themselves through ants to reach their definitive hosts. In the mysterious case involving *Rabbi-um paradoxus*, the presence of adults of a heteroxenous nematode in an ant raises the question of whether formicids can serve as sole hosts or this is just a case of precocity.

Fossils show that mermithids were infecting ants over 40 million years ago and tetradonematids and allantonematids had established parasitic associations with ants some 20–30 million years ago. Such fossils, which can be used to calibrate molecular clocks, provide minimum dates for the occurrence of nematode lineages and show the antiquity of nematode-ant relationships.

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## Review Article

# Ecto- and Endoparasitic Fungi on Ants from the Holarctic Region

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The ant-specific fungi *Aegeritella*, *Laboulbenia*, *Rickia*, *Hormiscium*, and *Myrmicinosporidium* in the Holarctic region—nine species—are reviewed. Present knowledge is highly biased geographically, as shows the single record for Holarctic Asia, and this is to solve. The phylogenetic position of *Aegeritella*, *Hormiscium*, and *Myrmicinosporidium* is unknown. Hosts seem to be also skewed phylogenetically although this may be a true pattern.

## 1. Introduction

Extensive, massive mycoses are an extremely rare instance in ants [1] and involve individuals, rather than whole colonies. A fortiori, documented population level attacks are practically nonexistent. A case concerning *Tetramorium caespitum* [2, 3] seems to be an isolate within ant literature. Here we deal with ecto- and endoparasitic fungi, and we limit our survey to those that are ant specific. We differentiate parasitic fungi, that are not deadly to ants, and pathogenic fungi, which kill the host. Thus, generalist entomopathogenic fungi like *Beauveria* and *Metarhizium* or ant specific like *Pandora myrmecophaga* (Figure 1) or *Telohannia solenopsae* are not included. Recent revisions of entomopathogens are those from Roy et al. [4], Kleespies et al. [5], Oi and Pereira [6] and, centred in social insects, in the seminal book by Schmid-Hempel [7]. We aim to review the knowledge of taxonomic and geographic distribution and, whenever possible, natural history and/or ecology of selected groups of fungi. The Holarctic is understood as comprising the nontropical parts of Europe and Asia, Africa north of the Sahara, and North America south to the Mexican desert region.

The fungi considered in this paper show a gradient of negative effects on the host. From a seemingly near absolute absence of any measurable—or measured—effect in some cases (*Aegeritella*, *Hormiscium*, and *Laboulbenia camponoti*), to a mild effect in other Laboulbeniales (reduced immunological response in *L. formicarum*; S. Cremer pers. comm.),

or a possible strong negative effect in *Myrmicinosporidium*). This effect may concern exclusively infested individual ants (*Myrmicinosporidium*) although in some cases, because of the fungus life cycle and the social nature of ants, with many physical contacts between colony members outside of the nest and in the nest galleries, this may be multiplied and traduced directly to the colony level (Laboulbeniales, or *Aegeritella*). This general absence of strong negative effects indicates probably a very old interaction with ants.

An unfortunate circumstance is the completely unknown phylogenetic position of some of those specific ant fungi, and this is calling for a dedicated, focused study, using molecular techniques. We stress the necessity of enhanced attention from the part of myrmecologists and mycologists towards this interesting group of ectoparasitic fungi. Just remembering their existence, and with a little care and open mind, many more instances of Laboulbeniales, *Aegeritella*, *Myrmicinosporidium*, and pathogenic fungi on ants should surface in ample areas within the Holarctic region.

## 2. Material and Methods

Apart from our current files, we did a search in the ant data base FORMIS (version 2011) [9]. Search terms are as follow: ectoparasitic, endoparasitic, fungus, fungi, Laboulbeniales, *Laboulbenia*, *Rickia*, *Aegeritella*, *Myrmicinosporidium*, and filtered out a posteriori by geographical region



FIGURE 1: *Pandora myrmecophaga* having killed a worker *Formica rufa*, from The Netherlands, showing the characteristic attachment to the distal part of a grass leaf caused by the summit disease [after [8]; Photo by H. Niesen; with permission].

(Holarctic). Within each fungus species, we give the country, ant species attacked, and reference. Taxonomical scheme and terminology follow Index Fungorum [10] (<http://www.indexfungorum.org/>).

### 3. Results

#### 3.1. Ectoparasitic Fungi on Ants

3.1.1. *Aegeritella* Bałazy & J. Wiśn. *Anamorphic Pezizomycotina*. Those fungi were first noted by Wiśniewski in 1967 [11] although its fungal nature was not proven then. The fungi grow over the cuticle like dark protuberances (= bulbils). On a first sight, they look like dirt, and its form is usually a dome, rounded in perimeter, and up to 400  $\mu\text{m}$  diameter (Figure 2). The number of bulbils may be from a single one to several hundreds. The distribution of bulbils on the body of ants is heterogeneous, being more abundant at the rear part [12–14]. The total number of bulbils is inversely related to ant size, with bigger ants having less bulbils than smaller ants [14]. Bulbils have been detected in workers and queens.

The ant-fungus relationship has not been properly ascertained although a reduced life duration or activity level has been suggested [15, 16]. In a similar vein, Bałazy et al. [17] note some workers with hundreds of bulbils, having immobilized bucal palps, all covered by hyphae. Nothing is known of the dynamics of infestation or transmission mechanisms of those enigmatic fungi, not even its phylogenetic position within the realm of Fungi.

- (1) *Aegeritella superficialis* Bałazy & J. Wiś. 1974.

#### Europe

Czech Republic: *Formica sanguinea* Latreille, *Formica rufa* L., *Formica polyctena* Förster, *Formica pratensis* Retzius, *Formica truncorum* Fabricius, *Formica lugubris* Zetterstedt, *Formica exsecta* Nylander [18, 19].

Germany: *Formica polyctena* Förster [16].

Italy: *Formica lugubris* Zetterstedt [20].

Poland: *Formica polyctena* Förster, *Formica rufa* L., *Formica pratensis* Retzius; *Formica truncorum* Fabricius, *Formica fusca* L. [21–24]; *Formica sanguinea* Latreille [25].

Rumania: *Formica rufa* group [26].

Spain: *Formica decipiens* Bondroit [12].

Switzerland: *Formica rufa* L., *Formica polyctena* Förster, *Formica lugubris* Zetterstedt, *Formica sanguinea* Latreille [15].

- (2) *Aegeritella tuberculata* Bałazy & J. Wiś. 1983.

#### Europe

Czech Republic: *Lasius distinguendus* Emery, *Lasius nitidigaster* Seifert (as *Lasius rabaudi*), *Lasius umbratus* (Nylander) [19].

Poland: *Lasius flavus* (Fabricius), *Formica fusca* L. [27].

Spain: *Lasius umbratus* (Nylander), *Lasius distinguendus* (Emery) [28], *Lasius umbratus* ([29], as *L. distinguendus*); *Formica pressilabris* Nylander [12]; *Formica rufa* L., *Formica rufibarbis* Fabr. [14]. Canary islands: Tenerife, *Lasius grandis* Forel [13].

#### North America

USA, Alaska: *Lasius pallitarsis* (Provancher) ([30], as *Lasius sitkaensis*).

- (3) *Aegeritella roussillonensis* Bałazy, Lenoir & J. Wiś. 1986.

France. On *Cataglyphis cursor* (Fonscolombe) [17].

- (4) *Aegeritella maroccana* Bałazy, Espad. & J. Wiś. 1990.

Morocco. On *Aphaenogaster baronii* Cagniant [31].

- (5) An unidentified *Aegeritella* was noted on two workers

*Polyergus breviceps* Emery from Arizona [30].

#### 3.1.2. *Hormiscium* Kunze, *Incertae Sedis* Pezizomycotina

- (1) *Hormiscium myrmecophilum* Thaxter, 1914.

The species was described from an Amazonian *Pseudomyrmex* and remained elusive since its original description until it was found in Europe eighty years later. The filamentous, somewhat dichotomic thallus is undifferentiated and grows directly out of different parts of the ant body, without any apparent attaching structure. Mycelia have a maximum length of 163  $\mu\text{m}$  and constant width of 10  $\mu\text{m}$ . (Figure 3). Spores are unknown.

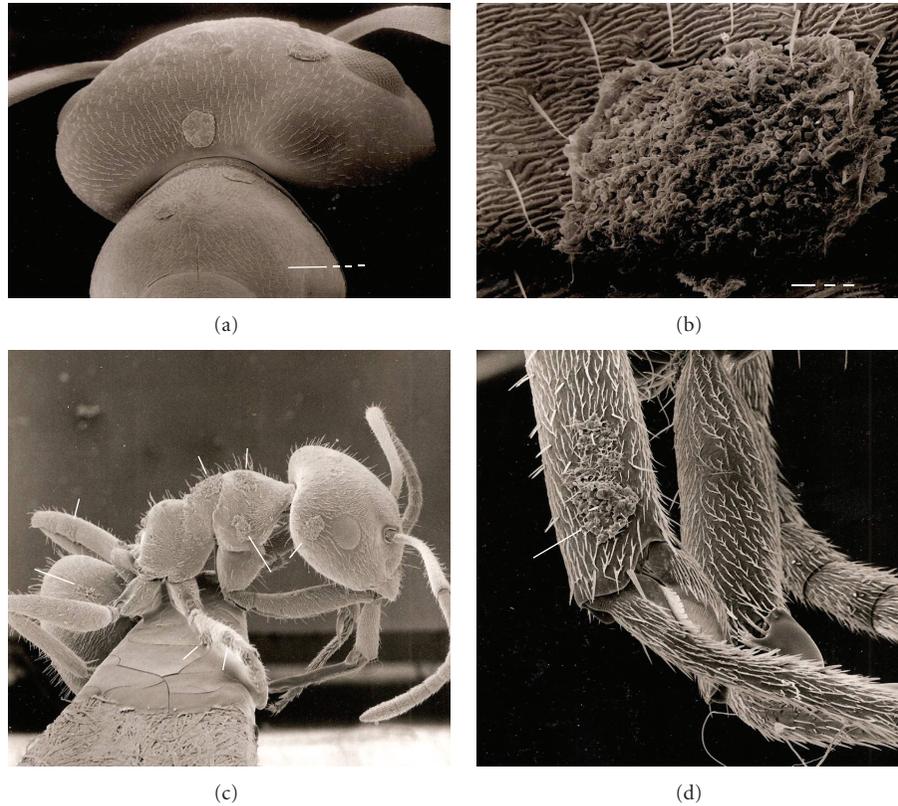


FIGURE 2: (a) *Aegeritella tuberculata* on *Formica pressilabris* (Spain). Two bulbils are in the pronotum, one at the back of head, (b) closeup of a bilbil; (c) *A. tuberculata* on *Lasius grandis* from Tenerife, Canary Islands; white arrows indicate bulbils; (d) closeup of bulbils in the first leg.



FIGURE 3: *Hormiscium myrmecophilum*. (a) hyphae on *Myrmica* sp.; (b) worker *Myrmica sabuleti* with hyphae on the head and lateral pronotum.

### Europe

Portugal. On *Myrmica* sp. [32].

Spain. On *Myrmica sabuleti* Meinert (present paper).

3.1.3. *Laboulbeniales* (*Ascomycota*). *Laboulbeniales* are unusual among fungi because of their limited thallus with determinate growth. They are obligate external parasites of arthropods, especially insects. One key peculiarity is the

ability to grow on their hosts without inflicting any noticeable injury. Ten orders of insects, in addition with millipedes and acari, may be affected although 80% of some 2000 species are recorded from beetles [33]. Only six are known to date infesting ants from the Holarctic region, and all castes are known to be susceptible to infestation.

(1) *Rickia wasmannii* Cavara, 1899.

The species is extremely characteristic in its microscopic morphological aspect (Figure 4) and is limited to several

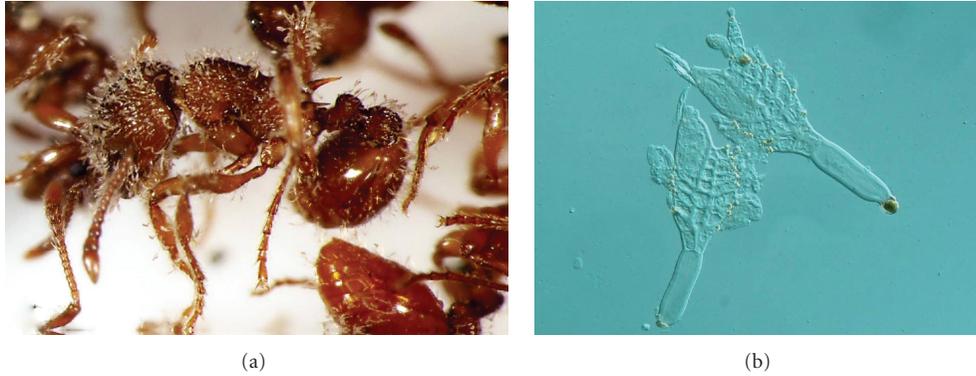


FIGURE 4: (a) *Rickia wasmannii* on *Myrmica scabrinodis* from Slovakia. Each "spatulate hair" is a thallus of *Rickia*. Photo by P. Bezděčka; with permission; (b) two mature thalli. Spores are oozing out of the perithecium on the specimen from the right.

species of *Myrmica*. Infested ants may harbour from a few thalli to several hundred thalli all over the body. Heavy infestations are visible to the naked eye and give a greyish shade, a pulverulent image to living individuals. Worker and queens may be infested.

#### Europe

- Austria: *Myrmica rubra* (L.) [34].  
 Bulgaria: *Myrmica scabrinodis* Nylander [35].  
 Czech Republic: *Myrmica slovacica* Sadil, *Myrmica scabrinodis* Nylander [36].  
 France: *Myrmica scabrinodis* (Nylander) [37].  
 Germany: *Myrmica rubra* (L.) [38].  
 Hungary: *Myrmica slovacica* Sadil (as *M. salina*), *M. scabrinodis* Nylander, *M. speciooides* Nylander, *M. vandeli* Bondroit [39].  
 Italy: *Myrmica scabrinodis* Nylander [40].  
 Luxembourg: *Myrmica rubra* L. [41].  
 Rumania: *Myrmica scabrinodis* Nylander [39].  
 Slovakia: *Myrmica scabrinodis* Nylander [42].  
 Slovenia: *Myrmica sabuleti* [41].  
 Spain: *Myrmica speciooides* Bondroit [28, 43]; *Myrmica spinosior* Bondroit ([43], as *M. sabuleti*).  
 Switzerland: *Myrmica rubra* (L.) ([44], as *M. laevinodis*).  
 United Kingdom: *Myrmica sabuleti* Meinert [45, 46].

#### (2) *Rickia* sp.1.

Greece: On *Messor* (unpublished observation: description is pending).

#### (3) *Laboulbenia camponoti* S. W. T. Batra 1963.

Under the binocular, the thallus looks like a distorted ant hair (Figure 5) and is found all over the body, albeit more abundant in dorsal surfaces and external surface of legs.

Density is much lower than in other ant-specific Laboulbeniales. In the Holarctic, it has been detected exclusively in *Camponotus* species, all six from the subgenus *Tanaemyrmex*.

#### Asia

Turkey: *Camponotus baldaccii* Emery [47].

#### Europe

Bulgaria: *Camponotus aethiops* (Latreille), *Camponotus universitatis* Forel, *Camponotus* sp. (as *C. pilicornis*) [35].  
 Spain: *Camponotus pilicornis* (Roger) [48]; *Camponotus sylvaticus* (Olivier) [49].

#### (4) *Laboulbenia formicarum* Thaxt, 1902.

This is one of the smallest Laboulbeniales (up to 0.3 mm total length). Thalli can be extremely abundant on infested workers (Figure 6), which go foraging seemingly unaffected amid noninfested workers.

#### North America

Canada: *Lasius alienus* (Förster) [50].  
 USA: *Formica argentea* Wheeler [51]; *Formica aserva* Forel ([52], as *F. subnuda*); *Formica curiosa* Creighton ([53], as *F. parcipappa*); *Formica incerta* Buren [51]; *Formica lasioides* Emery [54]; *Formica montana* Wheeler ([54], as *F. neocinerea*); *Formica neogagates* Viereck [51, 55]; *Formica pallidefulva* Latreille ([54], as *F. nitidiventris*; [56], as *F. schaufussi*); *Formica puberula* Emery [52]; *Formica subintegra* Wheler [54]; *Formica subpolita* Mayr ([52], as *F. camponoticeps*); *Formica subsericea* Say [54]; *Formica vincularis* Wheeler [54]; *Lasius alienus* (Förster) ([55, 57], as *L. americanus*); *Lasius murphyi* Forel [58]; *Lasius neoniger* Emery [51, 59]; *Lasius pallitarsis* (Provancher) ([30], as *L. sitkaensis*); *Myrmecocystus mimicus* Wheeler [60]; *Polyergus breviceps* Emery [54]; *Polyergus lucidus* Mayr [54]; *Prenolepis imparis* (Say) [54].

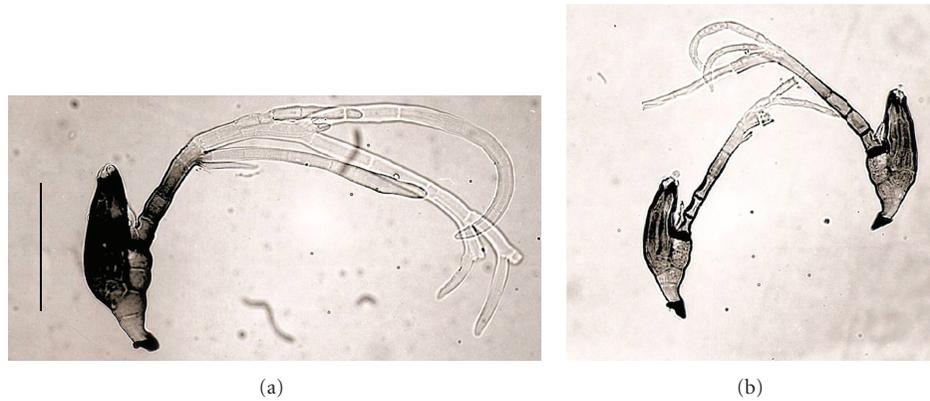


FIGURE 5: *Laboulbenia camponoti* from *Camponotus sylvaticus* (Spain); line: 1 mm. (a) A mature specimen; (b) two immature specimens.

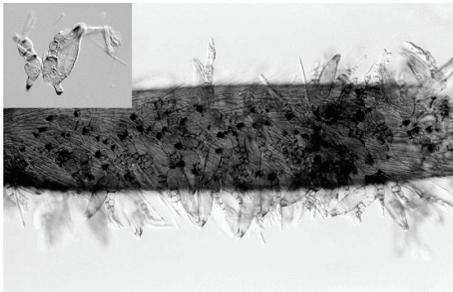


FIGURE 6: *Laboulbenia formicarum* on *Lasius grandis*. Worker tibia, showing full-grown thalli and dark spots which indicate attachment point of spores (more than 50 in the viewed side). Inset: one mature (right) and immature (left) specimens of *Laboulbenia formicarum*.

### Europe

France: *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy [61].

Portugal (Madeira): *Lasius grandis* Forel [62].

Spain: *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy [63].

### 3.2. Endoparasitic Fungi on Ants

#### 3.2.1. Incertae Sedis

*Myrmicinosporidium durum* Hölldobler 1933. Those fungi were first noted by Hölldobler [64, 65] although they were formally described later, in 1933 [66]. Its phylogenetic position is still unknown, and their true fungal nature has been only proved recently [67]. Infested ants are usually well detected because the darker spores are visible through the integument (Figure 7); spores number may be very low, but usually they reach more than one hundred in a single ant. The caveat here is that the fungus may be much difficult to detect in ants having fuscous or black colouration. As a consequence, host range is probably biased. The usual aspect of concave spores, with a bow-like depression, is an artefact of fixation in alcohol [68].

Although the infested workers are almost certainly killed by the fungus when spores begin producing hyphae, life span seems not to be curtailed [67]. Infested workers seem scarcely affected in its normal behaviour [67, 69], and infested queens may participate in swarming flights [69] and show normal fertility [68]. Males have been found infested too [70]. Life cycle and mode on infestation are unknown although reports of *Myrmicinosporidium* from callow workers in *Pogonomymex badius* indicate that the infection is carried over from immature stages [71]. It is perhaps significant that the majority of diseased ants were collected in late summer and fall. After hibernation, those infested workers die [69]. Its geographical distribution is ample as is also the range of hosts.

### Europe

Austria: *Plagiolepis vindobonensis* Lomnicki [67].

Croatia: *Temnothorax recedens* (Nylander), *Temnothorax affinis* (Mayr), *Temnothorax unifasciatus* (Latreille), *Plagiolepis pygmaea* (Latreille) [67].

France: *Solenopsis fugax* (Latreille), *Pheidole pallidula* (Nylander) [72]; *Temnothorax unifasciatus* (Latreille), *Temnothorax recedens* (Nylander) [68].

Germany: *Solenopsis fugax* (Latreille) [64, 65], *Temnothorax tuberum* (Fabricius) [66].

Hungary: *Solenopsis fugax* (Latreille), *Tetramorium caespitum* (L.), *Plagiolepis taurica* Santschi [73].

Italy: *Temnothorax unifasciatus* (Latreille) [67, 69], *Temnothorax albipennis* (Curtis) [67], *Temnothorax angustulus* (Nylander) [67], *Temnothorax exilis* (Emery) [67], *Temnothorax nylanderi* (Forster) [67], *Chalepoxenus muellerianus* (Finzi) [67].

Spain: *Pheidole pallidula* (Nylander), *Solenopsis* sp., *Strongylognathus caeciliae* Forel, *Tetramorium semi-laeve* (André), *Plagiolepis pygmaea* (Latreille) [70], *Temnothorax lichtensteini* (Bondroit), *Temnothorax racovitzaei* (Bondroit) [72].

Switzerland: *Solenopsis fugax* (Latreille) [68].

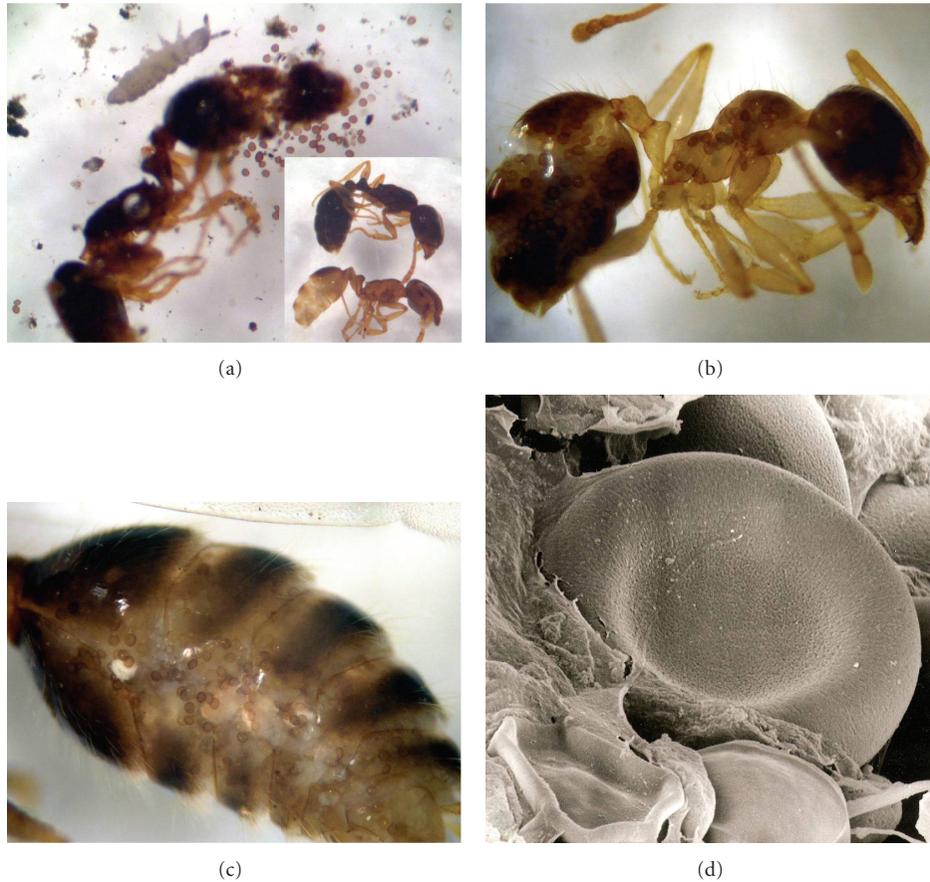


FIGURE 7: (a) *Myrmecinosporidium* mature spores inside workers *Tetramorium semilaeve* (inset: darker, infested worker, and normally coloured worker); (b) *Pheidole pallidula* with many spores on thorax, coxae, and gaster; (c) gaster of a male *Pheidole pallidula* with spores; (d) SEM image of a spore, showing the artifactual characteristic doughnut shaped form resulting from the alcohol fixation.

#### North America

USA: *Pogonomyrmex barbatus* (F. Smith) [67]; *Solenopsis carolinensis* Forel, *Solenopsis invicta* Buren, *Pheidole tysoni* Forel, *Pheidole bicarinata* Mayr, *Pyramica membranifera* (Emery), *Pogonomyrmex badius* (Latreille) [67]; *Nylanderia vividula* (Nylander) ([67], as *Paratrechina vividula*).

3.2.2. *Dubious Cases.* Across literature, two cases have been described but not identified. Although unproven, those are highly likely to belong in *Aegeritella* because of the macroscopic description given.

Bequaert ([56], page 74) wrote “A number of so-called ‘imperfect fungi’—incompletely developed, conidia-bearing or sterile stages of various Ascomycetes—have been recorded from ants. A nest of *Formica rufa* Linné, at Potsdam, Germany, was heavily infested with fungous growths, about the size of a pin-head and attached mainly to the thorax, more rarely to other parts of the body. The ants were apparently but little hampered by their parasites. From cultures obtained with these fungi, Bischoff concluded that they belonged to several species, among them a *Mucor*, a *Penicillium* and a yeast. Thaxter also found

in the vicinity of Cambridge, Mass., a fungus forming blackish incrustations on various parts of ants and giving rise to a few short, colorless, erect branches; the exact nature of this plant has not been determined, nor is the name of its host mentioned.”

Donisthorpe ([74], page 235 and Figure 86) commenting on *Lasius umbratus* var. *mixto-umbratus* Forel, [now *Lasius (Chthonolasius)* unrecognisable species] noted “On August 11th, 1912, when at Weybridge in company with Professor Wheeler, we found two colonies of this variety, very many of the ants of both being infested with a curious dark brown warty growth in patches on parts of the body and legs—this Wheeler thought might be a fungus which was unknown to him. I kept a number of these ants in captivity, and added uninfected workers of *umbrata* from other localities; the growth however did not increase nor spread to the new ants, but rather seemed to decrease. I sent some of the infested ants alive and others in spirit, to Dr. Baylis Elliot, and she considered the patches were colonies of unicellular organisms growing on the outside of the ants; eventually she came to the conclusion that they were not fungoid growths, but probably colonies of an alga.” Thus, albeit without a named host, *Aegeritella* is probably present too in the United Kingdom. A search with Donisthorpe’s collection and/or in the vicinities of Weybridge could confirm this.

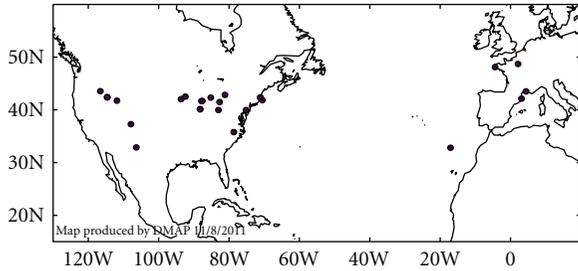


FIGURE 8: Distribution of *Laboulbenia formicarum*. North American records date from 1902 to 1979 and belong in 24 ant host species of five genera. European records date from 2003 to 2011 and imply two host species of *Lasius*.

## 4. Discussion

**4.1. On Fungus Taxonomy.** Laboulbeniales are taxonomically and nomenclaturally stable. There seems to be no major problem in morphological identification of the species involved. Perhaps, only, it would be worth examining the possibility of several species within *Laboulbenia formicarum* since its hosts belong in five genera, from three tribes—Formicini, Lasiini, and Plagiolepidini—in Formicinae.

*Aegeritella* is an especially difficult situation. Apart from its doubtful position within Fungi, bulbils are usually not in a perfect fruiting condition, and microscopic preparations are not easy to do since the bulbils are tightly attached to the ant's surface, anchored by the pubescence and hairs of the ant. The two most abundant species (*A. superficialis*, *A. tuberculata*) are well differentiated by the presence of hyphal elements in *A. superficialis* and by its absence in *A. tuberculata* [17].

*Myrmicinosporidium* is also an unsolved problem. All records but one are based simply on the presence of spores, which have a strikingly similar appearance across the two continents. Although they seem to be close to Chytridiomycetes [67], it remains to be studied where do those fungi belong within the phylogeny, and also the conspecificity of all so-called *M. durum* records. A similar situation is that of *Hormiscium*, from which only hyphae are known.

**4.2. Host Phylogeny.** A minimum of 13 subfamilies of ants are found in the Holarctic region. Only two (Myrmicinae and Formicinae) are noted with ecto- or endoparasitic fungi. Why should the distribution be so biased? If this is not a sampling artefact, it is noteworthy that the two subfamilies appear close together in the last comprehensive ant phylogenies [75, 76], thus indicating perhaps an ancestral susceptibility for both subfamilies.

*Aegeritella* is found on *Formica* and *Lasius*. *Laboulbenia* species infest exclusively ants from the subfamily Formicinae and *Rickia* infests Myrmicinae. This host specificity is not rare with Laboulbeniales [33]. Inasmuch *L. formicarum* is hosted by 24 ant species that belong in three tribes (Formicini, Lasiini, and Plagiolepidini), this calls for a dedicated evaluation (molecular and morphological) of the conspecificity of all populations of *L. formicarum*.

*Myrmicinosporidium* may be found in both ant subfamilies although the majority of cases belong in the Myrmicinae. We may speculate if the generic name is entirely appropriate or there is a detection bias of unknown origin towards Myrmicinae. Infested species belong in six tribes in Myrmicinae (Dacetini, Formicoxenini, Myrmicini, Pheidolini, Solenopsidini, and Tetramoriini), and one tribe in Formicinae (Plagiolepidini), widely scattered within ant phylogeny ([75], Figure 1; [76], Figure 1). Specificity is evidently not to uncritically assume in this fungus.

**4.3. Geographical Distribution and Host Number.** Knowledge is absolutely fragmentary and skewed. Asia in special, with a single record of ecto- and endoparasitic fungi, is a promising region to explore. The genus *Myrmica* with its many species should be searched for *Rickia*, and the genera *Formica* and *Lasius* for *Aegeritella*. Within Europe, countries such as Ireland, Belgium, The Netherlands, Denmark, Poland, or Portugal are obvious candidates for *Rickia*. The northernmost locale for *Rickia* seems to be Denbies Hillside, at 51°14'N [45]. Some cases, such as *Laboulbenia formicarum* (Figure 8) or *Myrmicinosporidium durum* (Figure 9) agree with the usual worldwide or wide-ranging specific distribution of fungi although others are only known from its original description, from a single locality (*Aegeritella maroccana*, *Aegeritella roussillonensis*).

With host number, the situation seems to be dichotomous. Some fungi are known from a range of hosts: *A. superficialis* 9 hosts, *A. tuberculata* 10, *L. formicarum* 24, *L. camponoti* 7, *R. wasmannii* 8, and *Myrmicinosporidium* 27, while other fungi are known from single hosts, in parallel with geographical range, likely reflecting a sampling artefact. Horizontal transmission to slave-making ants is possible, as attested by *Aegeritella* [30] and *Laboulbenia formicarum* [54] on *Polyergus*, and by *Myrmicinosporidium* in *Chalepoxenus* [67] and *Strongylognathus* [70].

In the USA, three species (*Pheidole*, and 2 *Solenopsis*) from a single farm in Houston Co., Alabama [71] were noted as infested with *Myrmicinosporidium*. In southern Hungary, three genera (*Plagiolepis*, *Solenopsis*, and *Tetramorium*) [73] were noted as hosts in a single locality. A similar situation is that of an organic citrus field in Spain [70], in which up to four different genera (*Pheidole*, *Plagiolepis*, *Tetramorium*, and *Solenopsis*) have been detected as hosts during several years, their nests being at distances of 5–20 m. The disease may qualify as chronic in the three localities. In this last locality, *Aegeritella* on *Formica rufibarbis* and *Laboulbenia camponoti* on *Camponotus aethiops*, *C. pilicornis*, and *C. sylvaticus* exist too. The single circumstance we can suggest for this “abnormal” abundance of parasitic fungi in this last site is the intensity—monthly samples—and duration—since 2002 and ongoing—of ecological studies with abundant insect collection. This is suggestive of a general low-prevalence but ample geographic distribution. Thus, we cannot but expect a growth of information if proper attention is directed to those ecto- and endoparasitic fungi of ants. Myrmecologists, please, be aware!

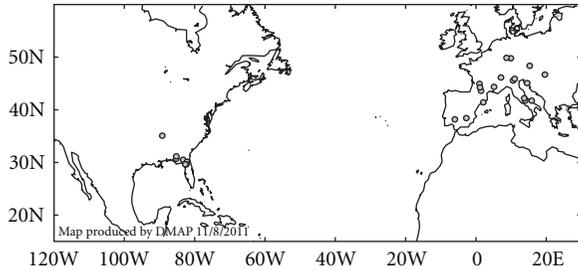


FIGURE 9: Distribution of *Myrmecinosporidium* sp. Eight ant host species are known from USA, and 19 from Europe.

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## Research Article

# Life History of *Aricoris propitia* (Lepidoptera: Riodinidae)—A Myrmecophilous Butterfly Obligately Associated with Fire Ants

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The immature stages of *Aricoris propitia* (Stichel) are described and illustrated for the first time, using both light and scanning electron microscopy. Females oviposit in at least seven host-plant families, always in the presence of fire ants (*Solenopsis saevissima* (Smith) complex), without being attacked by them. Larvae are tended by ants during all larval and pupal stages. From the fourth instar on, larvae feed at night and rest during the day inside underground shelters constructed by ants on the host plant roots, and where pupation occurs. Several observed features, including ant-mediated oviposition, persistent ant attendance throughout all instars, and high spatiotemporal fidelity indicate that *A. propitia* is a myrmecophile obligately associated with fire ants. We propose *A. propitia* as an extraordinary model for studies on ant-butterfly evolutionary history in the Neotropics.

## 1. Introduction

Symbiotic associations between butterfly larvae and ants have attracted the attention of early naturalists, both in Europe and North America, since the second half of the 18th century (see references in [1]). Nonetheless, these interactions are historically poorly studied in the Neotropical region despite their richness and abundance [2, 3]. An exception in this scenario is the classic paper by Bruch [4], which describes some aspects of the life history of an Argentinean species of *Aricoris* Westwood. In addition to being the first detailed description of a myrmecophilous larva from the Riodinidae family, the aforementioned study presents the first evidence of a butterfly larva living inside ant nests in the Neotropics. This behavior has been reported for a small number of Lycaenidae clades, such as the charismatic large blue *Maculinea* Van Eecke (*Phengaris* Doherty spp.), which parasitizes ant societies in Eurasia (see [5–7]). But unlike large blue butterflies, which today are model organisms in mutualism and parasitism studies, little progress has been

achieved on the biology of *Aricoris* since the initial work by Bruch [4] (but see [8–12]).

The riodinid genus *Aricoris* contains 24 described species [13, 14] typically found in open dry areas of South America [3]. *Aricoris propitia* (Stichel) is widespread in Central and Northern Brazil ([15], C. Callaghan, pers. comm.). Since its original description in 1910, no additional information was published for this species. The purpose of this paper is to fill that gap by presenting the natural history and morphological description of immature stages of *A. propitia*, with emphasis on their obligatory association with fire ants of the *Solenopsis saevissima* (Smith) complex (Formicidae: Myrmicinae).

## 2. Material and Methods

**2.1. Study Sites.** Four sites were sampled in central and northern Brazil (Figure 1): (1) cerrado *sensu stricto* and gallery forest areas in Alto Paraíso, Goiás (13°48'S, 47°54'W) (July 2009); (2) suburban areas of the city of Assis Brasil, Acre

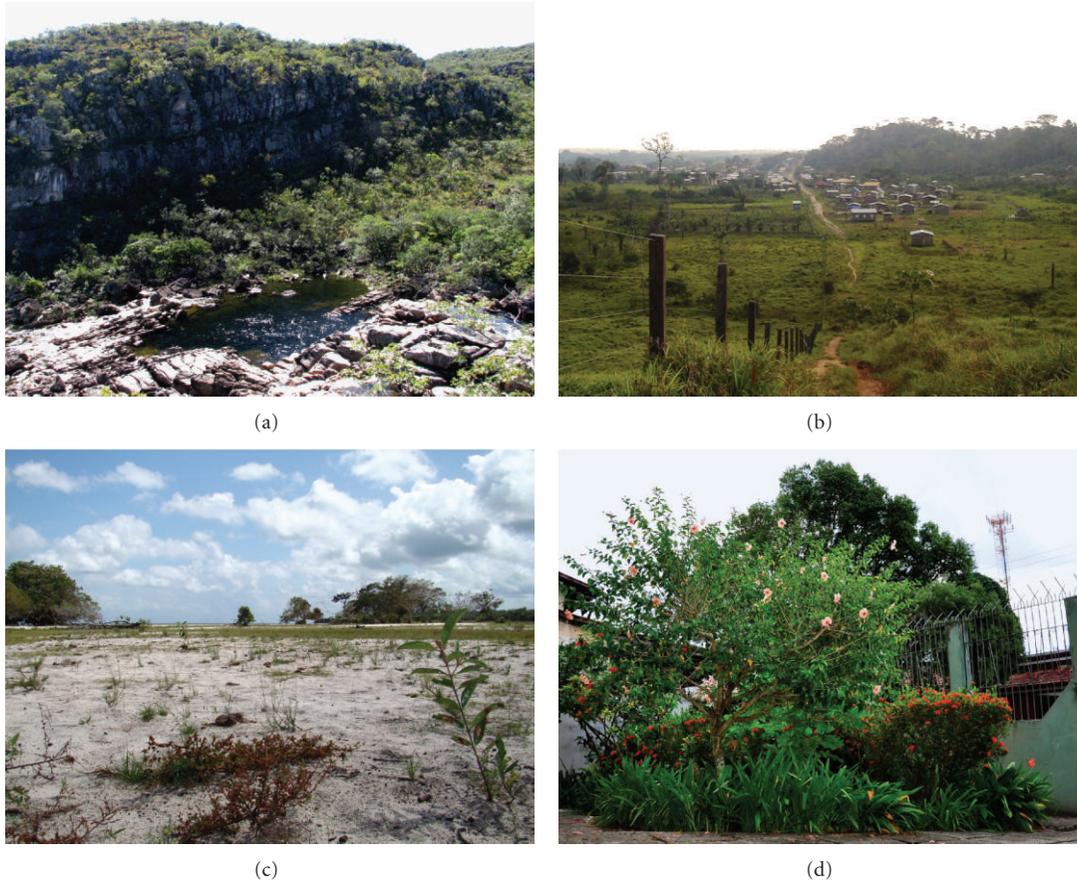


FIGURE 1: Overview of *Aricoris propitia* study sites in Central (a) and Northern ((b)–(d)) Brazil. (a) Cerrado *sensu stricto* area in Alto Paraíso, Goiás; (b) surroundings of Assis Brasil, Acre—note the bordering suburbs, pastures and forest; (c) sandy beach areas along the Xingu river, Porto de Moz, Pará; (d) small home garden in a neighborhood of Belém, Pará—note that within this small space the larvae were able to use three ornamental plant species (see Table 1).

(10°56'S, 69°33'W) (August–September 2006); (3) sandy beach and small-farm cultivation areas along the Xingu river, Porto de Moz, Pará (02°07'S, 52°15'W) (July 2010); (4) house garden in a neighborhood of Belém, Pará (01°25'S, 48°27'W) (several occasions between 2006 and 2009).

**2.2. Sampling, Rearing, and Behavioral Observations.** Available host-plants in the study sites were visually scanned for the presence of larvae and tending ants (as in [16]). Additionally, some potential host-plants with distinct signs of herbivory and visited by *S. saevissima* ants were excavated in search of larvae and pupae. Plants with immatures (eggs and larvae) were collected for identification, as well as the tending ants. We also recorded the presence of food sources that may promote ant visitation on the plants, such as extrafloral nectaries (EFNs) and/or honeydew-producing hemipterans (HPHs). The immatures of *A. propitia* used for morphological description were collected in the field and reared as follows: eggs were placed in Petri dishes and observed daily until eclosion; newly hatched larvae were reared individually in transparent 250 mL plastic pots under controlled conditions ( $25 \pm 2^\circ\text{C}$ ; 12 h L: 12 h D). Branches

of the same host-plant on which each larva was found were offered *ad libitum*, and larvae were checked daily for food replacement and cleaning when necessary. Immatures for morphological analysis were separated, fixed in Dietrich's solution, and then preserved in 70% ethanol. Shed head capsules were collected and preserved for measuring. Voucher specimens of the immature stages were deposited at the Museu de Zoologia “Adão José Cardoso” (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Behavioral interactions between *A. propitia* larvae and tending ants were observed *ad libitum* [17] in the field during the day (ca 10:00–16:00 h), and sometimes at night (ca 18:00–06:00 h), for the population of Porto de Moz. Additional observations on larval ant-organs and their role in the interaction with ants were obtained from larvae reared in plastic pots with their host ants or from larvae maintained in a terrarium together with a captive colony of tending ants (from a population of Belém).

**2.3. Morphology.** Measurements were taken and general aspects of morphology were observed using a Leica MZ7.5 stereomicroscope equipped with a micrometric scale. Egg

TABLE 1: Summary of recorded host-plants of *Aricoris propitia*, including liquid food source types available for ants (EFNs, extrafloral nectaries; HPHs, honeydew-producing hemipterans) and localities.

Host plants	Sources of liquid food	Localities
Chrysobalanaceae		
<i>Hirtella glandulosa</i>	EFNs, HPHs	Alto Paraíso (Goiás)
Fabaceae		
<i>Senna obtusifolia</i>	EFNs	Belém (Pará)
Malpighiaceae		
<i>Byrsonima</i> sp.	HPHs	Porto de Moz (Pará)
Malvaceae		
<i>Hibiscus rosa-sinensis</i>	HPHs	Belém (Pará)
Verbenaceae		
<i>Aegiphila</i> sp.	EFNs	Assis Brasil (Acre)
Rubiaceae		
<i>Ixora coccinea</i>	HPHs	Belém (Pará)
Simaroubaceae		
<i>Simarouba</i> sp.	EFNs	Alto Paraíso (Goiás)
Turneraceae		
<i>Turnera ulmifolia</i> *	EFNs	Campinas (São Paulo)

\*Lab-accepted host-plant.

size is given as height and diameter. Head capsule width of larvae was considered to be the distance between the most external stemmata; maximum total length for both larvae and pupae corresponded to the distance from head to posterior margin of the tenth abdominal segment in dorsal view (as in [18]). Measurements are given as minimum-maximum values. Scanning electron microscopy (SEM) was conducted using both JEOL JSM-5800 and Carl Zeiss LEO-1430VP microscopes, with samples prepared according to standard techniques (for details, see [19]). Terminology for early stage descriptions follows Downey and Allyn [20] for eggs, Stehr [21] for general morphology of larvae, Mosher [22] for pupae, and DeVries [23] for ant-organs.

### 3. Results

**3.1. Natural History of *Aricoris propitia*.** This butterfly is locally abundant in open areas, where it occurs close to its ant colonies. Adults can be observed flying fast near the ground, perching on the undergrowth where they become almost invisible. Males were observed defending small territories and visiting many wild flowers. Females were seen flying near host-plants infested by host ants (Figure 2(a)), which for all studied populations were ants of the *Solenopsis saevissima* complex. Oviposition occurred in the warmest period of the day, from 11 AM to 2 PM ( $n = 15$  oviposition events), a period when ants are more active. Females flew in circles around a host-plant occupied by ants before starting to oviposit (prealighting phase). After landing (postalighting phase), females frequently touched the plant surface with the tip of their abdomen, particularly on ant trails, but

were never attacked by the ants. Eggs were laid singly or in small clusters of two to five eggs (Figure 2(b)). Our host-plant records indicate that the larvae of *A. propitia* are polyphagous using at least seven families of plants, including ornamental (nonnative) species cultivated in urban gardens (see Table 1 and Figure 1(d)). Also, in the laboratory, larvae accepted and developed well on leaves of *Turnera ulmifolia* L. (Turneraceae). All observed host-plants of *A. propitia* provided some source of liquid food that could be potentially used by ants, such as honeydew-producing hemipterans and/or extrafloral nectaries (see Table 1). Other potential host-plants without fire ants or visited by other ant species were also examined at some of the study sites ( $n = 51$  at Assis Brasil,  $n = 15$  at Alto Paraíso), but no larvae of *A. propitia* were found.

All instars are ant-tended, and even the small first instar is equipped with functional tentacular nectary organs (TNOs). From the second instar on, other ant-organs appear or become functional (Figure 3). Ants antennate the larval body intensely, but especially the anterior region where a row of papilliform setae and the openings of the anterior tentacle organs (ATOs) are located (Figure 3(a)). When everted, these organs provoke clear alterations in ant behavior, such as opening of the jaws and a marked increase in activity and aggressiveness. In the early instars (first to third) the larvae can be found during the day feeding on the host-plant leaves (Figures 2(b)–2(d)). From the fourth instar on, they rest during the day inside underground shelters constructed by ants within the host-plant roots, and that is where pupation occurs. When night falls, the larvae leave the underground shelters and climb up to feed on the host leaves (Figure 2(e)), returning to the shelters by dawn. Large quantities of mature larvae and pupae can be found inside the underground shelters, which are permanently patrolled by tending ants (Figure 2(f)).

**3.2. Description of the Immature Stages.** The reared immatures from the four sites were very similar and went through five instars. Developmental time is based on material from Alto Paraíso, Goiás, reared on *Turnera ulmifolia* leaves. The egg description and measurements are based on material from Assis Brasil, Acre; the larval and pupal description and measurements are based on material from Porto de Moz and Belém, Pará.

**3.2.1. Egg (Figures 2(b) and 4).** Duration 6–7 d ( $n = 5$ ). Height 0.30–0.32 mm; diameter 0.54–0.58 mm ( $n = 3$ ). Color whitish-cream when laid, changing to beige before hatching. General spherical shape, with convex upper surface and flattened bottom surface; exochorion with smooth surface and hexagonal cells in lateral view (Figure 4(a)). Slightly depressed micropylar area; annulus present, and rosette surrounded by petal-shaped cells; micropyles at center of the micropylar area (Figure 4(b)). Aeropyles in tiny protuberances in the rib intersections (Figure 4(c)).

**3.2.2. First Instar (Figures 2(c) and 5(a)–5(c)).** Duration 4–5 d ( $n = 2$ ). Head capsule width 0.24–0.26 mm ( $n = 3$ ), total length 2.2 mm. Dark brown head, prothoracic and anal

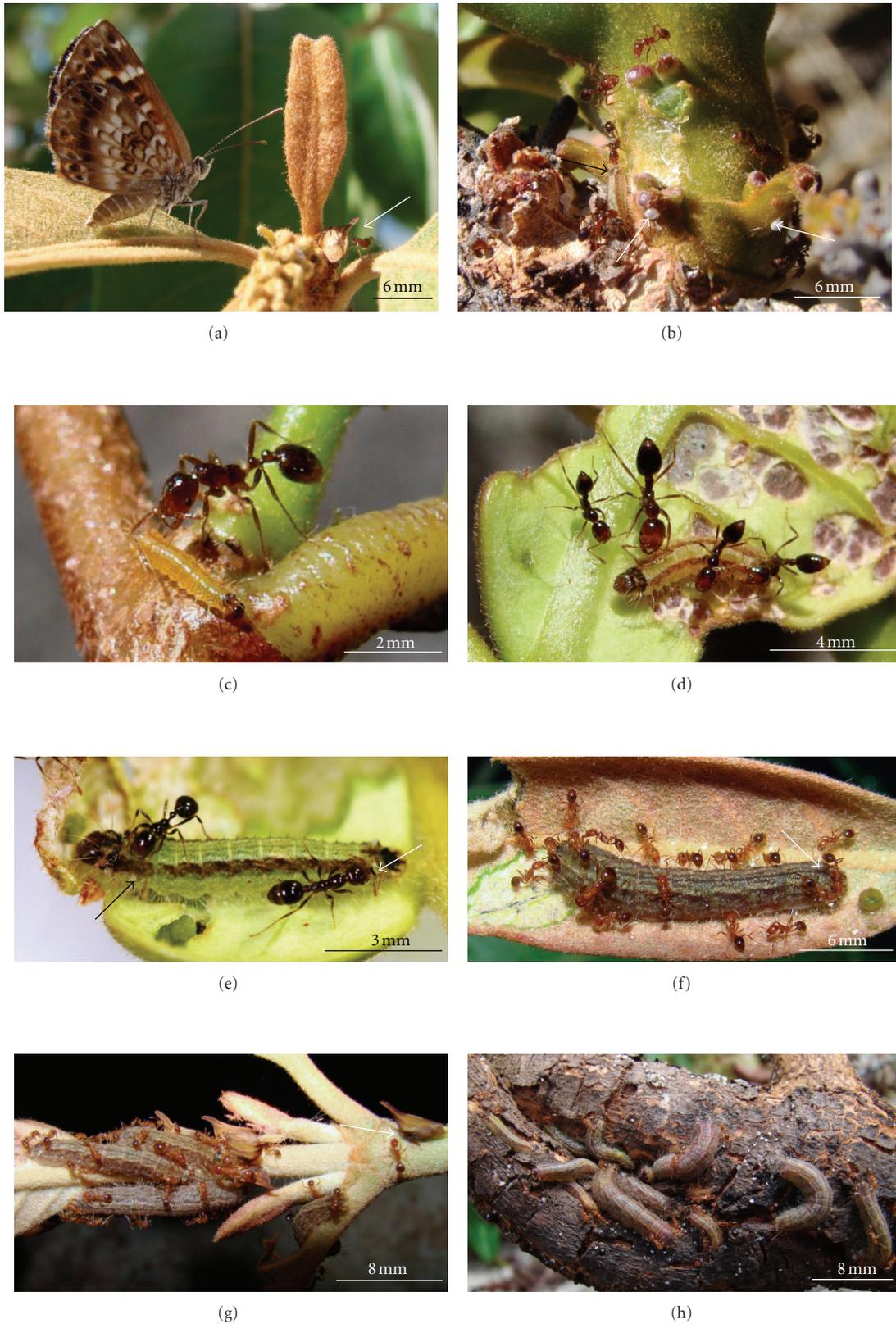


FIGURE 2: Life stages of *Aricoris propitia* tended by *Solenopsis saevissima* on *Byrsonima* sp. ((a), (d)–(f)), *Simarouba* sp. ((b), (d) and (e)), and *Hirtella glandulosa* (c). (a) Female at postlighting phase near an ant-tended treehopper aggregation (arrow); (b) eggs (white arrows) and a third instar larva (black arrow); (c) first instar tended by one worker; (d) second instar tended by ants; (e) third instar tended by ants, note that both anterior (black arrow) and tentacle nectary organs (white arrow) are everted; (f) nocturnal fifth (last) instar tended by several ants; (g) nocturnal group of larvae and treehoppers (arrow) tended by ants; (h) diurnal larval group inside a shelter in the host-plant roots.

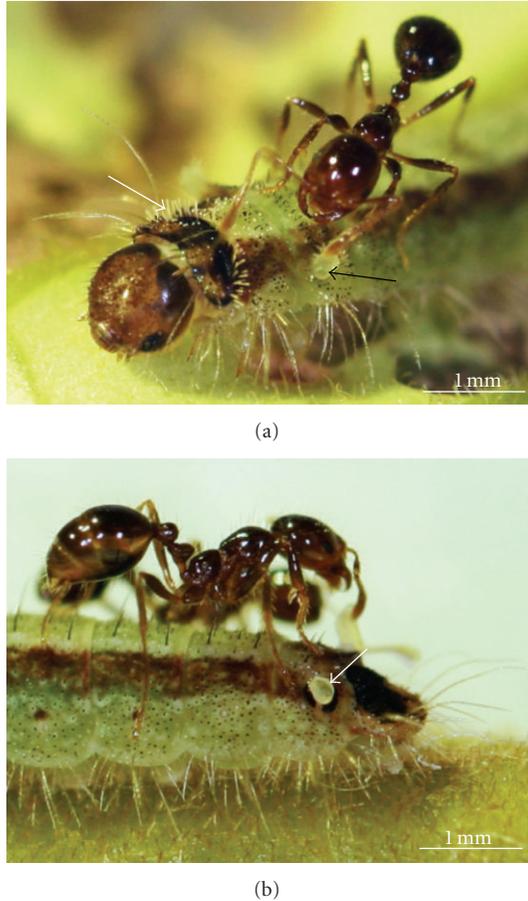


FIGURE 3: Sequence of interactions between *Aricoris propitia* third instar larva and *Solenopsis saevissima* ants. (a) Worker antennating the row of setae on the prothoracic shield (white arrow), note the everted anterior tentacle organ (black arrow); (b) everted nectary tentacle organ (white arrow) after repeated antennation by ant on the A8 segment.

shields; yellowish orange body with beige or translucent setae (Figure 2(c)). Epicranium and frontoclypeus with several setae, pores, and two pairs of perforated cupola organs (PCOs) in the adfrontal areas (Figure 5(a)). Body with long plumose setae in the lateral areas and in the prothoracic and anal shields; the remaining dorsal and subdorsal setae are short and dendritic, and PCOs are associated with these groups of setae. The openings of the anterior tentacle organs (ATOs) are present in the metathoracic segment, but these organs are apparently not functional (Figure 5(b)). Functional tentacle nectary organs (TNOs) are present in the A8 segment (Figure 5(c)).

**3.2.3. Second Instar (Figure 2(d)).** Duration 5–6 d ( $n = 2$ ). Head capsule width 0.44 mm ( $n = 2$ ), total length 3.1 mm. Dark brown head, prothoracic and anal shields; yellowish green body with two longitudinal light brown bands (Figure 2(d)). All ant-organs present, including ATOs, TNOs, PCOs, dendritic setae, and one pair of vibratory papilla on the anterior border of the prothoracic shield. A

dorsal row of papilliform setae is also present on the posterior margin of the prothoracic shield and is maintained in the subsequent instars (Figures 3(a) and 5(d)).

**3.2.4. Third Instar (Figures 2(e) and 3).** Duration 6 d ( $n = 2$ ). Head capsule width 0.72–0.84 mm ( $n = 2$ ), total length 6.2 mm. Brown head; black prothoracic and anal shields with beige spots; green body with two longitudinal brown bands (Figure 2(e)). General morphology is similar to the second instar's, but with more numerous and enlarged setae.

**3.2.5. Fourth Instar (Figures 2(g)–2(h) and 5(d)–5(e)).** Duration 6 d ( $n = 2$ ). Head capsule width 1.28–1.30 mm ( $n = 4$ ), total length 15.2 mm. Brown head; black prothoracic and anal shields with beige and grey spots; variegated body coloring with frosted brown and beige spots (Figures 2(g) and 2(h)). General morphology is similar to preceding instar's, but with more numerous and enlarged setae (Figures 5(d) and 5(e)).

**3.2.6. Fifth (Last) Instar (Figures 2(f)–2(h) and 5(f)–5(h)).** Duration 6–7 d ( $n = 2$ ). Head capsule width 1.76–1.87 mm ( $n = 5$ ), total length 2.1 cm. Coloring is similar to fourth instar (Figures 2(f)–2(h)). Mandibles with eight teeth and six setae (Figure 5(f)). Body covered with several types of setae, including prominent setae on the lateral areas, prothoracic and anal shields; two pairs of prominent dorsal setae in the same position as primary setae on the mesothorax to A8 segments; two types of dendritic setae and several perforated cupola organs (Figures 5(g) and 5(h)). The spiracle on the A1 segment is lateroventral, whereas those on segments A2 to A8 are in a dorsal position.

**3.2.7. Pupa (Figure 6).** Duration 10–12 d ( $n = 2$ ). Total length 1.29 cm, width at A1 0.33 cm. Variegated coloring with brown, beige, and dark spots (Figure 6(a)). Tegument is entirely sculptured, with irregular striations and lacking prominent tubercles (Figures 6(b)–6(e)). Prothorax bears dorsal clusters of papilliform setae (Figure 6(a)). Silk girdle crossing the A1 segment near one pair of small tubercles with several associated dendritic setae and PCOs (Figure 6(b)). Body with some small dendritic setae, and PCOs located in clusters on lateral areas close to spiracles (Figures 6(b)–6(e)); these clusters are absent on the A2 and A7 segments. The intersegmental area between the A4–A5 and A5–A6 abdominal segments features plates and files (Figure 6(f)) that may act as a stridulatory mechanism. The consolidated A9 and A10 segments constitute the ventrally flattened cremaster; with long crochets in a ventral position (Figure 6(g)).

## 4. Discussion

In general terms, the egg of *Aricoris propitia* resembles those described for other Nymphidiini genera in the Lemoniadini group (such as *Juditha* Hemming, *Lemonias* Hübner, *Synargis* Hübner, and *Thisbe* Hübner), with a semispherical shape, exochorion with hexagonal cells in lateral view, aeropyles in the rib intersections, and micropylar area centered on

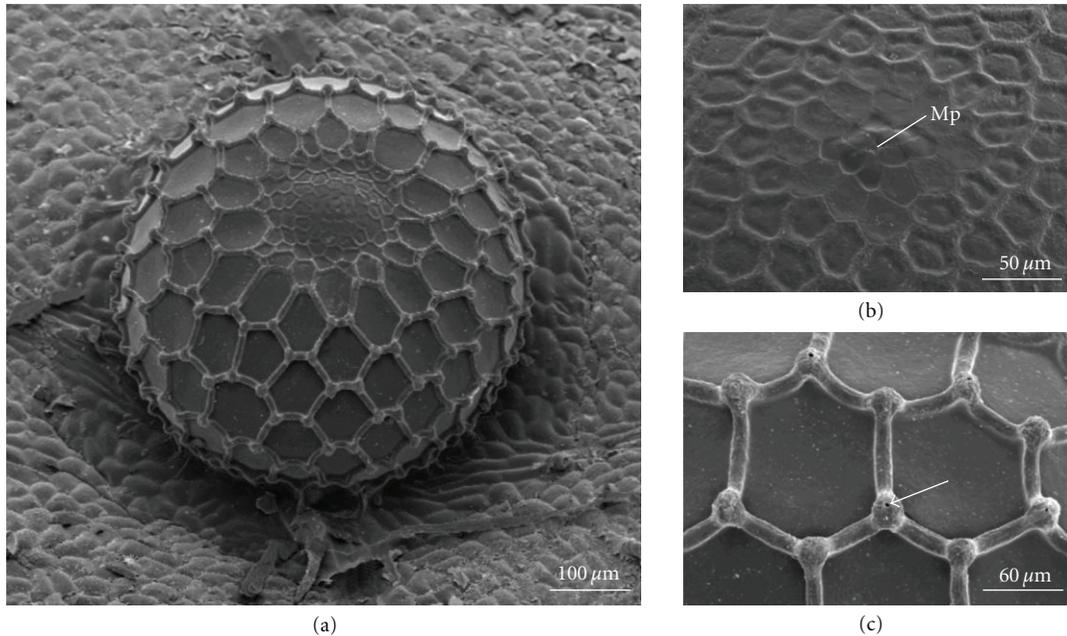


FIGURE 4: Scanning electron microscopy of *Aricoris propitia* egg (material from Assis Brasil, Acre). (a) Lateral view; (b) micropylar area (Mp); (c) hexagonal cells of the exochorion with aeropyles in the rib intersections (arrow).

the top surface (see [3, 24, 25]). However, it differs in that the limits of the micropylar area are slightly bounded; this pattern is shared with other *Aricoris* and *Ariconias* Hall and Harvey (L.A. Kaminski, unpublished). The first instar presents some characteristics of myrmecophilous larvae, namely, conspicuous perforated cupola organs, functional tentacle nectary organs, and short, dorsally located dendritic setae (see examples of riordinid first instar larvae in [3, 18, 26]).

Larvae of *A. propitia* present the typical pattern of Nymphidiini, with the first abdominal spiracle in a ventral position and vibratory papillae (VPs) on the prothoracic shield [27]. In addition to the sound producing organs (VPs), the mature larvae of *A. propitia* feature two other important types of riordinid ant-organs (see [3, 23, 24]): the anterior tentacle organs (ATOs) and the tentacle nectary organs (TNOs). The larvae also present another putative ant-organ: the row of papilliform setae on the prothorax, which had already been described for other *Aricoris* species [4, 8]. Tending ants frequently antennate these papilliform setae, and usually this palpation is accompanied by eversion of the ATOs. The way the ants react after ATO eversion suggests that the ATOs emit a volatile chemical similar to the ant alarm pheromone, as has been suggested for other Riordinidae [3, 23, 28]. The chemical compositions of ATO emissions by myrmecophilous butterflies are still unknown. In contrast, the chemical ecology of fire ants, including alarm pheromones and their role in interactions with other organisms, is relatively well known (e.g., [29]). Thus, the *A. propitia*/fire ants system may be helpful in answering some outstanding questions about the functioning of ant-organs in myrmecophilous butterflies.

The larvae of *A. propitia* can be considered polyphagous since they feed on at least seven families of host-plants. Polyphagy in obligate myrmecophilous butterflies, including Riordinidae, has been regarded as a consequence of ant-dependent oviposition [3, 12, 25, 30–32], and this seems to be the case for *A. propitia*. Aphytophagy, on the other hand, is quite rare in butterfly larvae [33], but it has been suggested for some species of *Aricoris* [3, 12]. It is believed that the larvae of these species are able to get food directly from ants, through regurgitations (trophallaxis) from ant workers or by preying directly on ant brood. Although *A. propitia* rest during the day inside underground shelters together with their tending ants, we do not have evidence that the larvae get some kind of food from the ants.

All known species of *Aricoris* seem to be engaged in obligatory associations with their tending ants. To date, *Aricoris domina* (Bates) has been associated with *Ectatomma* Smith [11], and seven *Aricoris* species have been associated with *Camponotus* Mayr [3, 8–12, 34]. So far, only *Aricoris hubrichi* (Stichel) and *Aricoris campestris* (Bates) have been reported to be associated with *Solenopsis* Westwood ants ([4], A.V.L. Freitas pers. comm.). Both *Aricoris* species are inserted within the derived “*epulus*-group” sensu Hall and Harvey [35]. Despite the high species richness and ecological prevalence, symbiotic interactions between butterfly larvae and *Solenopsis* are very rare ([36], L.A. Kaminski, unpublished). Apart from the association with fire ants, several natural history and morphological features of *A. propitia* are very similar to those observed for *A. hubrichi* and *A. campestris*, suggesting an evolutionary relationship among these species. As the life history of most species within the “*epulus*-group” is still unknown (see [35]), it is not possible to tell whether

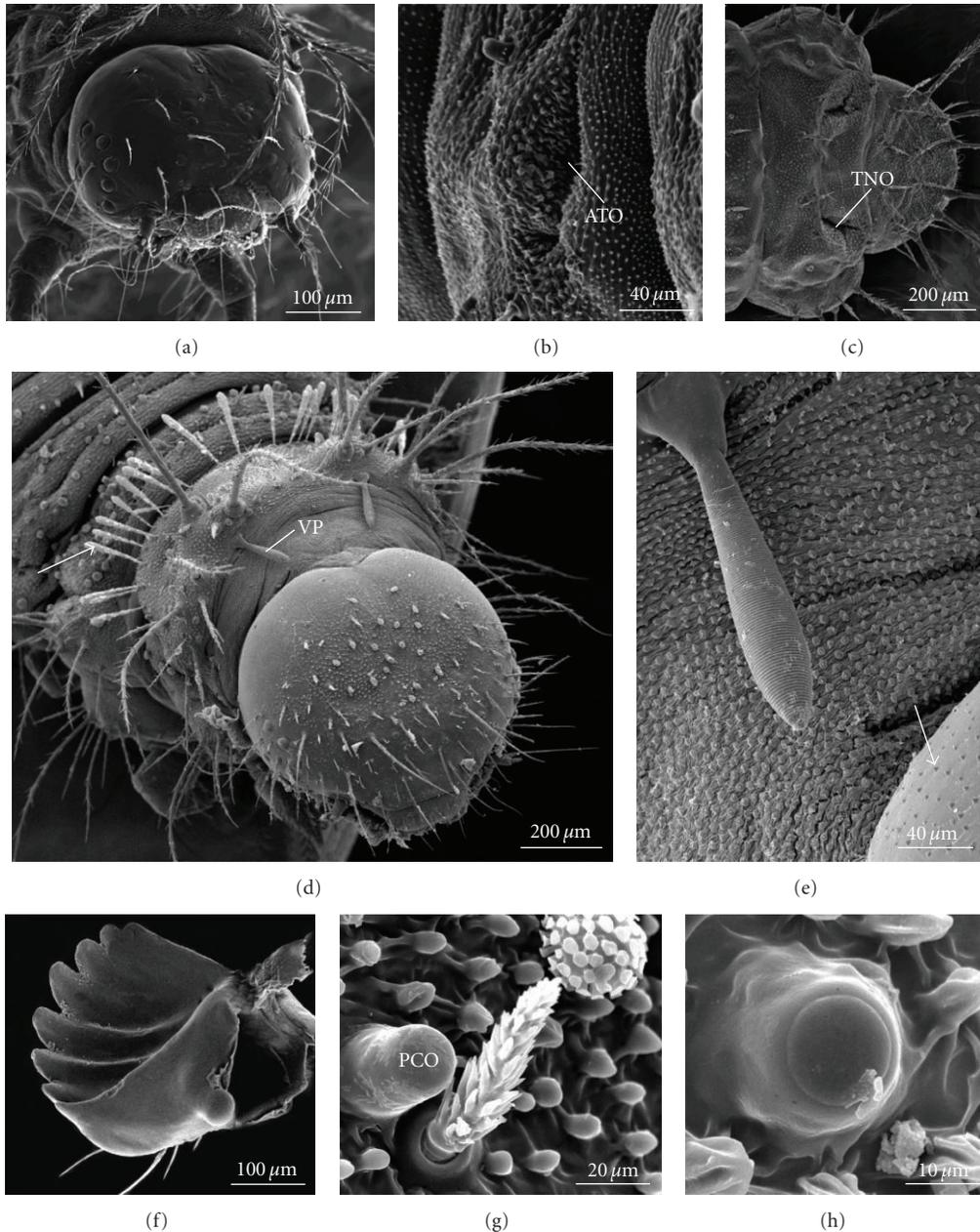


FIGURE 5: Scanning electron microscopy of first ((a)–(c)), fourth ((d) and (e)), and fifth ((g) and (h)) instar of *Aricoris propitia* ((a)–(e) from Assis Brasil, Acre, and ((f)–(h)) from Belém, Pará). (a) Head in frontal view; (b) opening of the anterior tentacle organ (ATO); (c) posterior abdominal segments showing the openings of tentacle nectary organs (TNOs); (d) head and prothorax in dorsofrontal view, note the dorsal row of setae (arrow) and the vibratory papillae (VP); (e) detail of vibratory papilla, note the epicranial granulations (arrow); (f) mandible; (g) two types of dendritic setae and perforated cupola organ (PCO); (h) perforated cupola organ.

interaction with fire ants has a single origin or has arisen more than once in these lineages.

The fire ants are highly dominant organisms and considered one of the most harmful bioinvaders ever known [37]. In their native range, from southern Brazil to Suriname, they are also considered pests in disturbed areas, especially in the Amazon (e.g., [38–40]). Although *A. propitia* occurs naturally in the Amazon (the holotype is from “Amazonas”), continual deforestation over the recent decades—especially

in the “arc of deforestation” (see [41])—could be providing a recent range expansion for this butterfly. Recent studies involving several molecular markers and morphological variation have revealed that *Solenopsis saevissima* belongs to a geographically structured complex of cryptic species [40]. How populations of *A. propitia* respond to ant host structure is an interesting and yet unanswered question. A recent study [42], for example, did not find a direct influence of host ants on the population structure of the obligate myrmecophilous

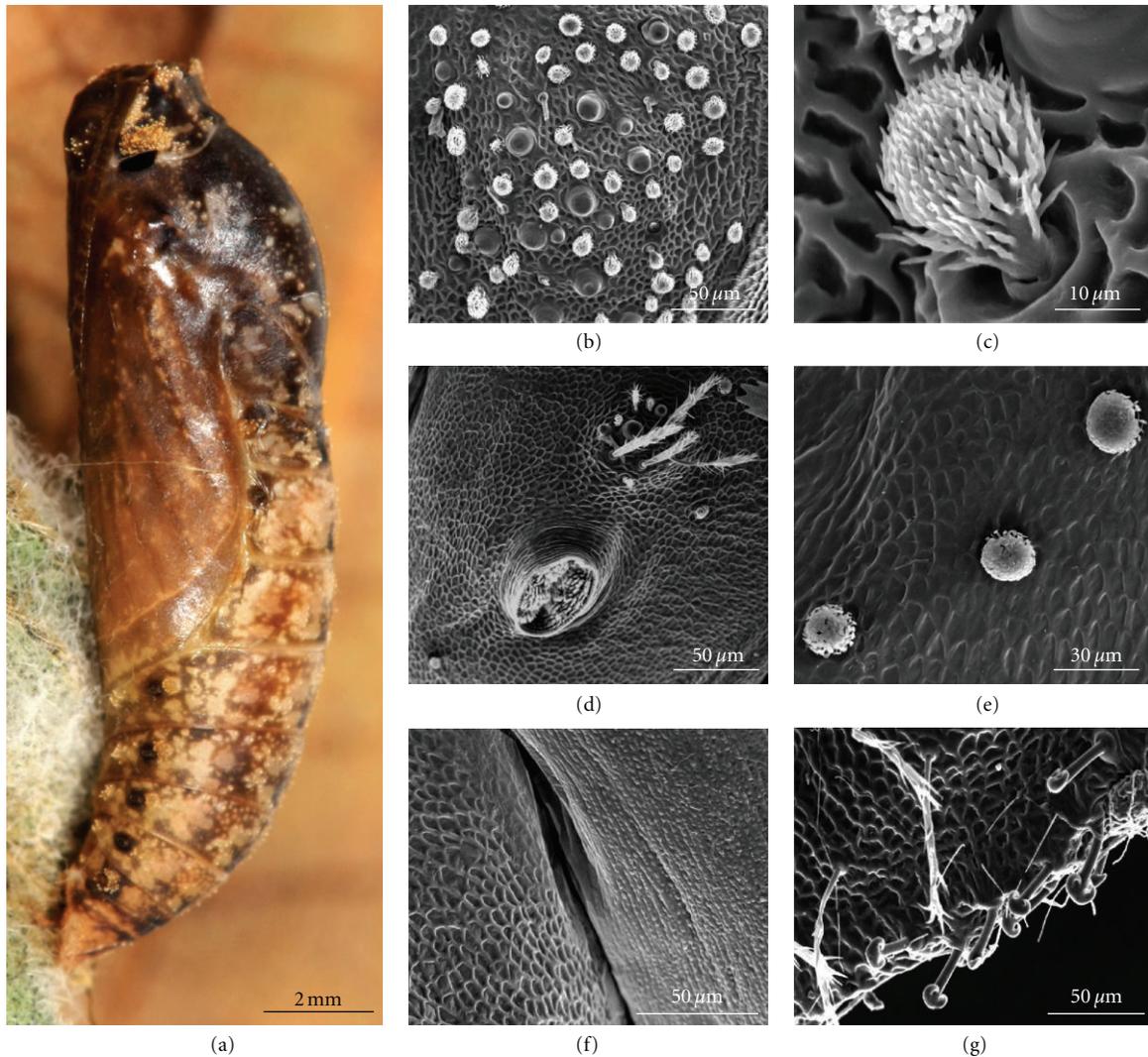


FIGURE 6: Pupa of *Aricoris propitia* in lateral view (a) and details ((b)–(g)) in scanning electron microscopy ((a), from Alto Paraíso and ((b)–(g)), from Belém, Pará). (b) Laterodorsal tubercle on A1 segment with dendritic setae and perforated cupola organs; (c) dendritic setae on A1 segment; (d) spiracle on A5 segment; (e) dendritic setae; (f) detail of putative stridulatory area between A4–A5 segments; (g) detail of cremaster crochets.

butterfly *Jalmenus evagoras* (Donovan) (Lycaenidae), but showed that biogeographical and host-plant aspects have an effect on that structure. *Aricoris propitia* may be a candidate system to elucidate the effects of ant attendance on the diversification of myrmecophilous butterflies.

The system involving *Aricoris propitia* and their tending fire ants presents several features of a model system, including: (1) it is common and widely distributed; (2) it is found in easily accessible environments (open and/or altered areas); (3) it adjusts well to laboratory conditions; (4) it has a short generation time; (5) the larvae accept many host-plant species; (6) the host fire ants have economic importance and various aspects of their biology are well known. Accordingly, we expect that the basic information provided in this work will encourage further studies on this interesting butterfly-ant system.

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## Review Article

# Current Understanding and Future Prospects of Host Selection, Acceptance, Discrimination, and Regulation of Phorid Fly Parasitoids That Attack Ants

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Phorid fly parasitoids (Diptera: Phoridae) have evolved a diverse array of cues used to successfully parasitize their ant hosts. Successful parasitism often involves (a) host habitat location, (b) host location, (c) host acceptance, (d) host discrimination, and (e) host regulation. In this paper we discuss our current understanding of how phorid flies use each of these steps to successfully parasitize ant hosts. We examine the wide variety of strategies and cues used by a multiple species of phorid flies within three separate genera that most commonly parasitize ants (*Apocephalus*, *Pseudacteon*, and *Neodohrniphora*) and discuss future directions within this field of study.

## 1. Introduction

Parasitoids have evolved effective and efficient methods of successful parasitism, many of which involve utilization of multimodal cues [1]. Many dipteran parasitoids in the family Phoridae use social insects as hosts due to the reliability of their intraspecific chemical communication signals that make for effective host selection cues [2–5]. Phorid fly adults parasitize ants by hovering over insect hosts and then diving down to insert an egg beneath the insect's exoskeleton [3, 6–8]. Phorid flies have direct parasitic effects on ants (i.e., cause ant mortality) and also significantly change ant foraging behavior by limiting host resource acquisition behavior, modifying ant competitive hierarchies, and dampening ant effects on herbivores [9–14]. There are phorids that attack ants from at least 22 genera across 5 subfamilies. Likewise, more than 20 genera of phorids attack ant hosts [3]. With such taxonomic diversification in ant-phorid relationships, the types of cues used by phorids to locate, select, and successfully parasitize ant hosts are also quite diverse.

Successful parasitism requires a series of interactions between a parasitoid and its host. The process can be categorized into five general and sometimes overlapping steps: (a) host habitat location, (b) host location, (c) host acceptance, (d) host discrimination, and (e) host regulation [1].

For phorid parasitoids, host location involves the use of both habitat and host cues. Host habitat location is the use of environmental cues by the parasitoid to select areas to search for potential hosts. These cues may be directly related to the preferred environment of the host itself (e.g., volatiles from plants commonly used by hosts) or related to the parasitoid's general habitat preferences (light, temperature, and humidity conditions within a given area) [1]. The host location process also requires that a parasitoid use long-range cues to be directed to its' host. However, unlike host habitat location cues, these cues come directly from the host itself. Ants communicate interspecifically by using complex pheromones. These pheromones often act as host location cues for parasitoids as they can be both reliable (with volatile pheromones highly conserved within a species or

genus) and detectable (ants, being eusocial, live in relatively high densities, and can produce large volumes of volatile pheromones) for the parasitoid [15]. Once a phorid parasitoid has located a potential host through long-range cues, the parasitoid requires host acceptance cues to trigger the parasitoid's oviposition behavior. Short-range cues such as movement, host size, and contact chemical cues have all been implicated in triggering phorid fly oviposition [7, 16–26].

In addition to the cues that are required for overall host selection, host discrimination cues, used by parasitoids to detect and reject potential hosts that have been previously parasitized, can be present. While these cues are not necessary for parasitism, they can increase the likelihood of offspring success [1]. Parasitoids can also increase the success rate of their offspring through host regulation, whereby parasitoids manipulate their hosts to promote the development of the next generation of parasitoids. Host regulation can involve altering the physiology of the host to facilitate growth and development of egg, larvae, or pupae of the parasitoid or altering host behavior to optimize nutrient intake or location within the external environment [27].

This paper focuses on our current understanding of the process by which phorid flies successfully parasitize ants. We examine the wide variety of strategies and cues used by multiple species of phorid flies within three separate genera (*Apocephalus*, *Pseudacteon*, and *Neodohrniphora*) to successfully parasitize ant hosts.

## 2. Host Habitat Location

Parasitoid habitat preference is a major factor that determines where parasitoids will search for hosts and therefore which hosts will be successfully parasitized. Some hosts are selected not because they have a greater degree of inherent suitability but because they happen to be in an environment where parasitoid abundance is greater or where parasitoids are better able to detect cues released by their hosts [1]. Light levels affect attack rates of several species of phorid flies. For example, *Neodohrniphora tonhascai* and *Neodohrniphora elongate* both attack *Atta sexdens* at significantly higher rates when in high-light-level laboratory conditions [28]. Field experiments with *Pseudacteon litoralis* and *Pseudacteon tricuspis* which attack ants in the *Solenopsis saevissima* complex show that these species prefer lower light levels (i.e., just after sunrise and before sunset) and higher light levels (midday sun), respectively [23]. Analogously, lab experiments with *Pseudacteon curvatus* show that the flies attack *Solenopsis* spp. ants on darker backgrounds at greater rates than ants on white or light backgrounds [29]. *Pseudacteon* spp. phorids that attack the *Solenopsis saevissima* also display habitat preferences based on environmental factors such as temperature, rainfall, photoperiod length, sugar availability, wind, humidity, and number of days with frosts [30–32].

Habitat complexity also affects phorid fly attack rates. Two species of phorid flies, *Apocephalus* sp. 8 and *Apocephalus* sp. 25 attack their host ants (*Pheidole diversiphilosa* and *Pheidole bicarinata*, resp.) at higher rates when leaf litter is less complex, most likely because the ants are able to take better refuge in more complex leaf litter [33]. Further,

*Pseudacteon* spp. attack rate on *Azteca instabilis* is higher in coffee plantations with lower shade tree canopy complexity although the exact set of habitat variables that create a preference for lower shade complexity remain unclear [34].

## 3. Host Location

The long-range cues used by phorid flies to hone in on potential hosts have been examined in several phorid-ant relationships. Some phorids travel at least 10–20 m to reach hosts and possibly up to 50 m, thus host location cues are likely generally volatile compounds, which can be detected by parasitoids well beyond the visual range of their hosts [42]. While sound cues have the potential to be long range and have been documented in some non-phorid parasitoid-insect interactions, to date no phorid flies have been recorded to use sound as a cue in ant host location [3, 43]. Paralleling the rich diversity of volatile ant pheromones, chemical host location cues used by phorid flies can vary widely in structure, glandular origin, and purpose in ant-phorid relationship (Table 1). Long-range cues for phorids derive from several glands (mandibular, pygidial, etc.) and represent a wide array of pheromone types (trail, alarm, etc.). Several specific examples of these cues for different ant-phorid relationships follow.

The first set of host location cues documented for phorids were in the “giant tropical ant” *Paraponera clavata* attacked by the phorid, *Apocephalus paraponerae*. Parasitism of *P. clavata* by *A. paraponerae* was first observed in 1958 by C. W. Rettenmeyer on Barro Colorado Island, Panama. Rettenmeyer originally suspected that the flies were attracted to audible stridulations made by *P. clavata* individuals when alarmed. However, field observations showed that *A. paraponerae* were attracted to mandibular gland extracts of *P. clavata* that contain alarm pheromone [2, 4]. The two major products of the mandibular glands of *P. clavata*, 4-methyl-3-heptanol and 4-methyl-3-heptanone (characterized in [35]), were tested individually and both attract *A. paraponerae* [4].

Another species of phorid fly that utilizes the alarm pheromones of its host is *Pseudacteon brevicauda*. Studies show that these phorid flies are attracted to mandibular gland extracts of their host, *Myrmica rubra* [36]. Within these glands are 3-octanone, 3-nonanone, and 3-octanol [37]. The two ketones were found to attract *P. brevicauda* from a distance [36]. While the alcohol, 3-octanol, did not attract flies from long distances, it was found to increase the “alertness” of the flies at a closer range, possibly indicating its synergistic role in host location or a possible role in host acceptance; however, further observations are needed to confirm the role of this compound [36].

Formic acid, a relatively common alarm and defense compound from the venom glands of ants, is the primary host location cue attracting *Pseudacteon formicarum* to the ants *Lasius niger* and *Lasius emarginatus* [38]. The use of formic acid is relatively common in ants, and previously *P. formicarum* was thought to be one of the only phorid flies with multiple hosts because these flies frequently arrive to areas where a wide variety of ants using formic acid are aggregated. However, it was recently discovered that *P. formicarum* is specific to ants in the genus *Lasius*, rather than all

TABLE 1: Chemical host location cues used by phorid flies in search of ant hosts.

Phorid species	Ant species	Cue	Source	Ant use	Ref.
<i>Apocephalus paraponerae</i>	<i>Paraponera clavata</i>	4-Methyl-3-heptanol and 4-methyl-3-heptanone	Mandibular glands	Alarm pheromone	[2, 4, 35]
<i>Pseudacteon brevicauda</i>	<i>Myrmica rubra</i>	3-Octanone and 3-nonanone	Mandibular glands	Alarm pheromone	[36, 37]
<i>Pseudacteon formicarum</i>	<i>Lasius niger</i> and <i>Lasius emarginatus</i>	Formic acid	Venom glands	Alarm/defense pheromone	[38]
<i>Pseudacteon</i> spp.	<i>Azteca instabilis</i>	1-Acetyl-2-methylcyclopentane	Pygidial Gland	Alarm pheromone	[17, 39]
<i>Pseudacteon litoralis</i>	<i>Solenopsis saevissima</i> complex	Unknown	Unknown	Unknown use at disturbed mounds	[20]
<i>Pseudacteon wasmanni</i>	<i>Solenopsis saevissima</i> complex	Unknown	Unknown	Unknown use at disturbed mounds	[20]
<i>Pseudacteon obtusus</i>	<i>Solenopsis saevissima</i> complex	Unknown	Unknown	Unknown use on trails	[20]
<i>Pseudacteon borgmeieri</i>	<i>Solenopsis saevissima</i> complex	Unknown	Unknown	Unknown use on trails	[20]
<i>Pseudacteon nuicornis</i>	<i>Solenopsis saevissima</i> complex	Unknown	Unknown	Unknown use on trails	[20]
<i>Pseudacteon solenopsidis</i>	<i>Solenopsis saevissima</i> complex	Unknown	Unknown	Unknown use on trails	[20]
<i>Pseudacteon tricuspis</i>	<i>Solenopsis saevissima</i> complex	2-Ethyl-3,6-dimethylpyrazine	Mandibular glands	Alarm pheromone	[40, 41]

ants that use formic acid, which indicates that these phorid flies must use other shorter-range cues in addition to formic acid to locate their hosts [44].

Three species of *Pseudacteon* phorid flies [45] use compounds from the pygidial gland of their host *Azteca instabilis* as long-range host location cues. The pygidial gland of *A. instabilis* is the source of the alarm pheromone. At least one compound present within the pygidial gland of *A. instabilis*, 1-acetyl-2-methylcyclopentane, attracts one or more of these phorid fly species to their host [17, 39], but further research is necessary to determine if all three phorid species are attracted to the same compound or suite of compounds.

The *Solenopsis saevissima* complex has one of the largest groups of congeneric parasitoids recorded, with more than 18 *Pseudacteon* spp. known to parasitize this host group. However, despite significant research on these interactions, the details of the host location cues used in these interactions have remained somewhat elusive. In an early study, several of these phorids were categorized based on whether they were more likely to be found near disturbed ant mounds or trails—with the general hypothesis that phorid flies attacking ants near disturbed mounds must use alarm or defense compounds released by the ants as host location cues, and trail pheromone as a cue if they attack near trails. *Pseudacteon*

*litoralis*, *P. tricuspis*, and *P. wasmanni* were all found attacking predominately near disturbed mounds or, in a few circumstances, trails where aggressive interspecies interactions were taking place between the ants. *Pseudacteon obtusus*, *Pseudacteon borgmeieri*, *Pseudacteon nuicornis*, and *Pseudacteon solenopsidis* were more often found attacking ants on trails [20, 46]. In another set of studies, *P. tricuspis* was attracted to the midden (consisting primarily of dead workers) of *Solenopsis invicta*, lending further evidence to the hypothesis that its host location cue is a volatile chemical from the ants themselves [47, 48]. Additionally shaken workers both elicit an alarm response in other workers and attract phorid flies [49]. Electroantennogram (EAG) experiments with *P. tricuspis* show that the flies are attracted to whole body extracts of workers, ant heads (including, to some extent, the mandible alone), and abdomens [49]. The same study confirmed that *P. tricuspis* is not attracted to the trail pheromone of *Solenopsis invicta*, (E,E)- $\alpha$ -farnesene [49]. The mandibular glands located within the head of *Solenopsis* spp. ants are the source of the ant's alarm pheromone, providing evidence that *P. tricuspis* likely uses a set of (rather than an individual) alarm pheromone compounds as a host location cue [50, 51]. Recently, 2-ethyl-3,6-dimethylpyrazine has been confirmed as an active alarm pheromone component from within the

mandibular glands of *S. invicta* and EAG experiments shows that this compound elicits a response in *P. tricuspidis*, though the compound has yet to be tested in the field [40, 41].

Yet, not all ant-phorid relationships appear to involve long-range chemical cues. In behavioral observations of *N. elongata* phorid flies and *A. sexdens* ants using a 50 cm<sup>3</sup> observation chamber, Gazal et al. (2009) concluded that these phorids do not have a volatile chemical cue involved in host location [18]. However, it is possible that these cues are essential when phorids are at a greater distance from potential hosts and behavioral observations of ants and phorids in small and contained areas underestimate phorid specificity [52].

#### 4. Host Acceptance

Short-range cues used by phorid flies to inspect potential hosts and determine whether they are suitable for oviposition can be visual or chemical or in some cases both (Table 2). Visual cues are often multifaceted, including several simultaneous or sequential features such as movement, host size, and host shape. The chemical cues used in host acceptance are generally less volatile compounds that can only be detectable at close range.

Movement of target ants is a common visual cue frequently used by the *Pseudacteon* spp. phorid flies that attack both *A. instabilis* and ants in the *Solenopsis saevissima* complex as well as by *N. elongata* phorid flies attacking *A. sexdens* [16–19]. *A. paraponerae* attacking *P. clavata*, however, prefer stationary ant hosts [5].

Size is also an important factor in phorid host acceptance. Variation in size preferences between phorid species attacking the same host is generally seen as an effective method of niche partitioning [16, 20, 22, 25]. Within the guild of phorids that attack the *Solenopsis saevissima* complex, *P. curvatus*, *P. nudicornis*, and *P. obtusus* attack small workers, *P. tricuspidis* and *P. wasmanni* prefer medium-sized workers, *P. borgmeieri*, and *P. solenopsisidis* tend to attack medium to large workers, and *P. litoralis* attacks large workers [7, 16, 20–24]. Size of the phorid fly is to a great extent a function of host body size [25, 55]. In the case of *P. obtusus*, the small and large biotypes that are otherwise morphologically identical proved to be genetically distinct enough to be different species likely due to a variation in host size preference [56]. Moreover, in *P. litoralis* and *P. tricuspidis*, sex ratio is determined by the body size of the host, where larger host ants yield female offspring and smaller host ants yield male offspring [55]. Phorid flies in other genera also use size cues in host acceptance. *N. elongata* only attack *A. sexdens* foragers with a minimum head width of 1.6 mm, and *A. paraponerae* prefer large *P. clavata* workers [5, 53].

The complexity of the visual stimulus related to shape has also been implicated in host acceptance. For example, *N. elongata* will inspect (i.e., hover over) moving visual stimulus of varying degrees of complexity from simple to complex: one model mass sphere, two linked spheres, three linked spheres, a plastic ant model, and the host ant. Yet, the phorids only attack the most complex visual stimulus, which in the experiments was the host ant. Indeed, in this set of

experiments, movement was unnecessary to trigger inspection if the visual stimulus was identical to the host, indicating that movement may act as a secondary cue to shape or visual complexity cues in order to enhance the speed and accuracy of attacks in this species [18].

Two classes of short-range chemical cues have been identified in phorid-ant interactions, cuticular hydrocarbons and low volatility venom gland secretions. While *A. paraponerae* flies are equally attracted to untreated ants and ants treated with hexane to remove cuticular hydrocarbons, the flies significantly prefer to lay eggs in ants with cuticular hydrocarbons [5]. Recent work on three *Pseudacteon* spp. phorid flies [45] that attack *A. instabilis* ants also show that these phorid flies may use cuticular hydrocarbons in host acceptance. When cuticular hydrocarbons of other ant species were applied to live *A. instabilis* ants, these *Pseudacteon* spp. phorid flies were much less likely to attack the ants than *A. instabilis* ants that were coated in additional *A. instabilis* cuticular hydrocarbons [54]. In experiments using electroantennograms and y-tube olfactometer bioassays, *P. tricuspidis* flies used venom gland secretions of *S. invicta* in host acceptance. These experiments show that several piperidine alkaloids, which are present in the ant's venom glands and used in defense, act as short-range attractants [26].

#### 5. Host Discrimination

The ability for parasitoids to distinguish between unparasitized potential hosts and hosts that have been previously parasitized is evolutionarily favorable as offspring from the same species within a single host are at a competitive disadvantage [1]. In fact, many parasitic hymenoptera can distinguish between parasitized and unparasitized hosts. Hymenopteran parasitoids use a variety of inhibitory cues in host discrimination including internal and external host-marking pheromones, or visual cues such as oviposition wounds [1].

In contrast, dipteran parasitoids, including phorid flies, appear to have high rates of superparasitism within populations [6]. For example, incidences of superparasitism by *Neodohrniphora curvinervis* on *Atta cephalotes* ants are relatively high at 19% in one field study [53]. Superparasitism by *N. elongata* on *A. sexdens* has been reported at 29.4% self-superparasitism and 49.5% conspecific superparasitism in a study conducted under lab conditions [57]. However, behavioral observations also show that once a *A. sexdens* host ant is parasitized, it is significantly less likely to be parasitized again by *N. elongata*, indicating that *N. elongata* are able to discriminate between parasitized and nonparasitized host ants but may in some circumstances (e.g., lab conditions) choose to superparasitize a host. Thus, it appears, however these *N. elongata* do have some, however imperfect, form of host discrimination, that despite the cues [57]. Dipteran parasitoids such as phorid flies do not have the accessory glands commonly used by hymenopteran parasitoids to produce host-marking pheromones [6, 58]. Thus, while more work is needed to determine the mechanism, it seems most likely that at least some phorid flies use visual cues from the ants' oviposition wounds in host discrimination.

TABLE 2: Host acceptance cues used by phorid flies to choose ant hosts.

Cue modality	Phorid species	Ant species	Cue	Source	Ant use	Ref.
Visual	<i>Pseudacteon</i> spp.	<i>Solenopsis saevissima</i> complex	Movement	—	—	[16, 19]
	<i>Pseudacteon</i> spp.	<i>Azteca instabilis</i>	Movement	—	—	[17]
	<i>Neodohrniphora elongata</i>	<i>Atta sexdens</i>	Movement	—	—	[18]
	<i>Apocephalus paraponerae</i>	<i>Paraponera clavata</i>	No movement	—	—	[5]
	<i>Pseudacteon nuicornis</i>	<i>Solenopsis saevissima</i> complex	Small-sized workers	—	—	[16]
	<i>Pseudacteon obtusus</i>	<i>Solenopsis saevissima</i> complex	Small-sized workers	—	—	[16]
	<i>Pseudacteon curvatus</i>	<i>Solenopsis saevissima</i> complex	Small-sized workers	—	—	[16]
	<i>Pseudacteon tricuspis</i>	<i>Solenopsis saevissima</i> complex	Medium-sized workers	—	—	[16]
	<i>Pseudacteon wasmanni</i>	<i>Solenopsis saevissima</i> complex	Medium-sized workers	—	—	[16]
	<i>Pseudacteon borgmeieri</i>	<i>Solenopsis saevissima</i> complex	Medium- to Larger-sized workers	—	—	[16]
	<i>Pseudacteon solenopsidis</i>	<i>Solenopsis saevissima</i> complex	Medium- to Larger-sized workers	—	—	[16]
	<i>Pseudacteon litoralis</i>	<i>Solenopsis saevissima</i> complex	Larger-sized workers	—	—	[16]
	<i>Neodohrniphora elongata</i>	<i>Atta sexdens</i>	Minimum head width of 1.6 mm	—	—	[53]
	<i>Apocephalus paraponerae</i>	<i>Paraponera clavata</i>	Large workers	—	—	[5]
Chemical	<i>Apocephalus paraponerae</i>	<i>Paraponera clavata</i>	Cuticular hydrocarbons	—	Nest mate recognition	[5]
	<i>Pseudacteon</i> spp.	<i>Azteca instabilis</i>	Cuticular hydrocarbons	—	Nest mate recognition	[54]
	<i>Pseudacteon tricuspis</i>	<i>Solenopsis invicta</i>	Piperidine alkaloids	Venom glands	Defense pheromone	[26]

In other ant-phorid fly relationships, superparasitism has only been observed in laboratory experiments where the phorid flies were relatively contained and the phorid fly to individual ant ratio was higher than what would commonly be seen in the field. In a study with *Pseudacteon tricuspis* and *Solenopsis invicta*, laboratory experiments showed the rate of superparasitism to be approximately 15.4%; however, these results do not accurately reflect the rate of superparasitism under natural conditions [8]. Thus, more studies are needed to determine whether superparasitism occurs in the field and whether it is a density-dependent phenomenon potentially affected by colony size or ant behavior.

## 6. Host Regulation

While relatively little is known about how phorid flies, in general, may manipulate their host's physiology in order to optimize the development of their offspring, strides have been made to understand the role of host regulation of *S. invicta* by *P. tricuspis* phorid flies. Like many other dipteran parasitoids, the developing phorid flies build respiratory structures in order to access fresh air through a hole in the integument of the host ant's head capsule [8]. Additionally, developing *P. tricuspis* is suspected to affect the neurophysiology of its ant hosts, as parasitized ants have

altered behavior whereby they remain safely within the nest until just before the phorid larvae decapitate their hosts. Shortly before decapitation, ants will leave the nest, presumably to find a suitable location for the phorid fly to continue pupation and emerge [59]. However, much remains unknown about the mechanisms by which these behavioral changes manifest in their host. Furthermore, there is nothing known about how any other phorid species are able to affect the behavior or growth of host ant species.

## 7. Conclusions and Future Prospects

In order to successfully parasitize a host, phorid fly parasitoids must undergo a multistep process to detect and interpret a wide range of cues from their ant hosts. These cocktails of cues, each of which may vary in degrees of host specificity and timing of detection (sequentially or simultaneously), allow the flies to find suitable hosts in a complex environment. Researchers often study the interactions between phorid flies and their ant hosts in order to address the role of phorid flies as potential biological control agents of ants [30, 46, 60–66]. However, understanding these interactions could potentially shed light on evolutionary and ecological processes as well as provide a better understanding of multimodal communication.

Cues used by phorid flies are often traits considered to be highly conserved within the host species. These conserved traits are highly reliable and thus adaptive to phorid flies. Yet, little is known about how phorid use of these cues impacts the adaptive nature of these traits within ants. For example, *P. clavata* was originally thought to have no alarm pheromone responses, as these ants are relatively primitive and therefore independent outside of the nests, not requiring the assistance of their sisters during foraging. However, some studies indicate that *P. clavata* does have fairly developed intraspecific interactions during foraging [67, 68]. As previously mentioned, *A. paraponerae* use the alarm pheromones, 4-methyl-3-heptanol and 4-methyl-3-heptanone, to locate its hosts. Though more intensive investigation is required, it is possible that the use of alarm pheromone by *P. clavata* has been selected against in order to decrease parasitism. On evolutionary timescales, perhaps phorid use of chemical and visual cues has affected ant morphology, behavior, and chemical communication.

While phorid flies are ubiquitous and conspicuous users of ant cues, a wide variety of other organisms are attracted to ants [69, 70]. Considering the context-dependent nature of successful parasitism discussed above, it seems likely that multiple myrmecophiles are utilizing similar cues and may thus affect the parasitism process. Indeed, only considering pair-wise interactions between organisms rather than interactions between a network of multiple parties with distinct cue preferences, perceptions, and responses can be misleading. For example, competitive interactions between male hermit crabs affect mating strategy decisions of how male hermit crabs approach females [71]. Additionally, ant-*Acacia* mutualisms are now better understood based on the overall fitness benefits to the *Acacia* plants via a network of ant species rather than summing the effects of individual ant

species separately and, in coffee agroecosystems, the nuances of multiple insect-interactions give insight into the overall effects of coffee pests [72–75]. Thus, a network approach should be taken and future work should be conducted to elucidate how other ant symbionts may affect these phorid-ant interactions. Additionally, as phorid fly behavior is often dependent on a wide array of factors that may be altered in laboratory observations, more studies should be conducted in the field to verify the results of lab experiments.

Finally, phorid flies are often both ecologically relevant species and have remarkably diverse strategies for using diverse arrays of multimodal cues within a complex environment to successfully parasitize host ants [5, 17, 18, 26, 30, 39, 41]. Thus, phorid-ant interactions are ideal systems to bridge the gap between model organisms used in integrated pest management and model organisms used in understanding the behavioral ecology of multimodal cue use.

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## Review Article

# Biological Control of *Solenopsis* Fire Ants by *Pseudacteon* Parasitoids: Theory and Practice

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*Pseudacteon* parasitoids are potential biocontrol agents of invasive *Solenopsis* fire ants. *Pseudacteon* species that parasitize the invasive *S. invicta* Buren and *S. richteri* Forel have been introduced to, and naturally dispersed across, the southeastern USA, although there is no evidence yet that *Solenopsis* host ant populations have decreased. The ability of introduced *Pseudacteon* species to regulate *Solenopsis* populations will depend upon the relative importance of top-down effects in the recipient communities. In this paper, I examine the characteristics of the *Pseudacteon/Solenopsis* parasitoid/host system and evaluate the extent to which research findings are consistent with top-down control. Laboratory and field experiments evaluating *Solenopsis* population regulation have been equivocal, and overall the available evidence provides little support for strong top-down effects in this system. Competitive exclusion may occur among introduced *Pseudacteon* species, and future efforts at biological control are likely to be more efficacious if they focus on other types of natural enemies.

## 1. Introduction

Many species of *Pseudacteon* (Diptera: Phoridae) are parasitoids of *Solenopsis* (Hymenoptera: Formicidae) fire ants. Several species of *Solenopsis* fire ants are invasive pests and others have the potential to be [1]. High densities of the invasive *S. invicta* Buren in North America are usually attributed to an escape from natural enemies [2]. Much recent research has focused on the potential use of *Pseudacteon* parasitoids as classical biological control agents to regulate *Solenopsis* fire ant populations, particularly *S. invicta* and *S. richteri* Forel in North America. Two South American *Pseudacteon* species—*P. tricuspis* Borgmeier and *P. curvatus* Borgmeier—have been released at multiple locations and dispersed naturally across the southeastern USA. It is estimated that *P. tricuspis* now occurs in 65%, while *P. curvatus* may occur in as much as 90% of the invasive *S. invicta/S. richteri* range [3]. Two other species—*P. litoralis* Borgmeier and *P. obtusus* Borgmeier—have been established in localized areas, *P. cultellatus* Borgmeier has been recently released in Florida, and releases of additional species are planned [3, 4]. In addition to the direct effect of mortality, *Pseudacteon* phorids may have indirect effects on their *Solenopsis* hosts, affecting their

behavior and potentially putting the host species at a relative disadvantage with competing ants [5].

There have been many studies conducted on various aspects of the *Pseudacteon/Solenopsis* parasitoid/host system, and the literature is in need of an objective, critical review. There is great interest in whether the introduction of *Pseudacteon* phorids can regulate invasive fire ant populations, and if so, to what degree. I conducted original research on this system for a decade, but have more recently pursued other avenues of study. The success or failure (perceived or actual) of this biological control program has no bearing on my obtaining funding, promotion, or tenure. Thus, I am in a good position to conduct a knowledgeable, yet detached review. I do not attempt to review all the *Pseudacteon/Solenopsis* literature, but focus on the potential of introduced *Pseudacteon* parasitoids from South America to regulate population densities of host *Solenopsis* ants in their invasive range in North America, through both direct and indirect effects.

## 2. The Species

**2.1. The Host.** The genus *Solenopsis* contains about 185 described species worldwide; the ~20 *Solenopsis* species known

as “fire ants” are all native to the new world [1]. Three of these fire ants are invasive pests: *S. invicta*, the most notorious, is native to South America but has invaded North America, the Caribbean, Australia, Taiwan, and China [6]. *S. richteri*, also native to South America, has invaded the southeastern USA, where it has hybridized with *S. invicta* [7]. *S. geminata* (Fabricius), whose natural range spans southern North America to northern South America, has been found at numerous low latitude sites around the globe [7]. The vast majority of *Solenopsis* fire ant research has focused on these three species. All three are characterized by polymorphic workers, which live between 2 and 8 months, depending on worker size and temperature [8]. The massive fire ant literature has been summarized by Taber [7] and synthesized by Tschinkel [1].

One important aspect of *Solenopsis* biology that is relevant here is the seasonal cycle of population abundance. In the southeastern USA, *S. invicta* reaches peak abundances in midwinter, and the lowest worker numbers occurring in midsummer are only about half that of winter highs [9]. *S. invicta* above ground foraging activity is highest in summer, however, and lowest in winter when soil temperatures are too cold to forage [10, 11]. Thus, the availability of hosts to *Pseudacteon* parasitoids is greatest in the summer months, even though *S. invicta* absolute densities are near their lowest.

Beyond the seasonal oscillations in abundance, *Solenopsis* population size may vary with other factors. The *Solenopsis* species in question are disturbed habitat specialists [1, 12]. Disturbances come in all degrees, however, and across a broad scale either too much or too little disturbance may result in lower fire ant abundances [13]. Disturbance regimes undoubtedly vary temporally and result in variability in *Solenopsis* populations. Climatic events (i.e., droughts, floods, and unusually cold weather) may also affect *Solenopsis* abundances [14, 15]. Fluctuations in abundance due to a variable disturbance regime or such climatic events could either amplify or dampen the inherent seasonal oscillations.

Finally, *Solenopsis* fire ants have the propensity to rapidly increase in abundance. After removing all *S. invicta* from experimental plots in Florida, for example, *S. invicta* recolonized the plots and in only two years reached abundances similar to control plots [16]. It is against this background of wide and potentially variable fluctuations in host population size, in addition to a strong potential for colony growth, that the regulatory effect of *Pseudacteon* parasitoids must be evaluated.

**2.2. The Parasitoid.** Although a number of taxonomic issues remained unresolved, over 20 *Pseudacteon* species are known to parasitize *Solenopsis saevissima* complex fire ants in South America [17]. Similarly, more than 20 *Pseudacteon* species parasitize *Solenopsis geminata* complex fire ants from North America to northern South America [18]. The basic biology and natural history of *Pseudacteon* phorids that parasitize *Solenopsis* fire ants have been summarized by Porter [19] and Morrison [20]. The life cycle of *Pseudacteon*, in brief, is as follows. A female *Pseudacteon* hovers near *Solenopsis* worker ants and inserts eggs into the thorax of hosts in aerial attacks with a specialized ovipositor. Three larval instars—the

second of which migrates to the head—precede pupation. At pupariation, the worker is killed by the parasitoid consuming all the tissue inside the head capsule, which is then used as a pupal case. Development from egg to adult takes from 5 to 12 weeks, depending upon temperature and the *Pseudacteon* species. *Pseudacteon* are solitary parasitoids, with only one larva able to complete development in each host. Each female *Pseudacteon*, however, may produce >200 eggs [21].

### 3. Theory

Whether or not *Pseudacteon* parasitoids control or regulate population densities of *Solenopsis* fire ants can be thought of as a function of the relative importance of top-down versus bottom-up effects in the communities in question. In food web terminology, bottom-up effects occur when the abundance of a resource affects the population of the consumer of that resource. The higher the abundance of resources at lower trophic levels, the higher the abundance or diversity that can be obtained at higher trophic levels. Top-down effects, on the other hand, occur when the population density of a consumer affects the abundance of its resource. Top-down control refers to the situation where the abundance or diversity of lower trophic levels is dependent on effects from consumers at higher trophic levels [22]. There has been much discussion in the literature over the relative importance of top-down versus bottom-up effects in arthropod communities, including the seasonal and spatial variability in such effects [23–29].

In a community with strong top-down effects, *Solenopsis* populations would be regulated by *Pseudacteon* parasitoids (or other predators or parasites). In contrast, in a community with strong bottom-up effects, *Solenopsis* populations would ultimately be regulated by the food resources available to them, and simply support *Pseudacteon* populations but not be controlled by them. Larger *Solenopsis* populations could support larger *Pseudacteon* populations. Obviously, biological control of host ants requires a system with relatively strong (and consistent) top-down effects.

Interspecific competition has traditionally been viewed as the primary mechanism organizing ant communities and limiting ant populations [30]. It has been suggested, however, that top-down processes such as parasitism may also play an important role in some ant communities [31]. Here, I make no attempt to evaluate the importance of parasitoids to ant communities in general, but rather to determine which characteristics of the *Pseudacteon/Solenopsis* parasitoid/host system are consistent with top-down control. I refer to the prevalence of such top-down effects as “strong control,” in reference to the goal of regulating invasive *Solenopsis* populations.

The relative importance of top-down effects can be illustrated by comparing two scenarios: in the first scenario—“strong control”—top-down effects prevail. This scenario is characterized by (1) a diversity of *Pseudacteon* species that exert a broad range of parasitism pressure on host *Solenopsis* ants, (2) consistently high abundance and activity of *Pseudacteon*, (3) high rates of mortality resulting from parasitism, (4) a lack of refuge for, or ability to behaviorally adapt in,

host ants, and (5) shifting of the outcome of interspecific interactions with competing ants. The second scenario, which I term “weak control”, is characterized by (1) low diversities of *Pseudacteon* species, (2) low or fluctuating abundance or activity of *Pseudacteon*, (3) low rates of mortality resulting from parasitism, (4) the presence of a refuge or the ability to behaviorally adapt by host ants, and (5) little or no effect on the outcome of interspecific interactions with competing ants.

These two scenarios more appropriately represent the ends of a continuum rather than two mutually exclusive states (or because multiple characteristics are involved, the margins of a multidimensional space). Moreover, the characteristics of each scenario are largely independent of each other. Evaluation of recent research results relative to these scenarios allows for a greater understanding of the degree to which *Pseudacteon* parasitoids may control or regulate population densities of *Solenopsis* fire ants.

Given the high background population fluctuations of host *Solenopsis*, the potential effects of *Pseudacteon* as described in the two scenarios above are illustrated as a conceptual model in Figure 1. In the strong control scenario (Figure 1(a)), broad parasitism pressure (direct and indirect) depresses fire ant populations consistently over time, resulting in peaks and troughs of fire ant population cycles that are lower than without the parasitoids. The mean *Solenopsis* abundance over time is also lower.

In the weak control scenario (Figure 1(b)), parasitism pressure is weak and may only affect host ant populations seasonally, or is otherwise greatly limited in intensity. *Pseudacteon* populations also fluctuate, reaching their highest abundances and thus exerting peak parasitism pressure in the fall, due to greater host availability in the summer (because of higher above-ground foraging activity by the ants). Because of low rates of mortality due to parasitism and the ability of ants to adapt behaviorally and lessen the indirect effects, colony fitness is only slightly affected, and this decrease comes at a time when overall colony size is peaking. Because of the overall cyclical nature of *Solenopsis* abundance and the added stochastic effects of disturbance and climate (depicted in this figure as irregular seasonal oscillations), the impacts of *Pseudacteon* may be relatively small. Over the long term, such effects may be washed out by the greater population variability due to other factors. Under optimum disturbance intensity and climatic conditions, host ants may regain previous population peaks. In this scenario, the long-term average *Solenopsis* abundance or range of fluctuations may change relatively little due to *Pseudacteon*.

## 4. The Evidence

**4.1. Diversity of *Pseudacteon* Species.** In most locations that have been studied, multiple *Pseudacteon* species have been found. This is true for *Solenopsis saevissima* complex fire ants in South America and *Solenopsis geminata* complex fire ants in North America [17, 32–35]. Host ants are partitioned among *Pseudacteon* species along several axes, including size of worker [36–38], host location preferences [33, 39], and time of day [35–40]. Thus multiple *Pseudacteon* species

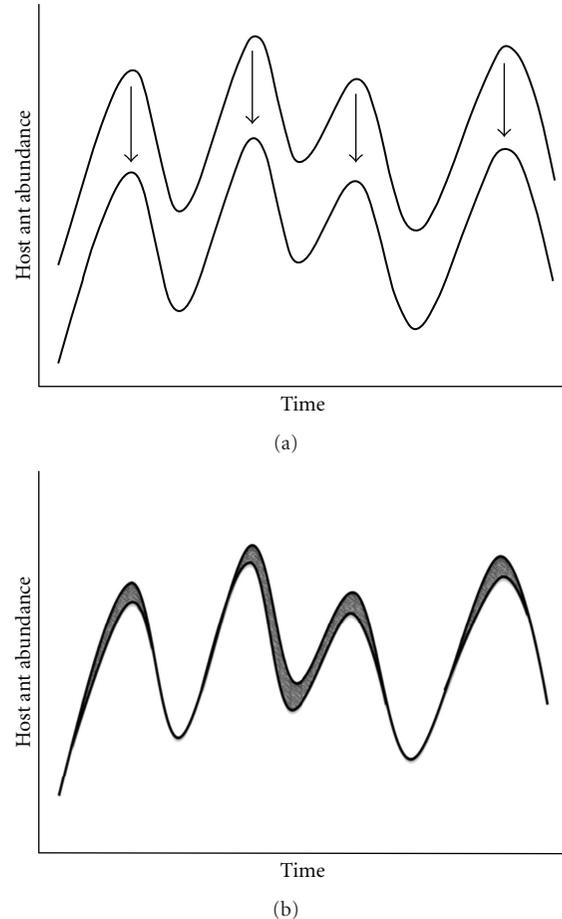


FIGURE 1: Two hypothetical scenarios of the potential effects of introduced *Pseudacteon* parasitoids on *Solenopsis* fire ant populations. (a) Strong control: parasitism pressure depresses fire ant populations consistently over time, resulting in peaks and troughs of fire ant population cycles that are lower than without the parasitoids. (b) Weak control: parasitism pressure primarily affects host ant populations seasonally, or to an otherwise relatively small extent, and ants rebound under optimum growth conditions. (Shaded areas indicate the amount of decrease due to the parasitoids.)

attack a greater size range of workers, engaged in a wider diversity of activities, over a longer period of time than a single *Pseudacteon* species would.

Although multiple *Pseudacteon* species may cooccur at a site, most species are usually relatively rare. This is also true both in North America [5, 34] and South America [32, 33, 35, 41–43]. The niche segregation observed is likely the result of competition among *Pseudacteon* species for hosts. There is now evidence that introduced South American *Pseudacteon* species are competitively displacing each other in North America [4, 44, 45], and a reanalysis of abundance data from South America suggests competitive exclusion exists there as well [44].

**4.2. Abundance and Activity of *Pseudacteon* Species.** Adult *Pseudacteon* live for only a few days [46], and reveal relatively great variability in abundance over time [34, 41, 47–49]. In

tropical and subtropical climates, *Pseudacteon* species are active year round, although relative abundance varies seasonally [32, 35, 41, 49]. *Pseudacteon* species are active from dawn to dusk, but diel variation exists among species [35]. In more temperate zones, flight activity of *Pseudacteon* is limited by cool air temperatures and adults may not be active in the winter months [34].

Introduced *P. tricuspsis* populations in the southeastern USA reveal cyclical patterns of seasonal abundance, with peaks in the fall and troughs in the spring [48, 50, 51]. *Solenopsis* above ground foraging activity, and thus host availability is highest in summer. Thus *P. tricuspsis* densities track host ant availability (with some lag time to be expected based on the 5–12-week-long development cycle) and greater availability of hosts (i.e., resources) is correlated with higher parasitoid (i.e., consumer) populations. Abundance patterns of *P. curvatus*, however, may differ, at least in some areas [S. D. Porter, unpublished data].

**4.3. Mortality due to Parasitism.** Parasitism rates (i.e., the percentage of *Solenopsis* workers in a colony infected with a *Pseudacteon* egg or larva at a given point in time) have been reported to be very low in a number of studies. In Texas, *S. geminata*—hosts of native North American *Pseudacteon* species—had parasitism rates of <3% [36]. Parasitism rates of *Solenopsis invicta* in its native range in Argentina by native South American *Pseudacteon* species were similarly <3% [35]. Parasitism rates of *Solenopsis invicta* in its exotic range in Florida from introduced *Pseudacteon* are generally  $\leq 2\%$  [48, 52, S. D. Porter, unpublished data]. These observed rates are an order of magnitude below the lowest parasitism rates associated with successful biological control programs (>30%) [53, 54]. These rates for successful biocontrol for other types of parasites, however, are based on the direct effect of mortality only.

Workers that are parasitized by *Pseudacteon* are not a random assortment from the colony, but rather represent primarily older ants with a shorter life expectancy. Fire ants exhibit a division of labor, in which the particular task engaged in by a worker depends on the age and size of that worker. In general, younger workers engage in relatively safe activities within the central colony, whereas older workers are found near the periphery of the nest, and the oldest workers engage in the most dangerous activity: foraging [55]. Mortality rates of foragers may be as high as 5% *per day* [1]. Because of the high mortality rates associated with foraging activities in ants in general, foragers have been described as a “disposable caste” [56].

*Pseudacteon* phorids attack host workers involved in foraging, interspecific interactions, and colony defense—all relatively dangerous activities. Thus most of the workers parasitized are engaged in high-risk activities and near the end of their natural lives, and colony fitness is affected to a lesser degree than if workers were parasitized at random with respect to age.

**4.4. Host Ant Refuges and Behavioral Adaptations.** A primary focus for research into the indirect effects of *Pseudacteon* has

been the reduction of foraging in host ants in the presence of these parasitoids. A number of studies involving different *Pseudacteon/Solenopsis* combinations in both North and South America have revealed that in the presence of these parasitoids, worker behavior changed and foraging at rich food resources dramatically diminished [5, 57–61]. The size of foraging *Solenopsis* workers has also been observed to decrease in the presence of *Pseudacteon* [52–64]. These studies, however, have almost exclusively focused on short-term effects of phorids at rich food resources, characterized by the recruitment of many workers in the absence of any shelter or refuge for the host workers and during ideal conditions for *Pseudacteon* flight activity.

Yet foraging in these *Solenopsis* species occurs under a great variety of conditions. In other words, these ants are characterized by a relatively broad foraging niche (*sensu*, [65]). They may forage by day or night [5, 66], as long as the soil temperature is at least 15°C [10, 11]. *Solenopsis* fire ants are omnivorous, with a very catholic diet [67, 68]. They excavate elaborate underground tunnel systems [69], and some unknown proportion of their food may be derived from underground sources (i.e., plant roots or root homopterans) [67]. The range of food items and types varies greatly; they may retrieve small items individually, but many workers may recruit to larger or long-lasting resources.

In contrast, *Pseudacteon* flies are not active after dark. Their aerial mode of attack makes it impossible to attack workers in underground tunnels (worker ants will quickly kill *Pseudacteon* flies if they can catch them). Additionally, *Pseudacteon* are unlikely to affect foraging of items that can be retrieved individually, although this has not been carefully studied. Some *Pseudacteon* species may not even affect foraging of relatively rich food resources if these are uncontested. Introduced *P. tricuspsis* in North America, for example, were not attracted to workers foraging at such resources, unless involved in interspecific interactions with other ants [39]. *P. tricuspsis* is attracted to the alarm pheromones and venom alkaloids that are typically released in such interactions [70, 71].

*Pseudacteon* native to North America are not active when air temperatures drop below 20°C [34], although some species in South America are active down to 14°C [41]. Thus there may be a narrow temperature zone in which it is warm enough for above-ground *Solenopsis* foraging, but not for *Pseudacteon* activity, although this appears to vary geographically and by species. Thus, *Pseudacteon* phorids will affect some unknown fraction of the overall broad spatiotemporal foraging niche of *Solenopsis*.

Furthermore, although the reduction in food obtained from such rich food resources may appear very large in the short term, in the longer term *Solenopsis* ants may be able to adapt behaviorally and still obtain much of the resource in question, if not removed by competitors. Field studies have shown that although forager numbers may decrease, *Solenopsis* workers do not usually completely abandon food resources in the presence of *Pseudacteon*, but some workers remain behind to guard the resource [34, 47, 59]. Foraging has even been observed to rebound to earlier levels after a depression by *Pseudacteon* [33]. Workers have been observed tunneling

beneath a rich food source and covering it with dirt and debris [5, 47].

Although most laboratory studies have constrained foraging to occur only in the presence of *Pseudacteon*, the design of one laboratory study allowed a colony of *S. invicta* to forage simultaneously in one arena with *P. tricuspis* and in a second arena without the parasitoid. Although less food was retrieved from the arena with *P. tricuspis*, the sum of food obtained from both foraging arenas was not different from that obtained in control trials without parasitoids [61]. Thus the colonies were able to compensate for decreased food retrieval caused by *P. tricuspis* harassment by simultaneously increasing food consumption when food was available elsewhere.

**4.5. Effects on Interspecific Interactions.** *Pseudacteon* species were never expected to have large direct effects of mortality on *Solenopsis* ants, and the expectation of this parasitoid's ability to regulate host ant populations was based primarily on the indirect effect on host behavior. These short-term behavioral effects would mean relatively little to colony fitness if they merely represented a delay in obtaining food resources. If, however, other ant species are able to secure a competitive advantage due to *Solenopsis*' parasitoid evasion behavior and are able to obtain relatively more food resources, then *Pseudacteon* could play an important role in mediating overall ant community dynamics and species abundance relationships.

Orr et al. [60], working in South America, observed *S. invicta* to lose food resources to competing ants in the presence of *Pseudacteon* phorids. Other studies, however, have found that host *Solenopsis* workers under *Pseudacteon* attack often did not lose control of the resource to competing ants. Studies of *Pseudacteon* parasitoids specific to *S. geminata* in Texas revealed that the presence of the parasitoids had no effect on the outcome of interspecific interactions involving *S. geminata* [5, 47]. A comparative study in Brazil revealed that *S. invicta*-specific *Pseudacteon* had no effect on the outcome of interspecific competition between *S. invicta* and other ants at two of three sites [72]. Thus, based on the available evidence, the ability of parasitoids to affect the outcome of interference competition among ant species appears to be too weak to be scientifically documented in many communities.

## 5. Long-Term Experiments

**5.1. Laboratory Experiments.** It has proven difficult to provide evidence for the success (or failure) of introduced *Pseudacteon* species in regulating host *Solenopsis* ants at the population level. Most evidence for a *Pseudacteon* effect, as discussed above, comes from short-term behavioral studies (minutes to hours in duration). In a relatively long-term lab study (28 days) incorporating both *Pseudacteon* phorids and a competing ant, *Forelius pruinosus* (Roger), Mottern et al. [73] reported a reduction in foraging in *S. invicta* due to *Pseudacteon* harassment, but no change in the colony growth rate of *S. invicta*. It is possible that the duration of this experiment was too short, or the method used to measure colony growth (i.e., photographing brood piles) was not precise

enough. The most likely explanation, however, is that ants were allowed to forage beyond the period that *Pseudacteon* attacked, and *Solenopsis* was able to compensate by increasing food retrieval when *Pseudacteon* were not active. Mottern et al. [73] state that such conditions would be "representative of those found in nature." No change in the growth rate of the competing ant was observed either, although this was not surprising because *F. pruinosus* never entered the communal foraging chamber.

In a longer lab study (50 days), Mehdiabadi and Gilbert [74] documented that the reduction of foraging in *S. invicta* due to *Pseudacteon* harassment did, as expected, eventually result in reduced colony fitness. In that study, the presence of *P. tricuspis* reduced the abundance of middle-sized workers, but not small-or large-sized workers. Worker ants, however, were always constrained to forage in small trays for limited periods in the presence of *P. tricuspis*, without any refuge or potential to adapt their foraging behavior. It is noteworthy that a combination of *P. tricuspis* phorids and a competing ant—this time *Forelius mccooki* (McCook)—had no greater effect on colony fitness than the competing ant alone [74]. As in the Mottern et al. [73] study, no increase was observed in the reproductive output of the competing ant [75]. (Interestingly, Mottern et al. [73] criticized the statistical analysis of Mehdiabadi and Gilbert [74], claiming that no significant differences existed for any of their treatments!)

Thus, the ambiguous results of these laboratory experiments provide little empirical support for the idea that *Pseudacteon* phorids could mediate competitive interactions that would ultimately lead to a decrease in *Solenopsis* populations, while allowing for a relative increase in competing ant populations. The general problem with such laboratory experiments is that the design can greatly influence the outcome. Given the complexity of fire ant foraging and the multitude of interactions with other species, any community-level laboratory experiment is destined to be an oversimplification of the natural world with limited inference.

**5.2. Field Experiments.** Field experiments, while more realistic, have their own limitations, in this case primarily logistical. Fire ant populations are undoubtedly affected by many factors, and while many of these variables can be controlled for in the laboratory, attempting to isolate the effect of one factor in the context of a broad field experiment is very difficult. Moreover, introduced *Pseudacteon* species spread naturally at a rapid rate; *P. tricuspis* dispersed at rates of up to 30 km/year for the first few years after establishment in north Florida [76], and at rates of up to 57 km/year over the following four years [77]. After three and a half years, *P. curvatus* had dispersed even farther in Florida than *P. tricuspis* did over the same period after initial release [78]. In Texas, small satellite populations of *P. tricuspis* have been found tens of km beyond the main expansion front; this jump dispersal was probably assisted by the prevailing winds [79].

Thus it is difficult to have true control sites that are not colonized within the time course of an experiment. Control sites would have to be placed so far away that there could be systematic differences in environmental variables between the treatment and control sites. This is almost a moot point,

as by now the vast majority of the invasive range of *Solenopsis* in the southeastern USA is estimated to have been colonized by at least one introduced *Pseudacteon* species [3]. Because of the tiny size of *Pseudacteon*, it is impractical to attempt to construct enclosures or exclosures, as these would include or exclude almost all other species (except microscopic ones).

The only published field experiment—including control plots (albeit 2 counties away) and spanning relatively large spatiotemporal dimensions—failed to find any measurable effect of introduced *P. tricuspidis* on *S. invicta* in Florida [50]. The study was ended after 3 years when *P. tricuspidis* dispersed to control plots. Relatively large variabilities were observed in fire ant activity and abundance, however, in both treatment and control plots. Thus the effects of this parasitoid would have had to be relatively large (perhaps reducing host ants by as much as 30%) to be detectable [50].

Studies are underway to gauge impact by comparing fire ant abundances before and after the introduction of *Pseudacteon*, in the absence of any control sites [3]. Such comparisons could be misleading, however, and should be interpreted with extreme caution. Fire ant abundances can and do change in response to many factors. *S. invicta* abundance, for example, was found to decrease by almost an order of magnitude over 12 years at a Central Texas site [80]. *Pseudacteon* species had not become established in Central Texas at the time, although other natural enemies of fire ants were present [80]. Additionally, numerous pathogens of *Solenopsis* fire ants are now known to be present in North America, and many have relatively high infection rates [52, 81, 82]. Finally, broad scale trends (i.e., climate cycles or directional change) may affect fire ant abundances independently of parasitoids.

## 6. Synthesis

**6.1. Summary of the Evidence in Relation to Theory.** Multiple *Pseudacteon* species frequently cooccur, although usually only one or a few species are very abundant. Overall, the *Pseudacteon* assemblage present at a given location may reach relatively high abundances at times, although populations fluctuate, and in the case of the introduced *P. tricuspidis* in Florida, in synchrony with host availability. Parasitism rates are usually very low, and most workers parasitized are probably near the end of their natural lives, so this direct effect of parasitism may be almost negligible. Host ants may engage in much of their foraging in the absence of *Pseudacteon* flies and have the ability to adapt their behavior in the presence of this parasitoid, so that in the long term, the overall reduction in resource retrieval is likely much less than that suggested in short-term observations. Finally, *Solenopsis* species often do not lose control of rich food resources to competing ants in the presence of *Pseudacteon*.

Thus the available evidence suggests that any impacts of *Pseudacteon* phorids on host ant populations are generally small, especially when measured over the relatively large population variability of *Solenopsis* fire ants. Moreover, given the ability of *Solenopsis* to rapidly increase in population size under ideal conditions, any depression in *Solenopsis* populations by *Pseudacteon* phorids could be ephemeral if parasitism pressure is not consistent. Thus, the effects are probably

much closer to the “weak control” scenario described above, although such effects could vary geographically and temporally. Experimental assessments of the impact of introduced *Pseudacteon* species have been few, and the results equivocal, although certainly no large impacts have been documented with any scientific rigor. Unfortunately, due to the constraints described above, we may never have a reliable, precise estimate for the effect of *Pseudacteon* parasitoids on *Solenopsis* fire ant populations in nature.

Thus the available evidence provides little support for strong top-down effects in this system. The accumulated data reveal that introduced *P. tricuspidis* in North America are positively correlated with *S. invicta* availability, both temporally [48, 50, 51] and spatially [51]. These findings are not inconsistent with the hypothesis that larger *Solenopsis* fire ant populations simply support higher abundances of *Pseudacteon* parasitoids, and that *Solenopsis* populations are primarily regulated by other factors.

**6.2. Host Specificity and Knowledge Gaps.** Although *Pseudacteon* phorids may have relatively small effects on fire ant populations, they possess two very desirable qualities of a biocontrol agent. They have been documented to be highly host specific, in a battery of tests conducted: (1) in the field in South America [83, 84], (2) in the lab prior to the release of South American *Pseudacteon* species to North America [85–88], and (3) in the field in North America after the establishment of introduced populations [89, 90]. Thus, *Pseudacteon* phorids appear to be safe (i.e., no documented adverse effects on any other species), which is the most important quality of any biocontrol agent. Additionally, once established, *Pseudacteon* species will persist as a permanent component of communities in which host *Solenopsis* are found, and naturally disperse to others.

Several aspects of *Pseudacteon/Solenopsis* interactions need more study. Some *Pseudacteon* are attracted to mound disturbances [33, 39], although the potential effects of *Pseudacteon* on mound disturbances are less clear than those of interruption of foraging. The presence of *Pseudacteon* may delay the mound rebuilding process, but only for a matter of hours, until *Pseudacteon* activity ceases with darkness. The mound is important in regulating brood temperature and thus development [91], and movement of brood out of the mound due to disturbance and delayed reconstruction could adversely impact colony fitness, although such an effect seems ephemeral and thus relatively small. The frequency of natural mound disturbance events is an important variable in assessing this impact, and this disturbance regime no doubt varies geographically.

Morrison and King [39] found that numerous *P. tricuspidis* flies were attracted to *S. invicta* mound disturbances when nonnestmate *S. invicta* were added, resulting in interspecific interactions, but on average fewer than one parasitoid was attracted in the absence of such interactions. Thus, most mound disturbances in the absence of interspecific interactions would probably be little affected by *P. tricuspidis*, although other *Pseudacteon* species may differ in this aspect of their behavior. *Pseudacteon* are also attracted to *Solenopsis* mating flights [92]. Although *Solenopsis* reproductives are

not suitable hosts [19], workers are very active on the mound surface before the reproductives take flight [93], and the presence of *Pseudacteon* could dampen this activity, potentially disrupting the mating flight. Large numbers of workers may be vulnerable to parasitism during mating flights or mound reconstruction, and such workers may be younger than workers engaged in foraging, thus representing a potential greater loss to overall colony fitness. Finally, *Pseudacteon* are carriers of pathogens that infect *Solenopsis* [94], although actual vectoring of diseases among colonies has not been demonstrated.

**6.3. Implications and the Bigger Picture.** Efforts are underway to introduce additional South American *Pseudacteon* species to North America. The question is how many additional species should be introduced? Because of the relatively high degree of niche partitioning observed in *Pseudacteon* species in South America, and the coexistence of multiple species at a site, the traditional wisdom has been that multiple *Pseudacteon* species will coexist at North American release sites, and that more *Pseudacteon* species will exert greater parasitism pressure on host *Solenopsis* ants. The species abundance patterns of *Pseudacteon* in both North and South America, and the recent, relatively unexpected finding of competitive exclusion among introduced *Pseudacteon* species, however, suggest only one or two introduced species may be abundant at any given location. Thus the introduction of additional *Pseudacteon* species may simply reduce the abundance of already-established species, without substantially increasing the density of the overall *Pseudacteon* assemblage.

An argument can be made that multiple *Pseudacteon* species are necessary because of the diversity in invasive *Solenopsis* populations in North America. Both *S. invicta* and *S. richteri* (and a hybrid) are present, and colonies may be either polygyne (i.e., multiple queen) or monogyne (i.e., single queen) [1]. There have been attempts to “match” or find the best combination of South American *Pseudacteon* species or biotypes with North American *Solenopsis* populations [95]. Ultimately, given the speed of dispersal of this parasitoid, after introductions of multiple *Pseudacteon* species to multiple locations in the *Solenopsis* invasive range, the flies may eventually sort themselves out. Not all *Pseudacteon* species may disperse at the same rate as *P. tricuspis* and *P. curvatus*, however. *P. litoralis* has spread much more slowly from a single release site in Alabama [4]. Although *Pseudacteon* species appear to have no detrimental effects, the time and effort involved in evaluating, rearing, and releasing these flies might be better spent evaluating other types of natural enemies. At this point, it seems likely that the marginal contribution of each additional *Pseudacteon* species released will be diminished as more species are added.

In South America, a diversity of parasites, pathogens, predators, and competitors affect *S. invicta* [1, 96]. Thus, introduction of a single type of natural enemy is unlikely to result in outsized reductions of invasive *Solenopsis* populations. It is more likely that regulation is incremental, and that each type of introduced natural enemy may have a relatively small effect, yet one that is cumulative so that overall

control becomes greater with the addition of more types of natural enemies. Thus, the continued search, evaluation, and introduction of other safe (i.e., host-specific) natural enemies of *Solenopsis* fire ants may eventually lead to measurable levels of fire ant population regulation.

Given the general lack of evidence for strong top-down control in this system, it is possible that other ants represent the greatest natural enemies of *Solenopsis*. The South American ant fauna contains more species that are strong competitors of *S. invicta* than does the North American fauna [72, 97, 98]. Many of these ant species would have their own deleterious impacts to recipient biotas, perhaps even greater than *Solenopsis* fire ants, and introductions of such natural enemies are not seriously contemplated. If competition from other ants is the primary reason that *S. invicta* is less abundant in South America, it follows that introductions of all possible other types of natural enemies will not result in a decrease of North American *S. invicta* or *S. richteri* populations to South American levels.

The conventional wisdom of *Pseudacteon* biological control is that the presence of these parasitoids may shift the competitive balance away from *Solenopsis* fire ants to native ant species, allowing for a relative increase in abundance of the native species at the expense of the invasive *Solenopsis*. Recent work on the effects of disturbance, however, challenges the conventional wisdom that *Solenopsis* fire ants are strong competitors that have displaced native ants primarily due to a competitive asymmetry. King and Tschinkel [99] obtained experimental evidence suggesting that native ants are first displaced as a result of habitat disturbance, and then *Solenopsis* fire ants—which are disturbed habitat specialists—move into the disturbed areas. Experimentally removing *S. invicta* from disturbed areas did not result in an increase of native species [100]. In this study, conducted in forest habitat in Florida, “disturbance” resulted in the simplification of habitat structure to a type that was more similar to the habitat where *S. invicta* is native (i.e., open areas with high insolation). Thus, at least in some areas, disrupting the behavior of *Solenopsis* fire ants or even reducing their abundances may have limited effects on native ant diversity, in the absence of restoring disturbed or simplified habitats.

Habitat type or disturbance alone, however, cannot adequately explain the high abundances of invasive *Solenopsis* fire ants in North America. *Solenopsis* species were found to be more abundant at the same type of disturbed (i.e., roadside) sites in North America relative to South America [2]. Thus the abundance of *Solenopsis* fire ants in an area is likely the result of a number of factors (and interactions of factors), including habitat type, degree of habitat disturbance, and the presence of natural enemies. Ultimately, efforts to reduce invasive fire ant densities would probably benefit by taking a broad perspective and include attempts at habitat restoration in addition to the introduction of an array of natural enemies.

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## Review Article

# Positive-Strand RNA Viruses Infecting the Red Imported Fire Ant, *Solenopsis invicta*

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The imported fire ants, *Solenopsis invicta* and *S. richteri* were introduced into the USA between 1918 and 1945. Since that time, they have expanded their USA range to include some 138 million hectares. Their introduction has had significant economic consequences with costs associated with damage and control efforts estimated at 6 billion dollars annually in the USA. The general consensus of entomologists and myrmecologists is that permanent, sustainable control of these ants in the USA will likely depend on self-sustaining biological control agents. A metagenomics approach successfully resulted in discovery of three viruses infecting *S. invicta*. *Solenopsis invicta virus 1* (SINV-1), SINV-2, and SINV-3 are all positive, single-stranded RNA viruses and represent the first viral discoveries in any ant species. Molecular characterization, host relationships, and potential development and use of SINV-1, SINV-2, and SINV-3 as biopesticides are discussed.

## 1. Introduction

The black imported fire ant (*Solenopsis richteri*) and red imported fire ant (*S. invicta*) were thought to have been introduced into the United States in 1918 [1] and sometime between 1933 and 1945 [2], respectively. *S. invicta* has clearly emerged as the most successful of the two ant species, largely displacing and relegating *S. richteri* to a roughly contiguous area in eastern Mississippi, western Alabama, and western Tennessee [2]. In contrast, since its introduction, *S. invicta* has expanded its range to infest more than 138 million hectares (Figure 1) from Virginia, south to Florida, and west to California [3]. Although both of these fire ant species are invasive, *S. invicta* is by far the most successful and considered the major pest species in the USA. Thus, in the strictest sense, the term “imported fire ants” (in the USA) refers to both *S. invicta* and *S. richteri*. However, in reality, efforts to study, understand, and control imported fire ants are focused nearly completely on *S. invicta*.

Introduction of these ants into the USA has had significant economic consequences. Damage attributed to *S. invicta* is quite diverse, including, physical damage to agricultural

commodities, livestock, and equipment, infrastructure (e.g., roads and electrical equipment), negatively impacting biological diversity, and even human health [4]. Costs associated with damage and control efforts are estimated to cost 6 billion dollars annually in the USA [5]. Although a number of highly effective insecticides are available to control *S. invicta* and *S. richteri*, they must be used regularly to provide sustained control. If insecticide use is discontinued, fire ant populations invariably re-inhabit these previously treated areas. In addition, because fire ants are so ubiquitous within the infested region, insecticide-based control is impractical, from both environmental and economic standpoints.

A number of comparative ecological studies have demonstrated that *S. invicta* nest density, nest volume, and population density compared with other ant species in the community are significantly greater in the USA compared with South America where the ant is native [6, 7]. These differences have been attributed to a lack of natural enemies in the USA as a result of a bottleneck event at the time of introduction [7]. The enemy release hypothesis [8] states that introduced species arrive without their complement of natural enemies, and release from these organisms confers

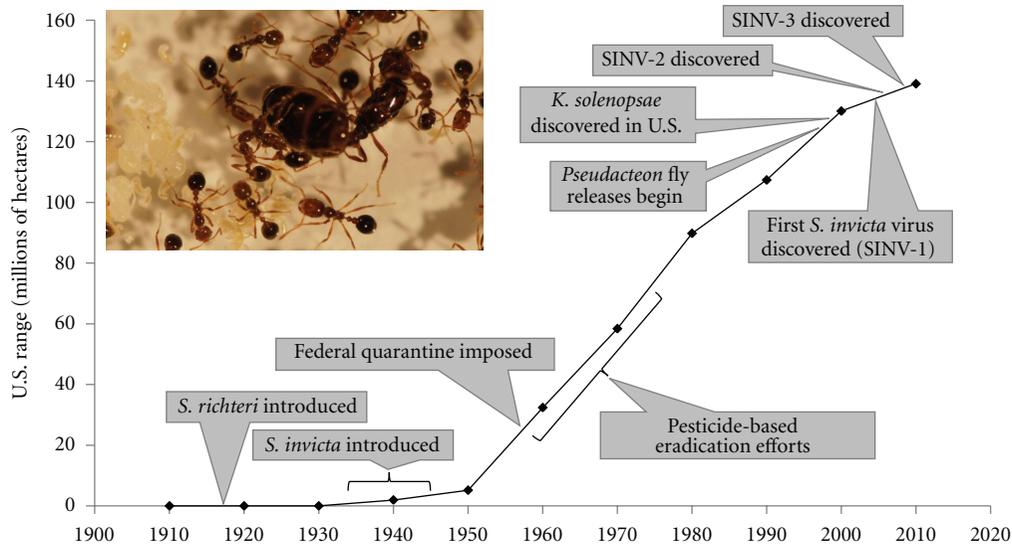


FIGURE 1: Seminal events during the invasion of *Solenopsis invicta* and *S. richteri* in relation to their USA quarantined range. Since the introduction of *S. invicta* and *S. richteri* into the USA, efforts to contain and eradicate the ants have been attempted [2, 4]. The USA federal government enacted a quarantine in May 1958 to limit the rate of range expansion of the ants. This quarantine is still enforced by the Animal and Plant Health Inspection Service (APHIS) today and prohibits movement of soil-containing products (e.g., sod, nursery stock, sand, etc.) or soil-moving equipment from a quarantined to a non-quarantined area unless first treated in an APHIS-specified manner to kill fire ants. The range values correspond to areas quarantined and do not relate changing population densities in the USA. Rather, the graph illustrates the expanding geographic range of these ants. Eradication efforts were attempted from 1957 through 1978 using several organochlorine insecticides (heptachlor, dieldrin, and mirex). Research efforts to discover, develop, and release pathogens and parasites as control agents in the USA resulted in identification and/or release of a microsporidian pathogen (*Kneallhazia solenopsae*) [18], viruses [24–26], and *Pseudacteon* parasites [28]. Inset: *S. invicta* queen surrounded by workers and brood. Quarantine data were provided by APHIS [3] and the figure was adapted from Lofgren [29].

superior performance and attainment of higher densities in the introduced region [9]. The *S. invicta* introduction into the USA exemplifies this hypothesis; at least 30 fire ant natural enemies have been identified in South America, but nearly all of these are absent among USA populations [7, 10–14]. Indeed, this premise has served as the impetus for research based on discovery, development, and use of pathogens and parasites because permanent, sustainable control of *S. invicta* across its USA range will likely depend on self-sustaining biological control agents as part of an integrated management strategy.

A limited number of pathogens and parasites of *S. invicta* have been detected or intentionally released in the USA. Currently, 2 species of endoparasitic fungi [15, 16], a microsporidian obligate parasite [17, 18], a neogregarine parasite [19, 20], a strepsipteran parasite [21], phorid flies in the genus *Pseudacteon* [22, 23], and 3 RNA viruses [24–26] comprise the known self-sustaining, biological control agents found in North American *S. invicta*. Discovery and exploitation of additional biological control agents, from either South or North American populations, could aid the control and suppression of fire ants and remain a key research topic for a number of academic and government laboratories [10]. Indeed, the number of natural enemies found in recently introduced *S. invicta* populations in Australia and China are even fewer than in the USA [27].

## 2. Virus Discovery

Although viruses can be important biological control agents against pest insect populations [30], until recently, no viruses had been shown to infect *S. invicta*. Indeed, no virus had been reported in any species of the Formicidae before the discovery of the fire ant virus, SINV-1. Extensive searches for pathogens, including viruses, of *S. invicta* have been conducted in the introduced (USA) and native (South America) ranges using traditional methods (identification of unhealthy ants followed by microscopic examination or simply examination of large numbers of healthy fire ant colonies) [11–14]. However, with the exception of “virus-like particles” observed in an unidentified species of *Solenopsis* from Brazil [31], no viruses had been described by these methods. Further complicating discovery of pathogens in fire ants by traditional methods is their fastidious nature [32]; sick or dying colony members are promptly removed from the nest precluding detection.

In an effort to identify virus infections of *S. invicta*, a metagenomics approach was employed [33]. The primary intention of this analysis was to utilize homologous gene identity to facilitate discovery of viruses infecting *S. invicta* that could potentially be used in pest management. A non-normalized gene expression library was created from a monogyne colony of *S. invicta* and a relatively small number (2,304) of clones

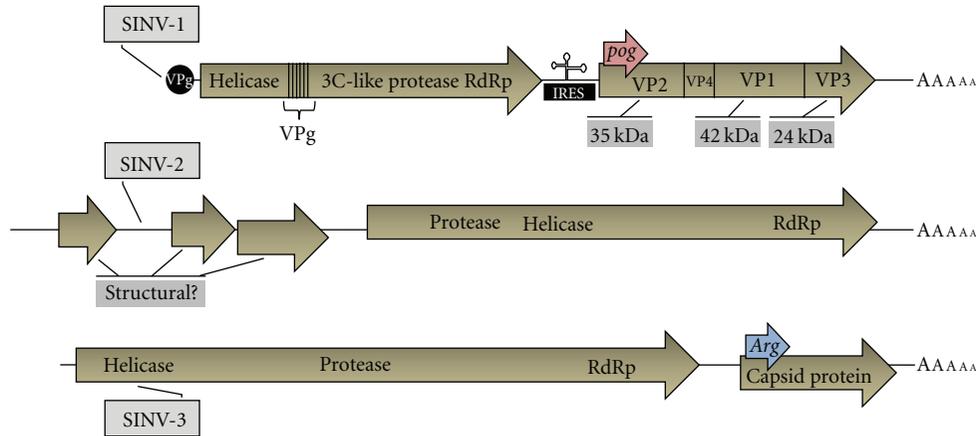


FIGURE 2: Comparative genome architecture of SINV-1, SINV-2, and SINV-3. Orientation of each genome is 5' to 3' (left to right). Relative positions of non-structural and structural proteins are indicated within each open reading frame (rectangles). ORFs are illustrated in different vertical positions to show their in-frame, comparative relationships. The SINV-1 genome is monopartite, dicistronic, and possesses 6 copies of the viral protein genome (VPg) peptide, an intergenic IRES, a predicted overlapping gene (*pog*) within ORF 2, and well-characterized capsid proteins (VPs) of known mass. The SINV-2 genome is monopartite with non-structural proteins encoded by the 3'-proximal ORF. The 3 small ORFs at the 5'-proximal end of the genome are presumed capsid proteins. The SINV-3 genome is monopartite with non-structural proteins encoded by ORF 1 (5'-proximal) and capsid proteins by ORF 2 (3'-proximal). An overlapping ORF (*Arg*) was identified within ORF 2 of a virus isolate from infected *S. invicta* ants from Argentina.

were sequenced. After assembly, 1,054 unique sequences were yielded and deposited into the GenBank database (accession numbers EH412746 through EH413799). Six sequences exhibited significant identity with RNA viruses. Subsequent analysis of these expressed sequence tags led to the discovery of three, positive, single-stranded RNA viruses, *Solenopsis invicta virus 1* (SINV-1), SINV-2, and SINV-3 [24–26].

### 3. *Solenopsis invicta* Virus 1

**3.1. Genome Characterization.** SINV-1, the first virus discovered in *S. invicta*, is the best characterized of the three currently described fire ant viruses [25]. Acquisition of the SINV-1 genome sequence was completed by a series of 5' and 3' rapid amplification of cDNA ends (RACE) reactions using expressed sequence tags identified from an expression library as anchor templates. The A/T rich genome is composed of 8,026 nucleotides excluding the poly(A) tail found on the 3' end (Genbank accession AY634314). Analysis of the genome (Figure 2) revealed 2 large open reading frames (ORFs) in the sense orientation (within frame) with an untranslated region (UTR) at each end and between the two ORFs. BLAST analysis [34] of ORFs 1 (5'-proximal) and 2 (3'-proximal) revealed identity to nonstructural and structural proteins, respectively, from positive, single-stranded RNA viruses. ORF 1 was found to exhibit a characteristic helicase, protease, and RNA-dependent RNA polymerase (RdRp) cassette ascribed to viruses in the *Picornavirales* [35] and ORF 2 the structural, or viral capsid, proteins. No large ORFs were found in the inverse orientation suggesting that the SINV-1 genome is a positive, single-stranded RNA virus. The 5', 3', and intergenic UTRs were comprised of 27, 223, and 204 nucleotides, respectively.

ORF 1 commenced at the first start AUG codon present at nucleotide position 28 and ended at the UAA stop codon at nucleotide 4,218 which encoded a predicted product of 1,397 amino acids with a molecular mass of 160,327 Da. ORF 1 conspicuously lacks a region thought to suppress host antiviral responses at the N-terminus—a characteristic exhibited by other dicistroviruses [36–38]. Thus, Nakashima and Shibuya [39] have suggested that SINV-1 lacks approximately 1,500 nucleotides at the 5' end of the genome. However, no empirical evidence for this suggestion has been reported. Sequence similarity analyses of ORF 1 identified domains consistent with a helicase, protease, and RdRp (Figure 2) [25]. Nakashima and Shibuya [39] identified the putative viral protein genome (VPg) sequence and location in SINV-1 ORF 1. The VPg is a peptide covalently linked at the 5' terminus of picornavirus genomes and serves as a primer for viral RNA genome replication [40]. Six copies of the heterologous 18 amino acid VPg peptide were identified between the helicase and 3C-like protease of SINV-1 ORF 1 (Figure 2), the most for any dicistrovirus [39]. Multiple VPg copies are thought to facilitate multiplication of dicistroviruses because fewer translation cycles of the non-structural polyprotein (ORF 1 for SINV-1) are necessary for viral replication to occur compared with the intergenic internal ribosome entry site-mediated production of the structural polyprotein (ORF 2). The 2C/3A and 3C/3D cleavage site positions were predicted within ORF 1 of SINV-1 [41].

ORF 2 was originally reported [25] to commence at nucleotide position 4,390 (canonical AUG start codon), however, it was later revealed empirically to actually start at codon GCU (genome position 4423–4425) encoding an alanine [42]. ORF 2 initiation at this noncanonical codon is a consistent characteristic of dicistroviruses [43] and its presence and location were predicted to occur in SINV-1 [44]

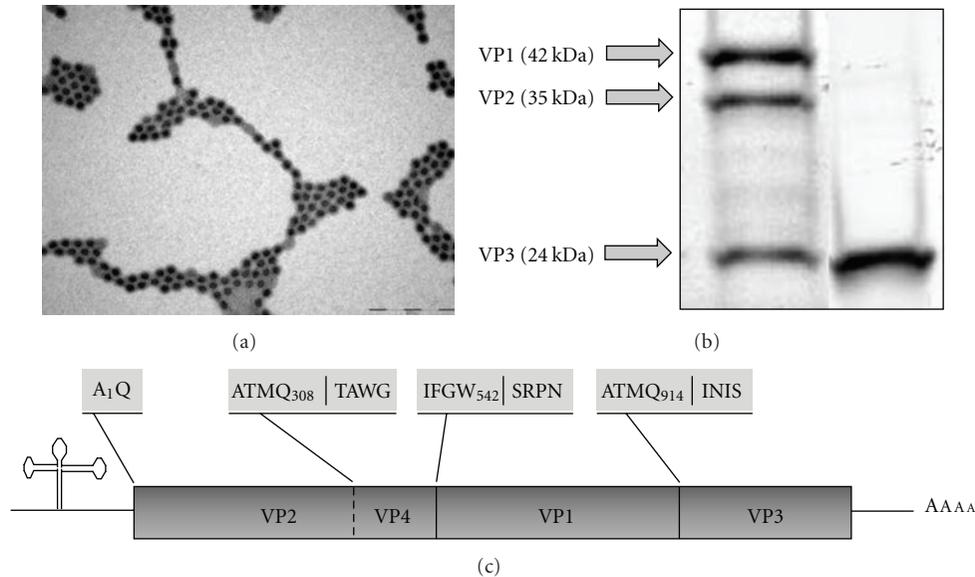


FIGURE 3: (a) Electron micrograph of purified SINV-1 particles. Scale bar represents 200 nm. (b) SINV-1 capsid proteins separated by SDS-PAGE (lane 1) and probed with polyclonal antibodies developed toward a portion of the predicted capsid protein, VP3 (lane 2). (c) Illustration of the intergenic region, IRES, and ORF 2 of SINV-1. Scissile bonds of each capsid protein of ORF 2 as determined by N-terminal sequence analysis of VP1, VP2, and VP3. Cleavage positions (subscript numeral) and amino acid residues about the cleavage site (vertical line) are illustrated.

before being empirically determined [42]. Thus, ORF 2 is comprised of 1,126 amino acids with a molecular mass of 126,434 Da. SDS-PAGE analysis of purified SINV-1 particles yielded 3 major and one minor protein band (Figure 3). The capsid proteins were labeled VP1, VP2, VP3, and VP4 based on mass and N-terminal sequence analysis of each of these proteins identified their respective positions within ORF 2 (Figures 2 and 3). Western analysis conducted with polyclonal antibodies developed from a peptide synthesized from the predicted amino acid sequence of VP3 (SRGGYRYKFFADDN) confirmed its location and synthesis from ORF 2 (Figure 3). The empirically determined and predicted molecular mass of VP0 (VP2 + VP4) (60.6 kDa), VP1 (41.8 kDa), and VP3 (24.0 kDa) were in agreement [42]. The positional organization of the capsid proteins of SINV-1 ORF 2 did not follow the pattern exhibited by most known dicistroviruses when based on mass (i.e., NH<sub>2</sub>-VP2-VP4-VP3-VP1). Although VP0 (VP2 + VP4) was at the N-terminus of ORF 2, and VP1 and VP3 were downstream of VP0, VP1 was found between VP0 and VP3. This organization (NH<sub>2</sub>-VP2-VP4-VP1-VP3) was also reported for deformed wing virus (DWV), an iflavirus [45]; SINV-1 and DWV both possess an unusually large VP1. The scissile bonds for VP0/VP1 and VP1/VP3 were located at amino acid positions 542/543 and 914/915, respectively. Amino acid residues at these junctions were consistent with other dicistroviruses and unclassified picorna-like insect-infecting viruses [46]. Amino acid residues G<sub>541</sub>, S<sub>543</sub>, and P<sub>545</sub> at the VP4/VP1 cleavage site and Q<sub>914</sub> at the VP1/VP3 cleavage site were conserved. These sites exhibited highest identities with Kashmir bee virus (KBV), Acute bee paralysis virus (ABPV),

and Israeli acute paralysis virus (IAPV), all of which infect honey bees [47, 48].

A third overlapping ORF of unknown function has been identified at the 5' end of SINV-1 ORF 2 in the +1 reading frame (Figure 2) [49]. The gene has been provisionally named *predicted overlapping gene (pog)*. Protein motif searches of *pog* revealed weak relationships precluding assignment of a potential function. Interestingly, all hymenopteran-infecting dicistroviruses in the *Aparavirus* genus (KBV, ABPV, SINV-1 and IAPV) feature the *pog* gene. However, neither a transcript nor protein encoded by *pog* has been detected.

The 5' and intergenic UTRs of dicistroviruses characteristically contain IRES regions that direct translation independent of a 7-methyl guanosine cap [50, 51]. SINV-1 has been shown to possess a Type II intergenic IRES based on sequence, structure, and homology within the dicistroviruses [44, 52]. Hertz and Thompson [53] have demonstrated that the SINV-1 IGR IRES is translation competent in yeast and mammalian cells.

Although positive, single-stranded RNA viruses, like SINV-1, do not synthesize a DNA template during any portion of their life cycle, portions of some positive, single-stranded RNA virus genomes (including a dicistrovirus) have been reported to be integrated into their host genomes. Interestingly, these integration events apparently afforded protection to the host from infection by the corresponding virus [54–56]. Because SINV-1 may be exploited as a microbial control agent, it was important to determine whether integration of a portion of the virus genome occurred in the host. A series of oligonucleotide primer pairs covering the entire

genome of SINV-1 were used to probe the genome of its host for integrated fragments of the viral genome [57]. Among 32 *S. invicta* genomic DNA samples collected from Argentina and the USA, no SINV-1 genome integration was detected.

**3.2. Host Specificity and Prevalence.** SINV-1 has been shown to infect *S. invicta* in the USA and Argentina [58–61]. Monogyne and polygyne *S. invicta* colonies [62] serve as hosts for SINV-1 [63]. However, SINV-1 infections appear to be more prevalent among polygyne *S. invicta* colonies [64]. *S. geminata*, *S. richteri*, the *S. invicta*/*S. richteri* hybrid, the *S. geminata*/*S. xyloni* hybrid (SMV unpublished) and *S. carolinensis* were also found to be infected with SINV-1 [58]. The infections in *Solenopsis* species other than *S. invicta* appear to be limited to areas in which *S. invicta* is sympatric and well established. SINV-1 was not detected in *S. xyloni* nor was it detected in *S. geminata* from southern Mexico (where *S. invicta* is not found currently), Hawaii, or Australia (SMV unpublished). Although still developing, these data suggest that *S. invicta* is the primary host of SINV-1 with other species in the *Solenopsis* genus serving as hosts occasionally (acquired from sympatric, SINV-1-infected *S. invicta*).

SINV-1 was distributed widely among *S. invicta* populations throughout the USA and Argentina [58, 60] with intercolony infection rates ranging from <10% [58] to >90% [63]. SINV-1 was detected in fire ants collected from all USA states examined with the exception of New Mexico. Although some dicistroviruses, like Cricket paralysis virus (CrPV), exhibit extremely wide host ranges, others, like Drosophila C virus (DCV), exhibit a genus-limited host range as observed for SINV-1 [50, 51, 65].

A strong relationship between temperature and SINV-1 colony prevalence was reported in two separate studies [58, 63]. Thus, time of collection (as it relates to temperature) must be considered when evaluating comparative prevalence data for SINV-1. This temperature dependency may be the result of more efficient IGR IRES activity. Hertz and Thompson [53] have shown recently that the SINV-1 IGR IRES exhibits increased activity at higher temperatures (3 to 5-fold). Further, the temperature-dependent enhanced activity resided in the ribosome binding domain of the IRES [53, 66]. So, the seasonally observed prevalence of SINV-1 appears directly related to the ability of the virus to replicate more efficiently at higher temperatures and not necessarily influenced by the behavior of the ant host.

Multiple genotypes of SINV-1 have been identified [25, 67, 68], and genomic diversity has been attributed to a high mutation rate characteristic of positive, single-stranded RNA viruses [69, 70]. Phylogenetic analysis of nucleotide sequences from the structural protein regions of the SINV-1 genome indicated divergence between isolates infecting North American and South American *S. invicta* [58] suggesting a prolonged duration of separation on the two continents. The analysis also indicated that North American SINV-1 had diverged more recently compared with those from Argentina. Indeed, a more extensive examination of the conserved RdRp region of the SINV-1 genome from *Solenopsis* hosts across the USA and northern Argentina revealed

clustering of Argentinean sequences, distinct from the USA sequences [59]. Thus, SINV-1 in North America likely arrived with one of the founding introductions of *S. invicta* from South America. This conclusion is supported by the lack of infection among other *Solenopsis* species (*S. geminata* and *S. xyloni*) in areas devoid (Mexico and Hawaii) or with incipient infestations of *S. invicta* (New Mexico, California, and Australia). SINV-1 infection of *S. geminata* and *S. carolinensis* (in Florida and Northern Mexico/Southern Texas) may have originated from introduced *S. invicta* or *S. richteri*.

SINV-1 was capable of being detected retrospectively in alcohol-stored arthropods for at least 2 years facilitating host specificity evaluations [71]. Pitfall collections of 1,523 ants from 16 genera (excluding *Solenopsis*) tested negative for SINV-1 from areas in Florida where SINV-1 was present in the *S. invicta* community [58]. Likewise, 282 other arthropods in four classes and ten families within the Hexapoda were negative for SINV-1. Even *Pseudacteon* parasitoids that complete development within *S. invicta* do not serve as hosts of SINV-1 [72]. Thus, SINV-1 appears limited to the *Solenopsis* genus with *S. invicta* likely the primary host.

**3.3. Stage and Tissue Tropism, Transmission.** Real-time PCR was employed to determine the presence of SINV-1 in tissues, individual ants, and among colonies of *S. invicta* by quantifying the genome of the virus [73, 74]. Initial experiments examined groups of tissues collectively to pinpoint the location of the virus infection. In workers, the abdomen contained the highest proportion of SINV-1; virus was also detected in the head and thorax of worker ants, but at very low rates (Figure 4). Among the remaining abdominal tissue groups, SINV-1 was detected occasionally in worker Malpighian tubules, the poison sac, hindgut and crop, but the greatest concentration of SINV-1 was in the midgut. Larval infection was also largely limited to the alimentary canal (Figure 4). SINV-1 specificity for the midgut of *S. invicta* is consistent with a number of other insect-infecting positive, single-stranded RNA viruses. Ingestion and the alimentary canal feature prominently in dicistrovirus infection acquisition and transmission processes [75]. Indeed, among the 14 described dicistroviruses, 12 exhibit a tissue tropism toward some part of the alimentary canal of their hosts [76]. Also, the gut contents of many hosts have been shown to contain high numbers of viral particles (e.g., Himetobi P virus (HiPV) [77], SINV-1 [73], DCV [78], and *Triatoma* virus (TrV) [79, 80]).

Electron microscopy of worker and larval gut homogenates revealed the presence of spherical virus particles with a diameter of 30–35 nm, consistent with SINV-1. The molecular and microscopic data suggest that SINV-1 replicates in gut epithelial cells of *S. invicta* and infectious viral particles are shed into the gut lumen [76, 81]. From there, the particles may be passed to nestmates by trophallaxis or substrate contamination by defecation [81]. Large quantities of SINV-1 detected in the gut contents of *S. invicta* larvae [73] suggest that this stage facilitates intra-colony dissemination of SINV-1. Late-instar *S. invicta* larvae digest all solid food for the colony which is redistributed in liquid form to nestmates

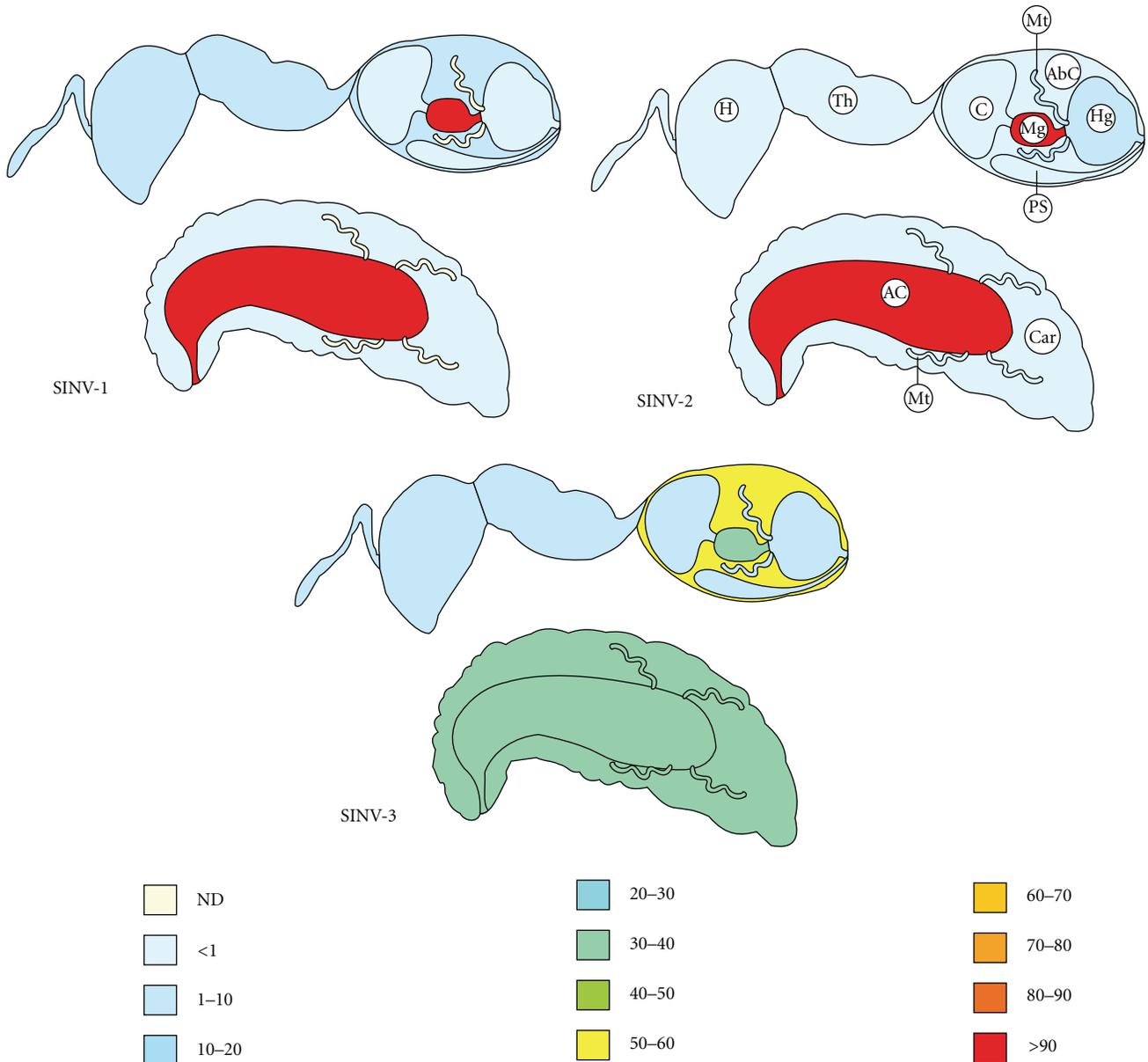


FIGURE 4: Tissue tropism of SINV-1, SINV-2, and SINV-3 among larval and worker *S. invicta* ants. Virus was quantified by real-time PCR. Color key reflects the proportion of the total for each virus detected in each tissue. (ND = not detected). Tissue key is identical for all viruses, H: head; Th: thorax; C: crop; Mg: midgut; Hg: hindgut; PS: poison sac; Mt: Malpighian tubules; AbC: abdominal carcass, AC: alimentary canal; Car: carcass.

[83]. Thus, larvae not only appear to serve as reservoirs for SINV-1, but also conduits for SINV-1 colonial dissemination (Figure 5).

Evidence for the importance of the alimentary canal in the horizontal transmission of dicistroviruses is further illustrated by the presence of virus particles in the excreta of infected hosts. The excreta serves as an important source of viral inoculum for *Plautia stali* intestine virus (PSIV) [84], Black queen cell virus (BQCV), Acute bee paralysis virus (ABPV), [85] Kashmir bee virus (KBV) [86], HiPV [87], and TrV [80]. The fecal-oral route of infection has even been

shown to play a prominent role in the infection process of many of the *Picornaviridae* in mammals [88].

SINV-1 was detected in all developmental stages of *S. invicta* including eggs and queens indicating vertical transmission of the virus [25, 73, 74]. Larval and worker ants generally exhibited the highest viral loads reaching levels of  $10^8$  to  $10^9$  per individual [74]. The SINV-1 titer was generally similar between larvae and workers collected from the same colony. A positive relationship was observed between the SINV-1 titer in individual ants and intracolony SINV-1 prevalence; colonies with higher intracolony

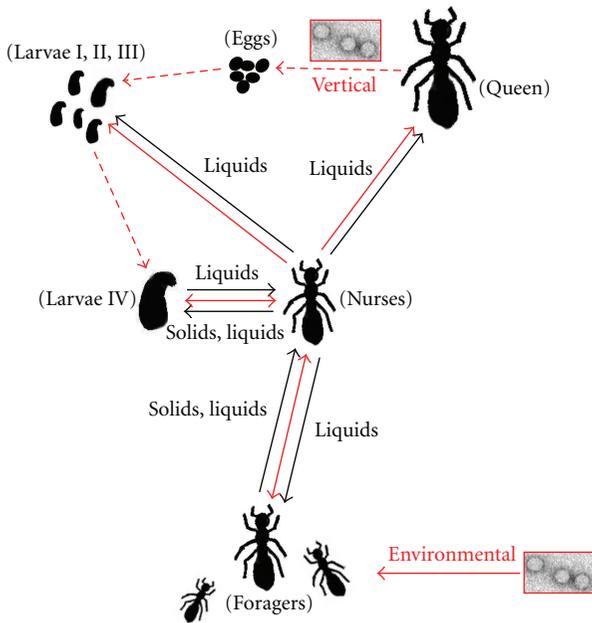


FIGURE 5: Routes proposed for SINV-1 (and possibly SINV-2 and SINV-3) colony acquisition and dissemination [81]. SINV-1 exhibits a tissue tropism toward the midgut, and large quantities of virus are detected in the midgut contents of adults and larvae of infected colonies. Thus, colony dissemination of SINV-1 appears to coincide with the food distribution route for the colony. Black arrows illustrate the flow of food throughout the colony and red arrows the corresponding movement of SINV-1. Two modes of virus acquisition are likely environmental (from outside sources entering the colony by way of foragers) and vertical (from infected queens). The vertical route of distribution is illustrated by broken red lines. The food flow diagram was adapted from Vinson and Sorensen [82].

SINV-1 prevalence exhibited higher SINV-1 titers among individuals [73].

**3.4. Colony Effects.** Initially, SINV-1 was associated with larval mortality in laboratory *S. invicta* colonies but no observable symptoms were detected among field populations [25]. A number of factors, including undetected pathogens and the inability to produce pure SINV-1 have limited the ability to directly infect colonies with SINV-1 and measure the impact of the infection on individual ants and colonies. SINV-1 appears to conform to the paradigm of many arthropod-infecting positive, single-stranded RNA viruses. Specifically, the virus persists as a chronic, asymptomatic infection that does not cause any overt signs or symptoms. However, under certain circumstances (e.g., environmental stress) the virus replicates rapidly causing overt symptoms and even death [89–91].

A recent series of experiments examined the ability of SINV-1-infected and -uninfected *S. invicta* ants and colonies to compete with native ant species *Pheidole fervens* and *Monomorium chinense* [92]. SINV-1-infected *S. invicta* were more quickly eliminated by *M. chinense* than healthy *S. invicta*. Direct confrontation tests confirmed these results;

*M. chinense* killed significantly more *S. invicta* minors from SINV-1-infected colonies compared with healthy colonies. Against *P. fervens*, SINV-1-infected *S. invicta* required significantly more time to eliminate competing *P. fervens* colonies compared with healthy *S. invicta*. The study revealed that SINV-1 infection weakened the competitive ability of *S. invicta* and made them more susceptible to elimination by some species of sympatric ants, like *M. chinense*.

#### 4. *Solenopsis invicta* Virus 2

SINV-2 is the second virus discovered that infects the red imported fire ant, *S. invicta* [26]. This virus possesses a genome structure that is unique and differs considerably from currently described positive, single-stranded RNA viruses. As with SINV-1, the SINV-2 genome was constructed by compiling sequences from successive 5' and 3' RACE reactions using an expressed sequence tag (Genbank accession EH413675) as the initial anchor template (Figure 2). The genome (Genbank accession EF428566) was monopartite, 11,303 nucleotides in length, polyadenylated at the 3' end, A/U rich (27.9% A, 28.9% U, 20.1% C, 23.1% G), and encoded 4 major ORFs (comprised of  $\geq 100$  codons) in the sense orientation (Figure 2). Untranslated regions were present on the 5' (nts 1–301) and 3' ends (nts 10917–11303) of the genome. Intergenic regions were also indicated between ORFs 1 and 2 (nts 1079–1828), and ORFs 3 and 4 (nts 3793–4454). ORFs 2 and 3 overlap a stop and start codon, respectively, and are not, therefore, interrupted by an intergenic region. ORF 3 was in the first reading frame, ORFs 1 and 2 were in the second reading frame, and ORF 4 was in the third reading frame. ORFs 1 through 4 encoded predicted proteins of 29,413; 31,160; 43,224; 246,845 Da, respectively. Blastp analysis [34] of SINV-2 ORF 4 identified regions with significant identity to RdRp, helicase, and protease conserved domains from positive, single-stranded RNA viruses [35] (Figure 2). Blastp analysis of ORFs 1, 2, and 3 yielded poor identity (expectation scores greater than 1) to corresponding capsid proteins from positive, single-stranded RNA viruses.

The monopartite, multiple ORF-encoding genome structure of SINV-2 is unique. Regions of the polyprotein encoded by SINV-2 ORF 4 exhibited identity with RdRp and helicase domains characteristic of positive, single-stranded RNA viruses. However, only a partial domain for a protease was recognized near the amino end of the ORF 4 polyprotein (amino acid residues 330 to 410). A similarly unique genome structure was reported for the Nora virus, an unclassified virus that persistently infects *Drosophila melanogaster* [93]. The Nora virus genome is also monopartite and encodes 4 major ORFs. In addition to genome structural similarities, Nora virus and SINV-2 contained truncated protease domains [93]. Amino acids thought to form the catalytic triad of the protease (H, E, C) and the consensus GxCG sequence motif were absent in all ORFs of SINV-2 [94, 95]. Additional differences between Nora virus and SINV-2 included positional relationships of the nonstructural proteins and relative ORF positions.

*S. invicta* colonies infected with SINV-2 did not exhibit any discernable symptoms in the field or consistently when reared in the laboratory. Occasionally, infected laboratory colonies exhibited brood die-off. Whether SINV-2 was responsible for this pathology was undetermined. The negative strand of the SINV-2 genome was detected in larvae and adults of *S. invicta* indicating that the virus was replicating [96].

SINV-2 host specificity evaluations have not been conducted. All developmental stages of *S. invicta* have been shown to be infected with SINV-2, including the queen and eggs suggesting vertical transmission of the virus [97]. Larvae and workers generally exhibited the highest viral load. Horizontal transmission of SINV-2 to uninfected *S. invicta* colonies was accomplished by feeding a homogenate of SINV-2-infected ants [97]. Tissue specificity of SINV-2 closely reflected that of SINV-1. The midgut of workers and alimentary canal of larvae possessed the highest quantities of SINV-2 (Figure 4).

### 5. *Solenopsis invicta* Virus 3

SINV-3 is the most recent virus to be discovered from *S. invicta* using the metagenomics approach [24, 33] and it also possesses features consistent with placement within the order *Picornavirales* [35]. As with SINV-1 and -2, the genome of SINV-3 was constructed by compiling sequences from successive 5' and 3' RACE reactions using an EST sequence (Genbank accession EH413252) as anchor template. SINV-3 possesses a genome that is 10,386 nucleotides in length, excluding the poly(A) tail present on the 3' end (Genbank accession FJ528584). Also consistent with SINV-1 and -2, the SINV-3 genome was A/U rich (70.9% A/U; 29.1% G/C). It encodes 2 large ORFs in the sense orientation with a UTR at each end and between the two ORFs (Figure 2). The 5' proximal ORF (ORF 1) began at nucleotide position 92 and ended at a UGA stop codon at nucleotide 7,834 yielding a predicted polyprotein of 299,095 Da (2,580 amino acids). The 3' proximal ORF (ORF 2), commenced at nucleotide position 8,308, terminated at nucleotide position 10,263 and encoded a predicted protein of 73,186 Da (651 amino acids). No large ORFs were found in the inverse orientation. The 5', 3', and intergenic UTRs were comprised of 91, 123, and 473 nucleotides, respectively. Blastp analysis [34] of the polyprotein encoded by ORF 1 identified conserved domains for RdRp, protease, and helicase (Figure 2). Blastp analysis of the ORF 2 polyprotein did not yield any sequences with significant identity.

For comparison, the genome of an Argentinean isolate of SINV-3 (SINV-3<sup>ArgSF</sup>) obtained from the Santa Fe region of Argentina was sequenced in entirety [98]. Argentina is thought to be the region from which the USA *S. invicta* population originated [99]. Excluding the poly(A) tail, the genome length of SINV-3<sup>ArgSF</sup> (Genbank accession GU 017972) was identical to the North American isolate (referred to as SINV-3). The SINV-3<sup>ArgSF</sup> genome possessed 3 major ORFs in the sense orientation; SINV-3 possessed only two ORFs [24]. Both isolates exhibited identical start and stop

codon positions for ORFs 1 and 2. Blastp analysis of the translated ORF 1 of SINV-3<sup>ArgSF</sup> recognized conserved domains for helicase, protease, and RdRp, and their corresponding positions were identical to those reported for SINV-3. ORF 3, unique to the SINV-3<sup>ArgSF</sup> genome (Figure 2), was located at nucleotide positions 8,351 through 8,827 and overlapped ORF 2. ORF 3 yielded a predicted protein sequence comprised of 158 amino acids with a molecular mass of 18.8 kDa. Blastp analysis of the translated amino acid sequence of ORF 3 revealed no significant similarity in the Genbank database.

The two SINV-3 isolates exhibited 96.2% nucleotide sequence identity across the entire genome [98]. The 5', 3' and intergenic UTRs of the genomes exhibited 100, 99.2, and 92.6% identities, respectively. The amino acid sequences of ORFs 1 and 2 exhibited 99.0 and 96.6% identities, respectively, indicating that the nucleotide differences between isolates were largely synonymous. Indeed, the proportion of amino acid residues that were similar in ORFs 1 and 2 were even higher (99.6% and 98.2%, resp.).

Tracking changes in pathogen genomes (including viruses) can be a useful and indirect method of providing information about their hosts [59, 100] and have been employed to construct demographic histories of host populations [101]. Unlike SINV-1 [59], comparison of the genome sequences of SINV-3 isolates indicated that no significant directional selection has occurred despite separation of the host populations geographically (Argentina/United States) and temporally (approximately 70 years). Thus, SINV-3 may have been a relatively recent introduction into the North American *S. invicta* population. SINV-1, -2, and -3 may aid a number of phylogenetic-based studies and reveal information about movement and establishment of the *S. invicta* host population.

SINV-3 infects all developmental stages of *S. invicta*, including the queen and eggs suggesting that vertical transmission occurs. Unlike SINV-1 and SINV-2, SINV-3 exhibits a broad tissue tropism. SINV-3 was detected in all tissues of *S. invicta* queens, workers and larvae examined (Figure 4). Thus, the SINV-3 infection appears to be systemic. This systemic characteristic appears to coincide with the association between SINV-3 infection and significant mortality among *S. invicta* laboratory colonies [24]. Signs of infection included large midden piles of ants, brood mortality, and colony collapse. Dead, dried brood found on the midden piles exhibit a crystallized appearance. Some workers may remain alive for considerable periods after the initial die-off, and occasionally, if the queen survived, colonies will rebound exhibiting normal brood production.

Although SINV-1, SINV-2, and SINV-3 are all positive, single-strand RNA viruses infecting *S. invicta*, they exhibit differences in their genome organizations; SINV-1 and SINV-3 encode 2 ORFs, while SINV-2 encodes 4 ORFs. However, the most important difference between SINV-1, SINV-2, and SINV-3 is pathogenicity. SINV-1 and -2 appear to cause chronic, asymptomatic infections that may result in mortality under certain stressful conditions, as reported in honeybees [90]. Although SINV-1 and SINV-2 were regularly transmitted to healthy colonies of *S. invicta* ants by feeding,

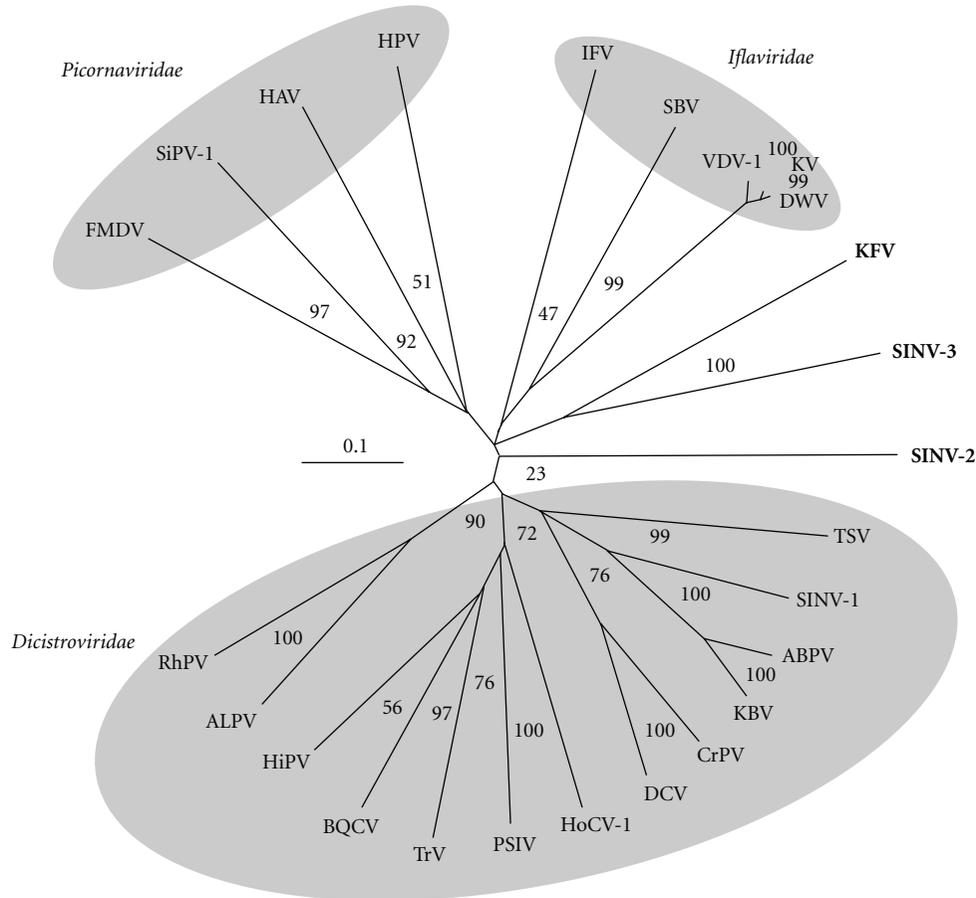


FIGURE 6: Phylogenetic analysis of the conserved amino acid sequences containing domains I to VIII of the putative RdRp from 13 dicistroviruses, 5 iflaviruses, 4 picornaviruses, and 3 unassigned viruses (bold). Virus abbreviation, Genbank accession number of the virus sequence, and amino acid residues of aligned sequences in a specific ORF (5' proximal ORF of dicistroviruses, otherwise an ORF number is specified) include the following. Aphid lethal paralysis virus (ALPV) [AF536531], 1661–1955; Black queen cell virus (BQCV) [NP620564], 1317–1585; Cricket paralysis virus (CrPV) [NP647481], 1423–1697; *Drosophila* C virus (DCV) [AF014388], 1415–1693; Himetobi P virus (HiPV) [AB017037], 1441–1710; *Plautia stali* intestine virus (PSIV) [NP620555], 1465–1739; *Rhopalosiphum padi* virus (RhPV) [AF022937], 1625–1916; *Triatoma* virus (TrV) [AF178440], 1446–1716; Acute bee paralysis virus (ABPV) [AAG13118], 1566–1837; *Homalodisca coagulata* virus-1 (HoCV-1) [DQ288865], 1446–1716; Kashmir bee virus (KBV) [AY275710], 1594–1864; *Solenopsis invicta* virus-1 (SINV-1) [AY634314], 1052–1327; Taura syndrome virus (TSV) [AF277675], 1770–2036; Infectious flacherie virus (IFV) [AB000906], 2618–2888; Sacbrood virus (SBV) [NC002066], 2522–2790; Deformed wing virus (DWV) [AJ489744], 2556–2826; Kakugo virus (KV) [AB070959], 2556–2826; *Varroa destructor* virus 1 (VDV-1) [AY251269], 2556–2826; Foot-and-mouth disease virus (FMDV) [AF308157], 2011–2264; Hepatitis A virus (HAV) [NC001489], 1904–2161; Human parechovirus (HPV) [AJ005695], 1871–2117; Simian picornavirus 1 (SiPV-1) [AY064708], 2119–2368; *Solenopsis invicta* virus 2 (SINV-2) ORF 4 [ABQ01575], 1814–2081; Kelp fly virus (KFV) [YP415507], 3015–3272; *Solenopsis invicta* virus 3 (SINV-3) ORF 1, 1848–2107.

mortality among recipient colonies was an occasional event [25]. On the other hand, SINV-3 was associated consistently with ant mortality and a correspondingly high SINV-3 titer ( $>10^9$  viral particles in a single dead ant carcass). Furthermore, SINV-3 was detected systemically—unlike SINV-1 and SINV-2 which were largely limited to the gut [73, 74, 97].

SINV-3 is readily transmitted to healthy colonies in the laboratory by exposure to homogenates of SINV-3-infected ants and by simply being confined in areas containing SINV-3-infected colonies (SMV unpublished). SINV-3-containing fire ant body parts become friable and airborne, contaminating surrounding areas. Disinfection of contaminated areas is

extremely difficult. However, hypochlorite solution is an effective means of disinfection.

## 6. *S. invicta* Virus Phylogenetic Analysis

Phylogenetic analysis of the conserved amino acid sequences containing domains I to VIII of the RdRp from representative dicistroviruses, iflaviruses, picornaviruses, and unassigned positive, single-stranded RNA viruses revealed a phenogram with SINV-1 clearly part of the *Dicistroviridae*, SINV-2 forming its own unique clade, and SINV-3 and Kelp fly virus (KFV) comprising a unique group (Figure 6). SINV-1 has

been placed formally into the *Dicistroviridae* family [102] and the recently proposed *Aparavirus* genus (pending approval from the International Committee for the Taxonomy of Viruses). Bootstrap values between the major clusters and SINV-2 were relatively low indicating an uncertain common ancestor for this virus. This independent placement of SINV-2 is further supported by phylogenetic results for the helicase region of ORF 1 [26]. SINV-3 is also unique, but exhibits a relationship with KFV. Both of these viruses exhibited a small virion size ( $27.3 \pm 1.3$  nm diameter) with apparent surface projections [103] and a high buoyant density ( $1.39 \pm 0.02$  g/mL). They also appear to possess only 2 major capsid proteins (VP1 and VP2) as opposed to 3 or 4 which is typical of the *Iflaviridae* and *Dicistroviridae*. The capsid proteins of KFV and SINV-3 exhibited poor comparative sequence identity (<10%).

## 7. Potential as Control Agents

SINV-1, -2, and -3 represent the only known viruses infecting any ant (Hymenoptera: Formicidae) species. As stated, the intention of virus discovery in *S. invicta* was to utilize viruses as novel control agents against this ant pest. Development and use of positive, single-stranded RNA viruses as insect control agents has been proposed [104, 105] and successfully demonstrated for a number of insect pests. CrPV was evaluated against the olive fruit fly, *Dacus oleae*, and shown to cause up to 80% mortality [106]. CrPV was also reported to be an effective control agent for adult Mediterranean fruit flies, *Ceratitis capitata* [107]. High rates of mortality were observed in laboratory and field tests of RNA viruses against *Epicerura pergrisea* and *Latoia viridissima* in Côte d'Ivoire [108, 109]. Unfortunately, a major limitation of the use of RNA viruses in insect control is large-scale production. This problem can be ameliorated when virus growth is supported by a cell line. However, insect host cell lines supporting viral production are available for only a handful of viruses. Indeed, the absence of a fire ant cell line has hampered investigation and development of the *Solenopsis invicta* viruses as microbial control agents. However, alternative methods of virus production have been demonstrated.

Production of infectious RNA transcripts [110–112] and *in vitro* baculovirus-driven expression of insect-infecting positive, single-stranded RNA viruses have been reported [113]. These methods facilitate study of virus biology and provide a means for their large-scale production. Development of a SINV-3 construct and subsequent *in vitro* expression of SINV-3 has been underway in our laboratory for the last year. Successful transcription of the SINV-3 genome has been accomplished, but production of encapsidated SINV-3 genome has not been observed (SMV unpublished). Indeed, transcript production and translation have proven extremely limited in this system. Because SINV-3 is associated with significant mortality among *S. invicta* colonies (reminiscent of colony collapse disorder of honeybees), our research is focused on studying this virus, including production, host specificity, efficacy, dose responses, mechanisms of action, and development as a biopesticide. Although SINV-1 and

SINV-2 appear to cause chronic, asymptomatic infections, they might find utility as control agents once their biology is more fully understood. Sodium alginate microencapsulated formulations of SINV-1 have been demonstrated to successfully transmit virus to uninfected colonies [114]. SINV-1 and -2 may also be exploited in unique ways, for example, as delivery vehicles for toxins or RNA interfering molecules after modification. Only through additional research to characterize the biology of these viruses will their full potential as control agents be realized.

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## Research Article

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

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

## 1. Introduction

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

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

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

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

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

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

Here I tested *Portia fimbriata*, an Australian spider-eating (araneophagic) salticid that specialises on capturing other salticids as prey [30], with Asian weaver ants (*Oecophylla smaragdina*). I then compared whether their response toward ant-like salticids was similar to that elicited by *O. smaragdina* by testing *P. fimbriata* with males and females of *Myrmarachne bakeri* from the Philippines. This species is an imprecise ant mimic [19], and males are expected to be less

precise than females due to their enlarged chelicerae. Finally, I tested *P. fimbriata* with an unrelated, but accurate, ant-like salticid from North America, *Synageles occidentalis*. In this study I address two specific questions: (1) does the non-ant-like salticid *P. fimbriata* avoid ants? (2) does *P. fimbriata* avoid or stalk ant-like salticids, and does this predators' behaviour differ depending on the accuracy of the mimic?

## 2. Materials and Methods

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

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

The testing apparatus was a wooden ramp (see Figure 1 for dimensions) raised at a 20° angle, which was supported by a wooden pole, glued to a wooden base. The entire apparatus was painted with two coats of polyurethane and was wiped with 80% ethanol and allowed to dry for 30 min between each test to eliminate possible chemical traces from salticids in previous tests. The ramp was marked in a 5 mm grid to allow accurate distance measurements to be obtained. A thin piece of wood glued to the top end of the ramp served as a background against which the salticid saw the lure. The lure was placed 40 mm from the top end of the ramp, equidistant from both edges, and placed such that it was faced 45° away from the pit, enabling test spiders to view cues from both the body and the head or cephalothorax of the lure. Lures were made by immobilizing an arthropod with CO<sub>2</sub> and placing it in 80% ethanol. One day later, I mounted the arthropod in a life-like posture on the centre

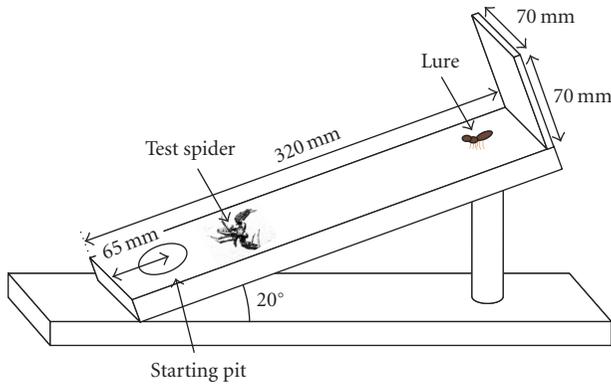


FIGURE 1: Ramp used for testing *Portia fimbriata* with lures of ants and ant mimics.

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

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

### 3. Results

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

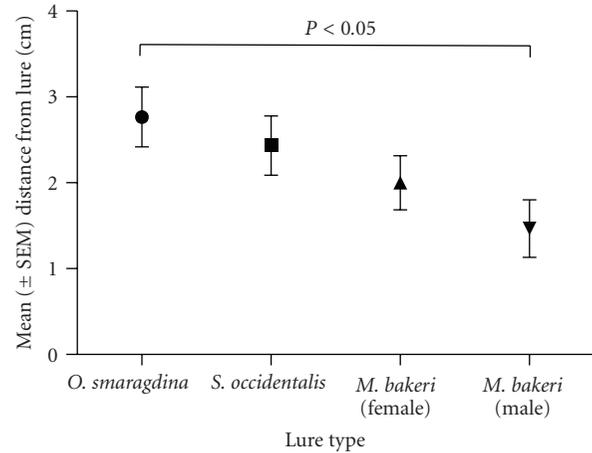


FIGURE 2: Mean ( $\pm$ SEM) approach distance by the spider-eating salticid *Portia fimbriata* to lures of ants (*Oecophylla smaragdina*) and ant mimics of varying degrees of accuracy of mimicry (*Synageles occidentalis* and male and female *Myrmarachne bakeri*).

### 4. Discussion

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

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

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

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

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