

## Review Article

# Leaf-Cutter Ant Parasitoids: Current Knowledge

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This review updates and summarizes the current knowledge about the interaction of leaf-cutter ants and their parasitoids by providing comparable data for *Acromyrmex* and *Atta* ants. First, an overview of the relevant aspects of the biology and taxonomy of leaf cutters and of their parasitoids is provided. Second, I show the peculiarities of the parasitoids attacking behaviors towards their host as well as the responses or ant defenses against the phorids exhibited by their hosts. Third, I discuss relevant aspects of the interactions between hosts and parasitoids. Finally, the review ends demonstrating why these phorids could be promising biological control agents of leaf-cutter pests and suggests priority lines of research for the future.

## 1. Introduction

Since the Feener Jr. and Brown [1] review discussion on flies as parasitoids, there has not been a comprehensive review on Phoridae (Diptera) parasitoids specialized on attacking adult ant workers. Phorids attacking fire ants are the ones most extensively studied due to their application in biological control. The literature is vast and dispersed although there is a review about *Pseudacteon* biology and interaction with fire ants [2]. Other scarce studies were done on other ant-phorid systems such as *Pheidole* [3, 4], *Azteca* [5, 6], and *Paraponera* [7]. Until more information is gathered, generalizations will not be possible for these groups. Hsun-Yi and Perfecto [8] have done an interesting review on indirect trait mediated effects of parasitoids on ants showing general patterns such as a reduction in ant's foraging activity, body sizes as well as the amount of food retrieved by colonies.

A compilation of leaf-cutter phorid species with their known and/or potential host species has been recently made [9]. The mentioned work includes some biological data about parasitoids of *Atta*, mainly from the laboratory, but a comprehensive review about their biology and ecological interaction with their hosts, including data of *Acromyrmex*, has not been done. Furthermore, Bragança [9] has not updated the scientific names of 14 species (called as *Neodohrniphora*) according to the status change of the subgenus *Eibesfeldtphora* to genus, proposed by Disney et al. [10]. Although the great majority

of data available is limited to the southern portion of South America and therefore more work is needed, it is enough to observe general patterns. This review will summarize the current information about this system and will identify key questions and gaps of knowledge where researchers should focus attention.

## 2. Leaf-Cutter Ants

The leaf cutters are a subgroup of the higher Attine fungus growing ants and are confined to two genera: *Acromyrmex* and *Atta*. *Acromyrmex* ants are the more diverse genus with 31 species with an additional 33 infraspecies [11]. Species that have more than 2 infraspecies, such as *Ac. coronatus*, *Ac. hispidus*, *Ac. lobicornis*, *Ac. lundii*, *Ac. octospinosus*, *Ac. rugosus*, and *Ac. subterraneus*, deserve to be studied in greater detail or using multiple techniques to avoid confusion and contradictory classification. *Atta*, on the other hand, exhibits less richness (14 spp.). *Acromyrmex* is more broadly distributed (by 10°N and S) than *Atta*, from 34°N to 41°S. Detailed maps of each species distribution can be found in Delabie et al. [11], and additional records for certain species from Argentina can be found in Elizalde and Folgarait [12].

*Atta* and *Acromyrmex* are larger Attines and are readily distinguishable from other ants because of their generally larger size, morphology, and behaviors. *Acromyrmex* ants are

easily recognized because all workers have at least 4 pairs of spines, 3 of which are on the thorax (promesonotum). The mesonotum spines are regular and smooth; also the frontal carinas in the head are short and never go beyond the eyes. The first abdominal tergite usually has tuberculous [13]. Their color varies from black to orange yellowish. On the other hand, *Atta* has 3 pairs of spines, 2 of which are in the promesonotum, the spines are generally curved, and the first abdominal tergite is smooth (Figure 1). Both genera are polymorphic, and although this trait is not as clear as in *Atta*, three castes of workers (tiny, small, and medium) can be differentiated in *Acromyrmex*; soldiers present in *Atta* are absent in *Acromyrmex* [14]. These ants have mass recruiting strategies, following a trail, more or less developed or clear, depending on the species, with 1 to several trails per nest, short or as long as 300 m. In *Atta* foraging trails are numerous and very conspicuous.

*Acromyrmex* colony nests can be completely hypogeous (underground, i.e., *A. striatus*, *A. aspersus*) with only small and few or variable number of entrances/exits or additionally have an epigeous mound (of variable height) such as in the case of *A. heyeri* or *A. coronatus*. Their foraging trails in general are not very conspicuous although this also depends on the taxa, the colony's age, and habitat. Although the nest's shape and appearance help render an ant's identification, more information is needed. The existent literature on the shape of *Acromyrmex* nests [15–17] is incomplete. Another complication is that certain species change greatly their type of nest in different habitats/regions (i.e., *A. lundii*, *A. lobicornis*) introducing confusion with others, such as *A. crassispinus*, *A. subterraneus*. For example, *A. lobicornis* epigeous nests are found in the southernmost part of its distribution while it barely has a mound in warmer areas (Folgarait, pers. obs.) such as in northern middle parts of Argentina. Another conspicuous feature that helps identify some species of this genus is the location of refuse dumps. Most *Acromyrmex* species have internal refuse dumps, although there are few exceptions where this characteristic is very helpful in identification (i.e., *A. lobicornis*, *A. crassispinus*, and *A. hispidus*). On the other hand, *Atta* nests are very distinctive as they create mounds of much greater size, that in general do not have vegetation on/or around them, and nests have loose soil with many holes on their surface. However, distinctions among species require an experienced eye that could also recognize key morphological characteristics of workers.

For *Acromyrmex*, climatic conditions can explain aspects of the mentioned differences regarding the presence/absence of a mound [18] and dump location either interspecifically (Farji Brener, pers. com.) as well as intraspecifically (Folgarait, pers. obs.), but other reasons such as colony sanitation and internal nest architecture may be additional factors, most likely all correlated with each other. Unfortunately, we know very little about the natural history of these species and the costs involved in dealing with trash and nest construction. For instances, is it less costly to lose additional workers by carrying the unsanitary trash outside to eliminate possible foci of infection or is it more energy efficient to close a trash filled internal chamber and not to maintain it? If the trash is internal, are these ants taking advantage of the nutrients

that mineralize within those trash-decomposition hot spots? Is the heat produced by internal refuse dumps utilized by the ants for colony or fungal thermoregulation? All these questions represent interesting lines of research, and the questions can be answered using C/N tracing techniques or manipulative field experiments.

### 3. Leaf-Cutter Parasitoids

*3.1. Richness, Distribution, and Characters Used to Distinguish among Genera.* Bragança [9] cites 30 species of phorids (Diptera: Phoridae) within 8 genera associated with *Acromyrmex* ants whereas 39 species in 5 genera were recorded on *Atta*. Also, he lists 7 cases of the same phorid species seen flying or sitting beside the nests of both genera. However, if only positive-sure cases (hosts from which parasitoids emerge or phorids seen pursuing and attacking ants) are considered, these numbers decrease for *Acromyrmex* to 15 species in 4 genera, for *Atta* to 25 species, and 4 genera with only 2 observations of phorids attacking both genera (*Apocephalus setitarsus* and *Myrmosicarius crudelis*), although these could well be mistakes or trials that were seen only once. Further observations for these two species should be specifically done as one of the references for each record is very old. In fact, Elizalde and Folgarait [12, 19] argue that leaf-cutter phorid parasitoids are very specific in the sense that those attacking *Acromyrmex* ants do not attack *Atta* and vice versa. Moreover, in many instances in which one phorid species is seen “ovipositing” an ant and this ant is reared, a different phorid species is obtained [20]. Therefore, these observations could be considered mistakes or tests made by the parasitoids. What really matters is the recurrent attack of a phorid species on the same host and its possibility of emerging from that host. According to this criterion, phorids that attack *Acromyrmex* or *Atta* ants are specific to that ant genus.

Despite the fact that phorids only represent 20% of known parasitoids, flies are the insect order that has the greatest range of hosts parasitized [21], and they are the only group known to attack adult ants [22]. Recently the subgenus *Eibesfeldtphora* was elevated to genus status [20], and a new genus with a single species has been described *Lucianophora folgaraitae* Disney [23].

So far, *Myrmosicarius* is the genus with the greatest geographical distribution ranging from 35°N to 41°S ([24]; Elizalde, Pers. Com.). However, *Eibesfeldtphora* is present in the largest number of countries [9].

Among the four most important genera attacking leaf-cutter ants, *Apocephalus* [26], *Eibesfeldtphora* [10, 27], *Myrmosicarius* [28, 29], and *Neodohrniphora* [10], it is difficult to say which one is most important. In the case of *Apocephalus*, the subgenera *Apocephalus* includes only ant-decapitating flies, and these flies are recognized for lacking tibial setae and possessing abdominal segments 7 to 10 fused to form an ovipositor, with which the eggs are inserted into the host. Segment 7 forms a rigid structure called oviscape. Another diagnostic character is the presence of a stylet comprised of segments 8 to 10 [30] (Figure 2). The mentioned subgenus has

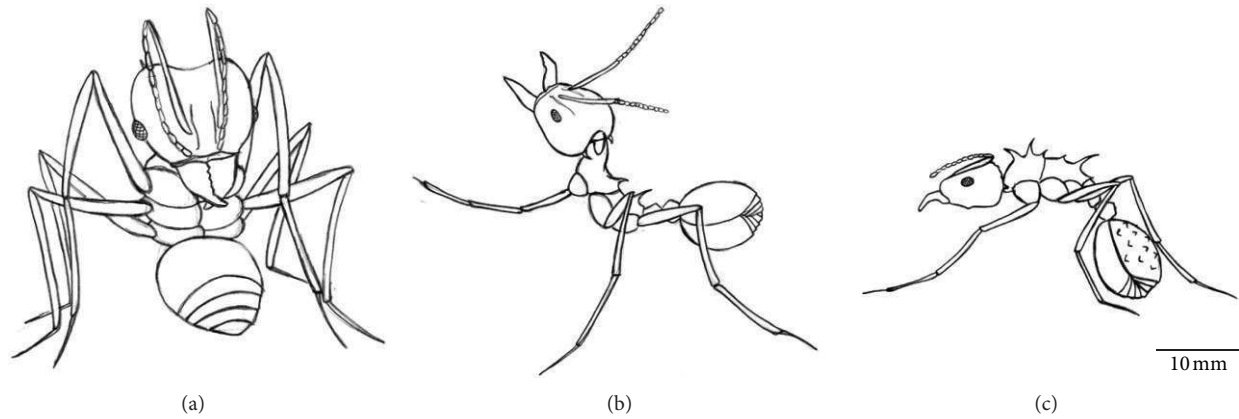


FIGURE 1: *Atta* (a, b) and *Acromyrmex* (c) morphological differences and exhibiting different body postures. (a) shows the C posture, (b) the alarm/attack phorid posture whereas (c) exhibits lowering the abdomen to avoid oviposition at the tip of the gaster.

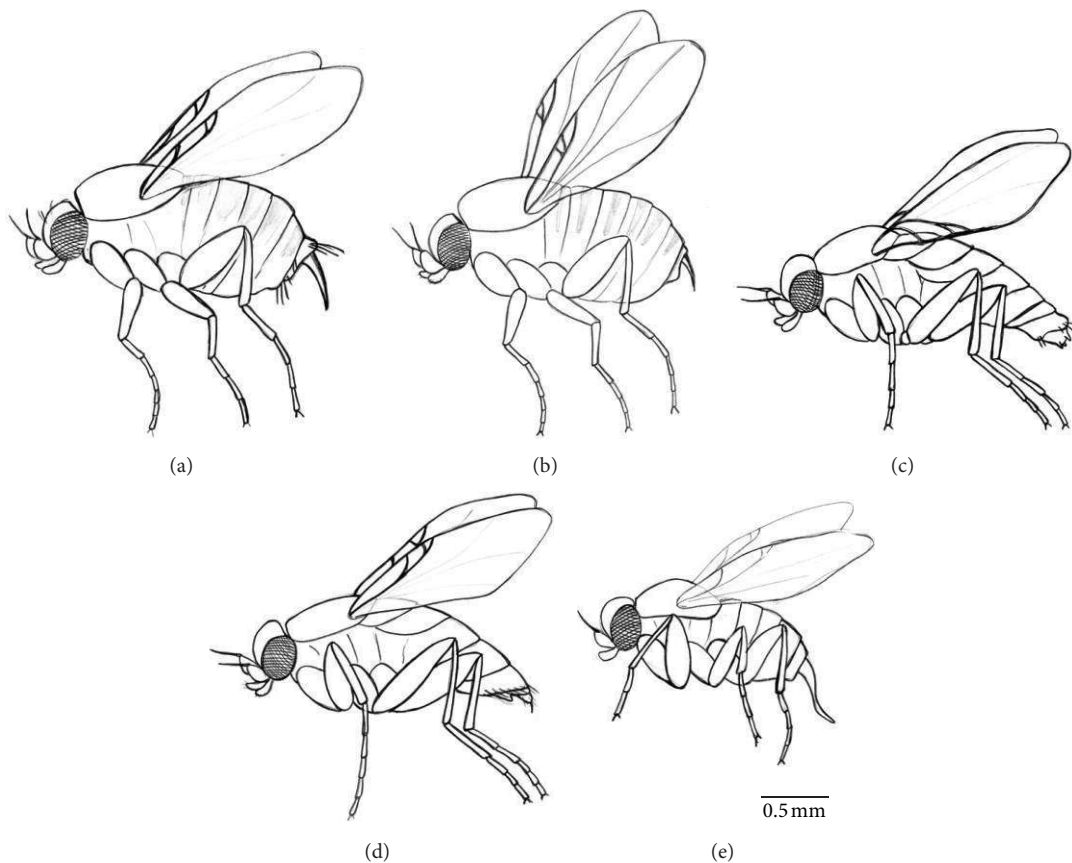


FIGURE 2: Schematic drawings of phorids showing details of the main characters that can be used to easily identify and distinguish among the main genera attacking leaf-cutter ants. Sizes represent real relative differences. (a) *Apocephalus* from the “attophilus” group and (b) from the “grandipalpis”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

subgroups specializing on different ant subfamilies. These are potentially monophyletic groups. The group “attophilus” is specialized on leaf cutters [26] and recognized because the apical sclerite is clearly separated posterior from the oviscape [30]. A few species from the “grandipalpis” group also attack *Acromyrmex* ants and are characterized by a short ovipositor, with a ventral sclerite wider than the dorsal one giving the

very distinctive effect of a rounded and lateral concavity in dorsal view [26]. *Apocephalus* flies attack both ant genera with 8 recorded species attacking *Atta* and 6 others that use *Acromyrmex* as hosts [9]. *Neodohniphora* at present has only two species attacking leaf cutters (*N. acromyrmecis* and *N. unichaeta*). This genus is distinctive because the front legs have 5 unusual fore-tarsal segments. Besides, abdominal

segment 6 is either reduced to hairs or has on its sides a transverse row of long hairs. Segment 7 also could be reduced to 2–4 hairy lobes or is basally articulated to form appendages. Beyond the ovipositor and below the tip of the abdomen is found a strongly sclerotized hook [31] (Figure 2). *Eibesfeldtphora* largely specializes as 9 out of 10 species are known to oviposit or develop on *Atta* hosts. It has yellow legs with dorsal enlarged hair palisade in all tibia. Fore leg with tarsomeres 4 and 5 fused, therefore with 4 distinctive tarsomeres. Abdominal segments are yellow ventrally (1–5), but segment 6 is mainly dark. Segment 7 has several lateral lobes darkly sclerotized. Segments 8–10 form at the end a pointed stylet [27] (Figure 2). There are 6 *Myrmosciarius* species that attack *Acromyrmex* whereas only 3 attack *Atta*. Females of the latter are recognized because the front tarsus is reduced to two segments; the sternite of the abdominal segment 6 is absent or vestigial and, by the characteristic oviscape tube, relatively nonornamented, that is, formed from abdominal segments 7 and 8 [29] (Figure 2).

Other features that help to identify among the mentioned genera are related to the pupae. While most *Apocephalus* species have a free pupae, the other genera have claustral pupation in the dead host head. *Apocephalus* do not decapitate their host and is unique in that more than one adult can emerge from a single host although this has not been recorded on *Acromyrmex* hosts. Also *Apocephalus vicosae* is the single exception for having a pupae coming out from the thorax. *Myrmosciarius* pupae are difficult to detect as the pupa is found deep in the head, below the tentorium arms, and the respiratory horns do not come outside of the head capsule; all these parasitoids decapitate their host. The other two genera pupae also develop in the head although they are easily seen and recognized by the exposed respiratory horns and sclerotized operculum (Figure 3); not all the species induce host decapitation [32].

### 3.2. Ecological Characteristics

**3.2.1. Generalities.** *Atta* parasitoids oviposit on workers while transporting leaves in the foraging trail or while potential hosts are cutting leaf fragments [33–36], sometimes using the load transported by the ant as a platform [37] or not [38, 39]. In the case of *Acromyrmex* parasitoids, not only these also attack ants on the foraging trail, those that are transporting a load or cutting leaves, but also while workers are repairing the nest or attending external refuse piles [19]. Both *Atta* and *Acromyrmex* parasitoids use either an ambush or an actively searching strategy and oviposit on different parts of the ant body such as through (on) the mandibles, in the head, thorax, legs, and anus [32, 38, 40]. Tables summarizing this information at the species level can be found for *Acromyrmex* [19] and for *Atta* [20].

*Eibesfeldtphora* females can use an ambush or active searching strategy, can land and oviposit on the head or abdomen, and always attack ants on the foraging trails while pursuing the host; in general they rest close to nest entrances. On the other hand, *Myrmosciarius* is mainly an active flyer while searching for its host. Some of them can fly onwards,

backwards, or sideward. They also land and oviposit in the head (mandible, clypeus, and occiput) and abdomen (tip) and can attack while on the trails, doing nest maintenance, or at refuse dumps. *Apocephalus* females attack using an ambush strategy, landing on the leaves carried by the ants, and ovipositing close to the mandible. *Neodohrniphora* are ambush or active searching parasitoids; there are too few records so as to generalize this genus. The four genera search hosts at foraging trails [19].

**3.2.2. Refuse Dumps.** Phorids attacking ants at refuse dumps were observed only for *Acromyrmex* ants [19]. This behavior was recorded consistently for *M. longipalpis*, *M. crudelis*, and *M. gracilipes* attacking *Ac. hispidus* for the first species and *Ac. crassispinus* for the latter two. The common factor seems to be the Monte habitat and inconspicuousness of the foraging trails of the mentioned hosts (either for being subterranean or otherwise covered with vegetation and being difficult to find). Therefore, the refuse piles could be a better place to spot the ants by these phorids in microhabitats with dense and high vegetation and low light. In fact, the mean light intensity at this habitat is 1 order of magnitude lower than for species attacking at other microhabitats [20]. Despite this capacity to oviposit at very low light levels, phorids attacking at refuse piles do not coincide with nocturnal ones (*M. brandaoi*, *M. gonzalezae*, *A. setitarsus*, and *A. longisetarum* for *Atta* and *M. cristobalensis*, *A. neivai*, *A. penicillatus*, and *A. necdivergens* for *Acromyrmex*). As nocturnal phorids are also diurnal, therefore an exact agreement between the phorid circadian rhythm and the microhabitat of attack may not be necessary. It is expected that refuse dump and nocturnal phorids rely more on close-range cues not associated with vision. This hypothesis, with the little knowledge that exists, disagrees with the data gathered for *Neodohrniphora elongata* [41]; however as it is a diurnal phorid (as far as it is known), it is reasonable that uses visual cues in motion for host location and recognition. On the other hand, another diurnal phorid, *Pseudacteon tricuspis*, uses short range chemical cues to locate their fire ant hosts [42]. This topic deserves further attention and research [43].

Phorid species that consistently attack at refuse piles such as *M. crudelis* and *M. longipalpis* seem to be very acrobatic flies, able to maneuver very rapidly, and are fast at flying forward as well as backwards, attacking the ants while being in front, back, or beside the host [32]. These abilities may be important in a small microsite, such as the refuse piles of these hosts, where many ants are together, carrying refuses and walking in a variety of directions (in comparison to the bimodal pattern on a foraging trail). Curiously, *M. crudelis* and *M. longipalpis* have the longest developmental periods recorded for leaf-cutter hosts (means of 49 and 52 days, resp.; these means are underestimated as it is not known when the oviposition occurred) [20]. Their developmental times are the longest recorded to date, even considering that developmental periods of phorids that attack *Acromyrmex* ants are longer than those coming from *Atta*. Furthermore, considering that these flies attack small ants [20], these lengthy developments are even more surprising as, in general,

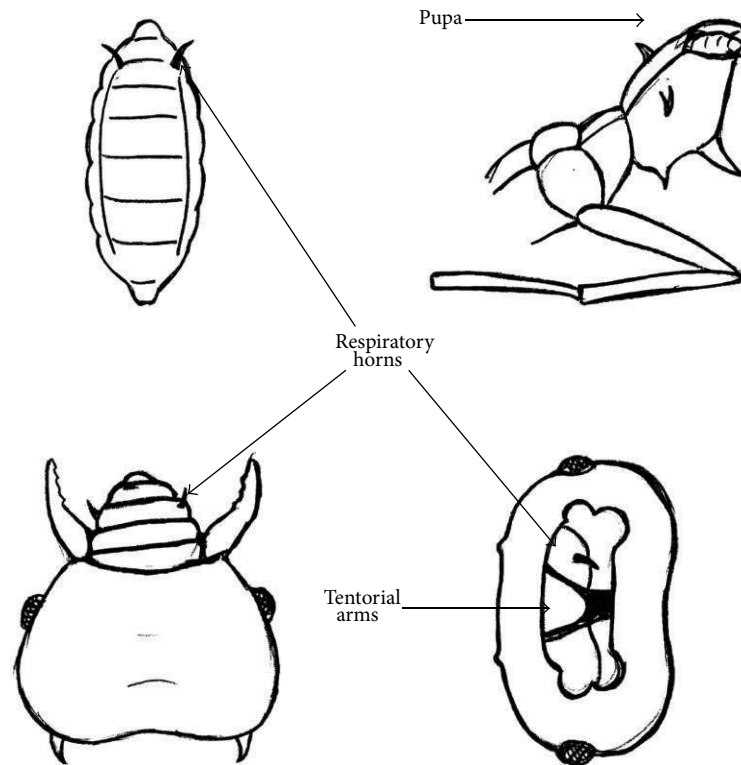


FIGURE 3: Schematic drawings showing different types of pupae according to the parasitoid genus. Top left: Dorsal view of a free pupae from *Apocephalus*, top right: claustral thoracic pupae from *Apocephalus vicosae*, viewed from ventral side, bottom left: claustral pupae from *Eibesfeldtphora* and *Neodohrniphora* coming out of the ant head between the mandibles (ventral view), and bottom right: claustral pupae from *Myrmosicarius*, viewed within ant head, under the tentorial arms (modified from [25]).

phorids attacking smaller ants develop faster than those attacking larger ones [25, 44, 45]. Probably, the ants involved in this task, such as carrying refuses plus working on them, are constantly dealing with infectious pathogens and may well be considered disposable ants from the colony point of view (either for being old or having a bad health) and, in turn, poor hosts from a phorid nutritional perspective. If this is the case, then a longer developmental time is expected.

#### 4. Leaf-Cutters Defenses against Parasitoids

**4.1. Generalities.** Phorids that parasitize leaf-cutting ants affect the ant behavior which translates to a negative effect on their foraging activity. The response behaviors of *Atta* ants against phorids include dropping their load [33], retreating to the nest [46], moving legs, antennae, and mandibles [37], outrunning the phorid [40], or adopting particular body postures in order to avoid oviposition such as lowering the tip of the abdomen, having a C posture, or making a ball with their whole body (Figure 1) [33, 39]. Similar behaviors were observed in *Acromyrmex* ants [19].

The presence of phorids was a significant determinant for the display of defensive behaviors by *Acromyrmex* ants. In fact, this chance was 5 times greater in the presence of phorids than in their absence [19]. It is particularly intriguing why phorids that attack *Atta* ants are not the same as those

attacking *Acromyrmex* [32] considering (1) that, in several cases, the ants are attacked by species from the same genus, (2) that hosts oviposited by different phorid species respond in such similar ways to the attacking flies, and (3) that both host genera could be present in the same habitat as well as their specific parasitoids. Besides, *Atta* parasitoids do not attack soldiers, a caste not present in *Acromyrmex* ants.

Although ant species varied in the incidence levels of defensive behaviors like the ones mentioned above, most ant species reacted against different phorids utilizing similar behaviors, as, for example, ants being attacked by an anus ovipositing fly typically lowered their abdomen, whereas ants being attacked by a head ovipositing fly adopted a C or biting posture (Figure 1). In contrast, parasitoids perform different behaviors when presented with multiple hosts [19]. Furthermore, *Acromyrmex* ants are generalist hosts in terms of being attacked by several phorid species, whereas phorids are mainly specialists (attack only one host species) [20], adding another level of asymmetry in the interaction. This pattern is not as strong for *Atta* ants [9]. As mentioned in Elizalde and Folgarait [19], parasitoids can choose their hosts whereas leaf cutters cannot easily reject or avoid a specific phorid species. Phylogenetic analyses of phorids that attack each genus may shed some light although immunological capacities could also help explain the lack of overlap. However, it will be more fruitful to first perform specificity tests offering different species of specialist parasitoids to a single

host species. Besides, it will be useful to evaluate, in long-term field studies, new communities where leaf-cutter hosts and nonhosts of several phorids species are present.

**4.2. Hitchhikers.** There has been a long standing controversy regarding the role(s) of hitchhikers, which are small ants riding on leaves that are transported by foraging workers. Despite the initial role proposed as defenders against parasitoids of the ants they ride [37], other functions are offered such as leaf microbes cleaners or sap ingestion from cut leaves [47–49]. Initially, it was also proposed that hitchhikers needed a flat surface where to ride [37] and were present only during the day because of the diurnal phorid activity [46]. However, in *Acromyrmex*, hitchhikers were found to ride on tips of monocots or pieces of grasses, they were present at night, and it was shown that nocturnal phorids exist [19, 35].

At present, hitchhikers are known for each of the 9 *Atta* species in which this behavior has been studied. *Acromyrmex*, however, do not have hitchhikers in about 1/3 (5 of 14 species) of the studied species; interestingly waste removers never carried hitchhikers [19]. The latter authors have shown that the chance for finding greater proportion of ants exhibiting hitchhikers was 2.5 times greater in the presence of phorids than in their absence.

## 5. Leaf Cutter and Their Parasitoids: Some Relevant Aspects of Their Interaction

**5.1. Parasitism Rates.** Natural parasitism from the same nests of *Atta* vary through time [25, 32], and these rates may reflect changes in health status of each colony or physiological tolerances of phorids to different weather conditions. For comparable data, percentages of natural parasitism in *Atta* are greater than in *Acromyrmex* in Argentina. Medians vary from 0.9–2.2% in *Acromyrmex* species to 3.8–20.2% in *Atta* [32]. However, the previous values include different species of ants and are medians. If we evaluate the parasitism rate by species and consider the maximum values, numbers are quite different. For example, a 12.5% was recorded in autumn for *A. lundii*, and a 35% maximum parasitism was found in *At. vollenweideri* in a mild winter. Evidently, parasitism rates not only change with seasons but also do across years. For example, for *At. vollenweideri* sampled at the same sampling site, maximum values range from 4% to 35% at different years [25, 32].

Rates of parasitism could also be related to the health status of the colonies, as discussed in Section 5.4.

**5.2. Host Sizes.** The parasitoid decision, about which host is good or not, should involve not only quality but also host size or amount of available food. In general, the larger the host selected, the bigger the resulting adult phorid [9, 25]. Host size is related to the amount of food available for the internal larvae to feed and be able to pupariate. Both, in *Atta* and *Acromyrmex*, several sizes are parasitized, but it is interesting to highlight that the ant size distribution available for parasitism does not differ statistically from that used for

oviposition in *Acromyrmex*, though it does in *Atta*; for the latter the smallest, biggest, or both extremes of the ant size distribution are not used as hosts [9, 32]. It is important to know the ant distribution available and that used by the phorids for two reasons: (1) a mean will not represent the most abundant size available relative to that used by the flies if the ant size distribution is not normal (which is typically left-skewed), and (2) without the ant distribution and that used by each phorid it is not possible to make inferences about phorid competition or segregation. Furthermore, speculations of ant competition/segregation should not be done considering either only one host and several phorids or the other way around, because several species in a particular area coexist, at least, at some months per year with other competitors and hosts. Therefore, community studies are necessary to make the best inferences and understand the community assembly rules involved for the species under study.

**5.3. Sex Ratios.** Data recorded so far [9, 25, 32] show that there is no sexual size dimorphism in adult flies nor in the size of the heads from which females and males emerge. This pattern holds for *Acromyrmex* as well as for *Atta* phorids. Possibly as a consequence of this, the sex ratio is near 1 or does not differ statistically from one in the many instances studied for phorids attacking leaf-cutter ants. This pattern is somehow unexpected because for many fire ant parasitoids females emerge from bigger head sizes whereas smaller heads produce males within a species [44].

The host size to adult fly size pattern is also very interesting because, on the one hand, the size of phorids is very different; for example, *Eibesfeldtphora* is double the size of *Myrmosicarius*, and two species of these genera attack the same size of the same host [32]. On the other hand, because of the great intraspecific plasticity of phorids, parasitoids coming from greater head sizes produce bigger phorids in comparison to those emerging from smaller ones [25, 32]. Three lines of research are needed in order to shed light on the two mentioned patterns; it will be important (1) to evaluate the sex ratios of phorids attacking monomorphic ants, (2) to discern if monomorphic or polymorphic ants and their specific phorid genera/species are more primitive or evolved, and (3) to study genetically the mechanism of sex determination.

**5.4. The Gestalt-Immunology Hypothesis.** A common pattern found in parasitoids attacking soil ants is that they parasitize ants from a few nests out of the total possible ones available in the same patch. Moreover, the same nests from which phorids emerged continue as such through time. Similarly, the percentage of parasitism could vary enormously from one colony to the other close by ([20]; Guillade unpublished). The fidelity and/or the great parasitism of a particular nest(s) through time represent(s) that the nest(s) in question is (are) better to complete the parasitoid's life cycle. How do phorids assess which nest is good? If the health of a colony or its suitability as a good host is linked with a particular taste, then phorids could choose one nest but not another using sensorial cues.

It has been shown in ants the importance of a chemical signature, given by their cuticular hydrocarbons, which is used by nestmates to differentiate self from nonself [50]. This implies that the particular chemical can be sensed by other ants also. We can extend this argument involving other organisms such as phorids. In fact, there is evidence from other systems that parasitoids can cue on the volatile compounds released by the plants due to having been fed by their herbivores [51]. Also, fire ant parasitoids use long-range olfactory cues to detect their hosts [42]. Then, if the gestalt (unique chemical signature shared by all members of a nest) of a colony is somehow related/linked to the health status of that colony, the consequence is obvious. Healthy colonies with vigorous ants will better nourish the parasitoid larvae than unhealthy ones which will have an altered gestalt. As the cuticular hydrocarbons are nonvolatiles, this information should be gathered by a phorid at very close range, in fact, by touching it. Following a sequence of events involved in host location, parasitoids first may use ant's alarm and/or trail pheromones as long-range cues to locate the ants (or their nests), second they may use intermediate-distance cues, such as visual ones, to determine which is the correct host size, and finally use taste-type cues to assess the health status of the ant/colony. This hypothesis can also help explain what is normally seen in fire ants, that is, where one colony is parasitized but not another one close by and surrounded by the same vegetation. In fact, cryptic sympatric species (haplotypes) are known of *S. saevissima* based on cuticular hydrocarbons and venom alkaloids [52]. Therefore, if there is a link between the cuticular hydrocarbons and the immunological status of the colonies, then a taste mechanism can be used to explain the parasitism rates discussed.

To my knowledge nothing is known about how the gestalt and immunological status of leaf-cutter ants (or any other) relate to each other and how these parameters could affect their relationships with natural enemies. De Souza et al. [53] evaluated encapsulation rates and cuticular hydrocarbon profiles in *Acromyrmex subterraneus* but did not relate one to the other because they were interested in answering another type of question.

**5.5. The Asymmetry Hypothesis.** The fact that hosts respond to phorids attack with similar behaviors, whereas phorids varied substantially among species in choosing and ovipositing their host, indicates that there is a great interspecific variation found in phorid behaviors but not in their host's responses giving support to the asymmetry hypothesis [54] in which the parasitoids can evolve different behavioral strategies as they can choose their prey but the hosts cannot evolve specific responses towards each parasitoid under the uncertainty of which one they will attack [32]. In addition, the high host specificity shown for most fly species with about 3/4 of taxa utilizing one host (30 in total, with 19 attacking *Atta* and 11 on *Acromyrmex*) and 13 different phorid species (6 attacking *Atta* and 7 *Acromyrmex*) using several species [9] is a pattern that somehow favors expectations from the asymmetry hypothesis. On the other hand, these host specificity ratios reflect data obtained from several regions

and seasons. It will be interesting to analyze the web of interactions at a local scale and from a richness point of view. If it holds, that is, finding more parasitoid species attacking a single species than attacking multiple hosts within each ant genus (where the immunological system might be more similar), then the asymmetry hypothesis could also help explain phorid speciation.

**5.6. The Conspicuousness-Abundance-Stability Hypothesis.** There might be a reason why every species of *Atta* has phorids attacking them while the same does not occur in *Acromyrmex*. One obvious hypothesis could be the conspicuousness and temporal-spatial stability of *Atta* which assures an enormous amount of resources available, relative to that for *Acromyrmex* [11]. If we define conspicuousness as any index that considers nest size, ant activity/trail, and number of trails, then a positive relationship could be expected among nests from different species that have different conspicuousness and the richness/abundance of phorids attacking them [55].

*Acromyrmex* species without known phorids are relatively inconspicuous with low number of individuals/colony. In fact, the species richness and abundance of hosts were the main determinants of phorid richness at the nest, hectare, and local scale, although, for the latter scale, climatic variables emerged in importance [12]. Moreover, the conspicuousness of the host was also important in explaining parasitoid richness [55]. In conjunction with the intriguing pattern that leaf-cutter phorids do not attack both genera of potential hosts, this latter result suggests that past competition could have led to segregation across different host niche axes [20, 25] whereas ecological conditions at local scales, with the availability of particular combination of hosts, may produce the final assembly that minimizes host overlap.

## 6. Biological Control of Leaf-Cutter Ants by Parasitoids

Leaf-cutter ant parasitoids exhibit several features that suggest they may become promising biological controls of leaf-cutter ants.

- (1) They are generally species host specific, with no intergenus parasitism to the extent that *Atta* and *Acromyrmex* phorids should be considered separate guilds.
- (2) They attack different sizes of hosts and in the case of *Acromyrmex* utilize most of the potential host size distribution which can assure the complete parasitism of all castes present in a colony.
- (3) The percentage of parasitism is high, in comparison to other analogous parasitoids such as fire ant *Pseudacteon* spp. In addition, they have a strong negative impact on ant foraging in the field.
- (4) The varied behavioral repertoire (attack strategies, presence throughout day and night and across seasons) and sites of attack (habitat and anatomical)

allow the selection of complementary species to promote broad spectrum parasitism.

- (5) The 1:1 sex ratio is extremely important to warrant matings in the laboratory as well as in the field.
- (6) The successful rearing of these parasitoids in the laboratory presents important baseline data that can be used to achieve mass rearing (Folgarait, unpublished).
- (7) The existence of a positive relationship between host size and phorid size could allow manipulation in the laboratory to produce females of greater size that might survive longer and have greater fecundity that would lead to higher attack levels.
- (8) The high resistance of some species to extreme weather and changes of climate [25] would allow for a larger area of biological control coverage.
- (9) The plasticity in host size selection makes these parasitoids less dependent on the varied size of hosts available [20].

However, it should be highlighted that the single use of parasitoids may not be able to control leaf-cutter ants. The hundred to million individuals involved in the nests of this successful group of ants will certainly need the use of a combination of different strategies to control them.

## 7. Promising Lines of Research

Over half of the 67 known species (38) have been described since Feener Jr. and Brown [1]. In addition, a great amount of information has been gathered on the basic biology of these newly discovered species, as well as that of longer known taxa. This information is also fundamental to any applied utilization of these parasitoids for biological control, including the descriptions of life cycles of many of the extant species, their host associations, the discovery of two guilds defined by the host genus, and the oviposition behaviors and response by their hosts under different circumstances. However, much waits to be studied and discovered about the fascinating interactions within this system. To help guide us through the many possible lines of research proposed within the body of this text, I list here the lines of research that I consider to be most important.

- (1) Examine how the physiological status of ant colonies, including immunological status, impacts on the performance of their parasitoids.
- (2) Identify the type of cues used by parasitoids to
  - (a) locate their host(s) at long and proximate distances,
  - (b) assess if hosts are already parasitized,
  - (c) determine if the colony is appropriate or not in order to be used as a source of ants to parasitize.
- (3) Understand the assembly rules involved in the leaf-cutter-parasitoid system at the community level.
- (4) Determine the place where parasitoid mating, late-stage infected host ants, and pupae are located, for at least 1 species from each host genus.
- (5) Develop a system by which ants can be parasitized in the laboratory without the need of the whole colony.

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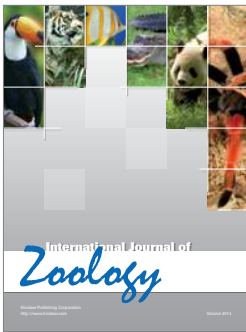
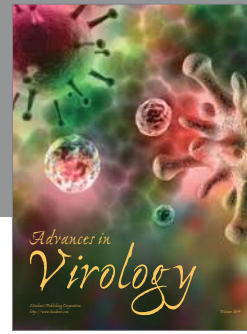
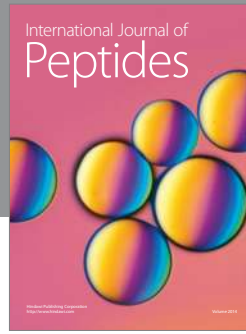
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