

Hermógenes Fernández-Marín · Jess K. Zimmerman ·
William T. Wcislo

***Acanthopria* and *Mimopriella* parasitoid wasps (Diapriidae) attack *Cyphomyrmex* fungus-growing ants (Formicidae, Attini)**

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Abstract New World diapriine wasps are abundant and diverse, but the biology of most species is unknown. We provide the first description of the biology of diapriine wasps, *Acanthopria* spp. and *Mimopriella* sp., which attack the larvae of *Cyphomyrmex* fungus-growing ants. In Puerto Rico, the koinobiont parasitoids *Acanthopria* attack *Cyphomyrmex minutus*, while in Panama at least four morphospecies of *Acanthopria* and one of *Mimopriella* attack *Cyphomyrmex rimosus*. Of the total larvae per colony, 0–100% were parasitized, and 27–70% of the colonies per population were parasitized. Parasitism rate and colony size were negatively correlated for *C. rimosus* but not for *C. minutus*. Worker ants grasped at, bit, and in some cases, killed adult wasps that emerged in artificial nests or tried to enter natural nests. Parasitoid secondary sex ratios were female-biased for eclosing wasps, while field collections showed a male-biased sex ratio. Based on their abundance and success in attacking host ants, these minute wasps present excellent opportunities to explore how natural enemies impact ant colony demography and population biology.

Introduction

The New World fungus-growing ants are abundant and diverse, and their nests contain significant caches of resources (Weber 1972). They are, therefore, attractive targets for numerous natural enemies. Excluding pathogens (see Currie et al. 2003), these enemies include predators

that attack colonies (e.g., Powell and Clark 2004; La Polla et al. 2002), agro-predators and social parasites that exploit worker labor (e.g., Schultz et al. 1998; Adams et al. 2000), and parasitoids that attack adult ants (e.g., Feener and Moss 1990; Feener and Brown 1993) or larvae (e.g., Loiácono et al. 2000). Despite their presumed importance, few quantitative data are available on the impact of these natural enemies (see Feener and Brown 1993).

Apart from several host records, little is known of the diverse diapriine wasps (Hymenoptera: Proctotrupoidea) that attack attine ants (see Masner and García 2002; Loiácono et al. 2000; J. Longino, personal communication). Here, we provide the first study on the biology and behavior of *Acanthopria* and *Mimopriella* wasps that attack *Cyphomyrmex* ants. These minute diapriid wasps are almost exclusively tropical, with the greatest diversity in lowland rainforests of Central and South America (Masner and García 2002), where attines are also most diverse. *Acanthopria* contains few described species, but Masner and García (2002) estimate that there are hundreds of undescribed ones.

Materials and methods

Overview of host biology The genus *Cyphomyrmex* is one of the more abundant and ecologically diverse taxon of fungus-growing ants which ranges from the southern USA to Argentina, including the Caribbean islands (Weber 1972; Snelling and Longino 1992; Mueller and Wcislo 1998). Colonies are relatively small, and nests are shallow and frequently common in the soil on the forest floor, in clearings, under leaf litter or rocks, and in cavities or rotten wood.

Ant demography and parasitism rates by diapriine wasps We studied four populations of *Cyphomyrmex minutus* in secondary forests on Puerto Rico from January to December 2003, and we studied one population of *Cyphomyrmex rimosus* located in grass in Gamboa (Colon Province), Panama, from May to November 2004. Ant colonies were

H. Fernández-Marín
Department of Biology, University of Puerto Rico,
P.O. Box 23360 San Juan, 00931-3360, Puerto Rico

H. Fernández-Marín (✉) · W. T. Wcislo
Smithsonian Tropical Research Institute, Apartado
0843-03092, Balboa, República de Panamá
e-mail: ic972735@rtpac.upr.clu.edu
e-mail: WcisloW@si.edu

J. K. Zimmerman
Institute for Studies of Tropical Ecosystems,
University of Puerto Rico,
P.O. Box 21910 San Juan, 00936, Puerto Rico

collected using an aspirator. We recorded nest contents and parasitism rates using a stereomicroscope. The number of eggs and small larvae may be underestimated because some may have been lost during aspiration. The percentage of parasitized larvae was almost certainly an underestimate because such larvae were identified easily at the last instar but not in younger larvae.

Field observations We collected wasps that were active near *C. rimosus* nests to study their natural abundance between 800 and 1800 hours from 9–29 October 2004 (total, 60 h of observations) using aspirators and nets placed over the ant nest entrance and adjacent grass vegetation. *C. rimosus* colonies were easily disturbed (e.g., by opening the nest), and usually, such disturbances induced the colony to relocate to a new nesting site. We recorded the behavior of *Acanthopria* wasps during induced relocations of 17 colonies of *C. rimosus*.

Laboratory observations We maintained *Cyphomyrmex* colonies within standard observation nests for approximately 2 weeks to record the development and emergence of parasitoids, numerical sex ratios, and to observe defensive behaviors by the ants. Most laboratory colonies did not accept garden substrata for cultivating fungi. Instead, during the first 2–4 days in the lab, ants seized parasitized or nonparasitized larvae and pupae for use as substrata, and after 6 days, the nests lost the majority of their juveniles. To ensure the emergence of adult wasps, we isolated immature ants in Petri dishes with wet cotton to maintain humid conditions. Under such conditions, and in the absence of tend-

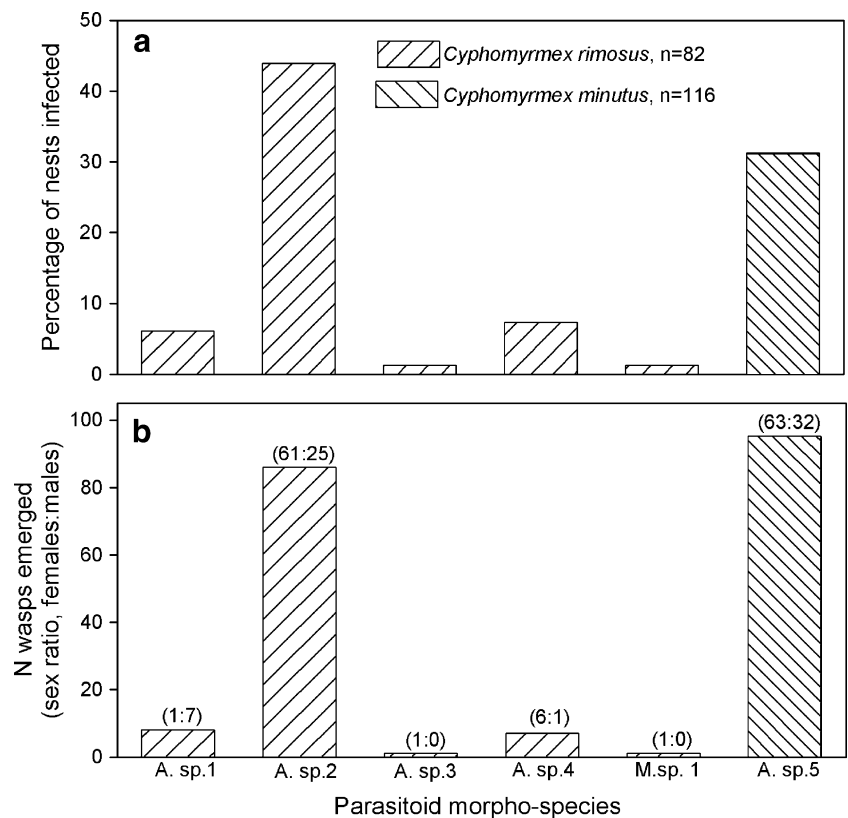
ing ants, some immatures were attacked by fungi. Thus, we assume that the number of wasps that emerged was low relative to the potential number of wasps that would emerge under natural conditions in colonies that were not food-stressed or stressed by artificial rearing conditions.

Wasp identifications and voucher specimens Wasps were identified to morphospecies; these were confirmed by Dr. M. Loiácono, who was describing them as new species. Means are given with their standard errors. Pearson correlation coefficients were calculated using Minitab. Voucher specimens were deposited in the Museo de la Plata, Argentina; Museo de Invertebrados de la Universidad de Panamá; and the Dry Reference Collection, Smithsonian Tropical Research Institute.

Results

In four populations of *C. minutus*, colonies contained zero to eight dealated female ants (1.9 ± 0.1 ; $n=144$ nests), 4–172 workers (60 ± 3 ; $n=144$), 0–95 larvae (12.22 ± 1.37 , $n=116$), and 0–52 pupae (12.21 ± 1.13 , $n=116$). Between 27 and 53% of the colonies were parasitized by one *Acanthopria* morphospecies, with considerable variation among populations (Fig. 1): Vega Alta, 39.3% infected ($n=28$ nests); Canovanas, 52.6% ($n=19$); Sabana 35.7% ($n=28$); and El Verde, 27.5% ($n=69$). The overall within-colony prevalence of *Acanthopria*, defined as the percentage of larvae parasitized per total number of ant larvae, for Puerto Rican populations was $16.6 \pm 2.4\%$ ($n=116$ nests; total number of

Fig. 1 **a** Percentage of *Cyphomyrmex* ant nests that were infested with *Acanthopria* or *Mimopriella* wasp morphospecies. **b** Number of *Acanthopria* or *Mimopriella* adults that emerged from *Cyphomyrmex* nests, with the emergence sex ratio given in parentheses (females to males)



larvae were not recorded for the Vega Alta site). Within populations, these percentages varied as follows: Canovanas, $21.8 \pm 5.5\%$, range 0–75%, $n=19$; El Verde, $15.1 \pm 4.4\%$; range 0–100%, $n=69$; and Sabana $15.5 \pm 5.3\%$, range 0–100%, $n=28$. Secondary sex ratios were female-biased (Fig. 1b).

In Panama, *C. rimosus* colonies contained from 0–4 dealated females (1.8 ± 0.3 , $n=82$ nests), 2–268 workers (55 ± 5.5), 0–129 larvae (14.52 ± 2.6), and 0–196 pupae (23.57 ± 3.3). Seventy percent of *C. rimosus* colonies were infested with one *Mimopriella* species and at least four morphospecies of *Acanthopria*, which differed from the *Acanthopria* sp. found in *C. minutus* (Fig. 1a). The overall prevalence of *Acanthopria* within colonies of *C. rimosus* was $34.3 \pm 3.3\%$ (range 0–100%, $n=82$). Based on adults that emerged from larvae in the laboratory, different morphospecies (and genera) can attack a single *C. rimosus* nest. Five *C. rimosus* colonies were infected with two different morphospecies: *Acanthopria* sp. 1 and *Acanthopria* sp. 2, each from two colonies; *Acanthopria* sp. 2 and *Acanthopria* sp. 3 from one colony; *Acanthopria* sp. 2 and *Acanthopria* sp. 5 from one colony; and *Acanthopria* sp. 2 and *Mimopriella* sp. from one colony. *Acanthopria* sp. 2 may have a higher impact on *C. rimosus* populations because the other four morphospecies were relatively rare (Fig. 1a). We did not observe cases of multiple parasitism within a single host individual.

There was no correlation between colony size (worker number) and the proportion of parasitized larvae for *C. minutus* (Pearson correlation=0.09; $P=0.36$, $n=116$), while there was a negative correlation for *C. rimosus* (Pearson correlation=-0.232; $P=0.029$, $n=82$). Infected colonies of *C. rimosus* had a larvae to pupae ratio of 1:2.3, whereas uninfected colonies had a larvae to pupae ratio of 1:1; the ratios for infected and uninfected colonies of *C. minutus* were 1:0.76 and 1:1, respectively.

When *C. rimosus* colonies (14 of 17 parasitized) were disturbed in the field, workers moved nests to new locations. No materials were observed in the old area, which indicates that parasitized larvae were also moved. On average, ants took 84 ± 10 min to relocate to a new site, which was 28.5 ± 21 cm distant from the old nest, and during relocations, 14 female wasps (*Acanthopria* sp. 2) were collected walking close to brood (<5 cm) within current and new nests, while 7 male wasps (*Acanthopria* sp. 2) were observed flying over grasses near the nests (<10 cm). We do not know whether these females were attempting to parasitize larvae in the emigrating colonies or were recently emerged and moving with the host colony. Wasps collected with nets at nesting sites tended to be males (6 females vs 47 males), in contrast to emergence sex ratios (Fig. 1b).

Each *Acanthopria* larva occupies almost the entire body of the late-instar ant larva, except the external cuticle. The presence of the parasite larva renders the cuticle gray rather than the normal cream color (Fig. 2a). The parasitized last-instar larvae are not modified in external form by the parasitoids, except for color, in comparison with nonparasitized last-instar larvae. Immediately after emerging, the wasp walks quickly away from the cocoon and any nearby immature ants and cleans the antennae and wings. In

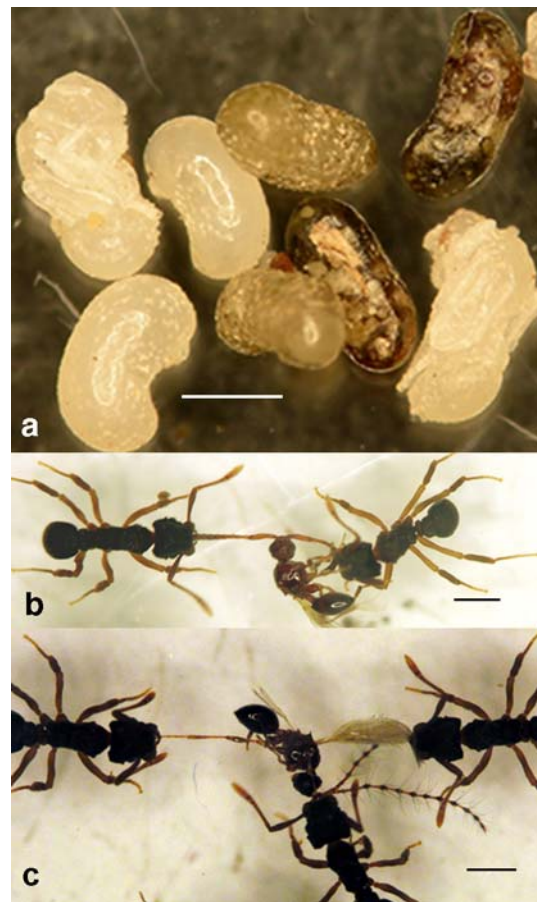


Fig. 2 a Parasitized brood of *Cyphomyrmex rimosus* ants can be recognized by the gray or black color of the cuticle, rather than the cream white color of healthy brood. Scale bar=1.7 mm. b, c *C. rimosus* worker ants attacking adult *Acanthopria* wasps (b, female wasp; c, male wasp). Scale bars=1.3 mm

artificial nests, wasps that emerged were repeatedly bit and seized by workers (Fig. 2b,c). Ants bit and curved their gasters toward active wasps, presumably as an aggressive behavior because multiple ants sometimes pulled wasps to pieces. Wasps that remained immobile were picked up with the mandibles and then frequently put back down without damage. Ants that were slightly disturbed usually released the wasps. Both female and male wasps that were found dead in Petri dishes showed signs of having been bitten by the ants, primarily on the antenna and wings. We infer that worker ants kill newly emerged wasps that they encounter, although we did not observe this directly.

Discussion

The *Acanthopria* and *Mimopriella* wasps in our study are koinobiont parasitoids of ant larvae of *Cyphomyrmex* ants, confirming previous hints that diapiirines are associated with Attini (Kistner 1982; Loiácono et al. 2000; Masner and Garcia 2002). Parasitism rates and abundance of *Acanthopria* show that these wasps sometimes inflict a heavy cost on *Cyphomyrmex* colonies. Nearly 16% of the

larvae in Puerto Rican colonies were attacked by parasitoids, while approximately 34% were attacked in Panamanian colonies. There are no detailed studies of colony growth rates as a function of the number of worker ants per colony for less derived attines. Due to the frequently substantial parasitism rates, we hypothesize that wasps may modify colony demographics with potentially strong fitness consequences, as proposed for eucharitid parasitoid wasps of some invasive ants (Heraty 1994). The negative correlation between *C. rimosus* colony size and parasitism rate may reflect the ability of larger colonies to better defend themselves against wasps. Alternatively, as suggested above, colonies are small because high levels of parasitism alter colony demographics and slow colony growth rates. The lack of a correlation in *C. minutus* and its occurrence in *C. rimosus* implies that different wasp species are using hosts as resources in different ways. Nothing is known about mechanisms of host recognition or discrimination, so we do not know if this implication is valid.

Presumably, adult wasps leave the nests and do not cohabit with the ants. This inference is based on the aggressive behavior of worker ants toward adult wasps, the presence of dead wasps in artificial colonies with wounds indicative of ant attack, and the lack of structures typical of myrmecophilous insects (Kistner 1982). If the ants remove dead wasps from the nest or use them or parasitized larvae as fungal garden substrata, it may be difficult to document wasp killing by the ants or parasitism by the wasps in natural nests. The primary defense against parasitoid wasps presumably is a behavioral response involving biting or killing adult wasps when they emerge or attempt to invade the nest. In turn, we infer that the wasps' primary defense is to avoid ants and, secondarily, remain immobile when attacked. The wasps quickly leave the emergence area, which supports our inference, because it reduces the probability that ants encounter them. We speculate that *Acanthopria* sex ratios were strongly male-biased in the field, in contrast to emergence sex ratios in the laboratory (Fig. 1b), because females were "walking" and not flying, unlike males, and so, the latter were more conspicuous. Differential mortality rates between males and females also would produce male-biased sex ratios, assuming that females are killed frequently by ants as they try to enter nests.

Different *Acanthopria* morphospecies can attack one single colony, similar to *Gynnopria* and *Trichopria* attacking *Acromyrmex* nests (Loiácono et al. 2000). In our study, we found at least four *Acanthopria* morphospecies and one *Mimopriella* sp. attacking host nests in a single population, although their relative abundance differs (Fig. 1a). Loiácono et al. (2000) collected 1,560 wasps (adults and immatures) from 430 parasitized larvae from three partial colonies of *Acromyrmex*, which shows how aggressive these wasps can be in attacking the ants. The impact of natural enemies on the demography and population dynamics of social insects is not as well known as individual behavior and the internal dynamics of colonies (Schmid-Hempel 1998), so relevant comparative data are scarce. Comparisons with other abundant attine parasitoids, like phorid flies

(Feener and Brown 1993), show that parasitism rates are 1–2% of individuals, which is substantially lower than we report. Based on their abundance and parasitism rates, these minute wasps present excellent opportunities to explore the hypothesis that parasitoid wasps regulate host population density and community structure both through indirect and direct effects (see Feener and Moss 1990; Feener and Brown 1993; Godfray 1994). Further studies of the adaptive radiation of these tiny wasps that utilize a single host may enhance our understanding of how interspecific competition relates to host specificity and other general aspects of life-history evolution (Godfray 1994). Finally, if further studies substantiate our results, then these minute wasps may have potential as agents for biological control of some attine ants.

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