RESEARCH ARTICLE

Biological notes on a fungus-growing ant, *Trachymyrmex* cf. *zeteki* (Hymenoptera, Formicidae, Attini) attacked by a diverse community of parasitoid wasps (Hymenoptera, Diapriidae)

**B. Pérez-Ortega · H. Fernández-Marín ·
M. S. Loiácono · P. Galgani · W. T. Wcislo**

Abstract A number of wasps in the family Diapriidae, subfamily Diapriinae (Proctotrupoidea), are parasitoids that specialize on ant larvae. These wasps are abundant and diverse in the Neotropics, but little is known about their biology. We studied parasitism rates by an array of diapriine wasps that attack the larvae of fungus-growing ants, *Trachymyrmex* cf. *zeteki*, in a single population (near Gamboa, Panamá). Relatively little is known about the biology and natural history of these ants, so we also present data on colony size and nest architecture. We excavated 136 colonies in central Panamá from June to September 2006, and 20 nests from July 2009. We reared six wasp morphotypes; two of them in the genus *Mimopriella* Masner and Garcia, one *Oxypria* Kieffer, two *Szelenyiopria* Fabritius and one *Acanthopria* Ashmead. The mean intensity of larval parasitism per ant colony was 33.9% (2006), and its prevalence across all ant populations was 27.2% (2006 and 2009). Parasitism rates were not positively correlated with host colony size. A single case of super-parasitism was documented in which two *Oxypria* males were reared from the same host larva.

Keywords Diapriinae · Fungus-growing ants ·
Parasitoid wasps · Adaptive radiations · *Trachymyrmex*

B. Pérez-Ortega · H. Fernández-Marín (✉) · P. Galgani ·
W. T. Wcislo (✉)
Smithsonian Tropical Research Institute, Balboa,
Apartado 0843-03092 Ancón, Republic of Panama
e-mail: hermogenes_f@hotmail.com

W. T. Wcislo
e-mail: WcisloW@si.edu

M. S. Loiácono
Museo de La Plata, División Entomología,
Universidad Nacional de La Plata, Buenos Aires, Argentina

Introduction

The New World fungus-growing ants (Hymenoptera, Formicidae, Attini) are especially diverse in the tropics (Weber, 1972). As is true for most social insects (Schmid-Hempel, 1998), they accumulate significant stores of resources within their nests, attracting a diverse array of predators, microbial pathogens, parasites, and parasitoids (e.g. Feener and Brown, 1993; Schultz et al., 1998; Loiácono et al., 2000; La Polla et al., 2002; Masner and García, 2002; Mueller et al., 2005; Powell and Clark, 2004; Fernández-Marín et al., 2006, 2009). Considerable attention has been given to the microbes attacking attine ants, but their insect enemies are less well studied, except for phorid flies (e.g. Feener and Brown, 1993).

Diapriine wasps (Hymenoptera, Proctotrupoidea, Diapriidae) are mainly Neotropical, and are frequently associated with ants; little is known of their biology and there are numerous undescribed species (Loiácono et al., 2000; Masner and García, 2002; Fernández-Marín et al., 2006). The subfamily contains an estimated ∼1,000 species in Costa Rica alone (Hanson and Gauld, 2006), so the taxonomy is difficult. Some diapriines apparently mimic their hosts' morphology (e.g. Loiácono et al., 2000; Masner and García, 2002), which presumably aids them in avoiding detection by host ants, but there are few behavioral data on host-parasitoid interactions.

We studied aspects of the intensity and prevalence of these little-known diapriine wasps that attack the larvae of a fungus-growing ant, *Trachymyrmex* cf. *zeteki* (Attini); we also provide notes on the ants' nest architecture, and host-parasitoid behavioral interactions. We discovered a remarkably diverse community of parasitoids within one host population near Gamboa, Panamá. High rates of parasitism should effectively slow the demographic growth rate of the ants,

by removing recruits to the work force, with potentially important consequences for understanding social biology.

Materials and methods

Nest collections

Established colonies of *Trachymyrmex* cf. *zeteki* were collected near Pipeline Road in Parque Nacional Soberanía, near Gamboa in central Panamá (9°07′38″N, 79°43′09″W). Collections included eight nests from June to August, 2005, which were used only to obtain taxonomic identifications; we collected 136 nests from June to September 2006 and 20 from July 2009. *Trachymyrmex* cf. *zeteki* were easily found on the banks of creeks where they were abundant, as well as on nearby slopes. Data are given as mean ± standard deviations. Voucher specimens of ants and wasps were deposited in the Museo de La Plata, Argentina; the Museo de los Invertebrados de la Universidad de Panamá, Panamá, and the Dry Reference Collection, Smithsonian Tropical Research Institute.

Nest architecture, demography and nesting biology

Nests were excavated using a hand pick and a small knife. During excavations, we recorded the following features of nest architecture. The auricle surrounding the entrance was measured as the longest vertical axis and the shortest horizontal axis, and its height was measured from the base to the highest part. We measured the diameter and length of the tunnel that led to the first chamber, and the width and height of each chamber. We also noted the positions of tunnels that connected adjacent chambers; the shape and location of any structures that supported the fungus gardens away from the soil chambers; the location of eggs and brood within the garden; and the location of the nest in the field. We collected the resident ants and brood using an aspirator. After excavating each nest the entire garden and all resident ants and brood were transported to the laboratory and maintained in a plastic box for 2 months using standard methods (Weber, 1972), and all brood that were present at the time of collection were repeatedly examined for evidence of parasitism until ants or parasitoids emerged as adults, or until the brood died, using methods given in Fernández-Marín et al. (2006).

Intensity and prevalence of wasp parasitism

For each nest we counted the number of queen(s), workers, eggs, larvae, and pupae using a stereomicroscope. The numbers of immatures and workers may be underestimates because some may have been lost during collections, if some immatures were buried in soil or adults escaped running. We recorded the number of older larvae parasitized by wasps, which were easily recognized by the dark coloration through the cuticle of an ant larva in which a wasp was developing (Fernández-Marín et al., 2006). For some nests, larvae from separate chambers were maintained in the laboratory in separate containers, in order to determine whether larvae in chambers that were closer to the entrance were more heavily parasitized. When a parasitized larva was identified, it was removed and maintained in a separate Petri dish until the wasp emerged or the larva died. Data on parasitism rates were pooled across morphospecies, because of difficulties in identifying morphotypes with a high degree of accuracy coupled with low survival rates of untended brood. These morphotypes will be described in a forthcoming paper with Dra. M. Loiácono (Museo de La Plata, Argentina).

Behavioral interactions between ants and parasitoids

Five nests of *T.* cf. *zeteki* were observed continuously in the field from 0800 to 1600 between 21 and 28 September 2006 (when nests contained parasitoids and hence adult females were active), each for 8 h to observe attempts by wasps to invade the nests. In the laboratory, behavioral interactions were recorded with a color CCTV camera attached to a stereomicroscope and illuminated with fiber optics, using minimal light levels. We subsequently viewed the recorded behaviors on a monitor. We observed six parasitized nests for ∼30 min each, and recorded the number of ant larvae within the field of view. Each recorded video was analyzed using scan sampling every 3 min: behaviors scored included contact with the larva or passing it; grooming the larva; and moving it.

Results

Nest architecture, demography and nesting biology

Nests were located along creeks and on slopes, below roots, stones and dead branches, which presumably provide protection against water run-off. Mature colonies of *Trachymyrmex* cf. *zeteki* were conspicuous due to an auricle at the entrance, made of packed soil (Fig. 1d). The auricle measured 28.6 ± 7 mm along the vertical axis, 21.3 ± 5.3 mm along the horizontal axis and 16.2 ± 8.5 mm in height ($n = 103$), and surrounded a single entrance that opened into a small antechamber, which in turn opened into a narrow tunnel that was 3.9 ± 0.5 mm in diameter and 12.1 ± 9.2 mm long ($n = 11$). There was a positive correlation between the number of workers and the number of chambers (Pearson's $r = 0.6$;
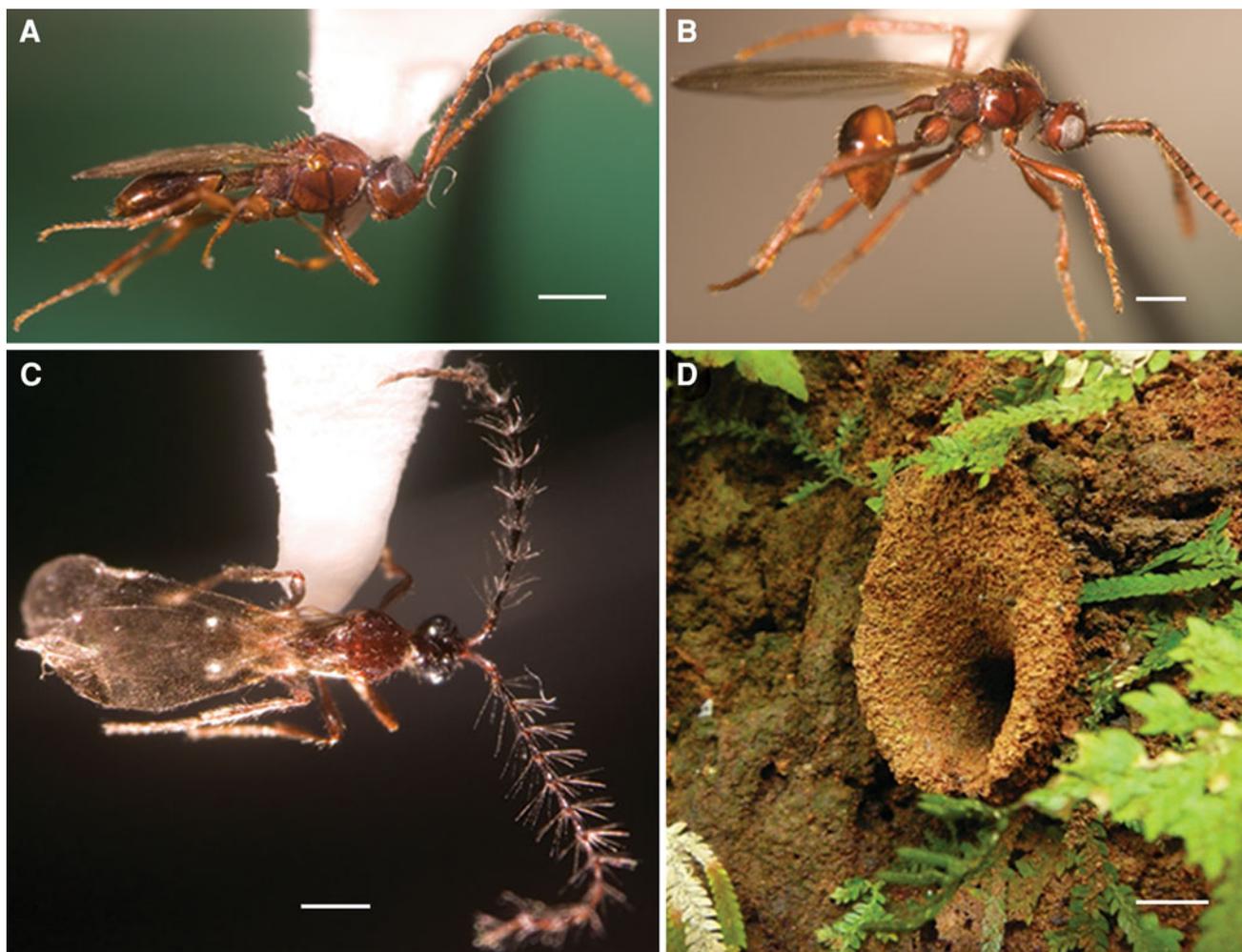
Fig. 1 a–c. Diapriidae parasitoids of *Trachymyrmex* cf. *zeteki* (Formicidae): **a** *Szelenyiopria* sp., female, *scale bar* = 0.6 mm; **b** *Mimopriella* sp., female, *scale bar* = 0.6 mm; **c** *Acanthopria* sp., male, *scale bar* = 0.7 mm; **d** nest entrance auricle of host ant, *scale bar* = 10 mm

$P < 0.0001$; $n = 125$). Nests with one or two chambers were the most common (45.6 and 36.8% of 125 nests, respectively), but some nests had three (16%) or four (1.6%) chambers. The chambers were connected through a tunnel that opened in the floor and led to an opening in the ceiling of the next chamber. The chambers were generally semi-spherical (width $= 35.3 \pm 10.2$ mm, height $= 30.4 \pm 6.0$, $n = 11$) with rootlets entering the chambers, which suspended the fungus gardens free from the soil in 50% of the cases; in 30% of the chambers the gardens were attached directly to the soil floor and in the remaining chambers (20%) the gardens were suspended by living rootlets but contacted the soil at one point on the floor. In all cases the juveniles, larvae and pupae were located in the periphery of the garden, while the eggs were distributed inside it ($n = 136$).

For 136 nests excavated, 83 nests had one queen, and ten nests had between two and four de-alate females; in 43 nests the queens were not found. The number of workers varied from 3 to 397 individuals per colony ($\bar{x} = 78.3 \pm 66.2$) and worker number correlated positively with queen number (Pearson's $r = 0.30$, $P = 0.003$, $n = 93$). Eggs and other brood were found in all months of this study and there was a positive correlation between colony size and total brood number (Pearson's $r = 0.55$, $P < 0.0001$, $n = 136$). The number of colonies with alate ants was greatest in June 2006; most of these ants were males, except one colony that had 1 female and 11 male alates. In July and September only one colony had alate ants and all were males (Table 1).

Intensity and prevalence of parasitism by wasps

There was no significant correlation between colony size and the proportion of parasitized larvae (Pearson's $r = -0.01$; $P = 0.9$, $n = 136$). Of 136 nests, 33 (24.3%) contained parasitized larvae. Within nests 3.1–100% of

Table 1 Demography of *Trachymyrmex* cf. *zeteki* from 136 nests excavated along Pipeline Road, Gamboa, Panamá. Data are given as mean ±SD with ranges given below in parentheses

Date (2006)	Colonies		N	N	N	N
	Collected	With alates	Eggs	Larvae	Pupae	Workers
June	46	8	6.57 ± 5.89	14.13 ± 13.32	12.38 ± 21.65	82.87 ± 79.28
			(0–28)	(0–52)	(0–67)	(4–397)
July	44	1	9.66 ± 6.48	13.89 ± 8.61	10.89 ± 10.82	64.75 ± 38.88
			(0–36)	(1–38)	(0–51)	(9–196)
August	19	0	10.47 ± 6.7	16.58 ± 12.18	11.89 ± 11.73	103.0 ± 80.67
			(2–26)	(3–56)	(0–43)	(13–376)
September	27	1	8.56 ± 6.63	13.19 ± 13.00	8.22 ± 10.32	75.15 ± 63.65
			(0–22)	(0–59)	(0–43)	(3–253)

Table 2 Intensity and prevalence of diapriine wasps that attack *T*. cf. *zeteki* fungus-growing ants along pipeline road, Gamboa, Panamá

Month (2006)	Colonies		Prevalence	Intensity
	Collected	Parasitized	(%)	(%)
June	46	10	21.7	47.4 (0–100)
July	44	15	34.1	33.3 (0–71)
August	19	3	15.8	17.1 (0–24)
September	27	5	18.5	19.3 (0–31)
July (2009)	20	9	45.0	–
Total	156	42	26.9	33.9

larvae were parasitized, and the overall mean intensity of parasitism was 33.9%. The nests with 100% parasitism rates had relatively few adult workers ($N = 4, 9,$ and 41). Prevalence and intensity per month are given in Table 2.

Six wasp morphotypes were reared from *Trachymyrmex* cf. *zeteki* larvae; two of them belong to the genus *Mimopriella* (Masner and Garcia), one to *Oxypria* (Kieffer), two to *Szelenyiopria* (Fabricius), and one to *Acanthopria* (Ashmead). Although our information is limited, some nests were parasitized by more than one parasitoid wasp morphospecies. In one nest we reared wasps of *Mimopriella* morphospecies 1 and *Szelenyiopria* morpho-species 2; another nest had wasps of three morpho-species including, *Mimopriella* sp. 1, *Szelenyiopria* sp. 1, and *Acanthopria* sp. 1. Based on nine colonies from 2009, we found one nest with one *Acanthopria* wasp; five nests with *Mimopriella* sp. 1; one nest with *Mimopriella* sp. 2; three nests with *Szelenyiopria* sp. 1, and one nest with *Szelenyiopria* sp. 2. We found one case of super-parasitism in which two males of one *Oxypria* morpho-species were reared from the same larva. Overall the wasp sex ratio reared from ants was female-biased (1:2.72, m:f, $n = 119$ wasps, 2006 only).

The nest entrance architecture was not associated with overall parasitism rates. All correlations between the size of the auricle and parasite intensity were insignificant (vertical axis: $r = -0.2$, $P = 0.324$; horizontal axis: $r = -0.12$, $P = 0.563$; height: $r = -0.23$, $P = 0.259$; $n = 27$). Of nests with multiple chambers, 18.2% had parasitized larvae in all chambers; 27.3% had them only in the outermost chamber; 12.1 and 3% had them only in the inner second and third chambers, respectively; and in 9.1% of nests we could not determine which chambers were infested because the chambers collapsed during excavations.

Behavioral interactions between ants and parasitoids

During 41.5 h of observations in the field we never observed diapriine wasps close to the ants' nests, despite rearing wasps from nests collected at the same time and despite observing wasps in the field. Observations in the field suggest that ants do not discriminate between parasitized and unparasitized larvae, because when the first chamber that contained brood was opened, workers moved both parasitized and unparasitized larvae and pupae to the next chamber. In the laboratory, however, worker ants contacted larvae with parasitoids more frequently than those without parasitoids (5.2 ± 2.87 and 1.9 ± 1.33 contacts per larva, respectively), and moved them more frequently as well (1.2 ± 1.73 and 0.58 ± 0.95, respectively) ($n = 40$; 3 min scans). Sample sizes were small and the significance of the behavior is unclear because the ants did not destroy the parasitized larvae.

Discussion

The diapriine wasps, including *Mimopriella, Oxypria*, *Szelenyiopria*, and *Acanthopria* in our study are koinobiont parasitoids of the larvae of *Trachymyrmex* cf. *zeteki* ants. This finding provides further evidence that diapriine wasps are associated with fungus-growing ants (Attini) (Kistner, 1982; Loiácono et al., 2000; Masner and García, 2002;

Fernández-Marín et al., 2006). Between ∼15 and 34% of ant colonies were parasitized by wasps, and in several cases involving small colonies, 100% of larvae were parasitized, suggesting these wasps inflict a heavy cost on the ants, roughly comparable to parasitism rates by diapriine wasps that attacked *Cyphomyrmex* ants (Fernández-Marín et al., 2006). To date, however, there are no comprehensive studies that examine colony growth rates as a function of the number of worker ants per colony, so the impact of these wasps on colony demography remains to be determined. We lack information concerning competition among parasites that attack *Trachymyrmex*.

Some features of nest architecture are frequently assumed to play a role in defending stored resources against natural enemies. Our data indicate that the nest entrance structure is not associated with parasitism rates, unlike the situation in species of sweat bees (Halictinae) or *Camponotus* ants (Formicidae), for example, which construct a narrow collar at the nest entrance that decreases the likelihood that predators and parasites enter the nest (e.g. Sakagami and Michener, 1962; Smith et al., 2003; Powell, 2008). The use of multiple brood chambers may render larvae in the outermost chamber more susceptible to parasitoids than larvae in inner chambers, although interpretations of data are complicated by the fact that ants sometimes move larvae between chambers. Some cavity-nesting stingless bees (Meliponini) construct an empty cavity as a false nest, adjacent to the nest entrance, and then construct a second cavity further in, which is thought to deter predators (Wille and Michener, 1973; for other taxa, see Tepedino et al., 1979; Munster-Swendsen, 2000; Asís, 2007).

Our scant data on behavioral interactions are inconsistent with respect to whether ants are able to detect parasitized brood, as inferred from rates of contact and movement involving parasitized and unparasitized larvae, but our sample sizes are too small to warrant firm conclusions. Ramos-Lacau et al. (2007) report that *Cyphomyrmex* ants do not discriminate between brood infested with *Acanthopria* parasitoids versus uninfested brood. In our study ants did not destroy parasitized larvae, suggesting the latter are not recognized, nor is there any evidence that parasitoids actively manipulate host behavior to enhance the fitness of parasitoids.

We reared six morpho-species from four genera of wasps in a single host population, which is a more diverse assemblage than observed for those that attack *Cyphomyrmex* (Attini) (Fernández-Marín et al., 2006). We found some nests with more than one morpho-species reared from the same colony, so the limited comparative data suggest super-parasitism may not be unique to *Trachymyrmex* (Loiácono et al., 2000; Fernández-Marín et al., 2006; Ramos-Lacau et al., 2007). *Trachymyrmex* cf. *zeteki* are found in

the same habitats in central Panama as is *C. rimosus*, and both have high parasitism rates, but they do not share diapriine morpho-species (compare Results with Fernández-Marín et al., 2006). Detailed studies are needed on host selection behavior to understand what factors might promote the coexistence of so many parasitoids in a single host population, and what factors preclude coexistence within host colonies (see "Discussion" in Fernández-Marín et al., 2006).

Combined with earlier work (Loiácono et al., 2000; Masner and García, 2002; Fernández-Marín et al., 2006), our study hints that these tiny wasps play a significant role in the population biology of attine ants, and thus warrant the attention of biologists studying the roles that natural enemies play in shaping the social evolution of attine ants (e.g. Fernández-Marín et al., 2009).

References

Asís J.D. 2007. The significance of the vestibular cell in trap nesting wasps (Hymenoptera: Crabronidae): Does its presence reduce mortality? *J. Insect Behav.* **20**: 289-305

Feener D.H. Jr. and Brown B.V. 1993. Oviposition behavior of an ant-parasitizing fly, *Neodohrniphora curvinervis* (Diptera: Phoridae), and defense behavior by its leaf-cutting ants host *Atta cephalotes* (Hymenoptera: Formicidae). *J. Insect Behav.* **6**: 675-688

Fernández-Marín H., Zimmerman J.K. and Wcislo W.T. 2006. *Acanthopria* and *Mimopriella* parasitoid wasps (Diapriidae) attack *Cyphomyrmex* fungus-growing ants (Formicidae, Attini). *Naturwissenschaften* **93**: 17-21

Fernández-Marín H., Zimmerman J.K., Nash D.R., Boomsma J.J. and Wcislo W.T. 2009. Reduced biological control and enhanced chemical pest management in the evolution of fungus-farming in ants. *Proc. R. Soc. London, Ser. B.* **276**: 2263-2269

Hanson P.E. and Gauld I.D. 2006. *Himenóptera de la Región Neotropical*. The American Entomological Institute. Gainesville, FL. 994 pp

Kistner D.H. 1982. The social insects' bestiary. In: *Social Insects*, Vol. III (Hermann H.R., Ed), Academic Press, New York. pp 1-244

La Polla J.S., Mueller U.G., Seid M. and Cover S.P. 2002. Predation by the army ant *Neivamyrmex rugulosus* on the fungus-growing ant *Trachymyrmex arizonensis*. *Insect. Soc.* **49**: 251-256

Loiácono M.S., Margaria C.B., Quiran E.M. and Corro Molas B.M. 2000. Diápridos (Hymenoptera) parasitoides de larvas de la hormiga cortadora *Acromyrmex lobicornis* (Hymenoptera: Formicidae) en la Argentina. *Rev. Soc. Entomol. Argent.* **59**: 7-15

Masner L. and García J.L. 2002. The genera of Diapriinae (Hymeno-
    ptera: Diapriidae) in the new world. *Bull. Am. Mus. Nat. Hist.*
    268: 1-138

Mueller U.G., Gerardo N.M., Aanen D.K., Six D.L. and Schultz T.R.
    2005. The evolution of agriculture in insects. *Ann. Rev. Ecol.
    Evol. Syst.* **36**: 563-595

Munster-Swendsen M. 2000. Interaction between the solitary bee
    Chelostoma florisomne and its nest parasite *Sapyga clavicornis*-
    empty cells reduce the impact of parasites. *Ecol. Entomol.* **25**:
    63-70

Powell S. 2008. Ecological specialization and the evolution of a
    specialized caste in *Cephalotes* ants. *Funct. Ecol.* **22**: 902-911

Powell S. and Clark E. 2004. Combat between large derived societies:
    a subterranean army ant established as a predator of mature leaf-
    cutting ant colonies. *Insect. Soc.* **51**: 342-351

Ramos-Lacau L.S., Delabie J.H.C., Bueno O.C., Villemant C., Prates-
    Luz H., Andrade-Miranda M.M., Pereira de Oliveira G., Rodri-
    gues Silva Jr M.R., and Lacau S. 2007. Estratégia comporta-
    mental de *Acanthopria* Ashmead (Hymenoptera: Diapriidae),
    parasitóide de *Cyphomyrmex transversus* Emery (Hymenoptera:
    Formicidae). *Biológico* (São Paulo), **69**: 451-454

Sakagami S.F. and Michener C.D. 1962. *The Nest Architecture of the
    Sweat Bees (Halictinae): A Comparative Study of Behavior.* Univer-
    sity of Kansas Press, Lawrence. 135 pp

Schmid-Hempel P. 1998. *Parasites in Social Insects.* Princeton
    University Press, Princeton. 392 pp

Schultz T.R., Bekkevold D. and Boomsma J.J. 1998. *Acromyrmex
    insinuator* new species: an incipient social parasite of fungus
    growing ants. *Insect. Soc.* **45**: 457-471

Smith A.R., Wcislo W.T. and O'Donnell S. 2003. Assured fitness
    returns favor sociality in a mass-provisioning sweat bee, *Mega-
    lopta genalis* (Hymenoptera: Halictidae). *Behav. Ecol. Sociobiol.*
    54: 14-21

Tepedino V.J., McDonald L.L. and Rothwell R. 1979. Defense
    against parasitization in mud-nesting Hymenoptera: can empty
    cells increase net reproductive output? *Behav. Ecol. Sociobiol.* **6**:
    99-104

Weber N.A. 1972. *Gardening Ants: The Attines.* The American Philo-
    sophical Society, Philadelphia. 146 pp

Wille A. and Michener C.D. 1973. The nest architecture of stingless
    bees with special reference to those of Costa Rica (Hymeno-
    ptera: Apidae). *Rev. Biol. Trop.* **21**: 1-278