

## A New Ectoparasitoid Species of *Pseudogaurax* Malloch, 1915 (Diptera: Chloropidae), Attacking the Fungus-Growing Ant, *Apterostigma dentigerum* Wheeler, 1925 (Hymenoptera: Formicidae)

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### Abstract

Fungus-growing ants (Attini) are abundant and diverse, yet only one taxon of flies (Phoridae) and one of wasps (Diapriinae) are known parasitoids, and the biology of most species is not well known. Here we describe the first evidence for an ant parasitoid in the family Chloropidae (Diptera), in which larvae of *Pseudogaurax paratolmos* Wheeler, new species, parasitize larvae of the ant, *Apterostigma dentigerum* Wheeler, 1925. Larval flies are solitary ectoparasitoids, each of which attaches to a single ant larva and develops from larva to pupa in ~2 wk, consuming nearly the entire host, and then ecloses as an adult ~1 wk later. Overall parasitism prevalence was 6.8% of 203 nests, and flies were active during both the dry and rainy seasons. Intensity of parasitism ranged from 18.2 to 100% of larvae attacked per parasitized nest. No other species of *Apterostigma* that nested in the same localities were parasitized by the flies, including *A. pilosum* (Mayr, 1865) ( $n = 93$  nests) and *A. auriculatum* (Wheeler, 1925) ( $n = 10$  nests). All immature ants, parasitized or not, as well as immature stages of *Pseudogaurax paratolmos*, were attended by adult ants that exhibited normal brood care behavior, including covering immatures with mycelia, grooming, and maintaining brood in the fungus garden.

**Key words:** demography, intensity, prevalence, Attini, parasite

Parasitoids influence numerous aspects of their host's biology, including their relative abundance and competitive ability (Feener and Brown 1997, Godfray et al. 1999, Lewis et al. 2002). Due to their diversity and abundance, parasitoids often promote complex and dynamic ecological interactions. Ants (Formicidae) are one of the dominant taxa of terrestrial invertebrates (Hölldobler and Wilson 1990), and are hosts to a large community of insect parasitoids (e.g., Feener and Brown 1997, Lachaud and Pérez-Lachaud 2012, Folgarait 2013). Remarkably, known Diptera parasitoids of ants have previously been documented only for three families: Tachinidae (Gösswald 1950), Syrphidae (Pérez-Lachaud et al. 2014), and Phoridae (Feener and Brown 1997); the latter contains the vast majority of known cases of fly parasitoids of ants (Lachaud and Pérez-Lachaud 2015).

Fungus-growing ants (Attini) are highly abundant and diverse, with colonies ranging from dozens to millions of workers,

representing a potentially large target for a diverse assemblage of natural enemies (Weber 1972). Many studies have focused on microparasites of attine ants (Currie et al. 1999, Fernández-Marín et al. 2006a), but their macroparasites are less well known (Weber 1972). These natural enemies include diverse flies (Phoridae) that attack foraging adults of leaf-cutting ants, *Atta* spp., usually at low rates (<0.02%; Feener and Brown 1993, 1997), and diapriine wasps that attack larvae of several attine species (Loiácono 1987, Fernández-Marín et al. 2006b, Pérez-Ortega et al. 2010, Loiácono et al. 2013).

*Apterostigma dentigerum* Wheeler, 1925 is a phylogenetically basal attine ant species that is broadly distributed from Brazil throughout Central America (Lattke 1997). Colony sizes range from 30 to 100 workers, with one to few dealate females, possibly functional queens, with worker length ranging from 1.18 to 1.22 mm (Lattke 1997). The nest is formed by a single fungus garden attached

as a veiled covering (see Munkacsi et al. (2004) and Dentinger et al. (2009), for details of the cultivar). The nests are partly exposed on slopes near rivers and creeks, and on the forest floor, under roots and rocks (Weber 1972, Fernández-Marín et al. 2004). Ant larvae and pupae are enveloped in the mycelium where the garden is attached to the soil or other structure (Armitage et al. 2012). Known natural enemies of *Apterostigma dentigerum* are agro-predators, *Megalomyrmex* spp. (Formicidae) (Brandão 1990) and diapiirine wasps that are parasitoids of ant larvae (González, Wcislo, Wheeler and Fernández-Marín, unpublished data).

Here we provide the first documentation of a new parasitoid–ant association, adding another family to the roster of ant parasitoids: a new ectoparasitoid in the family Chloropidae (Diptera), described here as *Pseudogaurax paratolmos* Wheeler, associated with *Apterostigma dentigerum* in Panama. This represents the fourth known case of a Diptera parasitoid attacking ant larvae, and the first known ectoparasitoid species of Chloropidae.

## Materials and Methods

### Nest Collections

In total, 306 *Apterostigma* ant nests were collected between June 2013 and March 2014 at different localities in and around Soberanía National Park (Plantation Loop road, 9° 04'27.47" N, 79 39'35.3" W; near the town of Gamboa, 9° 07'19.15" N, 79 42'43.13" W; and near Km 6.2 of Pipeline Road, 9° 09'39.44" N,

79 44'44.76" W), Panamá. During the rainy season (June to October 2013), 254 *Apterostigma* nests were collected: 155 nests of *A. dentigerum*, seven nests of *A. auriculatum*, and 92 nests of *A. pilosum*. During the dry season (January to February 2014), 52 nests were collected: 48 of *A. dentigerum*, three of *A. auriculatum*, and one of *A. pilosum*. Collected nests were transported to the laboratory at the Gamboa School House of the Smithsonian Tropical Research Institute, and were placed in petri dishes (60 × 15 mm, and 100 × 15 mm) containing wet paper towel for maintaining humidity, and fed with corn and rice two–three times per week. Using a Leica stereomicroscope (10×), we recorded demographic data from each nest, including numbers of eggs, larvae, pupae, workers, dealate females, alate females and males. The fungus garden was weighed, and each brood was observed carefully. We recorded the numbers of ectoparasitic fly larvae attached to the ant larvae, which were easily visible, and we searched the fungus garden to locate and count the number of pre-pupae or puparia attached to the fungus garden (Fig. 1).

Healthy ant larvae are white; their surface is clean and strongly textured. We used a Zeiss EVO 40 VP scanning electron microscope to examine larvae. Fly larvae are strongly segmented, in contrast to the attine larvae which are bean-shaped without protuberances on the surface (Fig. 1). Twenty-one adult flies that emerged in captivity were preserved in 95% ethanol. The behavior of the ants was observed while feeding, grooming, and tending brood to obtain information about how ectoparasites are handled inside the nests. Newly emerged adult flies were attacked by the worker ants in some cases

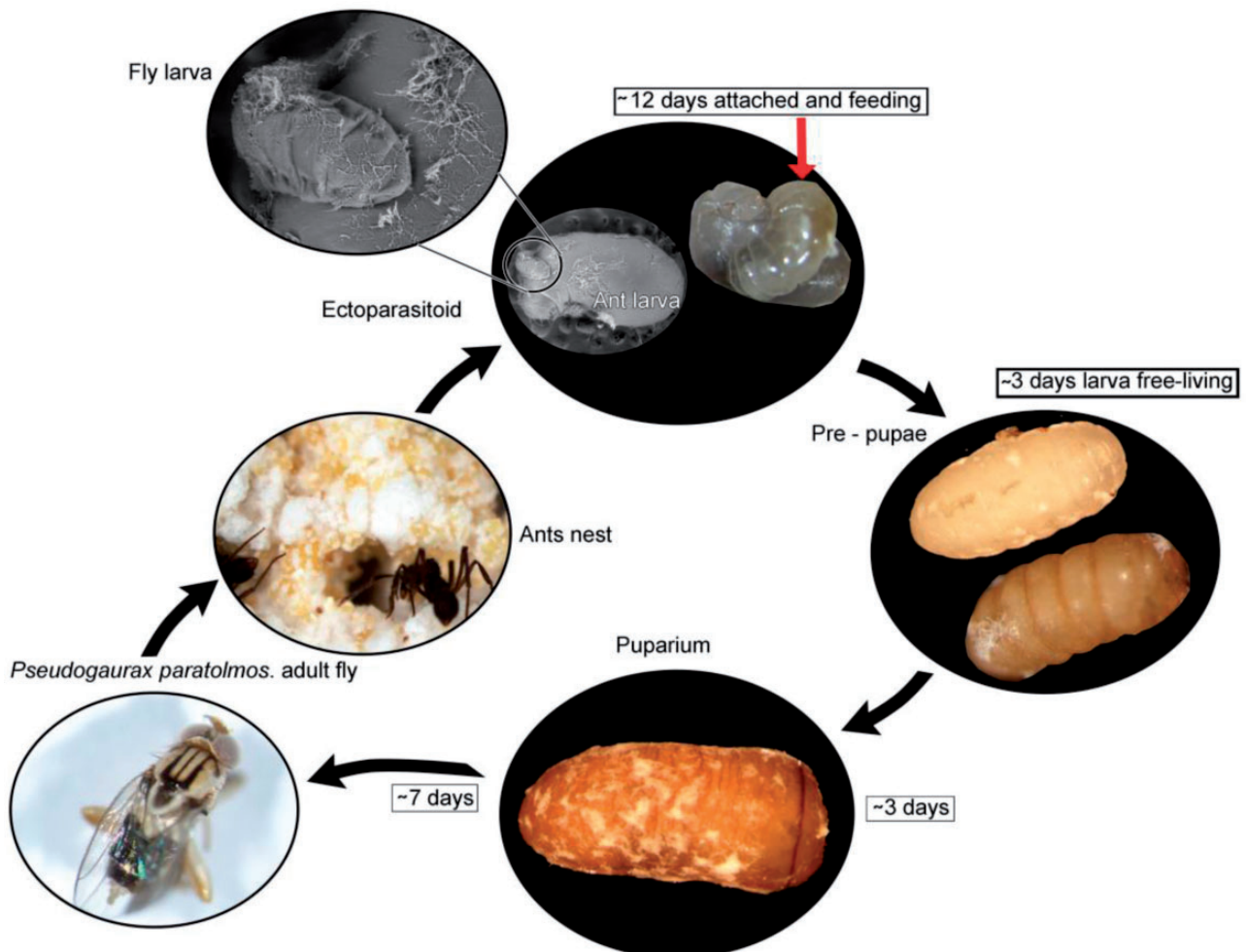


Fig. 1. Juvenile development of *P. paratolmos* (Chloropidae), an ectoparasitoid of larvae of *A. dentigerum*. (Red arrow shows fly larva on ant larva).

before they could be collected. We recorded parasitoid prevalence and intensity; prevalence is the percentage of the nests parasitized by *Pseudogaurax*, while intensity is the percentage of larvae infected from total ant larvae within attacked nests. Results are presented as mean  $\pm$  one standard deviation. Chi-square tests were used to assess demographic differences from parasitoids between dry and rainy seasons.

### Taxonomy

Adult specimens of Chloropidae preserved in ethanol were chemically dried using hexamethyldisilazane and mounted. Genitalic preparations were made by detaching the abdomen of mounted specimens, clearing in 85% lactic acid heated in a microwave oven for two periods of 10 s, separated by a cooling interval of 60 s. Cleared genitalia were transferred to glycerin for examination and later stored in glycerin in a plastic microvial attached to the specimen pin. Morphological terminology used in the species description follows Cumming and Wood (2009).

Type specimens are deposited in the Lyman Entomological Museum, McGill University, Ste-Anne-de-Bellevue, QC, Canada (LEMQ) and the Museo de Invertebrados G.B. Fairchild, Universidad de Panamá (MIUP). Additional voucher specimens of *Pseudogaurax* flies and *Apterostigma* worker ants are deposited at MIUP.

### Nomenclature

This paper and the nomenclatural act it contains have been registered in Zoobank ([www.zoobank.com](http://www.zoobank.com)), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:910BE30C-36B3-47F5-A766-B28CC99F14E6

## Results

### Taxonomy

#### *Pseudogaurax paratolmos* Wheeler, new species

(Figs. 2–5)

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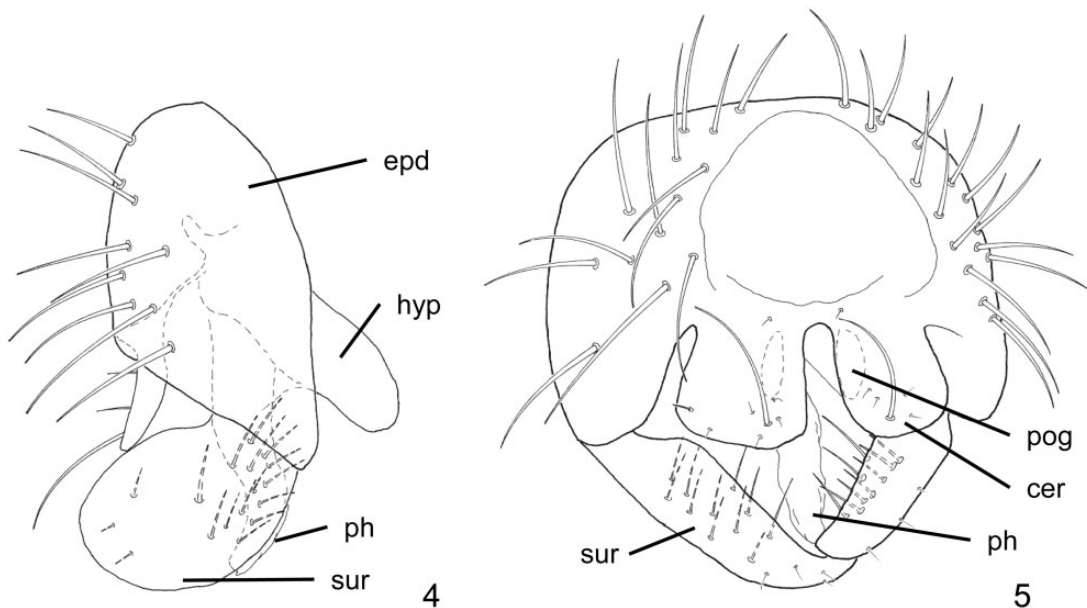
#### Description

*Male.* Total length (holotype and paratypes) 2.5–3.3 mm. Frons yellow, as wide as eye in dorsal view; frontal triangle two-thirds as long as frons, shining yellow, ocellar tubercle shining brown-black; 8–10 long, pale fronto-orbital setae; several pale interfrontal setae including single row of longer setulae bordering margin of frontal triangle; ocellar setae shorter than width of ocellar tubercle, reclinate; outer vertical and postocellar setae twice as long as ocellars, pale; inner vertical seta not distinguished from surrounding setulae; eye large, densely hairy; gena yellow, genal height 0.1 times eye height; vibrissa, subvibrissal, anterior genal setae long, pale; postgena yellow, narrow; occiput yellow; face flat, pale yellow; pedicel yellow, first flagellomere yellow, reniform, higher than long; arista brown, long pubescent, longest setulae three times basal diameter of arista; proboscis small, pale; palpus yellow, with pale distal and ventral setulae.

Thorax with pronotum distinct in dorsal view, pale yellow; postpronotum, notopleuron, and lateral margin of scutum pale yellow, scutum darker yellow medial to intraalar region, with medium brown stripe along dorsocentral line from just anterior to suture to posterior margin, lines curved medially anterior to scutellum; scutal setae pale, postpronotal and notopleural setae slightly darker; one anterior and two posterior notopleural setae; row of prescutellar



Figs. 2–3. *P. paratolmos*, dorsal view. 2. Male. 3. Female.



**Figs. 4–5.** *P. paratolmos*, male genitalia. 4. Lateral view. 5. Posterior view. Abbreviations: cer, cercus; epd, epandrium; hyp, hypandrium; ph, phallus; pog, postgonite; sur, surstylus.

setae slightly longer than other scutal setulae; scutellum yellow with medium brown spot anteromedially, rugose, with long pale setulae; apical scutellar setae pale, divergent, strong, lateral scutellar setae weak, pale (Fig. 2); thoracic pleurites pale yellow except for dark shining anteroventral spot on anepisternum and slight brown shading behind wing base. Legs pale yellow; mid-femoral organ absent; hind-tibial organ large, oval, pale. Wing typical of the genus; ratio of costal sectors 1–4: 2.4: 2.75: 1.75: 1, subcostal break indistinct, appearing as a narrowing of the costa, cell c broad; halter yellow.

Abdominal syntergite 1+2 yellow with brown posterolateral corners, tergites 3–5 brown with yellow lateral margins, tergites with long weak setae, especially laterally.

**Postabdomen (Figs. 4–5).** Epandrium pale yellow, broader than high in posterior view, with long posterior setae; surstylus oval, slightly clavate, projecting posteroventrally, setose on medial surface; hypandrium short and broad in ventral view; postgonites long, paddle-shaped; distiphallus long, pale, membranous; cerci quadrate, well-sclerotized, slightly diverging apically and separated by narrow ventral cleft, each cercus with long ventral seta and shorter setae and setulae; subepandrial sclerite simple, pale.

**Female similar to male except as follows.** Total length 2.5–4.0 mm (based on paratype specimens). Frontal triangle with brown shading anterolateral to ocellar tubercle. Scutum darker yellow laterally, with three brown-black striped extending entire length of scutum; median stripe narrow, dorsocentral stripes at least twice as broad as median stripe; dorsocentral stripes expanded laterally into a brown figure-8 shaped spot posterior to postpronotum and medial to notopleuron; scutal stripes coalesced posteriorly into a complete prescutellar band; scutellum with dorsal surface more extensively brown-black (Fig. 3).

**Postabdomen.** Tergite 6 broad, well-sclerotized; other tergites and sternites of segments 6–8 reduced; tergite 10 triangular, small; cerci well-sclerotized, elongate, narrow in dorsal view, brown, with long setulae; cerci not modified for piercing.

#### Type Material

**HOLOTYPE** ♂: PANAMA: Colon Prov; Soberania Nat Pk, Plantation road (09° 05.183' N, 79° 39.392' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 04.x.2013, emerged 01.xi.2013, C. González [20131004-179sp 1/11/13] (LEMQ). **PARATYPES:** Soberania Nat Pk, Plantation road (09° 05.183' N, 79° 39.392' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 04.x.2013, emerged 21.x.2013, Y. Christopher [20131004-180sp 21/10/13] (1♂, MIUP); Soberania Nat Pk, Plantation road (09° 05.183' N, 79° 39.392' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 04.x.2013, emerged 22.x.2013, C. Gonzalez [20131004-180sp 22/10/13] (1♀, LEMQ); Soberania Nat Pk, Plantation road (09° 05.183' N, 79° 39.392' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 04.x.2013, C. González [20131004-180sp] (1♀, LEMQ); Soberania Nat Pk, Plantation road (09° 05.183' N, 79° 39.392' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 04.x.2013, emerged 30.x.2013, C. González [20131004-177sp 30/10/13] (1♀, MIUP); Soberania Nat Pk, Plantation road (09° 05.031' N, 79° 39.449' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 03.ix.2013, C. González [BP20130903-134sp 30/10/13] (1♂, MIUP); Soberania Nat Pk, Plantation road (09° 05.031' N, 79° 39.449' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 04.x.2013, emerged 17.x.2013, C. González [20131004-191sp 17/10/13] (1♀, LEMQ); Soberania Nat Pk, Plantation road (09° 05.031' N, 79° 39.449' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 04.x.2013, C. González [20131004-191sp] (1♀, MIUP); Soberania Nat Pk, Plantation road (09° 05.031' N, 79° 39.449' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 23.i.2014, Y. Christopher [YG 20140123-236 sp] (1♀, MIUP).

#### Other Material Examined (Teneral or Damaged Specimens)

Soberania Nat Pk, Plantation road (09° 05.183' N, 79° 39.392' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 04.x.2013, C. González [20131004-176sp] (1♂, MIUP); Soberania Nat Pk, Plantation road (09° 05.183' N, 79° 39.392' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected

04.x.2013, emerged 21.x.2013, C. González, [20131004-179sp; 21/10/13] (1♀, MIUP); Soberania Nat Pk, Plantation road (09° 05.031' N, 79° 39.449' W), *Apterostigma dentigerum* nest, parasitoid of ant larvae, collected 23.x.2013, C. González [YG20140123-235sp] (1♂, 1♀, MIUP).

### Etymology

The species name is from the Greek *paratolmos* (foolhardy). Other *Pseudogaurax* species whose biology is known oviposit on egg masses of other arthropods, usually spiders, and the larvae are egg predators. This species lives in the more dangerous surroundings of ant nests.

### Comments

This species runs to couplet 4 in Sabrosky's (1966) key to New World *Pseudogaurax*, but does not key to either half of that couplet. The completely yellow legs correspond to *P. trifidus* (Duda), but the female scutal pattern corresponds to that of *P. tridens* (Sabrosky). The male scutal pattern is paler than either of those species. Other characters of *P. paratolmos* do not correspond to either *P. trifidus* or *P. tridens*. Sexual dimorphism in scutal color patterns has not been documented in other described New World *Pseudogaurax* species. Specimens of *P. paratolmos* may be distinguished from other described New World species of *Pseudogaurax* by the combination of the sexually dimorphic scutal color pattern, mostly yellow thoracic pleurites, completely yellow legs, and the apparent association with ant nests, unknown in other *Pseudogaurax* species.

### Parasitism by *Pseudogaurax paratolmos*

In total, 14 *A. dentigerum* nests contained ant larvae parasitized by *P. paratolmos* (Table 1) from 203 nests collected between 2013 and 2014. Of three *Apterostigma* species (*A. pilosum*, *A. auriculatum*, and *A. dentigerum*), only *A. dentigerum* was attacked by the fly. In the rainy season, the prevalence of nests infected by *Pseudogaurax* larvae was 5.9% ( $n = 155$  nests), while the intensity of parasitism was  $87.3\% \pm 18.1$  (range, 4–100%). In the dry season, the prevalence was 10.4% ( $n = 48$ ), and the intensity was  $62.6\% \pm 38.6$  (range 9.1–100%). No seasonal differences were found for prevalence ( $\chi^2 = 0.89$ ,  $df = 3$ ,  $P < 0.8$ ), nor intensity of parasitism ( $\chi^2 = 1.50$ ,  $df = 3$ ,  $P < 0.7$ ). The presence or absence of sexual ants during our collection was not associated with the presence of *Pseudogaurax* larvae ( $\chi^2 = 2.29$ ,  $df = 3$ ,  $P < 0.5$ ).

*Pseudogaurax* larvae were attached to different regions of ant larvae, generally perpendicular to the long axis (9 of 14 parasitized larvae). Each fly larva consumed nearly the entire ant larva ( $n = 14$ ) over the course of 9–14 d ( $12 \pm 2.6$ ,  $n = 14$ ). After feeding the fly larva transformed to a pre-pupa and remained in the fungus garden. No fly larva was observed to attack a second host larva. A fly larva that was mechanically removed from the host larva, and placed on a new ant larva, was apparently unable to attach successfully. After 2–3 d ( $2.6 \pm 0.6$ ,  $n = 4$ ), the fly pupated in the fungus garden ( $n = 14$ , Table 1). Following 5–7 d ( $6 \pm 1$ ,  $n = 14$ ) of pupation, adult flies emerged, both in the rainy and dry seasons. Hence, we estimated that development of immature flies takes ~20–28 d, based on daily examination of each parasitized nest and recording fly developmental stages (Fig. 1). This duration may be an underestimate since we do not know the time between hatching of the egg and when the fly larva attaches to the ant larva.

All immature stages of the flies were groomed by ants and received typical brood care. Fly larvae and puparia were covered by a similar amount of fungal mycelium (Fig. 1) used to cover ant larvae or pupae (Armitage et al. 2012), but this was not quantified. We lack systematic observations about the behavior of postemergence adult flies, the behavior of free-living adults, and how they gain entrance to the nests. Anecdotally, when adult flies emerged in our artificial nests, the ants bit and killed them ( $n = 3$ ). Some adult flies that were preserved for later identification have partially severed legs, apparently cut by ant mandibles. The sex ratio of adult *P. paratolmos* collected from *A. dentigerum* was 3:5 (female:male) ( $n = 16$  adults).

### Discussion

Chloropidae is one of the most ecologically diverse families of Diptera, with species differing in larval feeding habits, including larvae that are saprophagous, phytophagous, predaceous, cleptoparasitic, or parasitic (Wheeler 2010). Some chloropids are associated with Hymenoptera, including specimens reared from cells of spider wasps (Pompilidae) (Wcislo et al. 1988) and sweat bees (Halictidae) (Smith et al. 2008). Known larvae of *Pseudogaurax* are predators, mostly on spider egg masses, but have also been found in egg masses of mantids, Lepidoptera and Megaloptera, and cocoons of Lepidoptera (Sabrosky 1987, Barnes et al. 1992, Melo and Wheeler 2009). However, most Central American species of *Pseudogaurax*

**Table 1.** Information from 14 *A. dentigerum* nests parasitized by *P. paratolmos* from 2013–2014

Nests	Season	Eggs	Larvae		Pupae	Workers	Queen	Male	Alated female	Fungus garden weight (g)
			Healthy	Parasitized						
BP20130917-169 SP	Rainy	0	2	1	3	37	1	7	8	3.86
BP20130917-172 SP	Rainy	1	0	1	2	6	0	1	0	0.47
CG20131004-175 SP	Rainy	0	4	3	3	12	0	0	0	0.53
CG20131004-176 SP	Rainy	1	1	5	1	31	1	0	0	0.32
CG20131004-177 SP	Rainy	0	0	3	2	12	1	0	0	0.17
CG20131004-178 SP	Rainy	5	24	1	1	66	1	0	0	1.57
CG20131004-179 SP	Rainy	0	0	6	0	8	0	3	1	0.35
BP20131025-213 SP	Rainy	0	0	1	2	6	0	0	0	0.20
BP20131025-214 SP	Rainy	10	9	2	6	51	1	0	3	1.10
CG20140123-233 SP	Dry	2	0	1	0	8	2	1	0	0.51
YC20140123-235 SP	Dry	0	1	5	2	10	0	0	1	0.48
CG20140123-236 SP	Dry	0	0	4	1	2	0	0	0	0.30
YC20140123-241 SP	Dry	4	5	1	1	19	1	1	1	0.82
PG20140129-262 SCC	Dry	1	10	1	1	16	0	0	0	0.95

are undescribed (Wheeler 2010) and the larval habits of only a few species have been documented.

The impact of *Pseudogaurax* parasitoidism on host demography is unknown, and prevalence is relatively low compared to diapiirine wasps or phorid flies that attack other attine ants (Feener and Brown 1997, Fernández-Marín et al. 2006b, Pérez-Ortega et al. 2010). *Pseudogaurax* larvae appear to have high survivorship rates, at least in the laboratory, because almost all larvae identified and not manipulated emerged as adults. In contrast, the survivorship of other attine ant parasitoids, like diapiirine wasps, is often lower than 50% due to fungal infection and by cannibalization by worker ants (Pérez-Ortega et al. 2010, Fernández-Marín, unpublished data).

Parasitoid behavior is widespread in Diptera (Feener and Brown 1993, 1997). Curiously, dipteran parasitoids of ants are currently known only from four families, three of which have been documented only for a single species (Feener and Brown 1993, 1997; Folgarait 2013; Lachaud and Pérez-Lachaud 2015). Thus basic questions like degree of host specialization and host localization are unknown. Above all, *Pseudogaurax paratolmos* is the first known ectoparasitoid of fungus-growing ants, and only the second case of a dipteran ectoparasitoid of ant larvae (see Lachaud and Pérez-Lachaud 2015). This species is the first known ectoparasitoid chloropid fly, and this association with ants represents a novel host shift to exploit a diverse and abundant taxonomic group. There are published records of Chloropidae (an unidentified species of *Fiebrigella* Duda) as cleptoparasites of halictid bees, where they consume stored pollen in cells, resulting in smaller bee larvae emerging from cells with flies (Smith et al. 2008). Other Chloropidae attack solitary hosts and are predators of eggs or, rarely, pupae (Barnes et al. 1992, Melo and Wheeler 2009). However, the oviposition strategies used by these chloropid species are not known, and presumably those attacking social hosts have behavior different from those attacking solitary hosts. The discovery of *P. paratolmos* as an ectoparasitoid of social Hymenoptera with active nest defense suggests that there might be a number of behavioral and chemical changes in all life stages of this fly. *Apterostigma* ants care for their brood employing fungal-symbionts to envelop the brood; *Pseudogaurax* larvae or adults appear to bypass this physical defensive barrier (Fig. 1), but the chemical camouflage could help protect the parasitoids (Akino 2008). Eucharitid wasps, for example, appear to use ant cuticular hydrocarbons to escape from host nests (Pérez-Lachaud et al. 2015). Workers of some species of fungus-growing ants do not discriminate between unparasitized and parasitized brood attacked by diapiirine wasps, but emerging adult wasps are attacked and killed by ants (Fernández-Marín et al. 2006b, Pérez-Ortega et al. 2010). Similar behavior was observed toward the chloropid flies by *A. dentigerum* workers, based on limited observations. Detailed studies on the behavioral ecology of adult parasitoids are needed to fully understand the dynamics of host-parasitoid interactions among attine ants, as true for myriad other ant-parasitoid associations (e.g., Pérez-Lachaud et al. 2014, 2015).

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