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Parasitoid phorid flies (Diptera: Phoridae) from the threatened leafcutter ant *Atta robusta* Borgmeier (Hymenoptera: Formicidae)

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Abstract

Phorid flies are well known natural enemies of leafcutter ants, but there is no information on phorid species associated with the threatened species *Atta robusta* Borgmeier. During 2009 and 2010 we collected phorid parasitoids of *A. robusta* at Guriri Island, Espírito Santo (18°43'S; 39°45'W) and at Rio de Janeiro city (23°01'S; 43°28'W). Three new species were found: *Eibesfeldtphora breviloba* Brown, *E. digitata* Brown, and *Myrmosicarius exrobusta* Brown. These species, the attack behavior of the two species of *Eibesfeldtphora*, as well as the anti-parasitoid defense behavior of *A. robusta*, are described.

Key words: host-parasitoid interactions, *Atta*, *Eibesfeldtphora*, *Myrmosicarius*, Phoridae, Formicidae

Introduction

Parasitoid flies of the family Phoridae are among the natural enemies of leafcutter ants, genus *Atta* F. (Della Lucia, 1993), and various species of the genera *Eibesfeldtphora* Disney (some as *Neodohrniphora* Malloch), *Myrmosicarius* Borgmeier, *Apocephalus* Coquillett, and *Allochaeta* Borgmeier are recorded from Brazil, attacking *Atta bispharica* Forel, *Atta cephalotes* (Linnaeus), *Atta laevigata* (Smith) and *Atta sexdens* (Linnaeus) (Feener & Moss, 1990, Disney & Bragança, 2000, Brown, 2001). Five more *Atta* species occur in Brazil for which there is no information on associated phorid species, one of which, *Atta robusta* Borgmeier, was the object of this study. This is the only *Atta* species that occurs in “restinga” (sandbank) vegetation, with a geographic distribution apparently restricted to the States of Espírito Santo and Rio de Janeiro (Teixeira *et al.*, 2004; Teixeira *et al.*, 2003). Several years ago it was suggested that this *Atta* species could become extinct due to human encroachment on the restinga vegetation (Fowler, 1995; Fowler *et al.*, 1996) and recently *A. robusta* was included in the list of species threatened with extinction (Machado *et al.*, 2008).

Parasitoid phorid species of *Atta* may be species specific or parasitize more than one host species, and each *Atta* species may also be attacked by more than one phorid species (Brown, 2001). Field and laboratory studies on phorid-*Atta* interactions have shown that the parasitoid attack strategies, the host defense behaviors and the oviposition sites on the bodies of the workers of the various *Atta* species, are generally specific (Erthal & Tonhasca, 2000, Tonhasca *et al.*, 2001; Bragança *et al.*, 2003; Bragança *et al.*, 2009; Brown, 1999). The objectives of the present study were to search for phorid parasitoids associated with *A. robusta* and to observe and describe fly attack behaviors and host defense mechanisms.

Method and materials

Fieldwork was carried out by MALB, DSG, JMQ, and MCT. Nests of *A. robusta* located in the restinga of Guriri Island (18°43'S; 39°45'W), Espírito Santo state, were observed between 2–3 January 2009 and observations were also made at two locations of Rio de Janeiro city (23°01'S; 43°28'W) between June 2009 and October 2010. Approximately 25 nest entrance holes and their respective trails of eight colonies in the Guriri Island restinga and 36 entrance holes and their respective trails in the restinga of Marambaia and the Marapendi Park, both in Rio de Janeiro, were studied. The search for phorids in Rio de Janeiro was made at 18 entrance holes in open areas (sunny), more affected by human disturbance, but still considered restinga vegetation, and at another 18 entrance holes in closed areas (shaded), which were less disturbed.

Specimens were collected into alcohol and chemically dried using hexamethyldisilazane (Brown, 1993). Holotypes and most other specimens are deposited in the collection of the Museu de Zoologia, Universidade de São Paulo, Brazil (MZSP), with duplicates placed in the collection of the Natural History Museum of Los Angeles County (LACM) (collection codens from Arnett *et al.*, 1993).

Bar-coded data labels were placed on each specimen, and data entered in the LACM phorid database. The "LACM" prefix to such numbers indicate where the data are stored, not ownership of the specimens.

Terms are those used in the *Manual of Central American Diptera* (Brown, 2010; Cumming & Wood, 2009).

Results

Three new species of phorid flies were discovered during our field work. They are described below:

Eibesfeldtphora Disney, in Disney *et al.*, 2009

Neodohrniphora Malloch, 1914, in part.

Genus and species recognition. Disney (in Disney *et al.*, 2009) elevated this former subgenus of *Neodohrniphora* to generic stature. The latest identification key to species in this genus is that of Brown (2001), which included illustrations of the oviscapae of each species. Since then, two further new species have been described (Disney *et al.*, 2009).

Generalized description of adult females. Frons narrow, brown, median furrow present. 4-4-4 frontal setae present; lower interfrontal setae much lower on frons than lower fronto-orbital setae; supra-antennal setae absent. Three ocelli present; ocellar triangle black. Palpus and proboscis small, yellow. Flagellomere 1 slightly pointed, light brown; arista present. Scutum light brown; scutellum dark brown, with anterior setula and larger posterior seta. Pleuron yellow to light brown; anepisternum bare. Legs yellow; all tibiae with dorsal longitudinal row of enlarged setulae except absent on foreleg of *E. mexicana* Disney. Foreleg with only 4 separate tarsomeres (tarsomeres 4 and 5 fused). Wing well developed, with vein R_{2+3} present; halter dark brown. Abdominal segments 1–5 yellow (rarely light brown) ventrally, segment 6 dark gray becoming yellow posteriorly. Abdominal tergites dark grayish brown with posterior yellow band. Segment 7 (oviscapae) darkly sclerotized with various lateral lobes; membrane of intersegment 7–8 with small ventral sclerite; segments 8–10 form apically pointed stylet.

Species are relatively uniform, with differences largely confined to the female oviscapae. Thus, descriptions are short, but combined with illustrations, are sufficient to diagnose and recognize species.

Eibesfeldtphora breviloba Brown new species

Fig. 1.

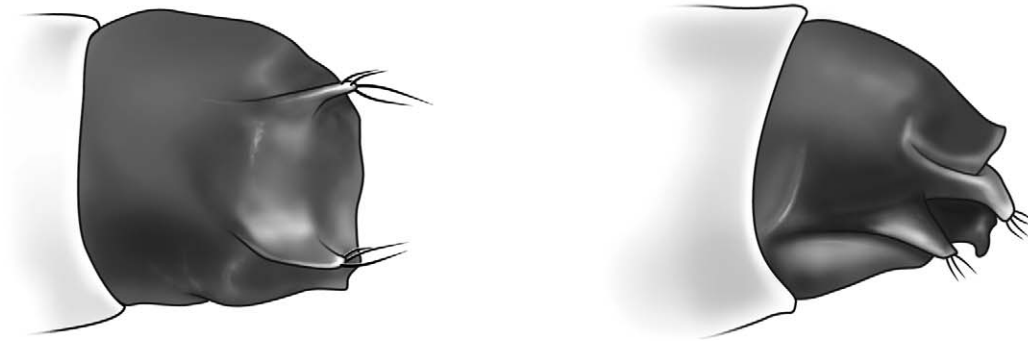
Diagnosis. This species is distinctive by the thin elongate dorsal process and shorter, but still thin ventral process of the oviscapae. It is most similar to *E. tonhascai* (Brown), differing in the much shorter ventral process, and to *E. declinata* (Borgmeier), differing by the thinner processes of the oviscapae. In the key of Brown (2001), *E. breviloba* keys to couplets 5, where it comes closest to *E. tonhascai*.

Description. Female. Body length 2.1–2.6 mm. Apex of foretarsomere 5 not narrowed. Costa ratio 0.41–0.44 wing length. Oviscape with two short processes; dorsal process dorsoventrally flattened in posterior view; ventral process rounded in posterior view, largely fused to oviscape. Both processes with fine apical setae.

Derivation of specific epithet. Named for the short lobes of the oviscape.

Holotype. ♀, BRAZIL: Rio de Janeiro, 23°01'S, 43°28'W), 10.i.2010, D. da Silva Gomes [barcode: LACM ENT 305110] (MZSP).

Paratypes. 2 ♀, same data as holotype (LACM, MZSP).



1 *Eibesfeldtphora breviloba* n. sp.

2 *Eibesfeldtphora digitata* n. sp.



3 *Myrmosicarius exrobustus* n. sp.

FIGURES 1–3. Female phorid fly oviscapes, lateral. 1. *Eibesfeldtphora breviloba*; 2. *Eibesfeldtphora digitata*; 3. *Myrmosicarius exrobustus*.

Eibesfeldtphora digitata Brown new species

Fig. 2.

Diagnosis. This species differs from others by the apically narrowed foretarsomere 5 with its greatly reduced claws. It further differs from other species with laterally flattened dorsal and ventral processes of the oviscape by their ventral curvature and relatively narrow size. In the key of Brown (2001), *E. digitata* keys to *E. isomorpha* (Brown) at couplet 8. It differs, however, in that the lateral processes of the oviscape are much smaller, less rounded and slightly ventrally curved. In *E. isomorpha*, the processes project straight posteriorly, are broad, and broadly rounded (compare with Brown 2001: figure 19).

The more recently described *E. saltensis* Disney is also somewhat similar, but has a broader dorsal lobe, and a much shorter ventral lobe (compare with Disney *et al.* 2009: figure 2).

Description. Female. Body length 2.2 mm. Apex of foretarsomere 5 narrowed, claws reduced. Costa 0.50 wing length. Oviscape with two well separated processes; dorsal process dorsoventrally flattened in posterior view, slightly ventrally curved; ventral process short, small, laterally flattened.

Derivation of specific epithet. Named for the fingerlike lobes of the oviscape.

Holotype. ♀, Brazil: Espírito Santo: São Mateus, i.2009, M. Bragança [barcode: LACM ENT 305108] (MZSP).

Myrmosicarius Borgmeier, 1928

Myrmosicarius exrobusta Brown new species

Fig. 3.

Diagnosis. This species is part of a group that have 3 notopleural setae present, differing by the shape of the apex of the oviscapae. In the latest identification key to species of this genus (Disney *et al.*, 2006), this new species keys to couplet 3, where the apices of the oviscapae of *M. grandicornis* Borgmeier and *M. brandaoi* Disney *et al.* are compared. The oviscapae of *M. exrobusta* is much less rounded than that of *M. grandicornis*, and less sinuous and differently shaped than that of *M. brandaoi*.

Description. Female. Body length 1.5–2.0 mm. Body color brown. Frons with 4-4-4 short frontal setae; ventral interfrontal setae arise nearer to eye margin than midline, strongly mediocline. Flagellomere 1 rounded-oval, laterally flattened. Palpus extremely short, brown, proboscis long, narrow; labella twice as long as labrum. Thorax with three notopleural setae. Legs with fore- and midtarsomere 5 elongate, narrowed, with claws reduced. Tarsomere 5 of hind leg not apically narrowed. Hind tibia with single dorsal setal palisade and posterodorsal row of small setae, most apical seta much larger. Costa 0.30–0.35 wing length. Vein R_{2+3} present. Halter brown. Posterior margin of abdominal tergites with minute setae only. Oviscapae only slightly sinuous, with bluntly rounded apex.

Etymology. Named for being “from” (*ex-*) the host ant species.

Holotype. ♀, BRAZIL: Rio de Janeiro, 23°01'S, 43°28'W, 10.i.2010, D. da Silva Gomes [barcode: LACM ENT 305112] (MZSP).

Paratypes. 2 ♀, same data as holotype (LACM, MZSP).

Natural history. The attack behavior of the two species of *Eibesfeldtphora* described herein consisted of an approach from the rear or from the sides of *A. robusta* workers and a landing on the hind part of their heads where, apparently, the phorid flies introduced their ovipositors. This behavior is similar to that of *E. tonhascai* (Brown) and *E. elongata* (Brown) females against the *A. sexdens* host (Bragança *et al.*, 2008; Bragança *et al.*, 2009) and *E. curvinervis* (Malloch) on *A. cephalotes* (L.) (Feener & Brown, 1993), but differs from that of *E. erthali* (Brown) and *E. bragancai* (Brown), which introduce their ovipositors into the gaster of *A. laevigata* and *A. bisphaerica* workers, respectively (Bragança *et al.*, 2003; Bragança *et al.*, 2002). The anti-parasitoid defense behavior of *A. robusta* against *Eibesfeldtphora* species was similar to that observed in *A. sexdens* against *E. tonhascai* and *E. elongata* (Bragança *et al.*, 2008; Bragança *et al.*, 2009): the workers ran towards the nest entrance holes, attacked the parasitoid with their mandibles or lowered their bodies and protected the head with their legs to avoid the fly's approach. The larval development of the new species of *Eibesfeldtphora* probably occurs inside the cephalic capsule of *A. robusta*, with a single puparium forming between the host's mandibles after its death and from which the adult parasitoid emerges, as observed for other *Eibesfeldtphora* species which attack *Atta* spp. (Bragança *et al.*, 2008; Bragança *et al.*, 2009). This sequence also occurs even when oviposition is in the gaster (Bragança *et al.*, 2003; Bragança *et al.*, 2002). Females of *Myrmosicarius exrobusta* also pursue *A. robusta* workers, approaching from behind to attack, but under field conditions it was impossible to verify exactly where this species oviposits in the host's body, due to the parasitoid's speed of attack and also to its small size. The defense behavior of *A. robusta* against this species was to run to the nest and attack the parasitoid with its mandibles.

It was also common to observe worker minims on plant fragments that were being transported to the nest. One of the functions of this behavior, called “hitchhiking”, seems to be as a defense strategy of *Atta* against parasitoid activity (Feener & Moss, 1990, Linksvayer *et al.*, 2002, Vieira-Neto *et al.*, 2006). Whether the presence of these minims served to protect *A. robusta* against phorid parasitoids could not be verified during the present study.

In Rio de Janeiro, the phorids were found attacking *A. robusta* workers both on the trails and at the nest entrance holes, in contrast to other species which choose specific places to attack their host (Bragança *et al.*, 2003). However, 85% of *Myrmosicarius* sp. individuals were collected from open areas whereas 96% of *Eibesfeldtphora* specimens were captured from closed areas. Elizalde and Folgarait (2010) discovered associations between phorid species and specific habitats due to the special climatic conditions generated for each one, including humidity and light intensity.

Just as *A. robusta* may be threatened with extinction, due to destruction of its habitat in the coastal areas of Espírito Santo and Rio de Janeiro states, this concern may also be extended to its parasitoid phorids. These flies appear to specifically parasitize *A. robusta*, as do the phorids *E. erthali* and *E. bragancai* that specifically attack the

ants *A. laevigata* and *A. bisphaerica*, respectively (Bragança *et al.*, 2003; Bragança *et al.*, 2002). Additionally, these phorids would not have alternative hosts if *A. robusta* became extinct, since no other *Atta* species were observed in the restinga vegetation.

Therefore, it seems certain that the three species of phorid parasitoids of *A. robusta* will also disappear if their host becomes extinct. This justifies the inclusion of these flies in the list of the Red Book of Endangered Brazilian Wildlife (Machado *et al.*, 2008). In order to increase our knowledge of these parasitoids, and also the chances of conserving them, we recommend basic biological studies on their biology be made to collect information on larval development, pupal formation, and adult fly emergence.

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