

**MORPHOLOGICAL NOTES ON THE WORKER AND QUEEN
LARVAE OF THE THIEF ANT (HYMENOPTERA, FORMICIDAE, MYRMICINAE) FROM BRAZIL**

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ABSTRACT

Young and mature larvae of queen and workers of the thief ant, *Solenopsis helena*, are herein described for the first time. Specimens were treated and described by usual methods of light and scanning electron microscopy. Many of the observed characteristics confirmed traits of the thief ant larva, reinforcing the assumption that they are a distinct group from fire ants. The larva of *S. helena* can be recognized from other close species by details of the mouthparts, but seem extensively similar to *Solenopsis molesta*. These findings are useful for taxonomic studies and phylogenetic inferences.

Key Words: Larval description, taxonomy, Solenopsidini, scanning electron microscopy, ant morphology

RESUMO

Larvas de diferentes tamanhos de operárias e rainhas de *Solenopsis helena* são descritas. Os espécimes coletados em São Paulo, Brasil, foram medidos e descritos por meios convencionais de microscopia ótica e eletrônica de varredura. As larvas desta espécie diferem de outras espécies próximas por detalhes das peças bucais, mas parecem ser muito semelhantes a descrições anteriores de *Solenopsis molesta*. Muitos caracteres distintos de larvas das formigas do antigo subgênero *Diplorhoptrum* foram aqui confirmados, sugerindo que realmente são um grupo distinto de formigas dentro do gênero *Solenopsis*. Estas informações são úteis em estudos maiores de filogenia e taxonomia do grupo.

Translation provided by authors.

Solenopsis Westwood (Hymenoptera: Formicidae) is a cosmopolitan ant genus with about 371 species and subspecies worldwide (Bolton et al. 2006) of which 108 occur in the New World (Pitts 2002). The genus *Solenopsis* is famous as some species known as "fire ants" are invasive pests in the United States (Tschinkel 2006). Another group within *Solenopsis* comprises the "thief ants", which are relatively inconspicuous and live a lestobiotic lifestyle, stealing resources from surrounding ant nests (Pacheco 2007; Pacheco et al. 2007). Thief ants are small (usually monomorphic), usually subterranean and rarely collected, while fire ants are larger and more easily collected and studied (Tschinkel 2006; Pacheco 2007; Pacheco et al. 2007). Thief ants were traditionally grouped in the subgenus *Diplorhoptrum*, which is a junior synonym of *Solenopsis* (Bolton et al. 2006).

Larval descriptions of ants are important to taxonomy as some characters can be applied to genus-level identification and phylogenetic analyses (Wheeler & Wheeler 1976; Schultz & Meier 1995; Pitts et al. 2005). However, the larva has been properly described for only a few ant species. Since ant larvae stand at the base of the social structure of the ant colony (Hölldobler & Wilson 1990) a more detailed knowledge about larval morphology can cast light onto unresolved aspects of ant biology (Fox et al. 2007), as illustrated by recent works on the role of the larvae in whole colony nutrition (Cassill et al. 2005; Masuko 2008).

The larval stages of the thief ant *Solenopsis molesta* (Say) were first described by Wheeler & Wheeler (1955). Later, Petralia & Vinson (1979) revisited the larva of this species, added some aspects about the morphology and distribution of

the body pilosity, and illustrated the morphology with scanning electron micrographs.

The present study was based on the larva of *Solenopsis helena* Emery, collected for the first time in Brazil. Here we describe the morphology of some immature stages with the aid of light and electron microscopy. We also provided further images and added detailed information in order to identify distinctions in the morphology of the 2 thief ant species *S. molesta* and *S. helena*.

MATERIALS AND METHODS

Solenopsis helena immature workers and queens were obtained during soil excavations on the campus of São Paulo State University in Rio Claro, São Paulo, Brazil ($22^{\circ}2' 35.00''S$ $47^{\circ}32'32.28''W$) on 08-X-2008.

All collected samples were fixed in Dietrich's solution (900 ml distilled water, 450 ml 95% ethanol, 150 ml 40% formaldehyde, 30 ml acetic acid) for 24 h and were conserved in 80% ethanol. A total of 5 mature worker larvae, 3 queen larvae, 2 first instar larvae, and several eggs were analyzed.

With a stereomicroscope (Wild Heerbrugg, 40X) equipped with a micrometer, we sorted the immature stages and measured their lengths and widths. Morphological descriptions were made with a light microscope (Zeiss Axiolab, 1000X) and a scanning electron microscope (LEO 435 VP at 20.0 kV). Measurements are either given in mm or in μm , and, where applicable, are presented as mean \pm standard deviation (SD).

Samples for SEM analysis were dehydrated in an alcohol-graded series (80-100%; specimens being immersed for 5 min in each concentration), and critical-point dried (Balzers CPD/030). Dried specimens were then attached to aluminum stubs with double-faced conductive adhesive tape, and were gold-sputtered with a Balzers SCD/050 sputterer. Observations and images were obtained as soon as possible as to avoid deformations after sample preparation. Prior to analysis under the light microscope, the larvae were warmed for 10 min in 10% KOH and placed in a small drop of glycerin on a microscope slide.

Voucher specimens of the adult worker ants are deposited in the entomological collection of the Laboratory for Environmental Biology of the Centennial Museum, El Paso, Texas, USA. All terminology used in our larval descriptions follow Wheeler & Wheeler (1976).

RESULTS

Egg

Ovoid (Fig. 1A); yellowish; delicate translucent chorion separated from developing embryo. Mean

egg length 0.164-0.182 ($N = 40$), width 0.110-0.145 mm ($N = 40$). Length: width ratio 1.48

First Larval Instar

Few observations were made of this instar as most specimens were lost during sample preparation, because of their diminutive size. One specimen was analyzed by SEM, and one damaged specimen by light microscopy. Body profile attoid (Fig. 1B); 0.32 mm long and 0.10 mm wide; hairs simple, $9.58 \pm 3.75 \mu m$ long ($N = 15$). A few simple hairs could be observed near the occipital border of the head capsule. Cranium about 113 μm wide; clypeus indistinct. Head hairs distributed as follows: 6 hairs on the occipital border, 2 hairs on vertex, 2 hairs on the frons, 4 hairs bordering the ventral border of clypeus, 6 hairs on the gena. Tentorial openings short slits under each gena. Mouthparts: Generally reduced and rounded. Labrum indistinct from clypeus or head capsule, 60 μm wide and 28 μm long, slightly depressed mesad, with a row of 6 hairs at mid-height and 1 basiconic sensillum on each anterior half. Mandibles transparent, 30 μm long and 25 μm wide at base, triangular with blunt apical and subapical teeth. Maxilla a rounded lump 40 μm high, maxillary palpus a slight elevation with 2 setaceous sensilla, and galea a pair of basiconic sensilla. Labium poorly defined with 4 short hairs on the lower half, labial palps being clusters of 3 basiconic sensilla.

Last Larval Instar of Workers

It is worth noting that mature larvae of *S. helena* had characters considered diagnostic of prepupae of *S. invicta* (Buren) by Petralia & Vinson (1979): sclerotized mandibles and body with abundant bifid hairs. Body shape dolichoderoid (Fig. 1C), 1.10-1.50 mm long and 0.44-0.62 mm wide ($N = 4$); length through spiracles 1.79-1.83 mm ($N = 2$); 10 pairs of spiracles, the second pair larger (10 μm) than others (5 μm ; $N = 4$ measured specimens). Body hairs evenly distributed, numbering approximately 750 ($N = 3$), usually bifid and of 2 types: smooth with tip bifid (0.025 mm) and moderately bifid with tips curling in opposite directions (0.030 mm); there are a few simple hairs around the food basket area (0.012 mm) and also around the anus (Fig. 2C). Body hairs often with side ramifications, resulting in the varied subtypes illustrated in Fig. 3. Food basket with transversal short rows of minute spinules (Fig. 2B).

Head subelliptical (Fig. 2A); 0.145 mm wide; with 18-20 hairs of 2 types: simple (0.010 - 0.012 mm) or bifid (0.057 mm), distributed as follows: 4 to 6 occipital deeply bifid hairs, 1 or 2 bifid hairs on each side of vertex, 2 bifid hairs

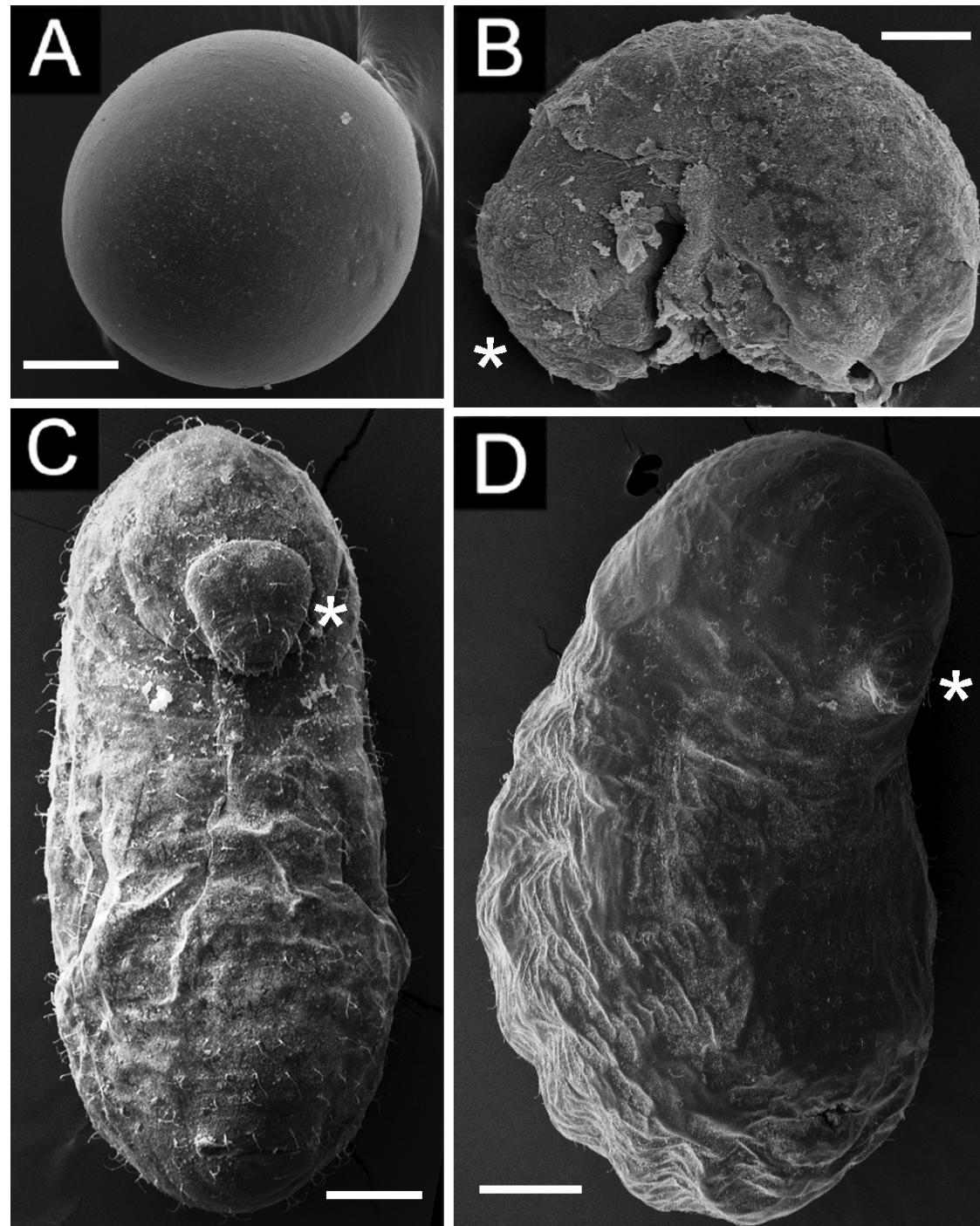


Fig. 1. Immature stages of *Solenopsis* (*Diplorhoptrum*). A-Egg; B-First instar larva partially covered with egg chorion; C-Mature worker larva; D-Mature queen larva. Asterisk indicates position of head capsule. Sizes of scale bars (μm): 50; 45; 110; 200.

on the frons, 4 simple hairs over each gena, 2 simple hairs on the dorsal border of clypeus (Figs. 2A and 2B). Only simple head hairs had

ramifications, always at the apex (see Fig. 3B and 3C); ramified simple hairs occurred on the gena and clypeus. Antennae are slightly ele-

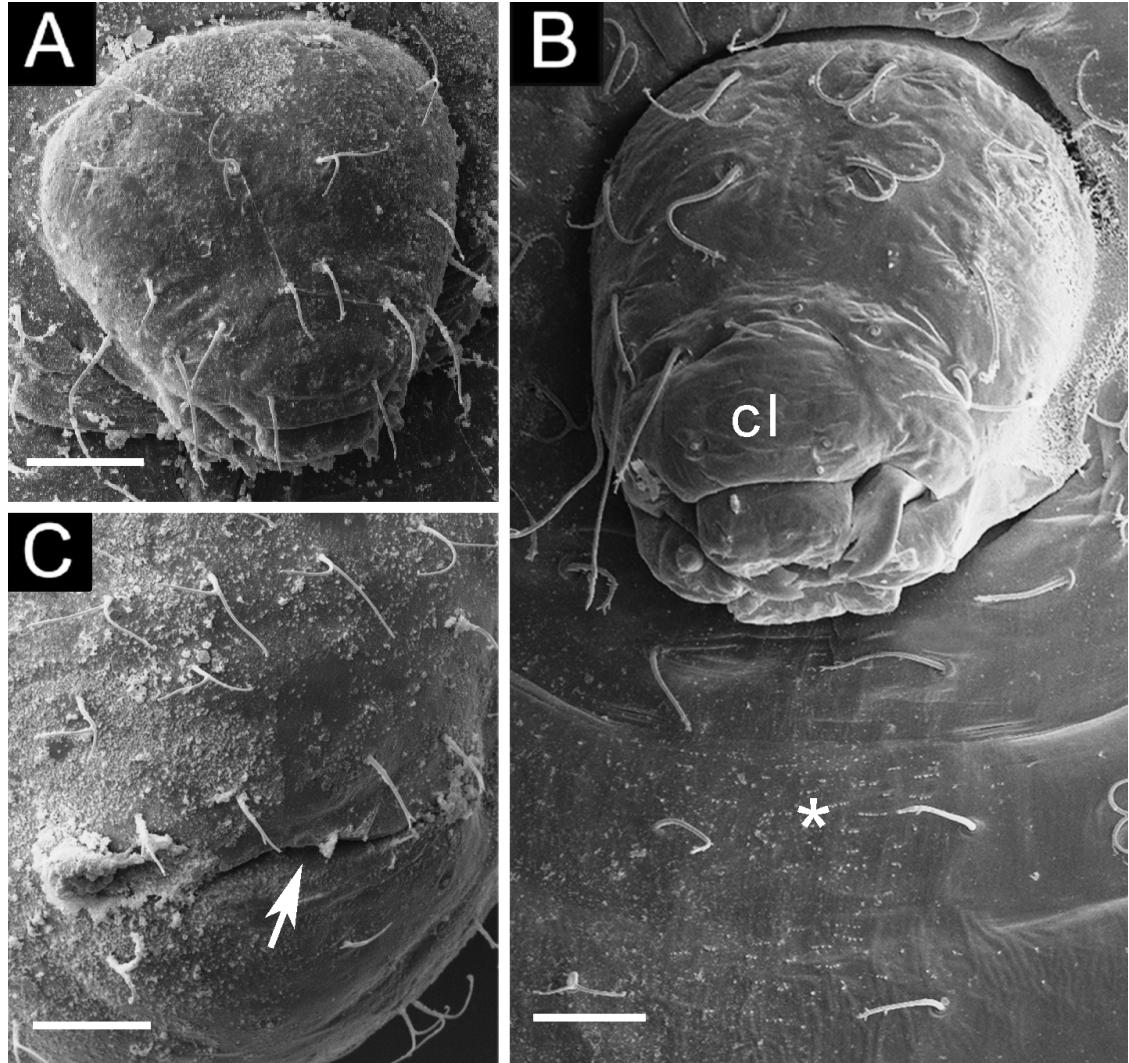


Fig. 2. Details of mature larvae of *Solenopsis (Diplorhoptrum) helena*. A-Worker larva in frontal view; B-Queen larva in oblique view; cl = clypeus, asterisk = food basket region, with rows of spinules. C-Posterior ventral body region of a worker larva; arrow = anus. Sizes of scale bars (μm): 40; 45; 50.

vated with 3 minute basiconic sensilla, which can be arranged in a row or a cluster. Sensilla can be so close to each other that they look like only 2 in optical microscopy observations. Clypeus rectangular (0.070 mm wide and 0.050 mm long; $N = 1$), fused with cranium.

Mouthparts: Labrum rectangular, slightly depressed mesadly (21.14-32.60 μm long and 40.10-42.00 μm wide; $N = 4$) (Fig. 4A), with row of 4 setaceous sensilla at midheight of anterior face; ventral border with 2 setaceous sensilla and spines pointing mesadly. Maxillae paraboloidal, 55.30-61.00 μm long ($N = 2$) and 30.00-35.00 μm wide ($N = 2$) (Fig. 4B), with 2 setaceous sensilla, one near base of palp and an-

other near galea; maxillary palp paxilliform (0.010 mm long) with two setaceous sensilla and one encapsulated sensillum on top; galea paxilliform (0.015 mm long) with 2 setaceous sensilla on top. Mandibles ectatommoid, 58.00-63.00 μm long ($N = 2$), with 2 prominent apical teeth and 1 subapical tooth followed by a blade. Labium rectangular, about 75 μm wide, with 1 setaceous sensillum on each side and sparse clusters of spinules; labial palp a round elevation with 1 basiconic sensillum and 1 enclosed sensillum. Opening of sericteries a transversal slit 18.00 μm long ($N = 1$); labial region above opening with scattered clusters of short spines towards mouth entrance.

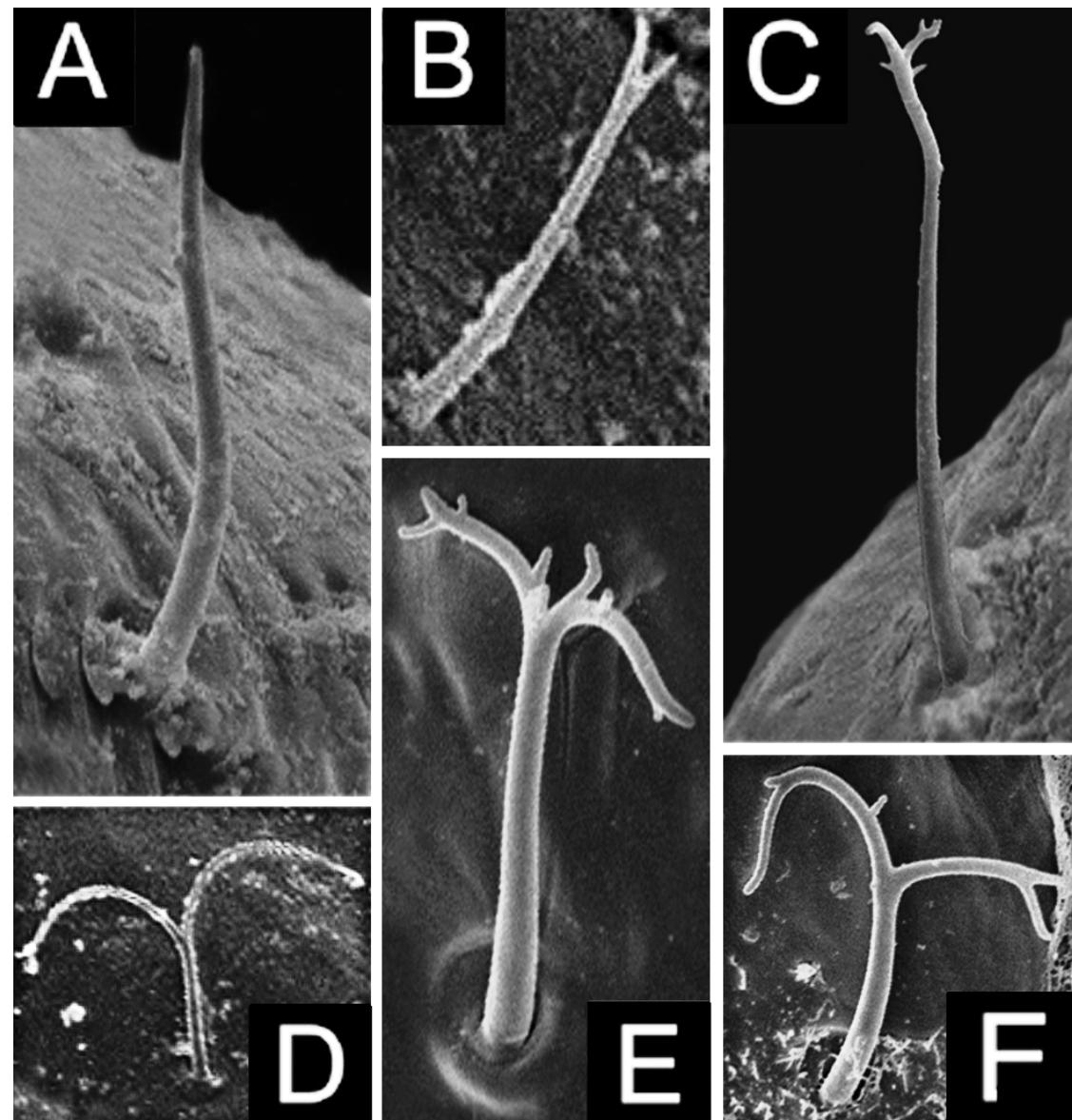


Fig. 3. Types of hairs found on the larvae of *Solenopsis helena*. A-Unbranched, smooth simple hair (may be greatly curved on the labrum of mature larvae); B-Unbranched with tip denticulate (from one to many denticles, see Fig. 3C); C-Distally multifid; D-Moderately bifid with tips curling in opposite directions; E-Superficially bifid hair with denticulate tips.; F-Moderately bifid with tips denticulate, curling in opposite directions (may or may not present uncinate ends).

Mature Queen Larva

Similar to worker larva in color and morphology, but larger and with the following differences: Body shape pheidoloid (Fig. 1D); 1.30-2.50 mm long and 0.63-1.1 mm wide ($N = 3$). Antennae usually formed by 3 conspicuous setaceous sensilla, with lower sensillum noticeably longer than oth-

ers (Fig. 3C); 1 specimen had 4 setaceous sensilla on 1 antenna.

Queen Pupa

Always exarate, without a cocoon, wings well developed (Fig. 5). Yellowish, integument surface

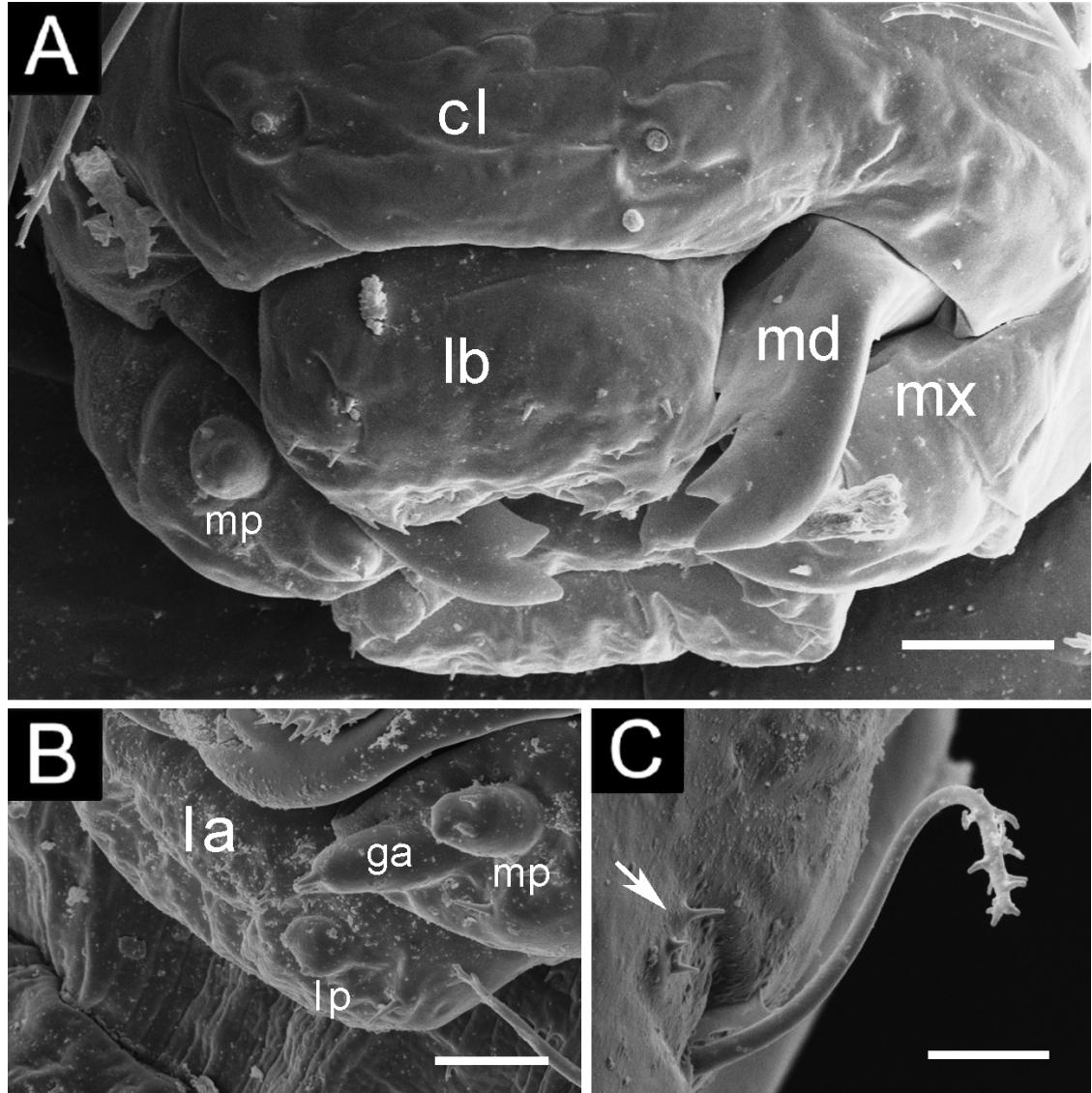


Fig. 4. Ultrastructural details of the head capsule of mature larvae of *Solenopsis helena*. A-Mouthparts of a queen larva; cl = clypeus, md = mandibles, mx = maxilla, mp = maxillary palp, lb = labrum; B-Oblique view of lower mouthparts of a worker larva; md = mandibles, mp = maxillary palp, lp = labial palp; ga = galea. C-Detail on region of antenna of a queen larva; arrow = antennal sensilla. Sizes of scale bars (μm): 13; 10; 10.

(i.e., pupal skin) rugose, papillae abundant. Mean length 1.50-3.73 mm and maximum width 0.49 mm ($N = 9$).

DISCUSSION

The mature worker larva of *S. helena* is slightly smaller but morphologically similar to the larvae of *S. molesta* (Wheeler & Wheeler 1955). The larva of *S. helena* is only slightly larger than the larvae of *Solenopsis picta* Emery, the smallest *Solenopsis* larva hitherto described

(compare Wheeler & Wheeler 1955, 1960). The larvae of *S. helena* presented the lowest number of head hair recorded in the literature (other *Solenopsis* have 22-26; Wheeler & Wheeler 1955, 1960), possibly as a consequence of their small size. It should be noted that Wheeler & Wheeler (1955) did not state number of head and body hairs of *S. molesta*, thus a direct comparison with that species is not possible; number of body hairs were seldom given in their descriptions. Like previous observations of thief ant larvae, the mesothoracic spiracle of *S. helena* was the largest

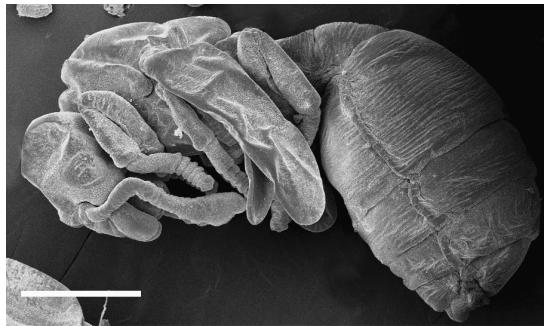


Fig. 5. Pupa of queen. Size of scale bar: 600 µm

(for a complete list see Wheeler & Wheeler 1955, 1960). This seems to be typical of thief ant species, except for *Solenopsis pergardei* Forel, where the first spiracle was reported to be the largest. In fire ants the first pair of spiracles has been reported as the largest (Wheeler & Wheeler 1960; Pitts 2002; Fox et al. 2012).

First-instar larvae of *S. helena* have a few body hairs, differently from fireant larvae, which are born naked (Petralia & Vinson 1979; Fox et al. 2011). The "very young" larva of *S. molesta* described in Wheeler & Wheeler (1955, 1960) also had body hairs, but the authors did not know if that was a first-instar. It is nonetheless possible, that larvae of thief ants are born with body hairs, which would be an interesting particular trait. This requires deeper investigation.

Regarding the mouthparts, *S. helena* have relatively small maxillary palps in comparison to other described *Solenopsis* larvae. Moreover, differently from *Solenopsis geminata* F. and *Solenopsis xyloni* McCook (Wheeler & Wheeler 1955), the anterior labral surface of *S. molesta* had fewer sensilla, while the posterior surface was smooth (see Wheeler & Wheeler 1960), which is also apparently typical among *Diplorhoptrum* larvae. These might represent specializations of the group that reflect feeding habits different from the fireants. Both the maxillary and labial palps of other described *Solenopsis* had more sensilla, possibly because of their greater body sizes (Wheeler & Wheeler 1955, 1960).

In conclusion, larvae of *S. helena* share many morphological characters with those of the thief ant *S. molesta* (Wheeler & Wheeler 1955; Petralia & Vinson 1979), and additional studies may well show this entire group within *Solenopsis* has a distinctive larval morphology.

ACKNOWLEDGMENTS

We are indebted to Scott Solomon and Roxana Jorens for help in providing the specimens, and to Elliot Watanabe Kitajima and Francisco André Ossamu

Tanaka (NAP/MEPA ESALQ/USP) for granting us access to the microscope facility. Christian Rabeling provided the specimens used and valuable comments on the narrative. The comments of two anonymous reviewers improved the final version of this paper.

REFERENCES CITED

- BOLTON, B., ALPERT, G., WARD, P. S., AND NASKRECKI, P. 2006. Bolton Catalogue of Ants of the World: 1758-2005, Harvard University Press, Cambridge, Massachusetts. CD-ROM.
- CASSILL, D. L., BUTLER, J., VINSON, S. B., AND WHEELER, D. E. 2005. Cooperation during prey digestion between workers and larvae of the ant, *Pheidole spadonia*. *Insectes Sociaux* 52(4): 339-343.
- FOX, E. G. P., SOLIS, D. R., JESUS, C. M., BUENO, O. C., YABUKI, A. T. AND ROSSI, M. L. 2007. On the immature stages of the crazy ant *Paratrechina longicornis* (Latreille 1802) (Hymenoptera: Formicidae). *Zootaxa* 1503: 1-11.
- FOX, E. G. P., SOLIS, D. R., ROSSI, M. L., DELABIE, J. H. C., SOUZA, R., AND BUENO, O. C. 2012. Comparative immature morphology of Brazilian fire ants (Hymenoptera: Formicidae: *Solenopsis*). *Psyche* (in press).
- HÖLLODIBLER, B., AND WILSON, E. O. 1990. The ants. Harvard University Press, Cambridge, Massachusetts.
- MASUKO, K. 2008. Larval stenocephaly related to specialized feeding in the ant genera *Amblyopone*, *Lepitanilla* and *Myrmecina* (Hymenoptera: Formicidae). *Arthropod Structure & Development* 37(2): 109-117.
- PACHECO, J. 2007. The New World Thief Ants of the Genus *Solenopsis* (Hymenoptera: Formicidae). Dissertation. The University of Texas at El Paso. 565 pp.
- PACHECO, J., HERRERA, H., AND MACKAY, W. 2007. A New Species of Thief Ant of the Genus *Solenopsis* from the Galapagos Islands (Hymenoptera: Formicidae). *Sociobiol.* 50(3): 1075-1086.
- PETRALIA, R. S. AND VINSON, S. B. 1979. Developmental morphology of larvae and eggs of the imported fire ant, *Solenopsis invicta*. *Ann. Entomol. Soc. Am.* 72: 472-484.
- PITTS, J. P. 2002. A Cladistic analysis of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae). Ph.D. Dissertation, Department of Biology, University of Georgia, Athens. 266 pp.
- PITTS, J. P., HUGH, M. C. J., AND ROSS, K. G. 2005. Cladistic analysis of the fire ants of the *Solenopsis saevissima* species-group. *Zool. Scripta* 34: 493-505.
- SCHULTZ, T. R., AND MEIER, R. 1995. A phylogenetic analysis of the fungus-growing ants (Hymenoptera: Formicidae: Attini) based on morphological characters of the larvae. *Syst. Entomol.* 20: 337-370.
- TSCHINKEL, W. R. 2006. The Fire Ants. Harvard University Press. 730 pp.
- WHEELER, G. C., AND WHEELER, J. 1955. The ant larvae of the myrmicine tribe Solenopsidini. *Am. Midl. Nat.* 54: 119-141.
- WHEELER, G. C., AND WHEELER, J. 1960. The ant larvae of the subfamily Myrmicinae. *Ann. Entomol. Soc. Am.* 53(1): 97-110.
- WHEELER, G. C., AND WHEELER, J. 1976. Ant larvae: Review and synthesis. *Mem. Entomol. Soc. Washington* 7: 1-108.