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On the immature stages of the crazy ant *Paratrechina longicornis* (Latreille 1802) (Hymenoptera: Formicidae)

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Abstract

The ant *Paratrechina longicornis* is a cosmopolitan ant species that has been accidentally spread by commerce around the world, and now is a pest of houses and hospitals. The morphology of its larval stages has been previously described without knowledge of the number of instars. The present study revisits the original description of *P. longicornis* larvae by adding differences observed among the three larval instars. Compared to other *Paratrechina* species studied so far, *P. longicornis* presents smaller larvae, less evident variation in mandible morphology, and clear variation in the morphology of head hairs between the analysed specimens of the second and third instar larvae. Ultrastructural images of the eggs, larval spiracle ornamentation, and pupae are also presented for the first time.

Key words: ant, morphology, larval instars, Formicinae, *Paratrechina longicornis*

Introduction

The genus *Paratrechina* Motschulsky comprises about 118 described species, some of which have been spread throughout the world by commerce, becoming important invasive pest ants in buildings (e.g. houses and hospitals) of many countries. The most economically important species of the genus in the Americas are *Paratrechina bourbonica* (Forel), *Paratrechina fulva* (Mayr), *Paratrechina longicornis* (Latreille) and *Paratrechina vividula* (Nylander) (Trager 1984). In Brazil, the species *P. longicornis* is regarded as a mechanical carrier of pathogenic microorganisms in hospital environments (Bueno & Fowler 1994; Zarzuela *et al*. 2005). In spite of the pest relevance of the genus, there are still few studies of their biology in tropical regions.

The importance of larval descriptions to myrmecology has been emphasized by Wheeler & Wheeler (1976; 1986), who have in fact described the larvae of over 700 ant species. One of their conclusions is that certain larval characters may be applied to species level taxonomy and systematics in various ant genera.

Some of these characters have been recently applied to phylogenetic studies (Schultz & Meier 1995; Pitts 2005), but for the vast majority of ant species — some 11,000 species (Agosti & Johnson 2005) — larval descriptions are unknown or were rather superficially described based on few specimens. Furthermore, deeper knowledge of immature morphology is also of crucial relevance in clarifying many aspects of ant biology and their social organization.

The larvae of *P. longicornis* have been previously described by Wheeler & Wheeler (1986), although without the knowledge of the number of larval instars and based only on five specimens. In this context, the present study aims at revisiting the original description armed with the knowledge of the number of larval

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instars present in the species (Solis 2006) and with the aid of electronic microscopy techniques for adding further details and clarifying vague aspects of their morphology.

Material and methods

The eggs and larvae were obtained from field-collected colonies in Campinas, São Paulo, Brazil (22º49'16.58''S e 47º05'20.33''W). They were fixed in Dietrich's solution for 24 h and preserved in 80% alcohol. The period in which the ant larvae were collected in the field was designed to be in the non-reproductive stage, which ranges from October to March in this region of Brazil (Solis 2006). Thus we assume that our samples did not include reproductive immatures (Hölldobler & Wilson 1990).

The number of larval instars of workers of *P. longicornis* has been previously established (Solis 2006), thus the larvae were separated in three instars according to their maximum head capsule widths. Measurements were made with a stereomicroscope (ZEISS, model MC80DX, maximum magnification power of 66x) equipped with an ocular micrometer. A total of 500 eggs and body lengths from 50 larvae from each instar were measured.

After separation and measurements, eggs (N=5) and larvae (N=10) from each instar were prepared for scanning electronic microscopy analyses as follows: samples were post-fixed in 1% osmium tetroxide, after which they were dehydrated in a graded acetone series and then critical-point dried (BALZERS CPD/030). Specimens were attached to aluminium stubs with double-face adhesive straps containing conductive silver paint, coated with gold (BALZERS SCD/050) and analysed under a PHILLIPS SEM-505 scanning microscope.

Additional larvae from each instar (N=10–15) were warmed for 15-min in an aqueous solution of KOH (10%) and placed in a small drop of glycerine on a microscope slide for observations under a ZEISS compound microscope. Digital pictures of preserved immature stages were taken using a ZEISS optical microscope with lateral illumination and a SONY digital camera placed over the lens.

All terminology used for the description of the larvae of *P. longicornis* follows Wheeler & Wheeler (1976). Although "hair" may be an inappropriate term for arthropods, Wheeler and Wheeler differentiated "hairs" and "setae" and we follow their usage here. Body hairs were measured at full length and body length is presented both in straight length (N = 50 for each instar) and length through spiracles, which was taken from only one larva of each instar still in good body shape after being mounted on the glass slide—this is the imaginary long axis of the larvae, used to compare sizes while maintaining the curvature of the body. Pupae lengths were measured on a straight line from the top of the head capsule to the tip of the abdomen. Concerning other measures taken (of the head capsule, mouthparts, hairs, etc), only those of one individual per instar are presented in the descriptions. All body measures are presented as mean±standard error.

Results

All observed specimens were whitish in colour and most third instar larvae presented a brown meconium showing through the interior of their digestive system. Mandibles, maxillary palpi and galeae were always light yellowish brown before being treated for microscopic analyses. It should be noted that the process of mounting on glass slides caused body segmentation to become indiscernible on all specimens.

Egg. The egg is oval in profile and presents a transparent thin corium, being markedly separated from the embryo (Fig. 1). The micropile is a slightly elevated punctured area at the anterior extremity (not shown). Length is 345±10 μm, range 279.3 μm–426.3 μm. Width is 212±10 μm, range 161.7 μm–279.3 μm (N=500). Egg length:width ratio for the species is 1.63 (ratio of the average length to average width).

First larval instar. Body—Whitish, slender in profile, rather pheidoloid in shape due the presence of a short neck formed by a more pronounced first thoracic segment; head bent ventrally (Fig. 2), anus a slightly subterminal transverse slit with the lower lip bigger than the upper lip (Fig. 3A and 3C). Head capsule proportionally large in relation to body size. One specimen had abundant body hair (Fig. 2B). However in all other analyzed specimens (n=19), there were only ten widely distributed body hairs (15 μm long), present at the anterior ventral surface of the thorax (Fig. 2C and 3A). Integument covered with minute spinules. Ten spiracles present, measuring about 1 μm in diameter; spiracle openings unornamented (Fig. 3 – detail). Body length 445.7±59 μm, range 323.4 –529.2 μm; width 203.1±18 μm, range 176.4–235.2 μm. Length through spiracles 617.5 μm.

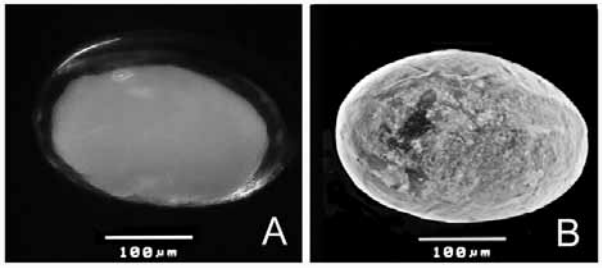

FIGURE 1. Eggs of *Paratrechina longicornis*. A — profile in optical microscopy with indirect illumination. B — Profile examined by scanning electronic microscopy

Head capsule—Cranium 86 μm high x 190 μm wide; roughly subhexagonal; antennae 11 μm wide slight elevations with three sensilla; head surface smooth; head hairs simple, about 20 μm long, six on the genal region, four on the vertex, and four circling the occipital area (Fig. 3B). Distribution of head hairs not always bilaterally symmetrical. Tentorial pits very well-demarked (Fig. 3B). A conspicuous pair of sensilla present on the genal region, near the base of the mandibles.

Mouthparts – Clypeus not clearly delimited from the cranium, upper surface of clypeus smooth, without sensilla; a distinct row of four simple hairs (40 μm long) lining the distal clypeal border (Fig. 3B). Labrum weakly bilobed, 105 μm wide, with about five sensilla over the anterior surface (Fig. 3B). Mandibles simple, sharp-pointed, roughly dolichoderoid in shape, 67 μm long and 20 μm wide at the base, lacking medial teeth, overlapping at the middle (Fig. 3D). Maxilla conical, 34 μm wide and 40 μm long. Galea a small hump culminating with two sensilla; maxillary palpus paxilliform (7 μm long x 12 μm wide), culminating with five sensilla (Fig. 3D). Labium pronounced and strongly elliptical, 50 μm wide, with seven widely spaced and asymmetrical setaceous sensilla; labial palpus a simple cluster of five sensilla measuring 4 μm in diameter. A conspicuous row of ten head hairs delimits the gula from the first thoracic segment.

Second Larval Instar. Body—Body profile as in the first larval instar, but more plump; head only slightly bent ventrally; anus clearly subterminal in position (Fig. 4A, 4B, 5A, 5C, and 5D). Body hairs are homogeneously distributed over the body surface and of four types: (1) 25 μm long, with four palmate branches, found sporadically on the lateral surface of the thorax; (2) 20 μm long, palmately trifurcate, short-branched , sparsely distributed over the entire body; (3) 32 μm long, deeply bifid, abundant over the entire body surface; and (4) 60 μm long, simple, circling the first thoracic segment (Fig. 4A and 5A). Body length 664.4±12.9 μm, range 470.4 –837.9 μm; width 272.2±41.0 μm, range 205.8 –338.1 μm. Length through spiracles 1240 μm.

Head capsule – Cranium 90 μm high x 205 μm wide; antennae distinct, 15 μm in diameter. Head hairs usually simple (62 μm long), distributed as follows: eight to ten hairs on the occipital border, four on the vertex, seven to nine on the genal regions, four on the gula (Fig. 4C and 5A). The following head hairs bifurcated in some specimens: the innermost two of the occipital hairs and any gulal or genal hairs, the latter distally trifurcated in some specimens (Fig. 5A and 5C). A conspicuous pair of sensilla near the base of the mandibles (Fig. 5B).

Mouthparts—Labrum markedly bilobed, 105 μm wide (Fig. 5A); mandibles camponotoid in general shape, 35 μm wide at the base, about 75 μm long, with a strong apical tooth and a clear medial tooth (Fig. 5B). Maxilla conical, 45 μm long, 43 μm wide, with three widely spaced 3 μm long setaceous sensilla and a single 25 μm long simple hair; maxillary palpus 31 μm long x 15 μm wide, bearing five sensilla: three laterally and two apically contiguous. Galea a simple 7 μm long x 12 μm wide skewed peg with two sensilla at the top. Labium stout and hemispherical, 52 μm wide with some scattered setaceous sensilla over the surface, the spinneret a transverse slit. Labial palpus a slight elevation, 5 μm in diameter, with five sensilla. Other characteristics as in the first larval instar.

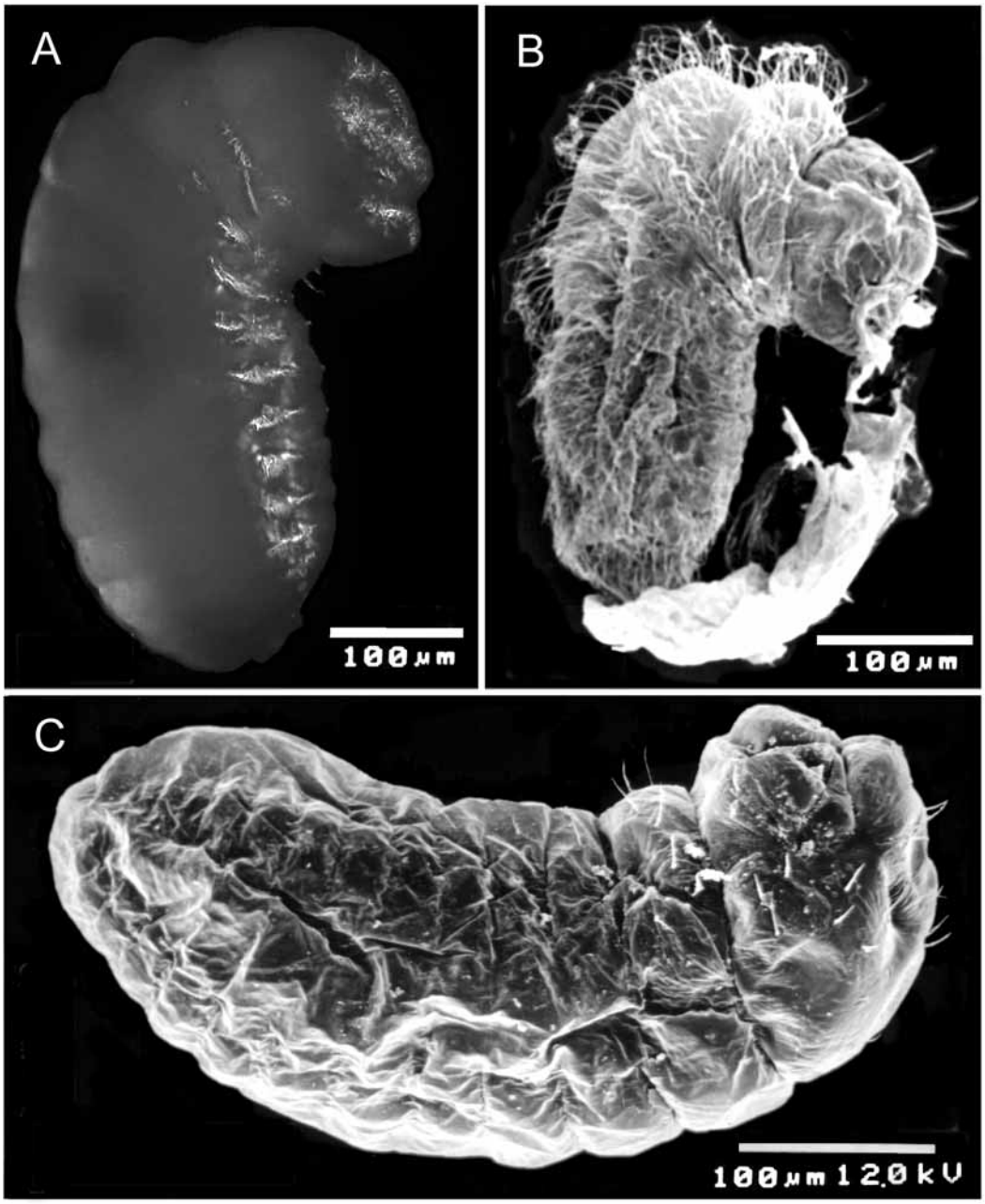

FIGURE 2. First larval instar of *Paratrechina longicornis*. A — profile in optical microscopy with indirect illumination. B — Hairy specimen apparently consuming egg chorion, examined by scanning electronic microscopy C — Profile examined by scanning electronic microscopy

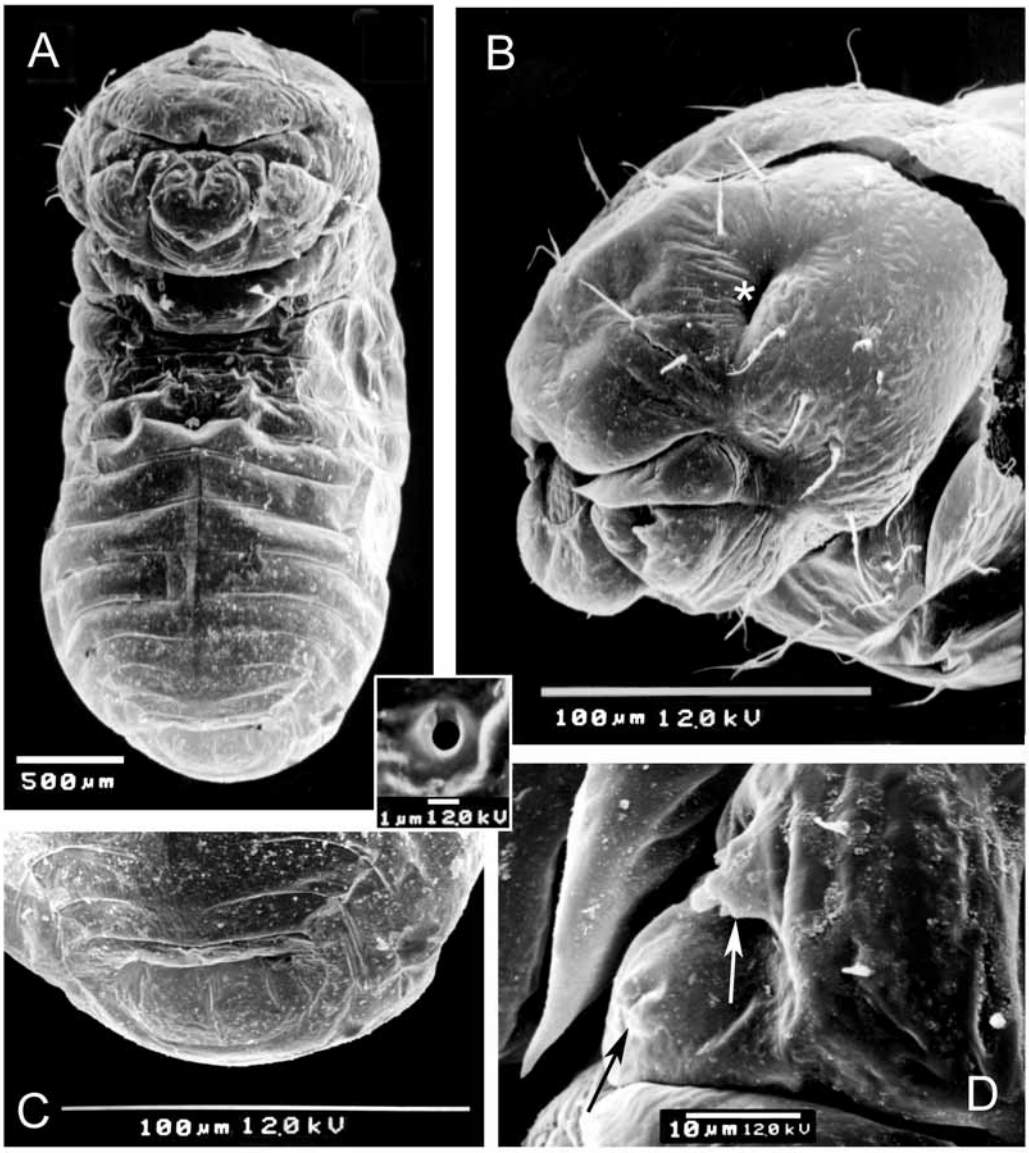

FIGURE 3. First larval instar of *Paratrechina longicornis*. A — Ventral view examined by scanning electronic microscopy. B — Head capsule; asterisk: tentorial pit. C — Detail of the terminal portion and transverse anus. D — Close-up of the left mandible and maxilla; white arrow: galea; black arrow: maxillary palpus. Figure detail in the middle: Entrance of the thoracic spiracle.

Third larval instar. Body – Clearly dolichoderoid in profile; anus subterminal (Fig. 6A). Body hairs denser over the posterior surface of the body; hairs over the dorsal surface of the body always simple, about 62 µm long; 37–62 µm long bifid hairs common over the rest of the body, most abundant on the ventral surface; 25 µm long trifid hairs predominant at the posterior end the body; one or two 20 µm long palmately 4-branched hairs present on the pleural region of the thorax. Spiracles about 23 µm in diameter, peritreme surrounded by short triangular spines (Fig. 6B). Body length 1431.60 µm±51.3 µm, range 779.1–1900.8 µm; width 552.4±20.8 µm, range 294.0 –720.3 µm. Length through spiracles 1478 µm.

Head capsule – Cranium 200 µm high x 250 µm wide. Distribution of the hairs over the head similar to second instar larva, but gula with one to three hairs (Fig. 7A) . All hairs simple, 62 µm-long. Antennae distinct shallow pits on upper half of cranium, about 20 µm in diameter and containing three sensilla (Fig. 7B).

Mouthparts – Clypeus not clearly delimited from the cranium, surface smooth, lacking sensilla; a distinct row of four 40 µm long simple hairs lining the distal border (Fig. 7A). Labrum about 115 µm wide and 60 µm long, with 6–8 setaceous sensilla over the anterior surface (Fig. 7A and 7D); ventral surface of the labrum

with abundant spinules concentrically arranged in rows, and with a conspicuous group of six asymmetrical sensilla mesad (Fig. 7C); labrum weakly spinulose laterally, lacking chiloscleres. Mandibles 90 μm long and 50 μm wide, culminating with an apical tooth that varies in prominence, occasionally with a weak medial tooth. Maxillae about 65 μm long and 50 μm wide at base, with three 5 μm long setaceous sensilla and a single 30 μm long simple hair at the base. Maxillary palpus paxilliform, 20 μm wide and 10 μm long, bearing five sensilla: three lateral and two apical (Fig. 7E). Galea 9 μm wide and 4 μm long with two sensilla at the top (Fig. 7E). Inner apex of maxilla covered with spiny papillae to base of galea (Fig. 7E). Labium 63 μm wide, with scattered setaceous sensilla over the surface, densely covered by spiky papillae along the sides and around the base of the labial palpus; labial palpus a 4 μm wide, slightly elevated cluster of five sensilla, two of which are larger and contiguous (Fig. 7E). Spinneret a transverse slit; a conspicuous and well demarked region over the spinneret densely covered with imbricate spiky papillae; an isolated basiconic sensillum present immediately below each side of the spinneret (Fig. 7E — detail). About eight scattered sensilla over the lower half of the labium. Epipharynx with sparse internal inconspicuous asymmetric sensilla, densely lined with imbricated spiky papillae (Fig. 7F). Other characteristics as in the second instar larvae.

Pupae. Pupae are milky-white when young, eyes getting black and the body gradually darkening to a deep brown as they develop into imagos; exarate, with no cocoon (Fig. 8), 2.44±0.02 mm long, range 2.00 mm–2.80 mm (N=50). Only white pupae were measured.

Discussion

The fact that mounting the larvae on glass slides caused body segmentation to become invisible should be considered. Descriptions based on mounted larval specimens should be complemented by examination of specimens that are live or preserved in fluid.

The present description confirms general aspects originally observed by Wheeler & Wheeler (1986), but has also found some marked differences. We have observed variation in hair distribution, especially over the head capsule (Fig. 4D), between different specimens within an instar. This variation was not reported by Wheeler & Wheeler (1986) because their description was made from only five specimens. Moreover, when analysing their drawing of the head capsule of *P. longicornis* larvae, the hair bifurcations are clearly illustrated, and the very conspicuous row of four hairs bordering the clypeus was not made evident and is not mentioned in the text (Wheeler & Wheeler 1986). They only roughly represented spinules over the labrum surface, while these are reported as absent in the text description. The labrum is represented as semicircular without any text reference to it, and in our specimens it was clearly bilobed. The antennae are rather exaggeratedly drawn, since they are actually very feeble. As a consequence, their drawing of this species' larva head capsule is considerably different from our illustrations. Although Wheeler and Wheeler left the impression that their drawing of the larval body profile was based on a last instar larva, we suggest that the drawing was actually based on a second instar larva. More than once during their descriptions, the authors mentioned an isolated sensillum between the labial palpus and the spinneret, which could "prove taxonomically useful some day" (Wheeler & Wheeler 1986). We have herein observed it to be a conspicuous basiconic sensillum in this species (Fig. 7E).

The observed variation in types of head hairs over the larval head capsule is especially relevant in a cladistic context. This was one of the characters considered in calculating the "specialization indices" proposed by Wheeler & Wheeler (1986). Furthermore, the presence of bifurcations in head hairs has been recently proposed as a character of considerable importance for separating species in the genus *Solenopsis* Westwood (Pitts 2005). We suggest that intraspecific variation in types of head hairs might occur in other ant species as well. Therefore, carefully revisiting other previously described ant larvae (especially when based on few specimens) is warranted.

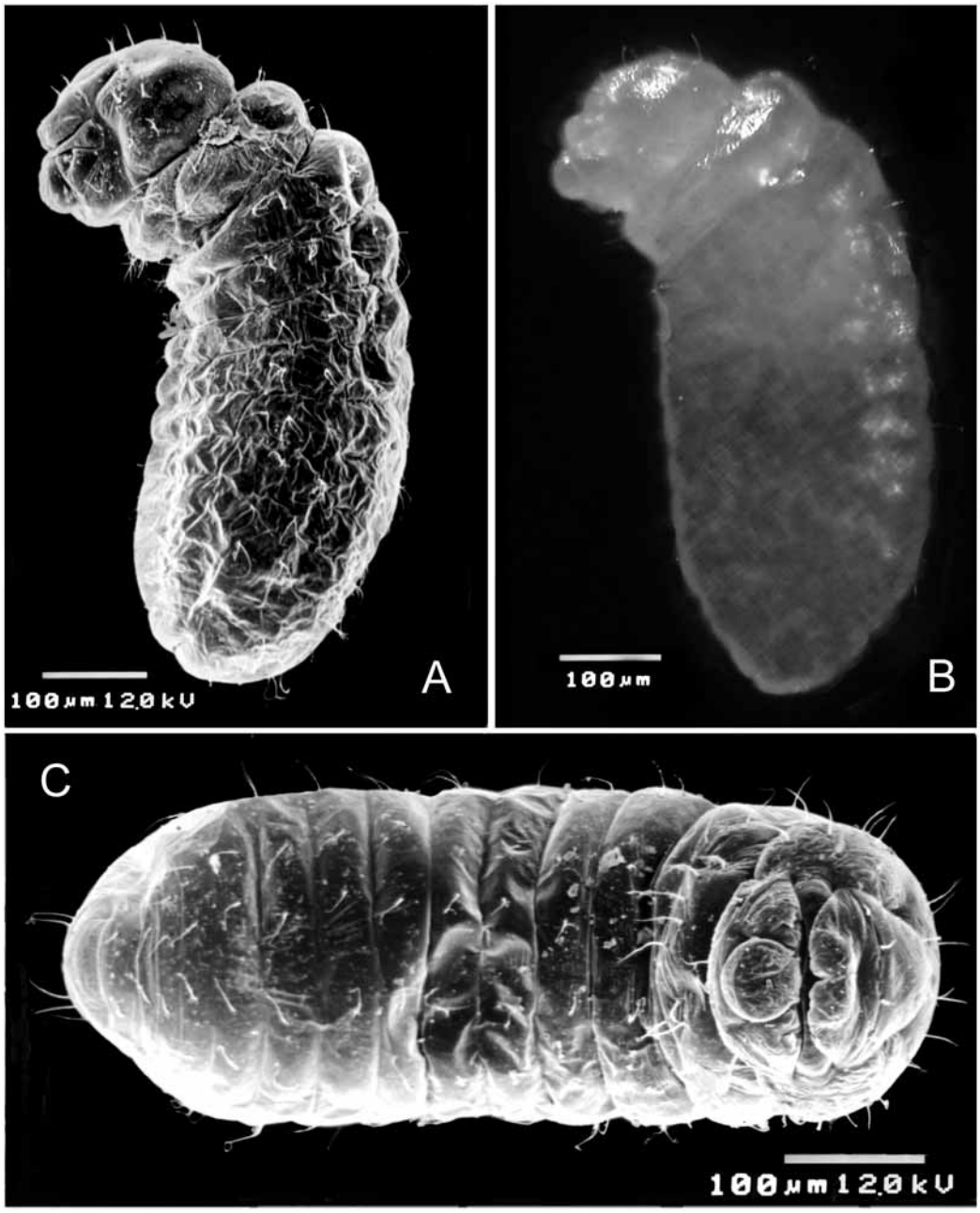

FIGURE 4. Second larval instar of *Paratrechina longicornis*. A — Side view examined by scanning electronic microscopy. B — Side view examined by optical microscopy with indirect illumination. C — Ventral view examined by scanning electronic microscopy.

The first instar larva of Fig. 2B had a pattern of body pilosity that was different from all other first instar larvae among our sampled specimens. It is possible that this larva was a sexual larva, but further studies are needed to confirm this. Male larvae of both *P. fulva* and *Monomorium pharaonis* (L.) have been reported to be distinct from worker larvae in terms of body hair coverage (Arcila *et al*. 2002, Peacock *et al*. 1954). In *M. pharaonis*, nursing workers in queenright nests have been observed exterminating sexual larvae during non-reproductive stages of the colony (Edwards & Abraham 1991), and sexual larvae were distinguished from other larvae through differences in size and body hair distribution (Peacock *et al*. 1954). Identification of sexual larvae by nursing ants has been reported in fire ants, but in this case it is apparently based on chemical traits (Hölldobler & Wilson 1990). Moreover, considering that the larva of Fig. 2B was apparently consuming the egg chorion, it must have been a hatchling that had not been identified by the nursing workers yet.

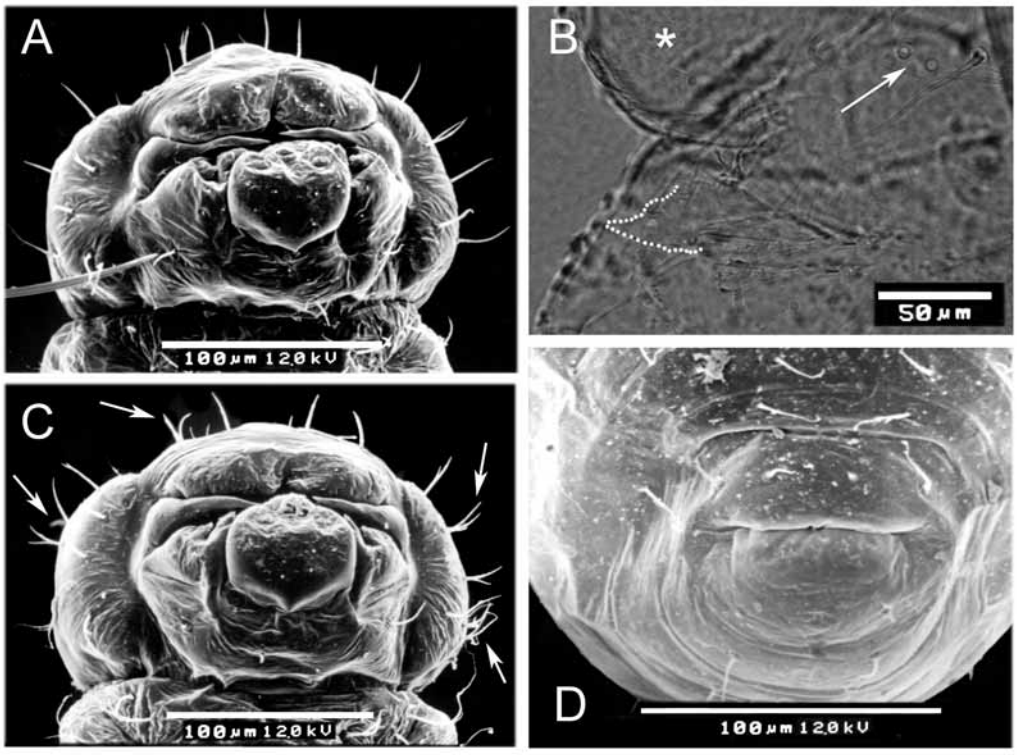

FIGURE 5. Second larval instar of *Paratrechina longicornis*. A — Frontal view of the head capsule with simple hairs. B — Optical microscopy of the mouthparts; asterisk: labrum in side view; white arrow: conspicuous pair of sensilla; dotted line: mandible. C — Frontal view of the head capsule with bifurcated hairs (marked with arrows). D — Detail of the terminal portion and anus.

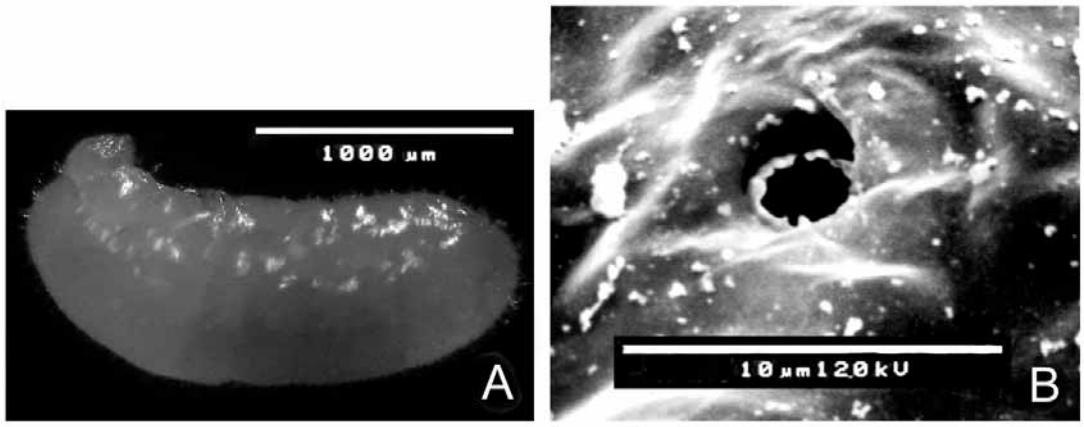

FIGURE 6. Third larval instar of *Paratrechina longicornis*. A — Side view by optical microscopy with indirect illumination. B — Scanning electronic microscopy of a thoracic spiracle.

Usually, the pupae of Formicinae have a cocoon (e.g. *Camponotus*), but pupae in this species have no cocoons, like other exceptions in the subfamily (Wheeler & Wheeler 1976) and in the genus *Paratrechina*—pupae of *P. fulva* are also devoid of cocoons (Arcila *et al*. 2002).

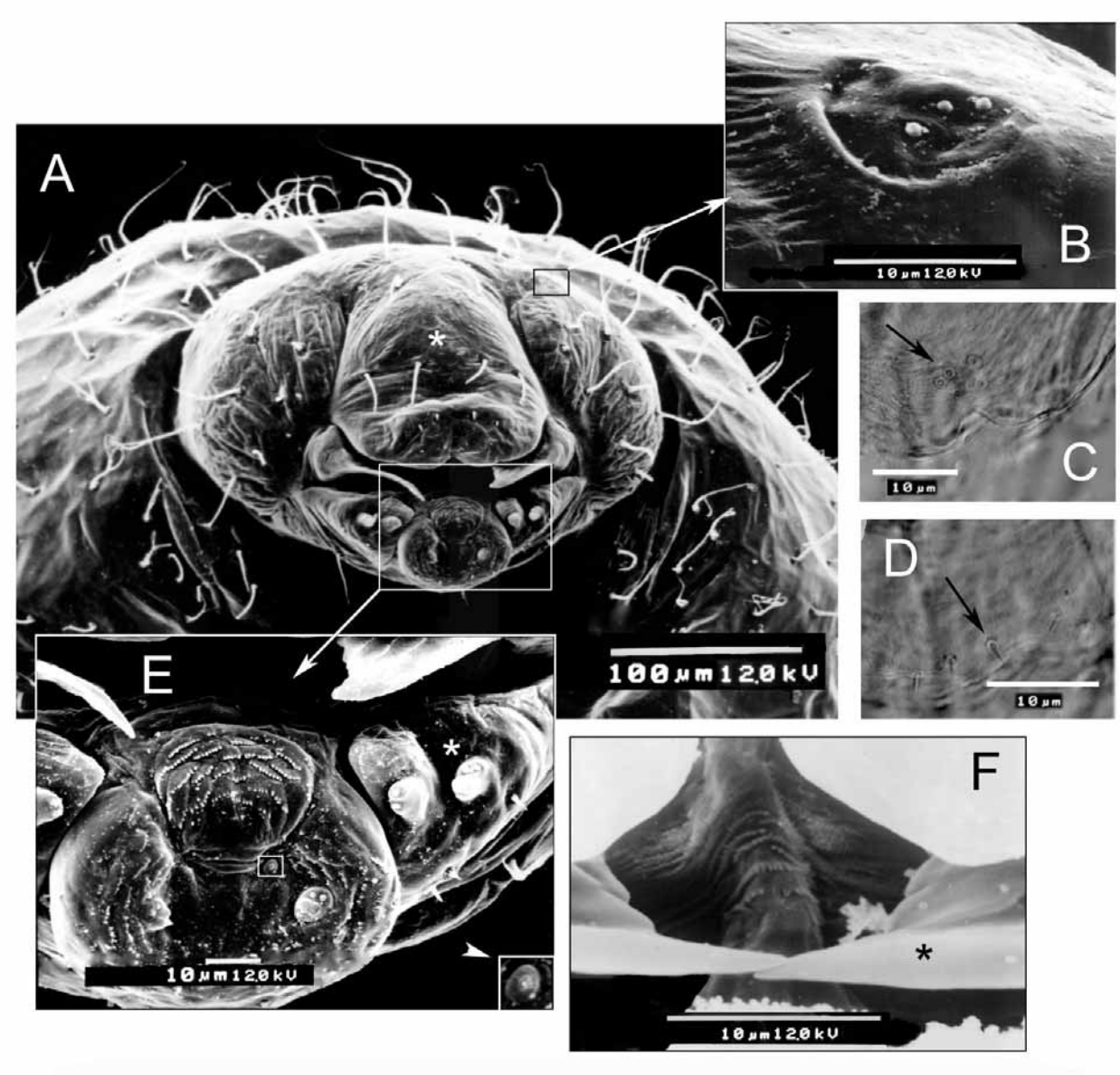

FIGURE 7. Third larval instar of *Paratrechina longicornis*. A — Frontal view of the head capsule; asterisk: clypeus. B — Close-up of the left antenna. C — Optical microscopy of the lower surface of the labrum; arrow: group of sensilla. D — Optical microscopy of the anterior surface of the labrum; arrow: row of setaceous sensilla. E — Detail on the mouthparts; asterisk: left maxilla and galea; arrowhead: detail on the basiconic sensillum demarked in the picture. F — Entrance of the mouth and spinulose epipharynx; asterisk: left mandible.

Arcila *et al*. (2002) described the larvae of *P. fulva* and observed considerable differentiation of mandibles between each larval instar. They considered mandible morphology a feasible character for identification of the larval instars in the species. In *P. longicornis*, there is noticeable difference between the mandibles of the first and last larval instars (Fig. 5B and 2B), but the second instar has mandible morphology very similar to that of the third instar (Fig. 5B, and see Wheeler & Wheeler 1986). We would not counsel relying on mandible morphology for separating instars within this species. Third instar larvae of *P. fulva* were considerably larger (about 3,000 µm long) than those of *P. longicornis*, although curiously the adult ants and the first instar larvae of both species are of approximately the same size (Arcila *et al*. 2002).

Wheeler & Wheeler (1953; 1968; 1986) have also described larvae from *Paratrechina bruesi* (Wheeler), *Paratrechina melanderi* (Wheeler) and *Paratrechina parvula* (Mayr); unfortunately there are no available drawings for the last one (Wheeler & Wheeler 1968). As a whole, all mature *Paratrechina* larvae described

seemed to present spinulose sensilla over the anterior surface of the labrum, spinulose ventral surfaces of labrum and hypopharynx, and similar numbers of sensilla over the galea, maxillary and labial palpi, while differing mainly in patterns of head hair distribution and occurrence of head hair bifurcations and trifurcations. Particularly the "young larva of *P. bruesi*" (supposedly a first or second instar larva) presented different body morphology from *P. longicornis* young larvae, with a protuberance on the ventral middle region of the body (Wheeler & Wheeler 1968).

Spiracular ornamentation has never been analysed in *Paratrechina* ant species before this study. We found that *P. longicornis* have simple and generally unornamented spiracles, as was observed for most other ants (Wheeler & Wheeler 1976).

Only *P. fulva* had its eggs previously studied in the genus, which were found to be slightly larger than those of *P. longicornis* (390±20 μm long x 250±10 μm wide) and presented a length:width ratio of 1.56 (Arcila *et al*. 2002), a bit more rounded than eggs of *P. longicornis*.

We have added further details to the description of the larvae of this species, while bringing attention to intraspecific variation in the morphology of head capsule hairs. Recognizing this variation affects inferences in ant systematics and phylogeny. The occurrence of distinct variants in young larvae is also of interest and warrants further investigation.

FIGURE 8. Pupa of *Paratrechina longicornis* examined by scanning electronic microscopy. Scale bar = 1 mm.

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