Morphology of the eggs and larvae of *Cyphomyrmex transversus* Emery (Formicidae: Myrmicinae: Attini) and a note on the relationship with its symbiotic fungus

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Abstract

The morphology of the eggs and larvae of the ant *Cyphomyrmex transversus* Emery is here described and discussed for the first time. Some morphological characteristics of the symbiotic fungus that covers the eggs, larvae, and pupae are also examined.

Key words: Formicidae, Myrmicinae, Attini, *Cyphomyrmex transversus* Emery, immature stages, morphology, symbiotic fungus

Introduction

The tribe Attini Smith, 1858 (Formicidae: Myrmicinae) includes 13 genera, among which *Cyphomyrmex* Mayr, 1862 is the second most diverse, with 37 valid species (Brown, 2000; Fernandez, 2003; Price et al., 2003; Bolton et al., 2006; see Table 1). This genus is usually regarded as monophyletic (Hölldobler & Wilson, 1990; Mayhé-Nunes, 1995; Schultz & Meier, 1995), but its exact phylogenetic relationships inside the tribe remain unclear (Schultz & Meier, 1995). Klingenberg (pers. com.) recently noted that several putative synapomorphies would justify the placement of *Cyphomyrmex morschi* Emery in the genus *Mycetophylax* Emery. However, the monophyly of the remaining *Cyphomyrmex* species is more secure. All *Cyphomyrmex* species are Neotropical, except *Cyphomyrmex wheeleri*, which is Nearctic (Kempf, 1972 & 1966; Bolton, 1995), and *Cyphomyrmex rimosus* and *Cyphomyrmex minutus*, which occur in the Nearctic and Neotropical regions (Longino, 2007).

Two informal species groups are recognized (Kempf, 1964 & 1966): the *strigatus* group and the *rimosus* group. The *strigatus* group is the smaller of the two (Table 1) and accounts for 16 species spread throughout South America and the Antillean islands near Venezuela (Kempf, 1964; 1972). The *rimosus* group includes 21 species that are geographically concentrated between Central America and the Northern part of South America (Snelling & Longino, 1992). Schultz & Meier (1995) demonstrated that the *strigatus* group is paraphyletic in relation to the *rimosus* group.
The colonies of *Cyphomyrmex* are generally small-colonies of the *strigatus* group and are comprised of less than 300 workers (Fowler *et al*., 1986)—but some species of the *rimosus* group have colonies with more than 4,000 individuals (Price *et al*., 2003). The nest may be found in several types of habitat, such as rotting wood on the forest floor, simple cavities formed between leaves in the litter, or underground (Snelling & Longino, 1992).

As in other Attini, *Cyphomyrmex* species cultivate a typical attine leucocoprineaceous symbiotic fungus (Basidiomycota: Agaricaceae) inside their nest as the main food source of the colony (Hölldobler & Wilson, 1990; Mueller *et al*., 2005). Several types of substrate, such as seeds, insects or body parts of dead arthropods,
are used as food for the fungus. However, Murakami (1997) showed that millipede frass is the preferred substrate, at least for *C. rimosus*, and that ants gather and store nectar in their crops, which is subsequently regurgitated onto their fungus garden. *Cyphomyrmex* is a genus of particular interest in the tribe Attini for two reasons: first, its phylogenetic position seems intermediate, morphologically speaking, between the lower and higher attines (Kusnezov, 1949; Schultz & Meier, 1995; Schultz, 1998; Schultz, 2000; Villensen et al., 2002); second, it is unusual because two distinct forms of fungus are cultivated (Schultz et al., 2002); the typical leuco- coprineaceous attine fungus (*auriculatum* group) and two kinds of pterulaceous fungi (non-*auriculatum* species) (Villensen et al., 2004). All species in the *strigatus* group and a few from the *rimosus* group cultivate fungus with a typical mycelial form (as in all other attines), while all other species farm a unicellular yeast-like form of fungus (Wheeler, 1907; Kempf, 1964; 1966; Weber, 1972; Schultz & Meier, 1995; Mueller et al., 2001, Schultz et al., 2002; Price et al., 2003). Within the tribe Attini, *Apterostigma* is the only other genus known to farm more than one kind of fungus (Price et al., 2003). These phylogenetic and ecological characteristics make *Cyphomyrmex* an important case-study for understanding the evolution of fungus-growing habits (Kusnezov, 1949; Schultz & Meier, 1995).

As is the case for nearly all ants, one aspect of *Cyphomyrmex* biology that remains very poorly known is the post-embryonic development and the immature morphology. Within the ants, only recently have a few studies described the number of larval instars by considering discontinuous variation in larval size and/or weight, which is a reliable morphological criterion for external instar discrimination (Baratte et al., 2005). According to Masuko (1990), Zara & Caetano (2001) and Fox et al. (2007), the number of larval instars has been studied in at least seven ant subfamilies (Amblyoponinae, Dolichoderinae, Ectatomminae, Formicinae, Myrmiciniae and Ponerinae), corresponding to 25 genera and 35 species. These studies indicate that the number of larval instars varies in ants between three and six, with three or four instars being the most common. However, care must be taken in interpreting these studies because often it is unknown if the larvae were gynes or workers. Torre-Grossa et al. (1982) observed that gyne larvae of *Acromyrmex octospinosus* have a higher number of instars than worker larvae. For this reason, it remains very difficult to compare results between the different published studies. In *Cyphomyrmex*, larval morphology has been partially described for eight species (four each in the two species groups, Table 1). The descriptions are often superficial, limited to only one instar, and lack indication of which instars are being studied. Moreover, the exact number of larval instars has yet to be established for any species of the genus.

The present study considers, for the first time, the immature morphology of *Cyphomyrmex transversus* Emery, 1894, a species of the *rimosus* group. The frequency distribution of larval cephalic capsule width suggested that, in our sample, five morphometric classes could be distinguished. We describe here their external morphology, as well as that of the reproductive eggs. Morphological features of the brood symbiotic fungus are also described.

**Materials and methods**

*Biological material.* Immatures of *Cyphomyrmex transversus* Emery, 1894 (*rimosus* group) from six colonies were collected at Ponta da Tulha (14°35′S 39°02′W), in the municipality of Ilhéus (Bahia state, Brazil). Three colonies were collected in April-May 2003, and the others in January 2004. The locality is on the coast, and the vegetation is known as “restinga,” the typical seashore ecosystem of the Brazilian Atlantic Forest biome. The area is subject to human activity and planted with spaced coconut trees (*Cocos nucifera* L.). Locally, the *C. transversus* colonies are very abundant, nesting mainly under and/or inside dry coconuts lying on the ground (Ramos et al., 2003). After collection, the colonies were reared in artificial nests at the Center of Social Insect Studies (UNESP, Rio Claro, Brazil). Seeds of Poaceae and fecal pellets of insects were supplied as substrate for fungus-cultivation.
Morphological study. A total of 359 immature specimens of *C. transversus* were measured (63 eggs, 19 mature embryos and 277 larvae of diverse sizes). Eggs and larvae were fixed in Dietrich solution [96% ethanol (30 ml), formaldehyde (10 ml), acetic acid (2 ml) and distilled water (60 ml)] during 48 hours and then placed in 70% ethanol; their external layer of fungus was removed with a pin. Measurements were made at 64x using the ocular micrometer of a *ZEISS Stemi SV 11* stereomicroscope; they were recorded to the nearest 0.001 mm but rounded off to the second decimal. The maximum length and width of the eggs, as well as the maximum width (frontal view) of the cephalic capsule of the larvae and mature embryos was measured, by placing each individual inside a Petri plate, slightly buried in a colored sand layer in order to get the best orientation under the microscope. The minute first-instar larvae hidden inside the egg piles and fungus masses were too difficult to manipulate, so we considered that a mature embryo (visible through the egg chorion) was biometrically equivalent to a newly hatched (first instar) larva. Plotting the frequency distribution of the cephalic capsule width of the larvae revealed five modes (Fig. 1). At least 3 individuals from each modal size were selected for more detailed examination. For light microscopy, larvae were fixed in 92% ethanol and cleared in a hot solution of lactic acid (80°C, 12 h) and colored using an alcoholic solution of “black chlorazol” (Carayon, 1969). Morphology was studied under a *ZEISS Jena* microscope (100 to 400x), and microphotographs taken using a *SONY MVC-CD 330* numeric camera affixed to the microscope. For scanning electron observations, larvae were fixed in a modified Karnovsky's solution [2.5% glutaraldehyde, 2.5% paraformaldehyde in phosphate buffer, cacodylic acid, sodium phosphate 0.05 M, pH 7.2] and then washed with a cacodylic buffer (0.1 M) in order to remove excess lipids from the cuticle. They were then gradually dehydrated in ethanol at 50%, 70%, 90%, 95% and 100%, critical point-dried (Balzers CPD-030) and then metallized with gold in a Sputtering SCD-050 (Balzers). The microphotographs were made with a *SUPER ROLEX* camera coupled to a *PHILIPS SEM-505* microscope at the Institute of Biology of the UNESP (Rio Claro, Brazil) and a *JEOL JSM-840* microscope at the Microscopy Service of the MNHN (Paris).

![Figure 1](image_url)

**FIGURE 1.** Frequency distribution of the maximum width of the cephalic capsule of mature embryos and larvae of *C. transversus* (n=296).
Ethological observations. The brood care by the workers (including fixation of fungal tufts on the larva body) was filmed in five colonies of *C. transversus*, during 20 hours, using a VPC 1100 micro-camera coupled with the stereomicroscope.

Terminology and nomenclature. For the larval morphology we followed Wheeler (1949), Wheeler & Wheeler (1976), and Schultz & Meier (1995). Generally, three edges are recognized in the mandibular shape of ant larvae: basal, apical (masticatory) and external margins. In this study, we define the “articulatory margin” as the edge where the basal portion of mandible is articulated with the cephalic capsule (very distinct in light microscopy). Systematic nomenclature follows Bolton *et al.* (2006).

Morphological descriptions

Reproductive egg (E; figs. 2–8). Average width (n=63) 19.49±13.2 µm (range 16.18–22.06 µm). Average length (n=63) 25.82±17.9 µm (range 20.59–27.94 µm) (there was no correlation between width and length, r² = 0.345). Typical shape of myrmicine egg, the body in lateral view very short and slightly oval (about 1.3 times longer than wide), with the transverse section nearly circular. Anterior pole (ap, fig. 5) slightly more convex than the posterior pole (pp, fig. 5), with the micropyle (mi, fig. 5) distinct and forming a discrete prominence in lateral view; surface of chorion smooth; color milky white.

First larval instar (fig. 9). Body in lateral view very short and robust, circa 1.5 times longer than height; cephalic capsule (CC, figs. 12–13 and 27) very close to the most anterior point of the body; occiput succeeded by a small tegumentary fold (tf, fig. 9) formed by the anterior margin of the prothoracic segment (T1: fig. 27); maximum distance between occiput and anus less than 1.5 times the maximum body length; ventral surface very short, nearly flat and without any protuberance; body with a long and very convex dorsal profile; caudal part of the body in lateral view forming a clear protuberance; body segmentation weakly visible in lateral view (only the spiracles visible), the intersegmental limits only distinct at the dorsal and ventral surfaces; the six last abdominal segments with a dorsal intersegmental fold in lateral view; height of body greatest in the middle, slightly and equally diminishing in the direction of each extremity; mesothoracic, metathoracic and abdominal segments each with a lateral pair of small circular spiracles (sp, fig. 27), positioned on the anterior half; succession of the spiracles forming a “J” figure in lateral view, their diameters decreasing posteriorly; mesothoracic spiracle (msp, fig. 10) twice the size of the metathoracic spiracle (mtp, fig. 10); body in dorsal view without constriction between thorax and abdomen, the two parts with a similar width; maximum diameter of the body at the first abdominal segment.

Head positioned antero-ventrally to the body axis, its volume large relative to the rest of the body; cephalic capsule in lateral view short and well rounded, its dorsum convex; head shape in full-face view “subelliptic” (*sensu* Wheeler & Wheeler, 1976: 30), wider than long; occipital margin (om, figs. 12–13) weakly convex; frons large (fr, figs. 12–13) and genal lobes absent; antennae (at, figs. 12–13) small, positioned dorso-laterally at the mid-length of the cephalic capsule, in the form of discoidal depressions (dda, fig. 26) bearing three sensillae (se1-3, fig. 26); tentorial orifices present and distinct; clypeus (cl, figs. 12–13) with the posterior and lateral margins indistinct, and its anterior margin slightly convex; two supraocular setae present, basiconic and positioned symmetrically at the mid-length of the sclerite; mouthparts small and poorly sclerotised; mandibles (M, figs. 12–13 and 17–18) short and robust, not crossed when closed; shape typically “attoid” (*sensu* Wheeler, 1949: 669): subtriangular and subconical, slightly falciform (apical part strongly curved medially); apex more sclerotised than the basal part; gnathobasis absent; articulatory margin large (arm, figs. 18–19); external margin (em, fig. 18) convex in dorsal view, its base well rounded; apical margin (apm, fig. 18) long and concave, the denticulation as follows: a fine and acute apical tooth (apt, fig. 18) followed by 2–3 short denticles, indistinct and more or less acute, similar in shape to the spines that cover the whole surface of the mandibles (spi, fig. 25); basal margin (bm, fig. 18) short and slightly convex, forming an...
obtuse and rounded angle at its contact with the apical margin; labrum (lab, figs. 12–13 and 17–18) trapezoidal, narrow and thick, its anterior part descending and the anterior margin distinctly convex; eight papilliform sensillae on the labrum, distributed as follows: one basal pair positioned medially and 3 lateral ones aligned at the distal margin; maxilla (max, fig. 17) moderately developed, relatively long and thick, its basal part fused with the head; maxillary articulation (ma, fig. 17) visible between the palp and the galea; maxillary palp (mp, figs. 17 and 22–23) well developed, strongly shifted ventro-laterally and distant from the galea (ga, fig. 17), and with accessory sensillae (asmp, fig. 23); galea reduced, forming a simple plate bearing two coeloconic sensillae (se, fig. 17); apical part free and with discrete spines, very short and sparse; two maxillary setae (ms, figs. 17 and 22–23) present between the palp and the galea; labium (la, figs. 17–18) short in frontal view; no sericteries; labial palps (lp, fig. 17) very reduced, only indicated by three setae positioned closed together ventro-laterally.

Thorax (T, fig. 27). Segments long, forming together about one third of the whole body surface; prothorax with an indistinct lobe in medio-ventral position, its ventral part smooth; mesothorax without ventro-median protuberance; diameter of mesothoracic spiracle (mts, fig. 28) 14.2 µm (n = 1).

Abdomen (A, fig. 27). No ventro-median protuberance; anus positioned ventrally and distant from the posteriormost point of the body; anal orifice (ao, figs. 11 and 31) forming a transverse slit, with a dorsal fold, prominent and rounded at the ventral face of tenth abdominal segment in lateral view.

Pilosity according to the diagnosis of Wheeler & Wheeler (1976: 26) for Attini larvae: body nearly glabrous, with sparse setae concentrated at anterior part of head, as well as on ventral surface of thorax and on first and last abdominal segments. Setae simple, relatively fine, slightly curved and gradually pointed apically; setae biggest on the head, of variable size on thorax and shortest near the anus; cephalic capsule with four pairs of sub-antennal setae (sas, figs. 12–13 and 24), transversally aligned at midlength between the anterior margin of clypeus and the level of antennal insertion; no supra-antennal setae; ventral faces of thoracic segments and of the three first abdominal segments, each one with two pairs of setae, the whole setae drawn two external and two internal longitudinal lines of setae delimiting a narrow glabrous territory, positioned postero-ventrally to the head; ventral surface of ninth abdominal segment with a pair of long setae; ventral surface of tenth abdominal segment with a lateral pair of long setae and an internal pair of short ones.

Sculpture. Body smooth, but covered by very numerous microspines that form concentric lines all over the body surface; microspines more dense at the ventral parts of the thorax and of the first and last abdominal segments; head smooth, except the mouthparts: mandible very sculptured, the external face entirely covered by short and acute denticles, well defined and individualized; labrum entirely covered by acute microspines, isolated or aligned; maxilla with microspines isolated and aligned; labium bearing microspines transversally aligned at its postero-dorsal surface.

Colour. Head and body naturally white-opaque; apex of mandible slightly yellowish.

**Second larval morph** (figs. 10 and 11). Morphology similar to the first larval instars, with the exception of the following characteristics: body slightly bigger and less compressed; last abdominal segments in lateral view with the dorsal intersegmental fold more discrete; mouthparts slightly more sclerotised.

**Third larval morph** (figs. 12). Morphology similar to the second larval morph, with the exception of the following characteristics: body in lateral view short and robust, slightly longer; dorsal intersegmental fold of the last abdominal segments weakly distinct in lateral view; head in lateral view distant from the more anterior point of the body and positioned slightly more ventrally in relation to the body; body in lateral view with the ventral face slightly more concave. Cephalic capsule in lateral view longer and with the dorsum less convex; cephalic capsule in frontal view wider than long, with a “subelliptic” to “sub-trapezoidal” shape (sensu Wheeler & Wheeler, 1976: 30); genal lobes present but poorly developed and slightly rounded; tentorial orifices not observed; mandibles more sclerotised and with the external margin forming a continual convexity in dorsal view; labrum with distal margin strongly convex. Prothorax without lobe.
FIGURES 2–8. Egg morphology of Cyphomyrmex transversus. 2: eggs interconnected by their fungus layers. 3 (detail of fig. 2): egg habitus with detail of its fungus layer. 4 (detail of fig. 3): cellular organization of the fungus layer. 5: egg habitus after removing of its fungus layer. 6: detail of the vitelline membrane, with epidermal cells visible through the chorion. 7: eggs and first instar larvae forming a brood package. 8: egg habitus after removing the fungus layer id 6. Abbreviations as follows: ap, anterior pole (fig. 5); C, cell (fig. 6); cf, fungus layer (figs. 2–3); ch, chorion (fig. 5); E, egg (figs. 2–4 and 7–8); fi, fungus filament (mycelium) (fig. 8); il, intercellular limit (fig. 6); L, first instar larva (fig. 7); mi, micropyle (fig. 5); pp, posterior pole (fig. 5); tuf, fungus tuff (fig. 8); vm, vitelline membrane (fig. 5); yc, yeast-like cell (fig. 4).
FIGURES 9–14. Larval morphology of *Cyphomyrmex transversus*. 9: first morph (1st larval instar), habitus in lateral view. 10–11: second larval morph, habitus in lateral view. 12: third larval morph, head in full-face view. 13: fifth larval morph, head in full-face view. 14: third larval morph, head in ventral view. Abbreviations as follows: at, antenna (figs. 12–13); CC, cephalic capsule (figs. 12–13); cl, clypeus (figs. 12–13); fr, front (figs. 12–13); lab, labrum (figs. 12–13); gl, genal lobe (fig. 12); M, mandible (figs. 12–13); msp, mesothoracic spiracle (fig. 10); mtp, metathoracic spiracle (fig. 10); om, occipital margin (fig. 12); sas, sub-antennal setae (figs. 12–13); tf, tegumentary fold (fig. 9).
FIGURES 15–16. Fifth larval morph of *Cyphomyrmex transversus*. 15: habitus in lateral view. 16: habitus in ventral view.
FIGURES 17–21. Fifth larval morph of *Cyphomyrmex transversus*. 17: head in ventral view. 18: head in full-face view, detail of the clypeo-mandibular region. 19: detail of the left mandible, external view. 20: detail of the labrum in dorsal view. 21: head and anterior region of the body, ventral view, detail of the pilosity. Abbreviations as follows: apm, apical margin (fig. 18); apt, apical tooth (fig. 18); arm, articulatory margin (figs. 18–19); bm, basal margin (fig. 18); co, condyle (figs. 18–19); em, external margin (fig. 18); ga, galea (fig. 17); gl, genal lobe (fig. 17); la, labium (figs. 17–18); lab, labrum (figs. 17–18); lp, labial palp (fig. 17); M, mandible (figs. 17–18); ma, maxillar articulation (fig. 17); max, maxilla (fig. 17); mp, maxillar palp (fig. 17); ms, maxillary setae (fig. 17); s, seta (fig. 17); se, sensilla (fig. 17); sp, spiracle (fig. 17); tr, trachea (fig. 17).
FIGURES 22–26. Fifth larval morph of *Cyphomyrmex transversus*. 22: detail of the maxillary palp, lateral view. 23: idem, full-face view. 24: detail of the left sub-antennal setae. 25: right mandible, antero-dorsal view. 26: right antennae, antero dorsal view. Abbreviations as follows: **asmp**, accessories sensillae of maxillar palp (fig. 23); **dda**, discoidal depression of the antenna (fig. 26); **gl**, genal lobe (fig. 24); **mp**, maxillar palp (figs. 22–23); **ms**, maxillary setae (figs. 22–23); **sas**, sub-antennal setae (fig. 24); **se1-se3**, sensilla (fig. 26); **spi**, spine (fig. 25).
FIGURES 27–32. Larval morphology of Cyphomyrmex transversus. 27: Schematic representation in lateral view of a fifth larval morph, focusing on larval segmentation and spiracles’ disposition. 28: detail of an abdominal spiracle of a first-instar larva, full-face view. 29–32: fifth larval morph. 29: Detail of the left metathoracic spiracle, full-face view. 30: detail of the protuberance of the first abdominal segment, ventral view. 31: caudal region, lateral view: anal slit. 32: ventral part of the body, lateral view. Abbreviations as follows: A, abdomen (abdominal segments); ao, anal orifice (fig. 31); CC, cephalic capsule (fig. 27); cr, caudal region (fig. 27); mts, mesothoracic spiracle (fig. 28); so, spiracle orifice; sp, abdominal spiracles (fig. 27); T, thorax (thoracic segments) (fig. 27). Vap, ventro-median protuberance (fig. 32).
**Fourth larval morph.** Morphology similar to the third larval morph, with the exception of the following characteristics: body in lateral view moderately short and robust but bigger, with a typical “attoid” shape (*sensu* Wheeler & Wheeler, 1986: 686); head in lateral view more distant to the more anterior point of the body; body in lateral view with its ventral face distinctly longer; first abdominal segment in lateral view forming a distinct protuberance; caudal part in lateral view with no distinct protuberance; last abdominal segments in lateral view, without any dorsal intersegmental fold. Head/body volume ratio smaller; cephalic capsule slightly longer and with its dorsum less convex; cephalic capsule in frontal view with a clear “substrapezoidal” shape (*sensu* Wheeler & Wheeler, 1976: 30), wider than long; genal lobes well developed; antennae small and tentorial orifices well visible; mandibles more robust. Thorax in lateral view long, its length slightly shorter than half the body length. Abdomen in lateral view with a ventro-median protuberance well developed (*vap*, fig. 32), slightly inclined posteriorly. Mandibles more strongly sculptured and labrum with numerous acute microspines, multidenticulate and aligned transversally.

**Fifth larval morph** (*figs. 13, 15–16, 17–21, 22–26 and 27–32*). Morphology similar to the fourth larval morph, with the exception of the following characteristics: body in lateral view longer and more robust, with a “bean” shape, about two times longer than high; head in lateral view more distant from the more anterior point of the body; maximum distance between occiput and anus two times shorter than the maximum body length measured along its longitudinal axis. Head volume much smaller proportionally to the rest of the body. Mesothoracic spiracle with diameter (n=1) circa 15.8 µm. Abdomen with ventro-median protuberance (*vap*, fig. 32) moderately developed.

**Morphology of the symbiotic fungus and its importance for the post-embryonic development of *C. transversus***

The morphological study of the *C. transversus* eggs, larvae and pupae revealed that their integument is characteristically covered by a leucocoprineaceous symbiotic fungus. The fungus layer that covers the whole body of the larvae and pupae is formed by numerous discoid tufts (*tuf*, fig. 8) composed of two cellular types; some spherical glutinous yeast-like cells (*cf*, figs. 2–3) and some mycelial cells. The first one may correspond to some gongylidia-like cells (José Luis Bezerra, pers. com.). A detailed observation of the tufts reveals that their radial shape results from the formation of numerous elongated hyphae (Fig. 7). These tufts are separated by a few micrometers and interconnected by the mycelium (*fi*, fig. 8), forming together a compact network that covers the brood specimens’ body.

In adult colonies, we observed that an egg laid by a queen is systematically taken by a worker with its mandibles and immediately added to a cluster of eggs. The workers always assist the larvae during ecdisis. Eggs and newly-molted larvae are intensively licked by the workers, behaviour that precedes the fungus development on their integument. Using mouthparts, antennae and front legs, the workers scrape together a white fungus tuft and deposit it on the integument of the egg or larva. After some hours, the numerous fungus tufts deposited on the integument grow and interconnect with mycelia. This process permits the formation of a new fungus layer on the surface of a newly laid egg or an ecdised larva. We observed that the old larval exuvium and its fungus layer are systematically eaten by the adults of the colony. We noted that a fungus tuft deposited on brood has a white colour, whereas a tuft deposited on substrate has a light yellow colour.

**Discussion**

The morphometric study of *C. transversus* eggs showed no evidence of size dimorphism, suggesting that male and female eggs do not differ in size, or that no male eggs were being produced by the colonies. This result is
similar to the observations of Masuko (1990) on *Amblyopone silvestrii* (Amblyoponinae) who found no difference between male and female eggs.

In ants, the number of instars varies between three and six (Hölldobler & Wilson, 1990; Masuko, 1990; Fox et al., 2007) according to species, caste and sex. Torre-Grossa et al. (1982) showed that *A. octospinosus* (Reich) workers have four instars while reproductives (gynes and males) have five. Since *Acromyrmex* and *Cyphomyrmex* have close phylogenetic relationships, the five larval morphs we described in *C. transversus* could represent five instars. Nevertheless, further observations and measurements are necessary to corroborate it.

The morphology of the last morph of *C. transversus* agrees with the diagnosis of the Attini larvae of Wheeler (1949) and Wheeler & Wheeler (1976): it is “apodous”, “eucephalic” and of the “attoid” type. The main features of *C. transversus* larvae that agree with the attine diagnosis are: short, robust and thick body, bean-shaped, weakly enlarged in the middle and with a smooth surface; anterior and posterior extremities rounded; indistinctly segmented; ten small spiracles organized in a “J”-shape; head sub-trapezoidal, with the genal lobes well developed and ventral, considerably distant from the apex of the body; mouthparts discrete, with the mandibles short and covered by microdenticles; anus ventral; pilosity very weak, consisting of simples setae, inserted on the head, on the ventral face of the thorax and the anterior and posterior parts of the abdomen.

While conspicuous changes exist between the first and the fifth larval morphs (compare figs. 9 and 15), few differences can be evidenced between the first three. The larvae of the first and second morphs differ only in their size and volume. In the third morph, the head gets a clear ventral position and the intersegmental folds of the last abdominal segments disappear; the head capsule has an intermediate shape between the first two and the last two morphs (i.e., between elliptical and sub-trapezoidal). The genal lobes are also intermediate in size and distinctness. The main morphological transformation of the larva concerns the body form that, in the fourth morph, becomes truly attoid and bean-shaped, while the head is shifted downwards some distance from the anterior larval apex. Furthermore, a well marked protuberance (first abdominal segment in lateral view) becomes clearly distinct in the anterior abdominal region. As for the fifth morph, it does not greatly differ from the fourth.

O’Neal & Markin (1975) observed that the larval instars of *Solenopsis invicta* Buren varied in body form, head capsule form, and mouthparts. We found that the main differences in *C. transversus* morphs were also in body and head capsule form but not mouthparts. Mouthparts were relatively similar among morphs.


The ventral surface of the *C. transversus* larva shows a peculiar morphology (setae arrangement, ornamentation consisting of small spines), which seems typical of attine larvae that are fed with fungi (Wheeler & Wheeler, 1974). The ventral hairs, apparently functioning to anchor the fungal mass, keep it firmly in place near the mouthparts while the larva is feeding (Wheeler & Wheeler, 1974; Schultz & Meier, 1995). On the other hand, Petralia & Vinson (1979) observed such specialization on the anteroventral body region of *Solenopsis invicta* larvae; they suggested that it may facilitate feeding on the solid chunks of food placed on the larvae by adult workers.

The ventral abdominal protuberance observed in *C. transversus* is similar to that observed in all *Cyphomyrmex* species studied by Schultz & Meier (1995). Within the Attini, this structure occurs exclusively in the genus *Cyphomyrmex*. It probably functions as a brace for food particles in ventral feeding. In non-attines, ventral abdominal feeding structures have been reported by Petralia & Vinson (1980) for the ponerine *Leptogenys elongata* and the myrmicine *Pogonomyrmex barbatus*.

The eggs, larvae, and pupae of most Attini are covered with a fungus layer. This fungus layer is found in
all the genera of the Attini (Weber, 1956 & 1972; Schultz & Meier, 1995; Adams et al. 2000) except Atta. Several hypotheses have been proposed to explain the development of the fungus on the body surface. The fungal layer could be a direct consequence of the behavior of the workers that lick the surface of the larvae during the moult, with the saliva facilitating the fungal growth (Weber, 1956). We observed that the workers, in addition to licking the larvae, take fungus fragments from the garden and actively fix them on the larvae soon after the moult. The fungus cover could be beneficial to the larvae, in protecting them against pathogens, parasites or parasitoids via a physical or chemical barrier (Weber, 1972; Swartz, 1998; Schultz & Meier, 1995). LaPolla et al. (2002) discussed the hypothesis that the fungal coat may protect the brood of Attini during army-ant raids. A last hypothesis is that the fungus layer on the larvae facilitates the moulting process. As with other Cyphomyrmex species, C. transversus uses dead insect carcasses to cultivate its symbiotic fungus, and, possibly, the fungus layer on the larval cuticle promotes a superficial degradation of the old integument and facilitates the moult.

Despite the fact that knowledge of post-embryonic development represents a very important pre-requisite for biological, ecological and toxicological studies in ants (Wheeler & Wheeler, 1976; Hölldobler & Wilson, 1990), there is a shortage of information on larval morphology. The scarcity of such studies is especially surprising considering the great ecological and economic importance of ants (Petralia & Vinson, 1979). Studies of larval morphology in additional species and better methods of determining instar number are needed.

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