Description of the Immatures of Workers of the Ant *Linepithema micans* Forel (Hymenoptera: Formicidae)

ALINE NONDILLO,[1] DANIEL RUSS SOLIS,[1]* EDUARDO GONÇALVES PATERSON FOX,[2] MÔNICA LANZONI ROSSI,[3] MARCOS BOTTON,[4] AND ODAIR CORREA BUENO[1]

[1]*Centro de Estudos de Insetos Sociais, Instituto de Biociências, São Paulo State University (UNESP), Rio Claro, São Paulo, Brazil*
[2]*Laboratório de Entomologia Médica e Molecular, Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro (IBCCF/UFRJ), Rio de Janeiro, Brazil*
[3]*Laboratório de Histopatologia e Biologia Estrutural de Plantas, Centro de Energia Nuclear na Agricultura, University of São Paulo (USP), Piracicaba, São Paulo, Brazil*
[4]*Centro Nacional de Pesquisa de Uva e Vinho, Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Bento Gonçalves, Rio Grande do Sul, Brazil*

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ABSTRACT *Linepithema micans* Forel is a poorly studied species that is now considered a pest in vineyards in South Brazil. Larval characters have been used in a few phylogenetic studies, and their importance greatly depends in the amount of available information on different species. This study presents a complete panorama on the external morphology of the immatures of *L. micans* based on observations by light and scanning electron microscopy. The number of larval instars was estimated as three based on the frequency distribution of head widths of 965 larvae. Larvae of *L. micans* were similar to other *Linepithema* Mayr in the general format of body and mandibles, presenting a dorsal abdominal protuberance, nine pairs of spiracle, and unbranched hairs. On the other hand, *L. micans* was unique for having shorter hairs, predominantly denticulate, intraspecific variation in the number of antennal sensilla and in the types of sensilla on the labial palps were reported. *Microsc. Res. Tech. 74:337–342, 2011.*

INTRODUCTION

The ant genus *Linepithema* Mayr includes 35 described species and subspecies (Bolton et al., 2006) of small monomorphic ants that occur in the woods, grasslands and mountainous areas of the Neotropical region (Wild, 2007). The species *Linepithema humile* Mayr stands out for being known as an invasive pest tramp species of rural and urban areas (Giraud et al., 2002). Another species, *Linepithema micans* Forel, has been recently ascribed considerable importance as a pest in the vineyards of Southern Brazil, where it associates with the Brazilian ground pearl *Eurhizococcus brasiliensis* Hempel (Sacchet et al., 2009), considered the most serious pest of vineyards in this region (Botton et al., 2004).

About 800 ant species (Wheeler and Wheeler, 1988) out of a total of 11,477 (Bolton et al., 2006) had their larvae described over a long series of studies by George C. Wheeler and Jeanette Wheeler during the second half of the 20th century. Few studies with ant larvae were undertaken by other authors ever since. Still, larval characters can be used for phylogenetic studies with ants (Schultz and Meier, 1995) and can add important information about the general biology of many species (e.g., Peeters and Hölldobler, 1992). The fact that they are usually ignored in most studies is probably due to the difficulties involved in obtaining samples and a general inconsistent knowledge about the usefulness of larval characters (Schultz and Meier, 1995).

The present authors are currently engaged in producing a series of larval descriptions additive to the series of works by George C. Wheeler and Jeanette Wheeler at present focusing on ant species commonly found within the Neotropical region. The present study is part of this joint effort, and aimed at describing the immature stages of *L. micans* with the aid of light and scanning electron microscopy.

MATERIALS AND METHODS

Obtention of Samples

Seven nests of *L. micans* were obtained in 2009 in the municipality of Bento Gonçalves (29°09′48.16″S, 51°31′58.00″W), Rio Grande do Sul, Brazil, at the vineyards of Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Centro Nacional de Pesquisa de Uva e Vinho. 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 then conserved in 70% alcohol. Voucher specimens of eggs, larvae, and pupae are deposited in the "Adolph Hempel" entomological collection of the Centro de Pesquisa e Desenvolvimento de Sanidade Vegetal in Instituto Biológico, São Paulo, Brazil.

Aline Nondillo and Daniel Russ Solis contributed equally to this work.

*Correspondence to: Daniel Russ Solis, Centro de Estudos de Insetos Sociais, Instituto de Biociências, Universidade Estadual Paulista "Julio de Mesquista Filho" (UNESP), Av. 24-A, 1515, CEP 13506-900, Rio Claro, SP, Brazil. E-mail: entomo75@yahoo.com

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Determining the Number of Larval Instars

The number of larval instars was determined using the method described in Parra and Haddad (1989): the maximum head widths of a large number of the collected larvae ($N = 965$) were measured and the results were plotted on a frequency distribution graph, wherein every distinct peak was considered to correspond to a different larval instar. Then the obtained number of larval instars was tested against Dyar's rule (Parra and Haddad, 1989). It should be emphasized that the first larval instar and the last larval instar can be explicitly identified based on hatching larvae and prepupae, and thus were used as reference to bracket the others.

Description of the Immature Forms

The morphological descriptions were made based on 20 larvae of each instar (10 used in scanning electron microscopy and 10 used in light microscopy) belonging to the most frequent head width found for the assigned instar. The compound light microscope used was a Zeiss MC80 DX with a maximum magnification of 1000X and the scanning electron microscope employed was a LEO 435 VP run at 20.0 kV. By means of a stereomicroscope (Zeiss Stemi SV11, maximum magnification of 66X) equipped with a micrometric eyepiece, we could rapidly measure length and medial width of eggs ($N = 195$) and larvae ($N = 470$), and head width and body length of pupae ($N = 100$).

Samples to be analyzed under the scanning electron microscope were dehydrated in an alcohol graded series (80–100%; 10 min for each concentration), and critical-point dried (Balzers CPD/030); dried specimens were then attached to aluminum stubs with double-faced conductive adhesive tape and gold-sputtered with a Balzers SCD/050 sputterer. Observations and images were obtained as soon as possible after sample preparation. Samples to be analyzed under the compound microscope were warmed for 5–10 min (depending on size of larvae) in KOH 10% and placed in a small drop of glycerin on a glass microscope slide.

Terminology used in the larval descriptions was based on Wheeler and Wheeler (1976). Measures, where applicable, were given as mean ± standard deviation followed by number of observations (N). The following abbreviations were used: (l) length; (w) width.

RESULTS

Determination of the Number of Larval Instars

The frequency distribution of head capsule widths resulted in a multimodal distribution with three distinct peaks (suggesting the existence of three instars), being the first peak formed by first instar larvae and the third peak entirely formed by prepupae (Fig. 1). The obtained number of larval instars yielded a good fit with Dyar's rule ($R^2 = 0.85$).

In accordance with Dyar's rule, mean growth rate between the larval instars was 1.31, while between the first and the second instars was 1.38, and between the second and third instars was 1.23.

Morphological Description of the Immature Forms

Egg. Ovoid but slightly elongate in shape, with delicate translucent chorion (l = 0.22–040 mm; $w = 0.13$–0.29 mm; $N = 195$). Egg index (length/width ratio) = 1.32.

General Aspect of Larvae. Larvae of the three instars shared many characteristics, thus a general description is given below and differences are given in Tables 1 and 2.

Body. Body shape "dolichoderoid", defined by Wheeler and Wheeler (1976) as "short, stout, plump, straight or slightly curved, with both ends broadly rounded; anterior end formed by enlarged dorsum of prothorax; head ventral, near anterior end; no neck; somites indistinct" (Fig. 2A). Body covered with trans-

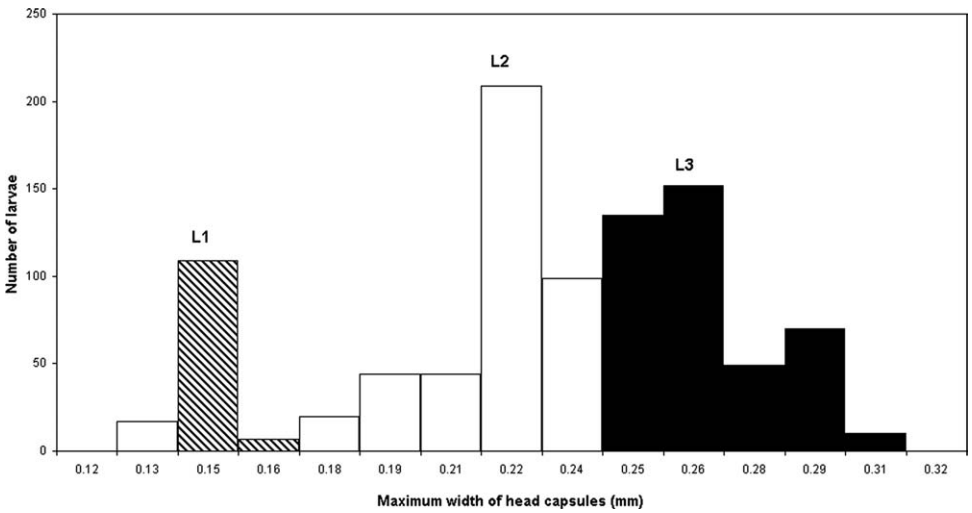

Fig. 1. Frequency distribution of the maximum widths of head capsules of worker larvae of *Linepithema micans*: (L1) first instar, (L2) second instar, and (L3) third instar. The hatched columns represent intervals in which mature embryos in the eggs were found, and the black columns represent the intervals in which prepupae were found.

TABLE 1. Bodily measures of larvae of three different instars of Linepithema micans

Measured part	Larval Instar		
	First	Second	Third
Body			
Length	0.46 ± 0.08 mm	0.80 ± 0.14 mm	0.66–1.75 mm (N = 152)
	0.28–0.66 mm (N = 109)	0.44–1.15 mm (N = 209)	
Width	0.15–0.29 mm (N = 109)	0.22–0.57 mm (N = 209)	0.35–0.67 mm (N = 152)
Length through spiracles	0.60–1.09 mm (N = 10)	1.20 ± 0.09 mm	1.74 ± 0.18 mm
		1.02–1.36 mm (N = 10)	1.37–1.96 mm (N = 10)
Length of body hairs	0.009 ± 0.002 mm	0.008 ± 0.001 mm	0.008 ± 0.001 mm
	0.005–0.011 mm (N = 50)	0.006–0.012 mm (N = 50)	0.007–0.012 mm (N = 50)
Spiracle diameter			
1st abdominal	0.004–0.007 mm (N = 10)	0.007–0.008 mm (N = 10)	0.008–0.009 mm (N = 10)
7th abdominal	0.001–0.004 mm (N = 10)	0.004 mm (N = 10)	0.004 mm (N = 10)
Others	0.002–0.006 mm (N = 70)	0.005–0.006 mm (N = 70)	0.005–0.006 mm (N = 70)
Length of dorsal protuberance	0.073 ± 0.018 mm	0.107 ± 0.008 mm	0.132 ± 0.007 mm
	0.055–0.110 mm (N = 10)	0.100–0.125 mm (N = 10)	0.125–0.145 mm (N = 10)

Values are given either as mean ± standard deviation or minimum-maximum interval, as applicable.

TABLE 2. Sizes of head capsule and mouthparts of larvae of different instars of Linepithema micans

Measured part	Larval Instar		
	First	Second	Third
Head capsule			
Maximum width	0.15 mm (N = 109)	0.22 mm (N = 209)	0.26 mm (N = 152)
Hair length	0.005–0.009 mm (N = 50)	0.007 ± 0.001 mm	0.006 ± 0.001 mm
		0.005–0.009 mm (N = 50)	0.005–0.008 mm (N = 50)
Length of labrum	0.042–0.045 mm (N = 3)	0.048–0.057 mm (N = 3)	0.048–0.052 mm (N = 5)
Length of mandible	0.046 ± 0.004 mm	0.058–0.060 mm (N = 10)	0.063–0.075 mm (N = 10)
	0.040–0.050 mm (N = 10)		
Length of maxilla	0.059 ± 0.006 mm	0.083 ± 0.009 mm	0.085 ± 0.010 mm
	0.049–0.069 mm (N = 10)	0.071–0.095 mm (N = 10)	0.072–0.095 mm (N = 10)
Length of labium	0.051–0.065 mm (N = 3)	0.071–0.084 mm (N = 4)	0.074–0.092 mm (N = 4)
Height of galea		0.007–0.009 mm (N = 6)	0.008–0.012 mm (N = 3)
Maxillary palp			
Length	0.014–0.015 mm (N = 2)	0.013–0.015 mm (N = 5)	0.014–0.016 mm (N = 5)
Width	0.009–0.011 mm (N = 2)	0.009–0.010 mm (N = 5)	0.008–0.011 mm (N = 5)
Labial palp			
Length	0.012–0.013 mm (N = 4)	0.012–0.016 mm (N = 7)	0.013–0.016 mm (N = 8)
Width	0.007–0.009 mm (N = 4)	0.009–0.010 mm (N = 7)	0.009–0.011 mm (N = 8)
Mouthpart hair length			
Maxilla	0.005 ± 0.001 mm	0.006 ± 0.001 mm	0.003–0.007 mm (N = 20)
	0.003–0.007 mm (N = 15)	0.004–0.009 mm (N = 20)	
Labium	0.004–0.006 mm (N = 4)	0.004–0.008 mm (N = 6)	0.004–0.007 mm (N = 8)

versal rows of spinules (Fig. 2B) and unbranched hairs (Fig. 2C), most of which denticulate. Smooth simple less frequently observed and only distinguishable from denticulate hairs by scanning electron inspection. Body hairs concentrated on thorax, equally-sized among body regions and different instars. Nine pairs of unornamented spiracles, being two thoracic and seven abdominal (Fig. 2D). Spiracle of same approximate size, except for the first abdominal pair which was greatest and the seventh abdominal pair which was smallest. All specimens with a clearly pronounced dorsal protuberance on the first abdominal somite (Fig. 2E). Anus subterminal. Head Capsule (Fig. 3A): Subhexagonal; antennae including three (rarely two or four) basiconic sensilla that may or may not be arranged in a series (Figs. 3B, 3C, and 3D). Head hairs always unbranched, either denticulate or smooth, slightly shorter than body hairs and equally sized among different instars (sizes given in Tables 1 and 2). Head hair distribution as follows: nine to eleven over each gena, four on clypeus, and none on frons, vertex, or occipital border. Clypeus clearly delimited. Mouthparts (Fig. 3E): Labrum bilobed with four setaceous sensilla (rarely three) on the anterior surface and one basiconic sensillum on each side of the ventral border; posterior surface with transversely arranged rows of spinules (Fig. 4A). Mandibles roughly "dolichoderoid" in shape, defined by Wheeler and Wheeler (1976) as: "basal part inflated and narrowed more or less abruptly to the distal part, which is slender and sharp-pointed; no medial teeth or blade," and sclerotized, but presenting two small teeth on the blade; two sensilla on base of mandibles. Posterior surface of mandible covered with long rows of spinules (Fig. 4B); present also on anterior surface of mandibles of third instars. Maxilla conoidal in shape with spinules arranged in transversal rows on apex, with one to three simple smooth hairs and two basiconic sensilla (rarely one); maxillary palpus a cluster of five sensilla, being one setaceous, three basiconic and one encapsulated; galea a skewed peg with two basiconic sensilla (Fig. 4C). Labium elliptical, with transversal rows of spinules above and on both side of the slit-like opening of the sericteries; two simple smooth hairs (rarely one), and one basiconic sensillum by each extremity of the sericteries, and about 4–6 sensilla (being 2–4 setaceous, and 2–3 basiconic) over the anterior surface (Fig. 4D). Labial palpus an elevated cluster of five sensilla, being 3 or 4 basiconic and 1 or 2

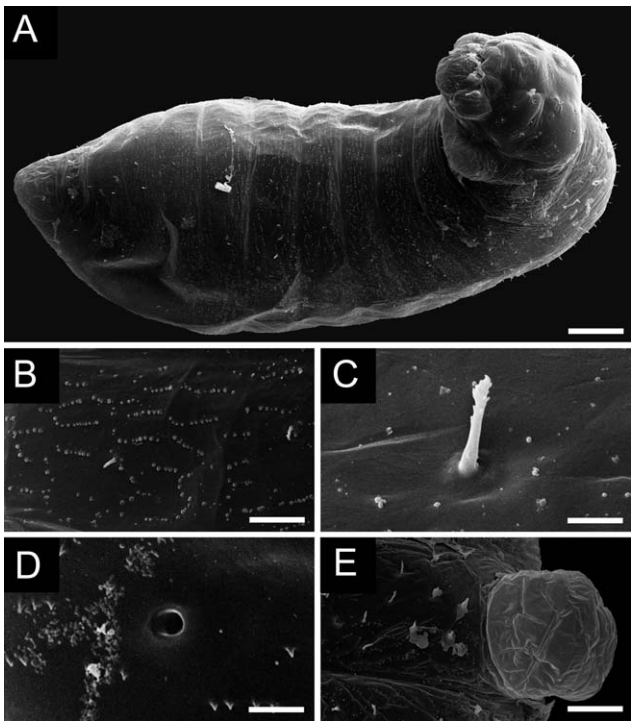

Fig. 2. Body of *Linepithema micans* larvae: (**A**) Second instar in ventral view; (**B**) rows of spinules on body of a second instar; (**C**) denticulate simple hair; (**D**) spiracle of a first instar; (**E**) dorsal protuberance of second instar. Sizes of scale bars: (A) 0.083 mm; (B) 0.020 mm; (C) 0.005 mm; (D) 0.004 mm; (E) 0.030 mm.

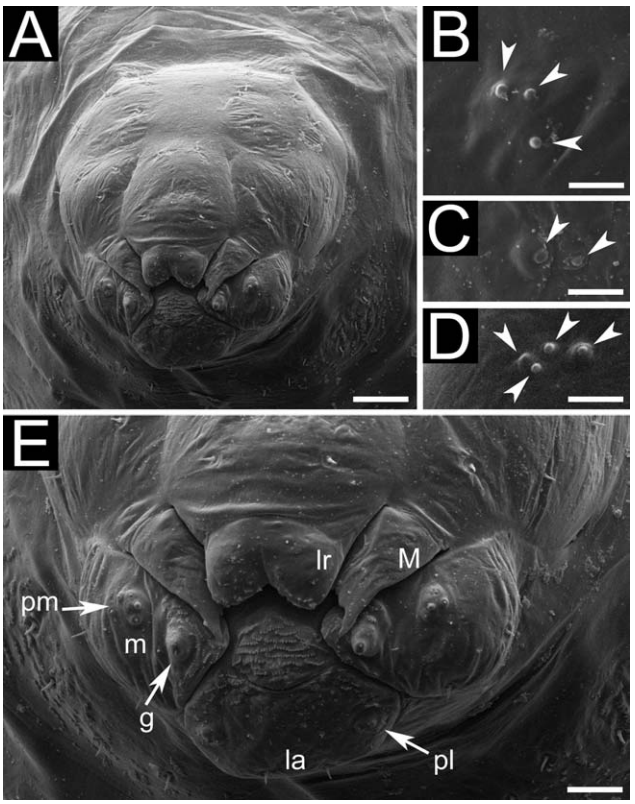

Fig. 3. Head of *Linepithema micans* larvae (sensilla indicated with arrowheads): (**A**) full frontal view of a third instar; (**B**) antenna of a second instar; (**C**) antenna of a first instar; (**D**) antenna of a first instar; (**E**) mouthparts of a third instar. Abbreviations: (g) galea, (la) labium, (lr) labrum, (M) mandible, (m) maxilla, (pl) labial palp, (pm) maxillary palp. Sizes of scale bars: (A) 0.042 mm; (B) 0.005 mm; (C) 0.007 mm; (D) 0.007 mm; (E) 0.020 mm.

encapsulated (Fig. 4E). Mouth entrance displaying hypopharynx covered with elongate spinules arranged in dense transverse rows (Fig. 4B).

Pupa. During early development, pupae are white, with eyes and body getting darker during metamorphosis. Pupae exarate with no cocoon (body: l = 1.63–1.92 mm; head: w = 0.47–0.59 mm; N = 100). Only white pupae were measured.

DISCUSSION

All obtained larvae had a dorsal abdominal protuberance, which is typically found on larvae of this genus (Shattuck, 1992). According with Solis et al. (2010b), the reproductive larvae of *L. humile* lack this protuberance. As *L. micans* is a similar species of the Humile species group (Wild, 2007), we believe the same probably occurs with *L. micans*, and thus indicating that our nests had no reproductive larvae. This could well be an indication that colonies of *L. micans* produce reproductive forms in a specific period, contrary to previous observations with *L. humile*, whose colonies produce alates over the whole year (Passera et al., 1988). This information may be invaluable to controlling this species, and thus deserves direct investigation.

Determination of Number of Larval Instars

Solis et al. (2010a) stated that the number of ant species that had their number of larval instars determined was 64, ranging from 3 to 5 instars depending on the species. Within Dolichoderinae, number of larval instars was determined to *Bothriomyrmex syrius* Forel (four instars; Thomé and Thomé, 1981), *L. humile* (three instars; Solis et al., 2010b), and *Tapinoma melanocephalum* Fabricius (four instars; Jesus and Bueno, 2007). The number of larval instars of *L. micans* is thus the same as *L. humile*.

Apart from head width, mandible had variable sizes among the different instars, thus they can be alternatively used to separate instars.

Morphological Description of the Immature Forms

Some typical traits of *Linepithema* larvae were confirmed with this species: general body and mandible shape, existence of a dorsal abdominal protuberance, nine pairs of spiracles, and simple hairs (Shattuck, 1992; Wheeler and Wheeler, 1976). Traits particular of this species would include: short denticulate hairs, intraspecific variation in the number of antennal sensilla and of types of sensilla on labial palps were herein observed for the first time in *Linepithema*. Intraspecific variations have to be emphasized as to avoid the utilization of unreliable characters for species-level or genus-level differentiation.

According with Shattuck (1992) the larvae of *Linepithema* can have simple and bifid body hairs. However,

only simple hairs were observed on larvae of *L. micans*, similar to *L. humile* (Solis et al., 2010b). For instance, larvae of *L. iniquum* can also present bifid hairs (Wheeler and Wheeler, 1973). Head and body hairs of *L. micans* are considerably shorter than those of *L. humile* and *L. iniquum* (Table 3). It seems thus that hairs are promising characters to separate species and species groups within larvae of *Linepithema*. The reliability of hairs as characters merits deeper investigation with other species.

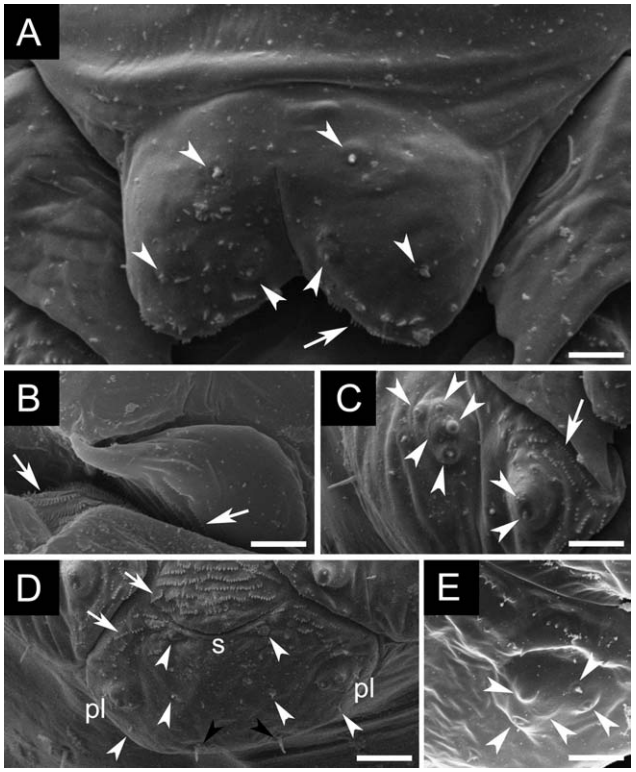

Fig. 4. Mouthparts of *Linepithema micans* larvae (white arrowheads indicate sensilla, black arrowheads indicate hairs, and white arrows indicated rows of spinules): (**A**) labrum of a third instar; (**B**) posterior face of mandible and mouth entrance of first instar, displaying hypopharynx covered with elongate spinules; (**C**) maxilla of a third instar showing galea and maxillary palp; (**D**) labium of a third instar showing opening of sericteries (s), and labial palps (pl); (**E**) Labial palp of a third instar. Sizes of scale bars: (A) 0.007 mm; (B) 0.006 mm; (C) 0.010 mm; (D) 0.013 mm; (E) 0.008 mm.

Solis et al. (2010b) did not report any denticulate unbranched hairs in *L. humile*. However, we recently revisited the larvae of that species, and indeed found denticulate body hairs over the dorsum; we think they were overlooked in the previous study because samples used in Solis et al. (2010b) were covered in debris.

The antennae of two first-instar specimens presented respectively two and four sensilla, demonstrating an intraspecific variation in the number of antennal sensilla within larvae of *L. micans*. Intraspecific variation in the number of antennal sensilla was observed by Wheeler and Wheeler (1976) and on some other ant species (Jesus et al., 2010; Solis et al., 2010c), thus the phenomenon is fairly widespread. In addition, the labrum of larvae of Humile-group species usually have four sensilla on the anterior surface, but one analyzed specimen of *L. micans* had three (Table 3). Thus, intraspecific variation in that trait is also possible. This serves to illustrate the importance of analyzing a large sample while searching for intraspecific variations.

Still concerning the labrum, the only fixed pattern within *Linepithema* seems to be a bilobed labrum with two sensilla on the ventral surface and spines on the posterior surface.

General mandible shape in *L. micans* is dolichoderoid, but with two denticles on the blade, as also observed in *L. humile* and differently from the observed with *L. iniquum* (Table 3). This character seems thus interesting for differentiating between larvae of both groups of species, yet still further species have to be described to see if it holds.

We hope the information provided herein proves useful to general ant systematics and taxonomy, and even in clarifying biological aspects and social organization of these ant other ant species in future behavioral studies.

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TABLE 3. Differing morphological traits of larvae from three ant species of Linepithema

	Species		
	Humile group		Iniquum group
Character	*Linepithema micans*	*Linepithema humile*[a]	*Linepithema iniquum*[b]
Types of body hairs	Simple (0.007–0.012 mm)	Simple (0.018–0.038 mm)	Simple (0.008–0.025 mm) Bifid (0.025–0.036 mm)
Number of antennal sensilla	3 (rarely 2 or 4)	3	3
Types of head hairs	Simple (0.005–0.008 mm)	Simple (0.023–0.045 mm)	Simple (0.013–0.025 mm)
Labrum	bilobed, 4 sensilla (rarely 3) on anterior surface, 2 sensilla on ventral border, rows of spinules on posterior surface	bilobed, 4 sensilla on anterior surface, 2 sensilla on ventral border, rows of spinules on posterior surface	bilobed, 2 sensilla on anterior surface, 2 sensilla on ventral border, rows of spinules on posterior surface
Mandible	Two teeth on blade	Two teeth on blade	One tooth on blade
Number of sensilla on maxillary palp	5	5	5
Number of sensilla on galea	2	2	2
Number of sensilla on labial palp	5	5	5

[a]Information on other species obtained from Solis et al., 2010b.
[b]Information on other species obtained from Wheeler and Wheeler, 1973.

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