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EXTERNAL MORPHOLOGY OF THE IMMATURES OF *POLYBIA PAULISTA* (HYMENOPTERA: VESPIDAE)

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ABSTRACT

The immatures of *Polybia paulista* Ihering were described using light and scanning electron microscopy and the results are compared with previous descriptions within the same or related wasps. This study is based on 2 whole nests collected in the municipality of Rio Claro, São Paulo, in Brazil. We have detected the existence of 5 larval instars. The main morphological alterations over development occur in the relative size of structures, yet certain structures appear with subsequent instars and become more evident later in development: increasing density in the number of body spines and papillae; the appearance of body setae in fifth-instar larvae; opening of spiracles upon second-instar larvae; 2 body shapes in fifth-instar larvae; the appearance of a lateral tooth on the mandibles of fourth instar; presence of spines on the maxillae of fifth-instar larvae; altered shape of galea and palps upon third-instar larvae from a cluster of sensilla to a conical elevation; and the appearance of spines on postmentum upon fourth-instar larvae. This way, the present study presents a detailed description of the immatures of *P. paulista*, and we hope the presented information can be useful to morphological, taxonomic, and phylogenetic studies.

Key Words: Larvae, Development, Polistinae, Epiponini, *Myrapetra*

RESUMO

Os imaturos de *Polybia paulista* Ihering foram descritos e comparados, com o auxílio da microscopia óptica e eletrônica de varredura. Foram analisados os imaturos provenientes de dois ninhos obtidos no município de Rio Claro, SP, Brasil. Verificou-se a existência de cinco instares larvais. Durante o desenvolvimento ocorrem principalmente mudanças no tamanho das estruturas. Além disso, certas estruturas surgem e se tornam evidentes com o desenvolvimento da larva: aumento da densidade de espinhos e papilas no corpo; presença de setas no corpo da larva de último instar; abertura dos espiráculos a partir do segundo instar; dois formatos de corpo na larva de último instar; a partir do quarto instar, a presença de dente lateral na mandíbula; presença de espinhos na maxila do último instar; mudança do formato da galea e palpos a partir do terceiro instar, de um agrupamento de sensilas para um cone; e o surgimento de espinhos na gula a partir do quarto instar. Desta maneira, o presente estudo efetuou uma caracterização detalhada dos imaturos de *P. paulista*, cujas informações poderão ser talvez úteis para estudos morfológicos, taxonômicos e filogenéticos.

The social wasps of the worldwide-distributed subfamily Polistinae are represented by about 958 species within 26 genera, most being native to the tropics (Pickett & Carpenter 2010); social wasps are quite common in Brazil, wherein 304 species in 22 genera are found (Carpenter & Marques 2001). The genus *Polybia* Lepeletier includes 60 species (Johnson 2012) of Neotropical

distribution, occurring from México to Argentina; *Polybia* wasps build a paper-like nest made of combs of superposed enveloped layers (Carpenter et al. 2000). The species *Polybia paulista* Ihering is a common wasp in Brazil, being found in the states of São Paulo, Goiás, Mato Grosso, Paraná, and Minas Gerais, also present in Paraguay and Argentina (Richards 1978), wherein they are re-

TABLE 1. SIZES OF STRUCTURES (MM) IN LARVAE OF *POLYBIA PAULISTA*.

Structure ^a	Larval Instar				
	1st	2nd	3rd	4th	5th
BL ¹	0.57-1.52 a (n = 22)	0.86-2.38 a (n = 61)	1.43-3.24 b (n = 71)	2.19-5.05 c (n = 122)	2.67-10.09 d (n = 431)
BW ¹	0.48-0.76 a (n = 22)	0.57-1.33 b (n = 61)	0.76-1.81 c (n = 71)	1.52-2.57 d (n = 122)	1.90-3.43 e (n = 431)
Ds ¹		0.010-0.025 a (n = 100)	0.020-0.040 b (n = 100)	0.025-0.055 c (n = 100)	0.050-0.095 d (n = 100)
R ²		1.41-2.00	1.37-1.76	1.34-1.59	1.16-1.37
LBH					0.008-0.047 (n = 8)
HW	0.44 (n = 22)	0.59-0.60 (n = 61)	0.85 (n = 71)	1.20 (n = 122)	1.60-1.65 (n = 431)
LR	0.171-0.185 (n = 3)	0.218-0.315 (n = 5)	0.335-0.400 (n = 10)	0.380-0.530 (n = 7)	0.550-0.700 (n = 10)
WR	0.090-0.105 (n = 3)	0.120-0.153 (n = 5)	0.143-0.190 (n = 10)	0.175-0.250 (n = 7)	0.200-0.310 (n = 10)
LM	0.057-0.080 (n = 6)	0.070-0.105 (n = 7)	0.155-0.195 (n = 10)	0.240-0.320 (n = 10)	0.380-0.430 (n = 10)
WM	0.069-0.095 (n = 6)	0.108-0.140 (n = 7)	0.130-0.200 (n = 10)	0.200-0.240 (n = 10)	0.210-0.270 (n = 10)
Lm		0.255-0.300 (n = 6)	0.240-0.300 (n = 10)	0.360-0.490 (n = 10)	0.450-0.610 (n = 10)
HG			0.025-0.040 (n = 8)	0.035-0.055 (n = 9)	0.060-0.085 (n = 10)
HPm			0.015-0.025 (n = 8)	0.030-0.040 (n = 9)	0.045-0.065 (n = 10)
LA	0.117-0.153 (n = 3)	0.132-0.156 (n = 4)	0.160-0.207 (n = 6)	0.230-0.270 (n = 4)	0.330-0.420 (n = 10)
WA	0.081-0.105 (n = 3)	0.089-0.130 (n = 4)	0.141-0.200 (n = 6)	0.151-0.280 (n = 4)	0.180-0.300 (n = 10)
HPa			0.015-0.025 (n = 8)	0.015-0.025 (n = 10)	0.030-0.045 (n = 10)
LMH					
	S	0.004-0.006 (n = 4)	0.005-0.012 (n = 12)	0.007-0.020 (n = 30)	0.006-0.037 (n = 30)
	B			0.009-0.017 (n = 4)	0.010-0.025 (n = 14)
	R				0.027 (n = 1)

^aStructure abbreviations are as follows: (BL) body length; (BW) medial width of body; (d) diam; (Ds) diam of spiracles; (HG) height of galea; (HPa) height of labial palp; (HPm) height of maxillary palp; (HW) head width; (l) length; (LA) length of prementum; (LBH) length of simple setae on body; (LMH-S) length of simple setae on mouthparts; (LMH-B) length of bifid setae on mouthparts; (LMH-R) length of 3-branched setae on mouthparts; (LM) length of mandible; (Lm) length of maxilla; (LR) length of labrum; (n) number of observations; (R) minimum and maximum values of product of the division of the greatest spiracle diam by the diam of other spiracles; (WA) medial width of prementum; (WM) width of mandible base; (WR) medial width of labrum.

¹Values followed by the different letter within a row are statistically different by Tukey's test ($P < 0.01$).
²These values do not represent a variable, as they were obtained by the dividing the diam of the largest spiracle by the diam of the remaining spiracles.

currently found in urbanized areas, often causing accidents when aggressively defending their nests upon any disturbance.

Carpenter et al. (2000) made a cladistic analysis of the subgenera of *Polybia* including also other wasp genera presenting similar nest architecture (termed phragmocytarus) based on morphological characters from the adults, larvae and nests. These authors remarked that larval characters are useful for phylogenetic studies with social wasps. Nelson (1982), in a study with

Polistes, also highlighted the importance of larval characters in phylogenetic studies. Kojima (1998) mentioned that existing larval descriptions with Polistinae and Vespinae are superficial, thus hardly provide any information useful to cladistic studies. In an attempt to remedy this issue, Kojima (1998) presents detailed descriptions of last-instar larvae of 146 species from 24 genera of Polistinae, and 14 species from 4 genera of Vespinae. Among these, 17 species of *Polybia* were included.

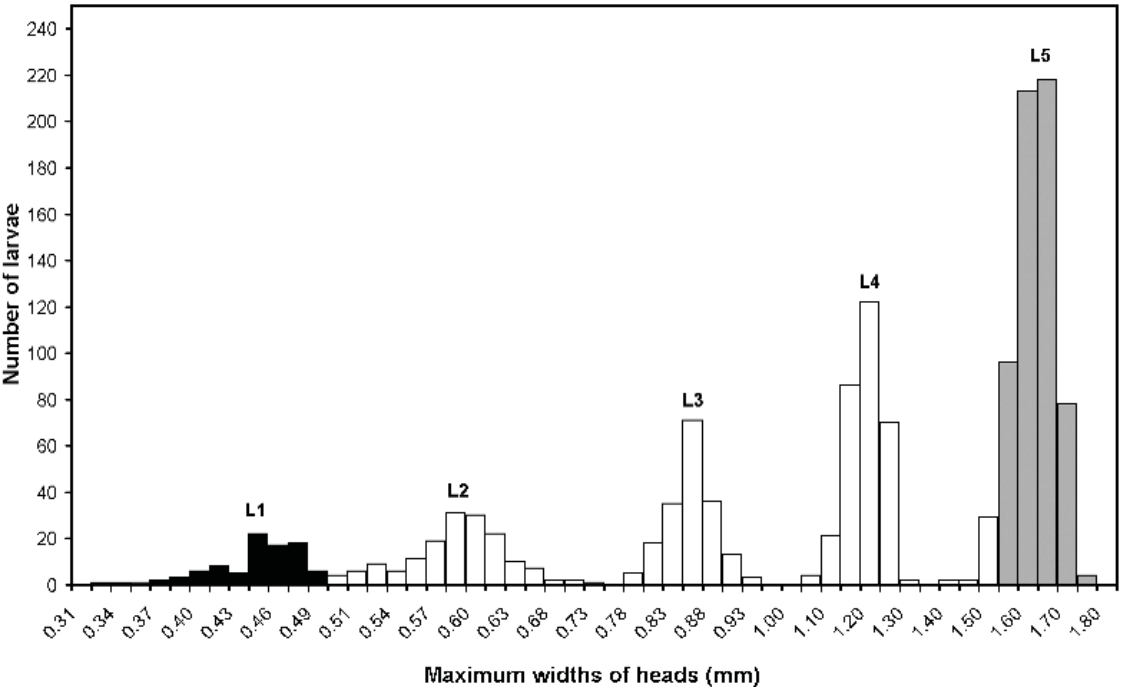


Fig. 1. Frequency distribution of the maximum widths of heads of larvae of *Polybia paulista*: (L1) first instar, (L2) second instar, (L3) third instar, (L4) fourth instar, and (L5) fifth instar. Black columns represent the size intervals of head widths of mature embryos inside the egg. Gray columns represent the intervals in which widths of heads in the prepupae were measured.

Most larval descriptions of social wasp larvae were limited to last-instar larvae and to determining the number of larval instars (Giannotti & Silva 1993, Giannotti 1995). Although there are several studies with *P. paulista*, the mature larva of this species was only superficially described by Dias Filho (1975), and the number of larval instars was originally determined by Carvalho & Silva (1975) and Machado (1983). In this context, the present study aims at gathering additional information about the external morphology of *P. paulista*, thus assessing prospective and proposed characters for morphological, taxonomic, and phylogenetic studies. We herein estimate again the number of larval instars, and describe each development stage (egg, larvae, and pupae)

by means of light and scanning electron microscopy, and compare our finds with published studies with the same and related species.

MATERIALS AND METHODS

Collection of Samples

Two nests of *P. paulista* were collected in the municipality of Rio Claro (22°23'44.09"S, 47°32'39.98"W), São Paulo state, Brazil. All collected samples were fixed and conserved in 70% ethanol. Voucher specimens of eggs, larvae, and pupae were deposited in the "Adolph Hempel" entomological collection of Centro de Pesquisa e De-

TABLE 2. TYPES OF SETAE ON *POLYBIA PAULISTA* LARVAE.

Type of seta	Characteristics	Location/Larval instar		
		Body	Cranium	Mouthparts
Simple	Unbranched smooth seta (Fig. 3B)	5th	Absent	2nd, 3rd, 4th, 5th
Bifid	Smooth seta with tip bifid (Fig. 5H)	Absent	Absent	5th
	Moderately bifid smooth seta	Absent	Absent	4th, 5th
3-branched	Moderately bifid smooth seta, with another ramification at a lower length	Absent	Absent	5th

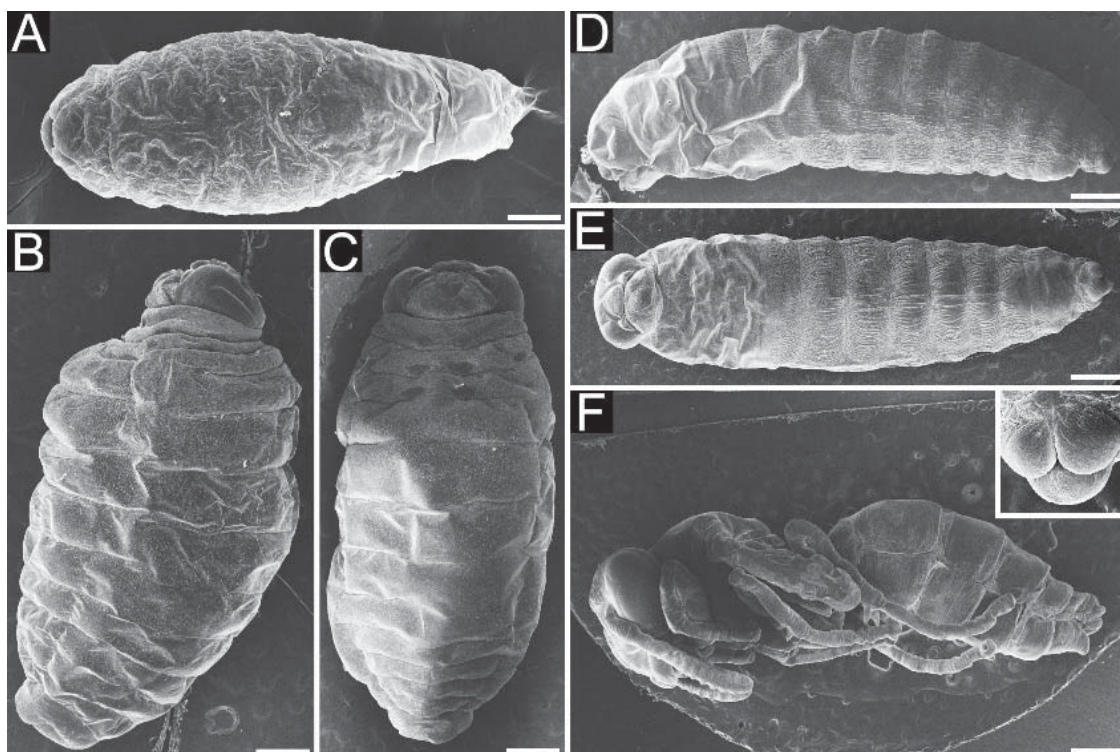


Fig. 2. Immature stages of *Polybia paulista*: (A) ventral view of a first instar larva; (B) side view of a fifth instar larva; (C) ventral view of a fifth instar larva; (D) side view of prepupa; (E) ventral view of prepupa, showing in the inset the last abdominal somite; (F) side view of pupa. Sizes of scale bars: (A) 0.185 mm; (B) 0.575 mm; (C) 0.650 mm; (D) 0.925 mm; (E) 0.925 mm; (F) 1.200 mm.

senvolvimento de Sanidade Vegetal of Instituto Biológico, São Paulo, Brazil.

Immature Descriptions

The number of larval instars of *P. paulista* was determined based on maximum head widths following the methods described in Parra & Haddad (1989) based on a sample of 1,378 larvae. Other measures presented were taken from 45 eggs, 707 larvae, and 75 pupae. Terminology used in the description was based on Kojima (1998). Details on sample preparation and the setting of microscopes were described in Solis et al. (2010).

The following abbreviations are employed: (BL) body length; (BW) medial width of body; (d) diam; (Ds) diam of spiracles; (HG) height of galea; (HPa) height of labial palp; (HPm) height of maxillary palp; (HW) head width; (l) length; (LA) length of prementum; (LBH) length of simple setae on body; (LMH-S) length of simple setae on mouthparts; (LMH-B) length of bifid setae on mouthparts; (LMH-R) length of 3-branched setae on mouthparts; (LM) length of mandible; (Lm) length of maxilla; (LR) length of labrum; (n) number of observations; (R) minimum and maximum values of product of the division of the greatest

spiracle diam by the diam of other spiracles; (WA) medial width of prementum; (WM) width of mandible base; (WR) medial width of labrum.

Statistical Analysis

All measures are presented with minimum and maximum values. In Table 1, measures are given in millimeter. When compared, measures from different larval instars (see Table 1) were subjected to analysis of variance (One-way ANOVA) followed by Tukey's test.

RESULTS

Determination of the Number of Larval Instars

The obtained frequency distribution of head capsule widths resulted in a multimodal distribution with 5 distinct peaks, suggesting the existence of 5 instars. The first peak includes width values for first-instar larvae found inside the egg chorion and the fifth peak includes width values of prepupae (Fig. 1). Intervals of head widths for each larval instar head width: first instar (0.32-0.49 mm; n = 90), second instar (0.51-0.73 mm; n = 156), third instar (0.78-0.93 mm; n = 181), fourth instar (1.05-1.30 mm; n = 305), and fifth in-

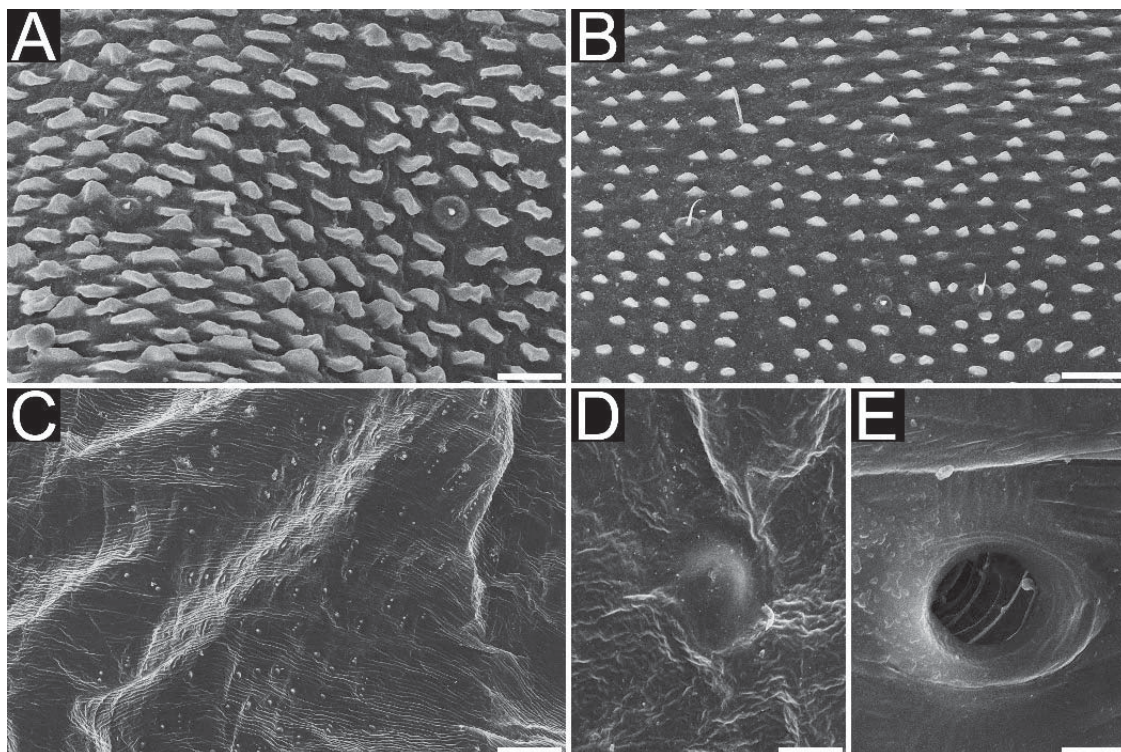


Fig. 3. Details of the body surface of *Polybia paulista* larvae: (A) thorax region of a fifth instar; (B) abdominal region of a fifth instar; (C) abdominal region of a third instar; (D) abdominal region of a first instar, displaying a closed spiracle; (E) abdominal spiracle of a fifth instar. Sizes of scale bars: (A) 0.016 mm; (B) 0.028 mm; (C) 0.020 mm; (D) 0.008 mm; (E) 0.010 mm.

star (1.40-1.75 mm; $n = 642$). The obtained number of larval instars yielded a good fit with Dyar's rule ($R^2 = 0.99$).

The mean growth rate between the larval instars was 1.39, while between the first and the second instars it was 1.34, between the second and third instars it was 1.44, between the third and fourth instars it was 1.40, and between the fourth and fifth instars it was 1.36.

Morphological Description of the Immature Forms

Egg. Elliptical in shape and white, found attached to the nest cell wall. Length and medial width ($n = 45$) respectively 0.93-1.23 mm and 0.31-0.49 mm, the ratio being 2.73.

Larvae. Obtained bodily measures from all instars are presented in Table 1. Body: 13-segmented, including 3 thoracic and 10 abdominal segments; segmentation upon first- and second-instar inconspicuous. Body shape short, robust, and slightly curved, with greatest diam upon 4 first abdominal segments, and the following segments gradually reducing in diam (Figs. 2A, 2B, 2C). Anus ventral. Fifth-instar present 2 different body shapes: the one described in the last sentence and an altered shape upon later develop-

ment (i.e. prepupa; Figs. 2D, 2E). The prepupa is more elongate, and upon the last abdominal somite presents a round protuberance with 3 lobes (Fig. 2E); during this stage, the nest cell is closed with silk produced by the larva. Body of larvae always grayish white, while prepupae are strongly white. The body tegument of all larval instars is covered with abundant spines and papillae, which increase in density on every subsequent instar (Figs. 3A, 3B, 3C), excepting on the last abdominal segments, where actually they reduce in density. Body setae are few and found only in fifth-instar (Table 2). Vestigial legs (3 pairs) are markedly evident on the ventral thoracic region of fifth-instar (Fig. 2C). There are 10 pairs of spiracles (2 thoracic and 8 abdominal, see Fig. 3E) distributed along a line at mid-width of body as seen from side view, placed near the intersection of segments. Body spiracles are closed in first-instar (Fig. 3D). First pair of spiracles always larger than the others. Cranium: Subelliptical in shape (Fig. 4A), horizontal diam larger, and sitting terminally on the anterior body extremity. Color tends to change from white to a yellowish white tonality over development. Parietal bands evident (Figs. 4A, 4B, 4C). Absence of setae, spines or papillae on the cranium. Antennal orbits circu-

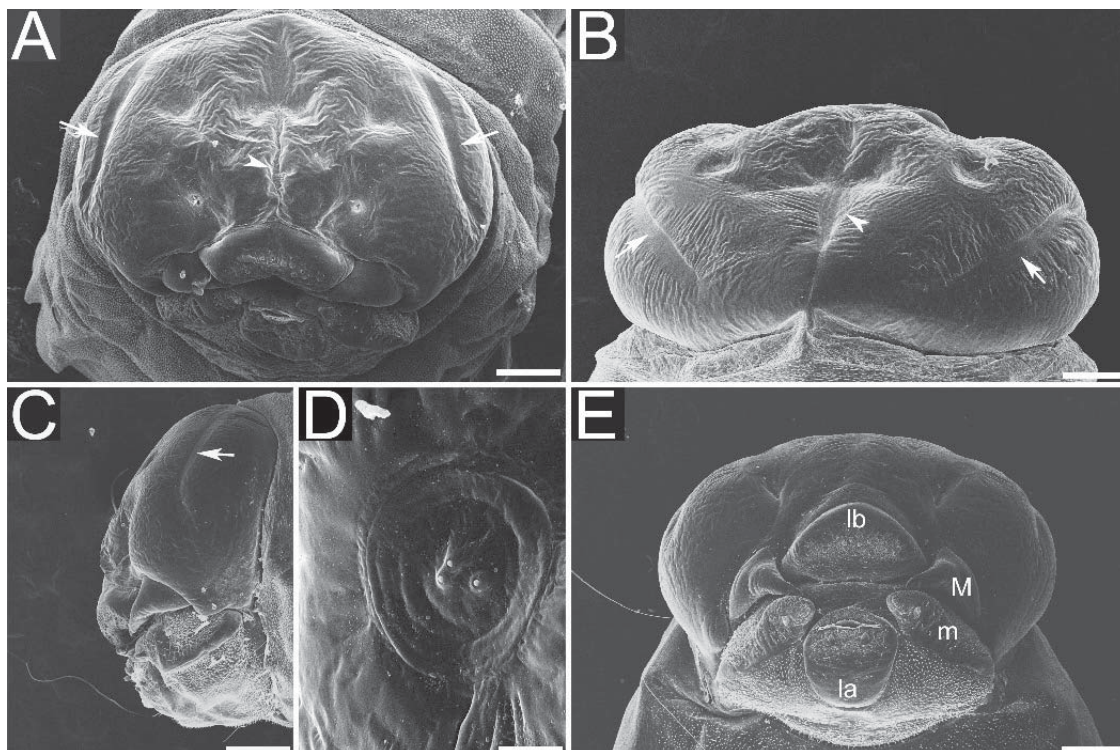


Fig. 4. Fifth instar larva of *Polybia paulista*, showing: (A) full frontal view of head; (B) dorsal view of head; (C) side view of head; (D) antenna; (E) mouthparts in prepupa. Abbreviations: prementum (la), labrum (lb), mandible (M), maxilla (m), ecdysial sulcus (white arrowhead), parietal band (white arrow). Sizes of scale bars: (A) 0.240 mm; (B) 0.178 mm; (C) 0.143 mm; (D) 0.028 mm; (E) 0.227 mm.

lar and concave (fifth-instar, d: 0.12-0.15 mm, n = 10) with 3 basiconic sensilla (Fig. 4D), which may or may not be arranged in line. In fifth-instar, the distance from the lower antennal orbit border to the anterior tentorial pit ranged 0.11-0.13 mm (n = 4), while in prepupae it was 0.22 mm (n = 1). Clypeus poorly delimited. Mouthparts (Fig. 4E): Labrum in anterior view trapezoid-shaped (Fig. 5A), with anterior surface smooth. In ventral view, the labrum presented 2 different shapes depending on larval instar: first- to third-instar and prepupae presented elliptical profiles (Fig. 4E), while fourth- and fifth-instar had strongly emarginate labrum profile (Fig. 5B). Ventral surface of labrum rough, with papillae (only in fifth-instar), 18-22 basiconic sensilla on conical protuberances (all instars) and 7-15 setae (all except first-instar). The palate of fifth-instar is slightly rough, with 6 basiconic sensilla disposed in 2 groups of 3 on the sides. Mandible always triangular; in first- and second-instar (Fig. 5C), mandibles are short and robust, wider at base than long, with apex rounded, and there is no lateral tooth. In third-instar mandibles are about as wide as long. In fourth and fifth-instar (Fig. 5D), mandibles are more elongate, with base width being less than

total length, and there is a lateral tooth near its pointed apex. Anterior face of mandibles presenting some papillae. Maxilla elongate, with setae on the anterior surface (absent in first-instar; at the number of 5-7 setae in second- and third-instar; 11-15 in fourth- and fifth-instar) which are concentrated on the distal half (near the apex). The maxilla of fifth-instar presents spines on the dorsal anterior region (Fig. 5E) covering the first proximal half (near its base). Galea cone-shaped with surface rough, being bifid at apex with each extremity bearing a basiconic sensillum (Fig. 5E). Maxillary palps cone-shaped, with surface smooth and bearing 2 basiconic sensilla and one encapsulated sensillum on top (Fig. 5F). In first- and second-instar, the galea and maxillary palps are simple aggregates of sensilla, with the maxillary palp of first-instar having only 2 basiconic sensilla. Prementum subcircular in shape (Fig. 5G), bearing papillae on the upper half of the anterior surface, above and below the opening of spinneret; on the lower half of prementum, surface is smooth. Presence of setae on the anterior surface: 13-14 setae in third-instar; 19-22 setae in fourth-instar; 24-33 setae in fifth-instar. In first- and second-instar, instead of setae, there

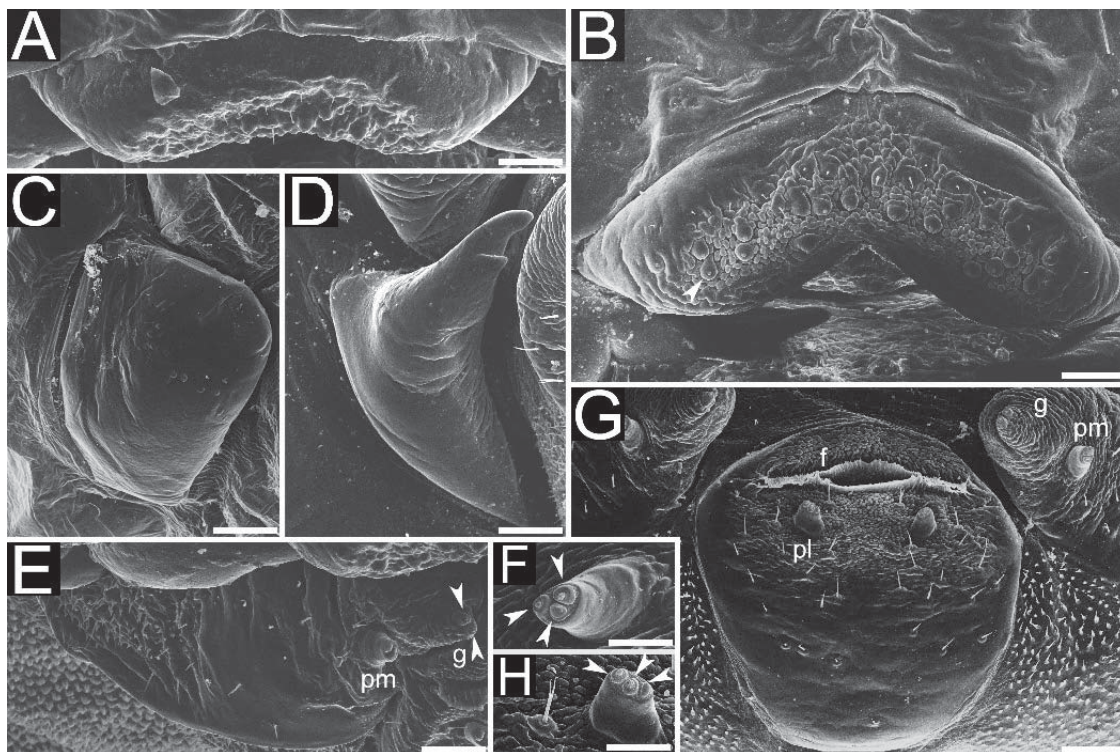


Fig. 5. Larval mouthparts in *Polybia paulista*, showing: (A) anterior region of labrum of fifth instar; (B) ventral region of labrum of fifth instar; (C) mandibles of second instar; (D) mandible of fifth instar; (E) maxilla of fifth instar; (F) maxillary palp of fifth instar; (G) prementum of prepupa; (H) labial palp of fifth instar. Abbreviations: opening of spinneret (f), galea (g), maxillary palp (pm), labial palp (pl), sensilla (white arrowheads). Sizes of scale bars: (A) 0.067 mm; (B) 0.060 mm; (C) 0.022 mm; (D) 0.058 mm; (E) 0.055 mm; (F) 0.024 mm; (G) 0.068 mm; (H) 0.028 mm.

are isolated spines on the prementum; first-instar has no papillae. Labial palps cone-like, with surface smooth and topped with 2 basiconic sensilla and one encapsulated sensillum (Fig. 5H). However, the number and type of sensilla varies: 3 basiconic sensilla or 3 basiconic sensilla with one encapsulated sensillum in second-instar; one basiconic sensillum and one encapsulated sensillum in fifth-instar. In first- and second-instar the labial palp is a group of sensilla. First-instar has no visible opening of spinneret, which are quite evident in fifth-instar (l: 0.200-0.236 mm, $n = 8$), in which they are fringed with an arrangement of fused spines (Fig. 5G). The postmentum of first- to third-instar is smooth, while fourth-instar (l: 0.60-0.62 mm, $n = 3$) have a few spines, and fifth-instar (l: 0.92-1.05 mm, $n = 3$) have it densely covered with spines, excepting the proximal ventral region on the prementum (Fig. 5G).

Pupa (Fig. 2F). Exarate, white but becoming darker over development, until transforming into black imagoes. Body length and head width of white pupae ($n = 75$) were respectively: 8.13-11.13 mm and 2.25-2.50 mm.

DISCUSSION

Determination of the Number of Larval Instars

In the subfamilies Polistinae and Vespinae, the registered number of larval instars is 5, while in Stenogastrinae the number varies between 3 and 5 instars (Kojima 1998). Carvalho & Silva (1975) and Machado (1983) found 5 larval instars in *P. paulista*, which was confirmed by our present observations. Interestingly, from comparing the mean head widths obtained in this study with the results of Carvalho & Silva (1975), we noted that the size intervals between instars are not the same. Size values obtained by Carvalho & Silva (1975) were larger: first instar (0.360-0.612 mm), second instar (0.648-0.972 mm), third instar (1.008-1.332 mm), fourth instar (1.368-1.836 mm), and fifth instar (1.872-2.340 mm). Consequently, the values of head widths of pupae in their study were also larger than our records. Parra & Haddad (1989) mentioned that the number of larval instars in

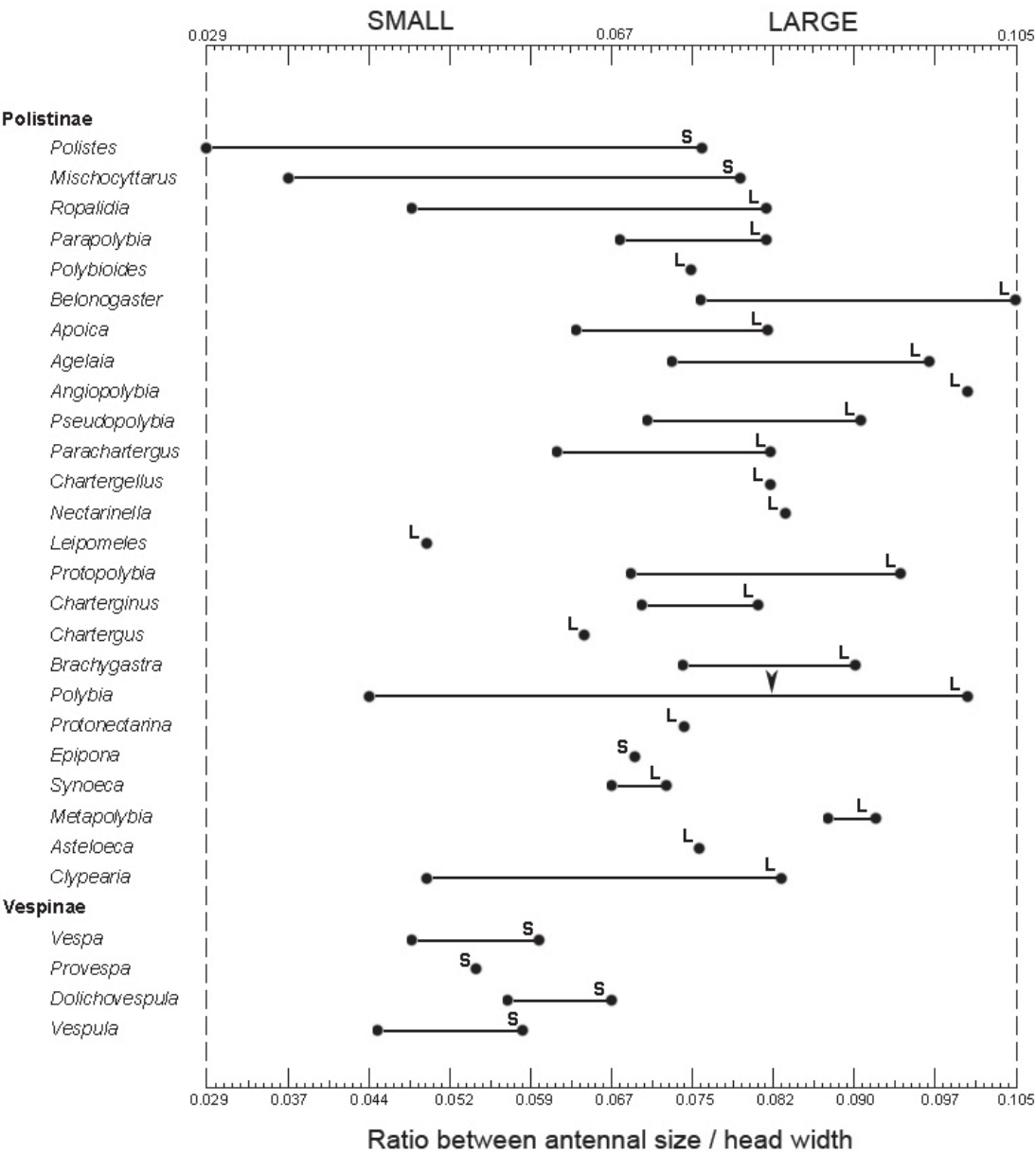


Fig. 6. Comparison of size ranges of antennal orbit size between *Polybia paulista* and among different social wasp genera analyzed by Kojima (1998). The ratios between antennal size and head widths were calculated based on images given in Dias Filho (1975) and Kojima (1998). As in Kojima (1998) did not declare any absolute or relative values for determining the character state in character 7, we decided employing the relative ratio to head width. Abbreviations of antennal size as defined by Kojima (1998): (S) for small, and (L) for large; the value given for *P. paulista* is an average figure, indicated with an arrowhead on the value column of *Polybia*.

a given species can vary, being dependent on biotic and abiotic factors such as hereditary traits, rearing methods, ambient temperature, and nutrition. It is very likely that such uncontrolled factors are responsible for the size differences observed, although the estimated final number of larval instars was not affected.

Morphological Description of the Immature Forms

Kojima (1998) based on 28 genera of Polistinae and Vespinae proposed 43 morphological characters of mature larvae for use in phylogenetic analyses. The morphological characters described by Kojima (1998) for *Polybia* were usually con-

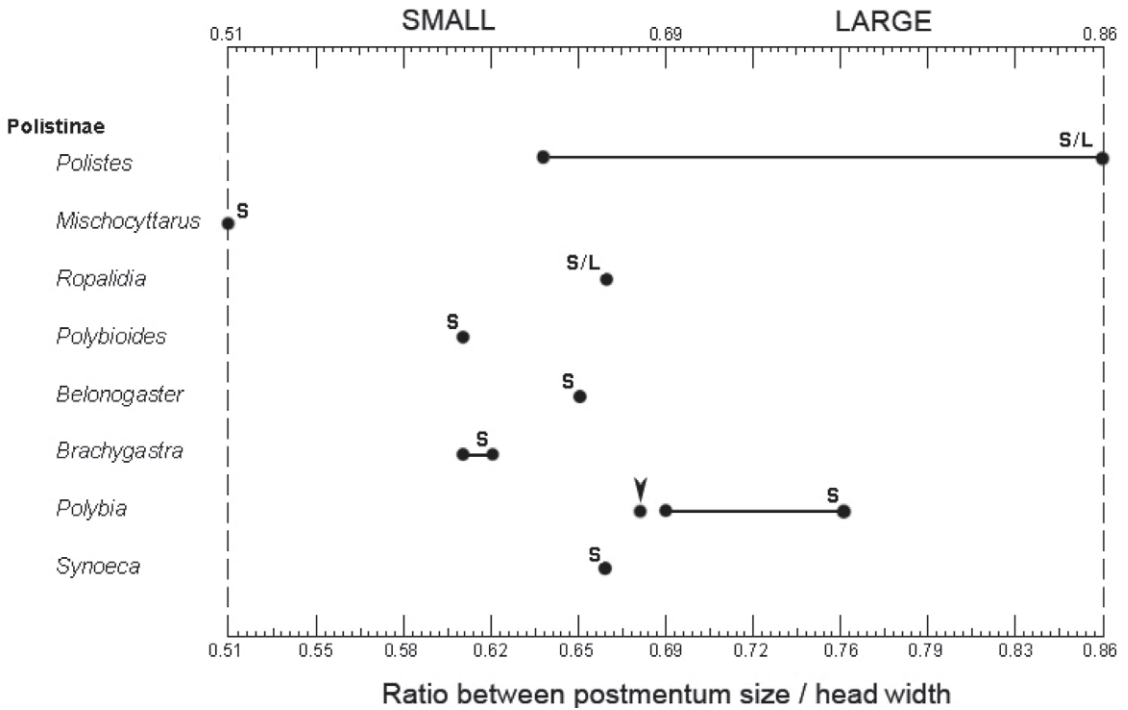


Fig. 7. Comparison size ranges of postmentum size between *Polybia paulista* and among different social wasp genera analyzed by Kojima (1998). The ratios between postmentum size and head widths were calculated based on images given in Kojima (1998). As in Kojima (1998) did not declare any absolute or relative values for determining the character state in character 33, we decided employing the relative ratio to head width. Abbreviations of postmentum size as defined by Kojima (1998): (S) for small, and (L) for large; the value given for *P. paulista* is an average figure, indicated with an arrowhead beside the *Polybia* column.

firmed by our observations with *P. paulista*. We found however some difficulties when applying a few of these suggested characters with *P. paulista*. Regarding the size of antenna (character 7), Kojima (1998) proposed 2 character states: small and large, with *Polybia* being presenting large antennae. The measures obtained in the present study with *P. paulista* fit in the interval estimated for the genus [0.107-0.208 mm, n = 10; obtained from images presented in Kojima (1998)]. However from analyzing the results with other genera (Fig. 6), we can see that some (e.g. *Mischocyttarus* and *Ropalidia*) received the character state of small or large antennae while presenting a broad variation between minimum and maximum size values, suggesting that both character states are present. In one instance, *Epipona* and *Synoeca*, 2 genera with the same proportional antennal size received different character states. We think such incongruent results with character 7 could be circumvented by adopting a clear-cut proportion to define relative size of antenna, as was done by Kojima (1998) with character 35, relative size of first spiracle. Based on Figure 6 and analysis of Kojima (1998), we would tentatively suggest that the value of 0.067 [ratio between of antennal orbit

/ head width; this limit value was set from analyzing 27 species illustrations from Dias Filho (1975) and 116 species from Kojima (1998)] be adopted as the borderline to separate relative antennal size, with the result that some genera (e.g. *Polybia*) would present both character states. Regarding the size of the postmentum (character 33), 3 character states were proposed by Kojima (1998): small, large, and large ventrally emarginate; in most genera the postmentum was described as small, including *Polybia*. The measures obtained in the present study with *P. paulista* fit in the interval estimated for the genus [0.96-1.48 mm, n = 4; values estimated from the illustrations in Kojima (1998)]. As with character 7, the value of 0.69 [ratio between size of postmentum and head; estimated based on 20 species illustrations analyzed in Kojima (1998)] could be used as a limit value between the character states small and large (Fig. 7). In *Polybia* both character states can be found. Further evaluations with other species might validate the proposed limit values, or even invalidate the use of these 2 characters because of substantial overlap between species.

Carpenter et al. (2000) employed 21 larval characters obtained from Kojima (1998) in their cladis-

tics analysis in differentiating between subgenera of *Polybia*. When analyzing their attributed states to the subgenus *Myrapetra*, within which *P. paulista* is included, we confirmed all characters. However, in cases in which more than one state is found in *Myrapetra*, only one character state was observed in *P. paulista*, namely: hypostomal ridge nearly straight (character 25), labrum almost as wide as clypeus (character 29), absence of spicules on palate (character 31) and mandibular corium (character 33), apex of maxillary palp flat (character 38), prementum subcircular (character 39), presence of spicules on the side and ventral regions of the postmentum (character 41).

From comparing our present finds with a previous description by Dias Filho (1975), we conclude that the author analyzed fifth instar larvae and we confirm all of his observations regarding: the shape of body, cranium, and mouthparts; and measures of body length and head width. However, few details on these structures were not provided by the author, such as distribution of spinules and setae, and number of sensilla on antennae and palps.

Moreover, we verified most modifications over development in larvae of *P. paulista* were an increment in size, and with the appearance and development of certain structures upon every subsequent instar, including: increase in the density of body spines and papillae; presence of body setae on fifth-instar larvae; the opening of body spiracles in second instar larvae; 2 types of body shape in fifth-instar larvae; the presence of a lateral tooth in the mandibles of fourth- and fifth-instar larvae; presence of spines on the maxilla of fifth-instar larvae; alterations in the shape of galea and palps upon third-instar larvae from a cluster of sensilla to a conical elevation; and the appearance of spines on the postmentum upon fourth-instar larvae. In other Vespidae species analyzed, besides the increment in general structural size there are other recorded morphological alterations over larval development, such as: appearance of ventral lobes on the first abdominal somite on third-instar larvae of *Mischocyttarus cassununga* (Ihering) (Giannotti & Silva 1993), and the development of a subapical tooth on second-instar larvae of *Polistes lanio* (Fabricius) (Giannotti 1995). By employing scanning electron microscopy and by minutely inspecting all development stages, further structural alterations are expected to be recorded.

Finally, the present study confirmed the existence of 5 larval instars in *P. paulista*, and described all external structural changes occurring over immature development. From comparing our finds with previous published information by Kojima (1998), we were able to confirm most of the proposed characters, yet we could not properly apply some of them: size of antenna and postmentum. We hope that the present description will be

followed by other detailed descriptions with larvae of social wasps, emphasizing on alterations that occur in immature development, which are information invaluable to understanding the biology and morphology of these species.

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REFERENCES CITED

- CARPENTER, J. M., KOJIMA, J., AND WENZEL, J. W. 2000. *Polybia*, Paraphyly, and Polistine Phylogeny. *Am. Mus. Novit.* 3298: 1-24.
- CARPENTER, J. M., AND MARQUES, O. M. 2001. Contribuição ao estudo dos vespídeos do Brasil (Insecta, Hymenoptera, Vespoidea, Vespidae). *Cruz das Almas: Universidade Federal da Bahia.*
- CARVALHO, G. G., AND SILVA, M. T. 1975. Alguns aspectos do desenvolvimento larval de *Polybia paulista* Richards (Hymenoptera - Vespidae). *Stud. Entomol.* 18: 555-568.
- DIAS FILHO, M. M. 1975. Contribuição à morfologia de larvas de vespídeos sociais do Brasil (Hymenoptera, Vespidae). *Rev. Brasileira Entomol.* 19: 1-36.
- GIANNOTTI, E. 1995. Immature stages of *Polistes lanio* (Fabricius, 1775) (Hymenoptera, Vespidae). *Rev. Brasileira Biol.* 55: 527-531.
- GIANNOTTI, E., AND SILVA, C. V. 1993. *Mischocyttarus cassununga* (Hymenoptera, Vespidae): external morphology of the brood during the post-embryonic development. *Rev. Brasileira Entomol.* 37: 309-312.
- JOHNSON, N. F. 2012. Hymenoptera Online Database. Available from: <http://hol.osu.edu> (accessed 07/22/2012)
- KOJIMA, J. 1998. Larvae of social wasps (Insecta: Hymenoptera; Vespidae). *Nat. Hist. Bull. Ibaraki Univ.* 2: 7-227.
- MACHADO, V. L. L. 1983. Análises morfométricas em colônias de *Polybia (Myrapetra) paulista* (Ihering, 1896) (Hymenoptera, Vespidae). *Naturalia* 8: 219-226.
- NELSON, J. M. 1982. External morphology of *Polistes* (Paper wasp) larvae in the United States. *Melandria* 38: 1-29.
- PARRA, J. R. P., AND HADDAD, M. L. 1989. Determinação do número de instares de insetos. Piracicaba: FEALQ.
- PICKETT, K. M., AND CARPENTER, J. M. 2010. Simultaneous analysis and the origin of eusociality in the Vespidae (Insecta: Hymenoptera). *Arthropod Syst. Phyl.* 68: 3-33.
- RICHARDS, O. W. 1978. The social wasps of the Americas, excluding the Vespinae. London: British Museum (Natural History).
- SOLIS, D. R., FOX, E. G. P., ROSSI, M. L., MORETTI, T. C., AND BUENO, O. C. 2010. Description of the immatures of workers of the ant *Camponotus vittatus* (Hymenoptera: Formicidae). *Florida Entomol.* 93: 265-276.