External Morphology of Immatures during the Post-embryonic Development of *Mischocyttarus nomurae* Richards (Hymenoptera: Vespidae)

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**Abstract**

We describe here the immatures of *Mischocyttarus nomurae* Richards from twenty colonies collected in the municipality of Rio de Contas in the Chapada Diamantina, Bahia State, Brazil. We determined the number of larval instars in post-embryonic development and measured 145 eggs, 353 larvae, 12 pre-pupae, and 59 pupae. Egg lengths varied from 0.80 to 1.33 mm and their widths from 0.33 to 0.50 mm. The average rate of growth of the larvae was 1.48. Cephalic capsule widths varied from 0.20 – 0.43 mm in the 1st instar larvae, from 0.47 – 0.63 mm in the 2nd instar, 0.67 – 0.83 mm in the 3rd instar, 0.87 – 1.20 mm in the 4th instar, and 1.37 – 1.73 mm in the 5th instar larvae. The cephalic capsule of the 1st instar larva was smaller than the width of the egg. The median widths of the 5th instar larvae and the pre-pupae cephalic capsules did not differ significantly. The species has two abdominal lobes that are highly projected forward, and the diameter of first thoracic spiracle is 3.1 times greater than the second. *M. nomurae* shows development patterns typical of the genus.

**Introduction**

The species *Mischocyttarus nomurae* was described by Richards in 1978 from two females collected in Fortaleza, Ceará State (CE), Brazil; it was included in the *Mischocyttarus cerberus* group in a review published by Silveira (2004). The individuals are black, with many pale-yellowish gaster markings, with distinct stripes and frontal margin of the proepisternum distinctly elevated (Richards, 1978). The males of that species were described by Silveira (2004) from samples collected in Lençóis (Chapada Diamantina), Bahia State (BA), Brazil. Among other features, the clypeus is covered by dense recumbent hairs with silvery reflections; the antenna is spirally rolled at the apex, with the last three segments elongated. Souza et al. (2015) recently recorded the occurrence of specimens of *M. nomurae* in Montes Claros, Minas Gerais State (MG), Brazil, captured in traps using passion fruit bait. These are the only references to the species in the literature.

Richards (1978) characterized the larvae of *Mischocyttarus* de Saussure representatives as having the first abdominal sternite with one, two or three lobes strongly projected, first thoracic spiracle twice as long as the others, and mandibles with only a single elongated tooth. Dias-Filho (1975) studied the 5th instar larvae of several wasp genera and characterized six species of the genus as having the labrum twice as wide as the mentum, tentorial pits located below the level of the antennae, and mandibles ending in a fine, single, curved tooth. Most of the species studied showed larvae with five instars (Giannotti & Fieri 1991; Giannotti & Trevisoli 1993; Cecílio et al., 2015) and two showed four instars (Raposo-Filho, 1981; Silva, 1984).

We describe here the external morphologies of the eggs and immatures of *Mischocyttarus nomurae* Richards during their post-embryonic development, and determine the number of larval instars of that species.
Material and Methods

The present study was conducted near the town of Rio de Contas in the Chapada Diamantina ecoregion, in south-central Bahia State, Brazil (SEI, 2011). The municipality of Rio de Contas covers 1082 km² (13°34′44″S x 41°48′41″W) and is located 612 km from the state capital of Salvador (IBGE, 2015). The regional climate is sub-humid to dry, with a mean annual temperature of 19.1 ºC and mean annual rainfall rate of 813.2 mm (SEI, 2011).

Twenty colonies were collected in February/2013 without adults, and were subsequently maintained in plastic flasks. Following their sacrifice at low temperatures, the immatures were removed from the nest cells (using tweezers and a desk magnifying glass) and then fixed in Dietrich solution for 48h and preserved in 70% alcohol.

Eggs, larvae, pre-pupae and pupae were measured under a stereoscopic microscope, with the aid of an ocular micrometer, in the Zoology Laboratory of the Federal University of Bahia, Campus Anísio Teixeira (IMS-CAT-UFBA). The following aspects were examined: 1) the largest widths and lengths of the eggs; 2) the greatest widths of cephalic capsules of the larvae, pre-pupae and pupae; and 3) the diameters of the first two spiracles of the last instar larvae (Giannotti & Silva, 1993; Giannotti, 1995; 1998; Prezoto & Gobbi, 2005).

Photographic records of immatures and the cephalic capsules were made using a digital camera coupled to a stereoscopic microscope in the Zoology Department of the Júlio de Mesquita Filho State University of São Paulo (UNESP-Rio Claro).

Scanning electron microscopy of prepared samples of larvae of 5th instar was undertaken in December/2014 at the Gonçalo Moniz Research Center of the Oswaldo Cruz Research Foundation (FIOCRUZ) in Salvador, Bahia (BA), Brazil. The samples were prepared according to standard protocols used at that research center: five colony samples were fixed in a 2% glutaraldehyde solution in 0.1 M sodium cacodylate buffer (pH 7.4) for 2h at room temperature, and subsequently rinsed in 0.1 M sodium cacodylate buffer (pH 7.4) (3 rinses of 5 minutes each, at room temperature). The samples were then post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1h at room temperature, and subsequently rinsed again with 0.1 M sodium cacodylate buffer (pH 7.4) (3 rinses of 10 minutes each, at room temperature). The samples were then dehydrated in an ethanol series (30%, 50%, 70%, 90% and 100% for 40 min. each) and then submitted to critical point drying. After this procedure, the specimens were mounted on stubs, sputter coated with gold, and observed using a JEOL 6390LV scanning electron microscope.

The growth rates of the larvae were calculated (Dyar, 1890), and the non-normally distributed quantitative variables were compared among eight groups using the nonparametric Kruskal-Wallis test, followed by Dwass-Steel-Critchlow-Fligner post hoc test to obtain the adjusted p-values for each pairwise comparison in multiple-group analyses. Normality was assessed by visual inspection of histogram plots and using the Shapiro-Wilk normality test. All statistical analyses were performed with R Statistical Software (R Development Core Team, 2011). A p-value of <0.05 was considered to be statistically significant, and all reported p-values are two-sided.

Results and Discussion

Morphometry and Larval Instars. 145 eggs, 353 larvae, 12 pre-pupae, and 59 pupae from twenty colonies were measured and characterized. The eggs, with narrow and transparent chorion, had an average length of 1.03 ± 0.12 mm (n= 145, 0.80 – 1.33 mm) and an average width of 0.40 ± 0.03 mm (n= 145, 0.33 – 0.50 mm) (Table 1). The 1st instar larvae differed in width from the eggs, being smaller (p<0.0001; Dwass-Steel-Critchlow-Fligner test) (Table 1, Fig 1). This pattern was similar to that reported for P. lanio (Fabricius) (Giannotti, 1995) and differed from three other species of the genus that showed 1st instar larvae with dimensions similar to egg diameters: M. cassununga (Giannotti & Fieri, 1991), M. drewseni (Giannotti & Trevisoli, 1993), and M. latior (Cecílio et al., 2015). The eggs were attached either to the side wall of the cell (Giannotti & Silva, 1993), or at the angle formed between two walls (Giannotti, 1995 and Prezoto & Gobbi, 2005).

The larvae specimens showed five instars, with differences in the median widths between the five groups (p<0.0001, Kruskal-Wallis test) (Table 1, Fig 1, Fig 2). This is similar to the situation observed with M. cassununga (von Ihering) (Giannotti & Fieri, 1991), M. drewseni Saussure (Giannotti & Trevisoli, 1993), and M. latior (Fox) (Cecílio et al., 2015). Other species of other genera likewise follow this pattern, such as Agelaia Lepeletier (Giannotti, 1988), Brachygastra Perty (Machado et al., 1988), Polistes Latreille (Giannotti, 1995; 1997; Prezoto & Gobbi, 2005), Polystia Lepeletier (Tech & Machado, 1989; Carvalho & Silva, 1975; Solis et al., 2012), and Protopolybia Ducke (Silveira, 1994). Giannotti and Fieri (1993) associated high numbers of instars to the long post-embryonic development of social wasps. It is

<table>
<thead>
<tr>
<th>Immatures</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>95% CI</th>
<th>Median</th>
<th>IQR*</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>145</td>
<td>0.40</td>
<td>0.03 (0.39; 0.40)</td>
<td>0.40 (0.40; 0.40)</td>
<td>0.33</td>
<td>0.50</td>
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<tr>
<td>L1</td>
<td>90</td>
<td>0.33</td>
<td>0.04 (0.32; 0.34)</td>
<td>0.33 (0.33; 0.33)</td>
<td>0.20</td>
<td>0.43</td>
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</tr>
<tr>
<td>L2</td>
<td>46</td>
<td>0.51</td>
<td>0.04 (0.50; 0.53)</td>
<td>0.50 (0.50; 0.53)</td>
<td>0.47</td>
<td>0.63</td>
<td></td>
<td></td>
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<tr>
<td>L3</td>
<td>54</td>
<td>0.72</td>
<td>0.05 (0.71; 0.74)</td>
<td>0.71 (0.67; 0.77)</td>
<td>0.67</td>
<td>0.83</td>
<td></td>
<td></td>
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<tr>
<td>L4</td>
<td>53</td>
<td>1.03</td>
<td>0.07 (1.01; 1.05)</td>
<td>1.03 (1.00; 1.07)</td>
<td>0.87</td>
<td>1.20</td>
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<tr>
<td>L5</td>
<td>110</td>
<td>1.60</td>
<td>0.08 (1.58; 1.61)</td>
<td>1.60 (1.57; 1.67)</td>
<td>1.37</td>
<td>1.73</td>
<td></td>
<td></td>
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<tr>
<td>PP</td>
<td>12</td>
<td>1.58</td>
<td>0.07 (1.53; 1.62)</td>
<td>1.60 (1.52; 1.63)</td>
<td>1.47</td>
<td>1.67</td>
<td></td>
<td></td>
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<tr>
<td>P</td>
<td>59</td>
<td>1.85</td>
<td>0.07 (1.83; 1.87)</td>
<td>1.83 (1.80; 1.93)</td>
<td>1.67</td>
<td>2.03</td>
<td></td>
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</tr>
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</table>

*Interquartile Range
therefore probable that the development time of *M. nomurae* is longer than that of *M. atramentarius* Z. (Silva, 1984) and *M. extinctus* Z. (Raposo-Filho, 1981), which develop four instars, although that has not been directly studied.

The average growth rate of the larvae was 1.48 (1.57 between L1 and L2, 1.38 between L2 and L3, 1.43 between L3 and L4, and 1.55 between L4 and L5). The highest rate, between L1 and L2, is similar to that observed in *M. latior* (Cecílio et al., 2015). The average growth rate is in accordance with Dyar’s rule. The species showed a higher average growth rate than the other species of the genus. The ascending order of growth was: 1.37 in *M. latior* (Cecílio et al., 2015), 1.38 in *M. drewseni* (Giannotti & Trevisoli, 1993), 1.42 in *M. atramentarius* and *M. cassununga* (Silva, 1984; Giannotti & Fieri 1991, respectively), and 1.46 in *M. extinctus* (Raposo-Filho, 1981).

No differences were observed in the median width values among 5th instar and pre-pupa cephalic capsules (*p* = 0.957; Dwass-Steel-Critchlow-Fligner test). The elongated 5th instar larva is considered pre-pupa, not an instar. The data on the widths of the pupae cephalic capsules are shown in Table 1 and Fig 1.

**General Morphology.** Like the other species of wasps studied by Dias-Filho (1975), the vermiform larvae of *M. nomurae* had a light-yellow color, thorax with three segments, and abdomen with 10 segments. The abdomen showed a pair of dorsal crests on each segment, ventral lobes, and lateral humps arranged below the spiracle line (Fig 2-A: L5).

The anal slot, which is transversal, is found on last segment (the anal segment) (Fig 3-G). The L1 kept the chorion of the egg and the L2 to L4 larvae kept the exuviae of the previous instar fixed to the final portion of the abdomen (that served as the attachment to the cell wall) (Figs 2-B and 3-H). This differs from the L5 larvae, which are fixed to the cell wall by the dorsal crests and lateral humps.

The head, formed by the cephalic capsule and buccal pieces, is more sclerotized in L5 larvae. It has a median suture and a pair of temporal bands that are seen in all instars (Fig 2-A). The tentorial pits are located below the level of the antennae sockets and in the upper portion of the lateral margins of the clypeus (Fig 2-C), as was observed by Dias-Filho (1975) for other species of *Mischocyttarus*. The
mandible is covered by the bilobate labrum in all of the instars, with only a single tooth, this being elongated, falcate, and thin, as is typical for the genus (Dias-Filho 1975; Giannotti & Silva, 1993). Both the pair of maxilla and the labium have a pair of elongated brownish papillae (Fig 2-C and 3-B).

The ten spiracles can be seen in Fig 3 (Larva L5). The first spiracle of the L5 larvae (located between the prothorax and the mesothorax) has a diameter of $0.26 \pm 0.04$ mm ($n=47$, $0.012 – 0.032$ mm), being 3.1 times larger than the second thoracic spiracle (between the mesothorax and the metathorax; $0.08 \pm 0.01$ mm [$n=47$, $0.06 – 0.09$ mm] (Fig 3-A to 3-D). It is therefore twice the diameter of the other spiracles (Richards, 1978). The other spiracles have similar diameters, with the third to the tenth being located in the 1st to the 8th abdominal segments respectively (Fig 3A, 3G).

The species shows two abdominal lobes on the first abdominal sternite that are strongly projected forward. They can be seen as small protuberances in L3, still little developed in L4, and fully developed in L5 (Fig 2-B). According to Silveira (2008), this character is widely observed in several

subgenera of *Mischocyttarus*. The fact that *M. nomurae* bears two lobes differs it from *M. latior*, which has only a single lobe on the first abdominal sternite – this being a character of the subgenus *Kappa*. These lobes are vestigial in the second instar larvae of *M. latior*, and also in the third instar of *M. drewseni* (Giannotti & Trevisoli 1993; Silveira, 1998; Cecílio et al., 2015).

The lobes show ornamentations on their cuticles and bristles that increase in density from the base to the apex, what could indicate a sensorial function (Fig 3-A, 3-B, 3-E, 3-F). Giannotti and Silva (1993) disagreed with the suggestions of Reid (1942) and Wheeler and Wheeler (1979) that the lobes could be used to hold food before its ingestion, as they observed that the workers of *M. cassumunga* delivered already macerated food directly to the buccal parts of the larvae. Jeanne (1972) reported larvae of *M. drewseni* using those lobes to help pump larval saliva to adults that had solicited it. Hunt (1988) designated this behavior “lobe erection”. Detailed studies of lobe ultrastructure have not yet been undertaken that could shed more light on its function(s).

As observed in *M. cassumunga* and *M. drewseni*, the transparent cuticle of the elongated pre-pupae allows the thoracic appendices of the pharate pupa (Giannotti & Silva, 1993; Giannotti & Trevisoli, 1993), and sometimes their compound eyes (Giannotti, 1995), to be seen. The lobes are now strongly projected rearwards as the larva prepares to metamorphosis (Fig 4-A).

The pupae become graded in color from milky white to dark as they became older, also becoming more sclerotized. Darkening initiated in the head and then advanced towards the thorax, while the eyes likewise darkened from reddish to dark brown, as had been reported for *P. lanio* (Giannotti, 1995). The wings, which were shaped like filaments below the gaster, then became free and positioned over the gaster (Fig 4-B).

Thus, with the exception of the 1st instar larva, which is much smaller than the egg, *M. nomurae* shows development patterns typical of the genus: with the presence of abdominal lobes strongly projected forward, but only fully developed in the 5th instar; mandibles with a single elongated tooth; and the first spiracle twice the diameter of the others, as reported by Richards (1978).

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**References**


