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Egg and Mature Larva of a Species of *Plebeia* with a Preliminary Overview of the Mature Larvae of the Meliponini Relative to Those of Other Corbiculate Taxa (Apoidea: Apidae)

JEROME G. ROZEN, JR.,[1] COREY SHEPARD SMITH,[1] DAVID W. ROUBIK[2]

ABSTRACT

The egg and mature larva of a presently unnamed species of the bee genus *Plebeia* are anatomically described, illustrated, and compared with those of other known Meliponini, based on abundant specimens collected from two nests recovered from a tree at the Yasuní Scientific Station, Orellana Province, Ecuador. A key to the four tribes (Apini, Bombini, Euglossini, and Meliponini) of known mature corbiculate larvae is then presented. It, in turn, is followed by a preliminary larval description of the Meliponini based on those taxa the mature larvae of which are known so far. The main feature distinguishing the mature larva of the Meliponini is in its having a tapering slender elongate mandibular apex, which contrasts with the much shorter, robust mandibular apex of the other three tribes. Furthermore, unlike in the other tribes, late stage larval Meliponini possess paired dorsolateral tubercles on most abdominal segments.

INTRODUCTION

This is another contribution by the first author exploring the developmental biology and immature stages of corbiculate bees (i.e., Euglossini, Bombini, Meliponini, and Apini). As explained by Michener (2007: 668), these four tribes "clearly constitute a holophyletic unit. . . the corbiculate

[1] American Museum of Natural History, New York.
[2] Smithsonian Tropical Research Institute, Republic of Panama.

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Apidae." In the past, the first author devoted much of his research efforts to investigating the immature stages and nesting biologies of numerous solitary and cleptoparasitic bees for this extremely large and diverse group, because it seemed understudied in contrast to the social taxa. For the last five years, however, it has become obvious that the developmental biology and immature stages of the corbiculate taxa also need further investigation. This is evidenced by the number of recent papers by the first author and coauthors exploring the nesting biology and immature stages of corbiculate Apidae, cited here arranged by tribe. **Euglossini:** Rozen, 2016, 2018; **Bombini:** Rozen et al., 2018a, 2018b; **Meliponini:** Rozen et al., 2019; **Apini:** Rozen et al., 2017.

Whereas the first part of this paper reports on the eggs and mature larvae of *Plebeia* "nan1," the second part provides a preliminary assessment of our current understanding of mature meliponine larvae relative to those of other corbiculate bees.

In this paper and in others to follow dealing with immature stages of meliponine bees, we anticipate that immatures of some will be described before their valid species name has been established. Under these circumstances, we propose that the generic name of the taxon should be followed by an acronym of "no available name, Arabic number," i.e., *Plebeia* "nan1," *Plebeia* "nan2," etc. These are temporary nomina, without nomenclatorial status. If it happens that the species is given a valid name before publication of the acronym, the reference to any and all acronyms should be eliminated from all earlier unpublished references. However, when the valid name appears after the publication of the acronym, it is important that a clear connection between the two be established since they link information concerning immatures to the adult of species.

PART 1. DESCRIPTIONS OF THE EGG AND MATURE LARVA OF *Plebaia* "nan1"

Source of Material and Methods

The immature stages of *P.* "nan1," described and depicted herein, were collected by D.W.R. from two nests discovered in separate branches of a single tree and taken to the lab. Five days later the entire contents of the two nests were combined and preserved in a single container and subsequently sent to the American Museum of Natural History for study. The specimens included an abundance of single cells separated from one another, most of which held pupae or mature larvae in cocoons. Other cells, presumably of wax, were attached to one another forming small irregular clusters. When opened these clustered cells were frequently found to hold provisions and not infrequently eggs, almost all of which appeared to have hatched or been preserved well after dying. Almost none contained immature larvae.

To be studied, preserved larval specimens were cleared in an aqueous solution of sodium hydroxide and stained with an ethanol solution of Chlorazol Black E and subsequently photographed with a Canon Power Shot A2300 camera, hand held to one of the eyepieces of a Leitz Wetzlar stereomicroscope and of a Carl Zeiss compound microscope. SEM micrographs of eggs were captured with a Hitachi S5700 SEM in the Microscopy and Imaging Facility of the American Museum of Natural History.

Egg of *Plebeia* "nan1"

Figures 1–3

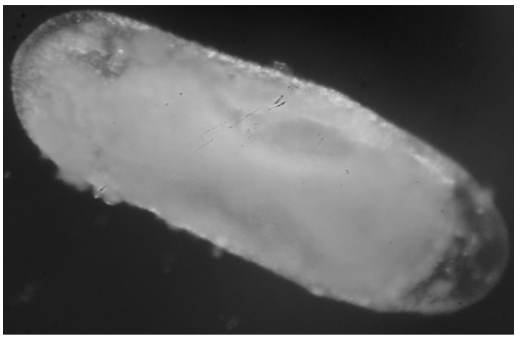


FIGURE 1. Microphotograph of entire egg of *Plebeia* "nan1," anterior end upper left.

Diagnosis: Because of its small size and lack of midbody swelling, the egg of this species is clearly different from known eggs of *Melipona* (Rozen et al., 2019: figs: 1–4, 9, 14). However, because of poor preservation, this egg cannot be reliably distinguished from those of smaller bee species (*Scaptotrigona pectoralis* (Dalla Torre) and *Tetragonisca angustula* (Latreille)), the eggs of which have been described and photographed with an SEM (Rozen et al., 2019: figs. 5–8, 19, 23) and which lack the "bowling pin" shape of described eggs of *Melipona*.

Description: Color white. Shape (fig. 1) elongate with sides lacking median bulge; comparison of symmetry between dorsoventral and lateral views uncertain; anterior end slightly blunter than posterior ends; shape similar to, but somewhat more elongate than that of *Scaptotrigona pectorialis* (Rozen et al., 2019: fig. 5) and to *Tetragonisca angustula* (Rozen et al., 2019: fig. 7) and somewhat shorter relative to width. Dimensions based on eight better-preserved specimens: length 1.125–1.35 mm; maximum diameter 0.35–0.45 mm.

When viewed with SEM, chorion at anterior end of egg with strongly expressed, elevated geometric pattern (mostly hexagons) that converge and narrow (fig. 2) as they approach micropyle; micropyle circular with pores central; chorion at posterior end of egg lacking geometric surface ridges (fig. 3).

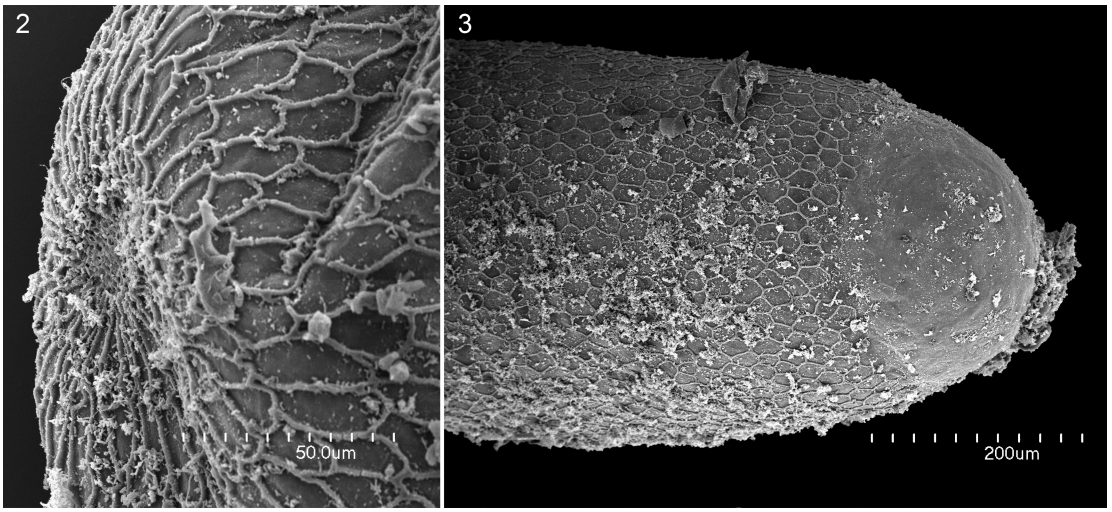
Material Studied: Eight well-preserved eggs selected from 50+ eggs: ECUADOR: Francisco de Orellana Prov.: Yasuní Scientific Station, PUCE (Pontífica Universidad Católica del Ecuador), collected from two nests. IX-24-2018 (David Roubik).

Although the shape and sizes of the known meliponine eggs vary from taxon to taxon, their chorionic geometric (hexagonal) ridges are markedly similar, when figs. 1, 2 are compared with the SEM's presented by Rozen et al. (2019).

Postdefecating Larva of *Plebeia* "nan1"

Figures 4–14

The following description is based on clearly postdefecating specimens of two phenotypes: (1) a brightly reflective white form (fig. 4) with only the mandibular apices strongly sclerotized and (2) a form also with strongly sclerotized mandibles but having a subdued grayish darker head and body revealing much of the subtle anatomical contours (fig. 4). This form is also farther along in development as judged by the more robust appearance of its mesosoma contrasting with a narrower metasoma. When, at first, only a few specimens were compared, it was uncertain whether these two phenotypes represented developmental differences between separate castes or



FIGURES 2, 3. SEM micrograph of **2.** front and **3.** posterior ends of egg of *Plebeia* "nan1" revealing geometric (mostly hexagonal) chorionic patterning at front and middle of egg and lack of patterning at rear (right).

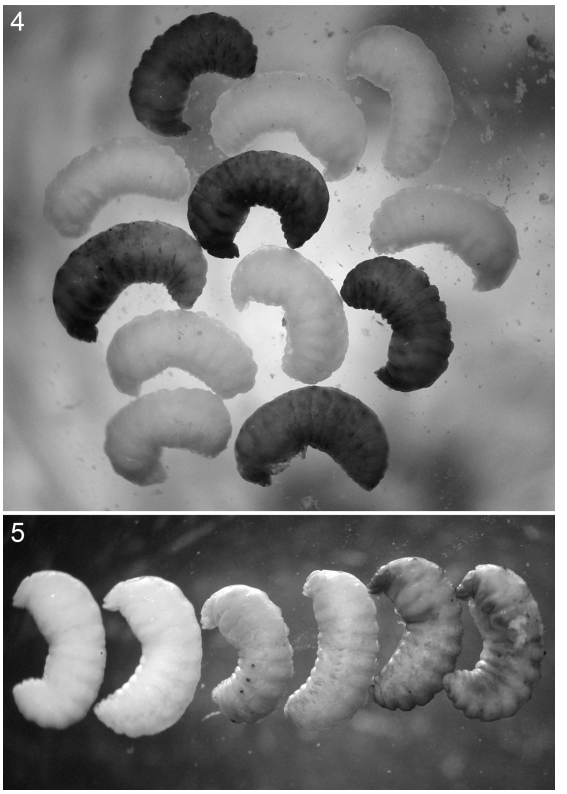
whether the differences were development steps that all individuals pass through. As more specimens were uncovered showing intermediate shades of gray, the darker forms now seemed more likely to be simple developmental steps characteristic of all. The following description encompasses both forms.

Diagnosis: The mature larva of this species is almost identical to that of *Plebeia droryana* (Friese) described and illustrated by Lucas de Oliveira (1965: figs. 1–3). The mandibles of the two were thought to differ at first. The ventral apical mandibular edge of that of *P. droryana* (Lucas de Oliveira, 1965: fig. 3 A, B) was described to have "micro-espiculos," while this edge seemed smooth in *P.* "nan1' these spicules were finely detected under high magnification.

Description: **Head:** Size moderate relative to body size (fig. 6); front of head in lateral profile relatively flat below narrow vertex, so that frons, clypeus, and labrum closely aligned and angling sharply from strongly projecting labiomaxillary region in lateral view (fig. 8); head capsule moderately broad; vertex not bilobed in frontal view (fig. 7). Condition of tentorium unknown; posterior tentorial pits normal in position; posterior thickening of head capsule narrow, scarcely bending forward medially as seen in dorsal view; coronal ridge absent; epistomal ridge on cleared head capsule (fig. 7) evident from anterior mandibular articulation to anterior tentorial pit but fading out immediately mesad of anterior tentorial pits; front of head capsule with transverse depression short distance above each antenna; integument of head capsule mesad of each parietal band unevenly swollen presumably because of weak sclerotization resulting from distortion in SEM preparation. Parietal bands evident (figs. 7, 8). Antennal papilla a somewhat large conical projection extending nearly as far as its basal diameter in profile; papilla bearing perhaps 3 sensilla; papilla (fig. 11) surrounded by membranous ring with radius about one-half basal diameter of papilla. Vertex rounded in lateral view; frontoclypeal area not projecting beyond labrum (fig. 8); apical surface of labrum bearing extensive patch of elongate, multipronged spicules (figs. 12, 13) medially intermixed with sensilla having hemispherical base and elongate, tapering apex (fig. 14: lower left corner).

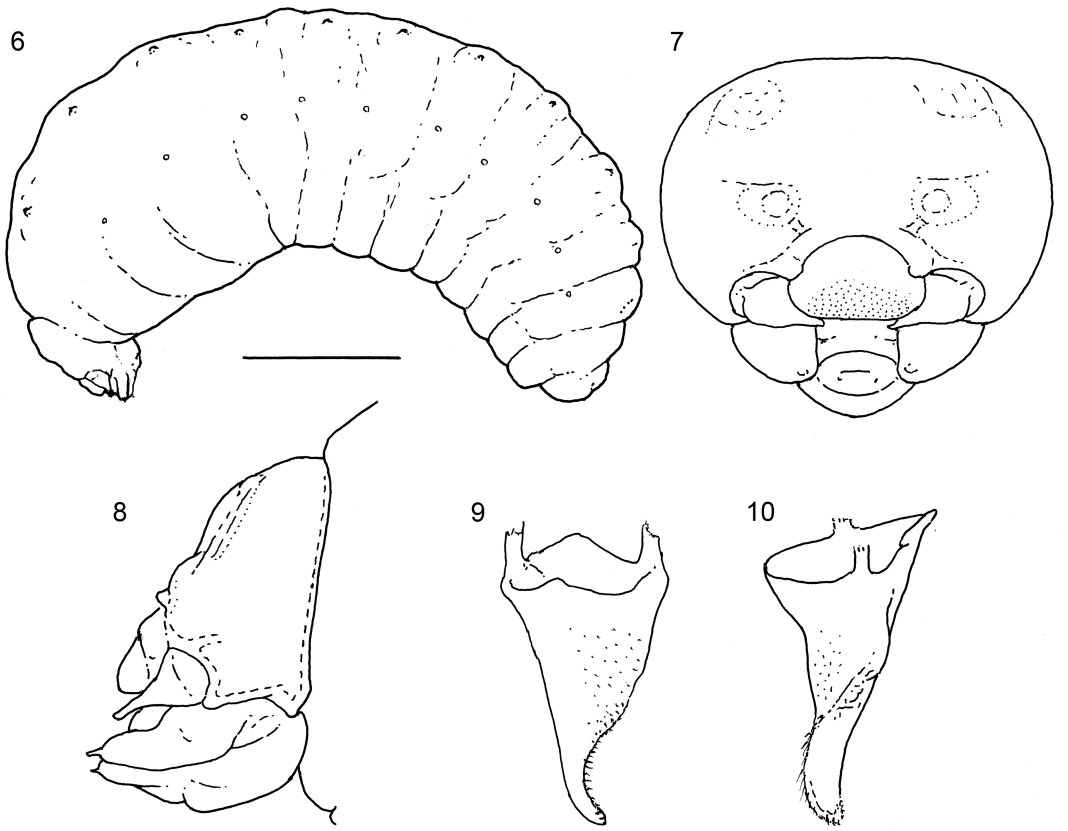
Mandibular apex (figs. 9, 10) pigmented; mandible seen in inner view (fig. 10) slender, elongate, narrowing evenly from base to pointed apex; apical concavity (fig. 10) narrow, considerably longer than distance from mandibular base at base of cusp; dorsal edge of apical concavity with numerous long slender sharp teeth as seen in inner view (fig. 10); ventral edge smooth; mandibular apex with several rounded teeth; cusp not projecting but inner surface uneven.

Labiomaxillary region variably projecting relative to head capsule in lateral view; integumental darkening pattern variable on older specimens; labium and maxilla extending more or less equally in lateral view (fig. 8). Maxillary apex not bent mesad; palpus apical in position, more than twice as long as basal diameter; galea evident at maxillary apex, bearing several sensilla; articulating arm of stipes questionably present; basal articulation of stipes to cardo evident; dorsal and inner apical surface of maxilla questionably spiculate. Labium divided into prementum and postmentum, bearing apically projecting broad lips of slitlike salivary opening, its width slightly less than distance between bases of labial palpi; length of labial palpus about twice basal diameter. Hypopharynx spiculate.



FIGURES 4, 5. Microphotographs of mature postdefecating larvae showing all white, presumable early form, and other various darker forms thought to represent subsequent developmental steps. **4.** Mixture of extremes in intensity of darkening. **5.** Selection of specimens showing gradations of darkening.

Body: Dorsal integument of body from posterior margin of head toward posterior end more or less densely covered with fine spicules that extend just laterad of each of paired dorsal body tubercles (identified below); density of spicules gradually diminishing toward rear of abdomen. Each thoracic segment with pair of small but distinct elevated dorsolateral tubercles on caudal annulet; abdominal segments 1 to 6 or 7 also each with paired tubercles, but tubercles becoming sequentially smaller toward posterior end of body; on darker (older) specimens these tubercles pigmented. When larva cleared and stained with Chlorazol Black E, these tubercles are seen to be positioned on paired, slightly elevated, transversely oblong stained surfaces of caudal annulets; abdominal segments 7, 8 with paired transverse oblong darkly stained areas small and lacking tubercles; surfaces of segments 9 and 10 each with darkly stained semicircles. Spiracles moderately large relative to body size, peritreme distinct; atrium shallow; atrial wall smooth or nearly so; primary



FIGURES 6–10. Diagrams of mature larva of *Plebeia* "nan1." **6.** Entire larva, with scale bar, lateral view, front end to left. **7, 8.** Head, frontal and lateral views. **9, 10.** Right mandible, dorsal and inner views.

tracheal opening a simple rim, smaller than atrial opening; subatrium moderately short, consisting of about six annulations; flexure collapsed into single, long narrow tube.

Material Studied: 10+ postdefecating larvae. Same locality and date as eggs.

PART 2. COMPARISON OF MATURE LARVAE OF THE MELIPONINI WITH THOSE OF OTHER CORBICULATE TRIBES

Key to Tribes of Corbiculate Bees Based on Mature Larvae

The following key is intended to permit tribal identification of the mature larvae of the four tribes of corbiculate bees.

1. Thoracic segments each with pair of elongate, transverse dorsolateral tubercles (Michener, 1953: fig. 275); mandibles short, lacking apical concavity (Michener, 1953: figs. 278, 279).....Apini
– Thoracic segments each with pair of pointed, more or less sclerotized, dorsolateral tubercles (fig. 6); mandibles short (Rozen et al., 2018b: figs. 56, 57) or attenuate (figs. 9, 10), with apical concavity..... 2

2. Primary tracheal opening a simple, circle without ornamentation; abdominal segments with or without paired dorsolateral tubercles; internal median section of epistomal ridge between anterior tentorial pits absent.....3
– Primary tracheal opening markedly ornamented with spines; abdominal segments without paired dorsolateral tubercles; internal median section of epistomal ridge between anterior tentorial pits well expres sed…….Bombini
3. Mandibles elongate, narrowing apically, with elongate, narrow apical concavity; prothorax not elongate; thoracic segments and at least first abdominal segment (but usually more) each with paired, pointed dorsolateral tubercles.....….....Meliponini
– Mandible short, robust, with apex narrowing only slightly (Rozen, 2018: figs. 23, 24); prothorax elongate anteriorly (Rozen, 2018: figs. 6–9); either thoracic segments alone with paired pointed dorsolateral tubercles (Rozen, 2018: figs. 6, 8, 10) or, in *Euglossa*, thoracic and first abdominal segments each bearing such tubercles (Rozen, 2018: fig. 9).....Euglossini

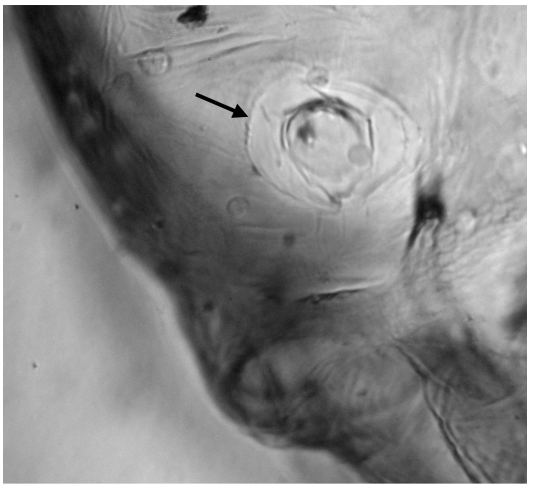
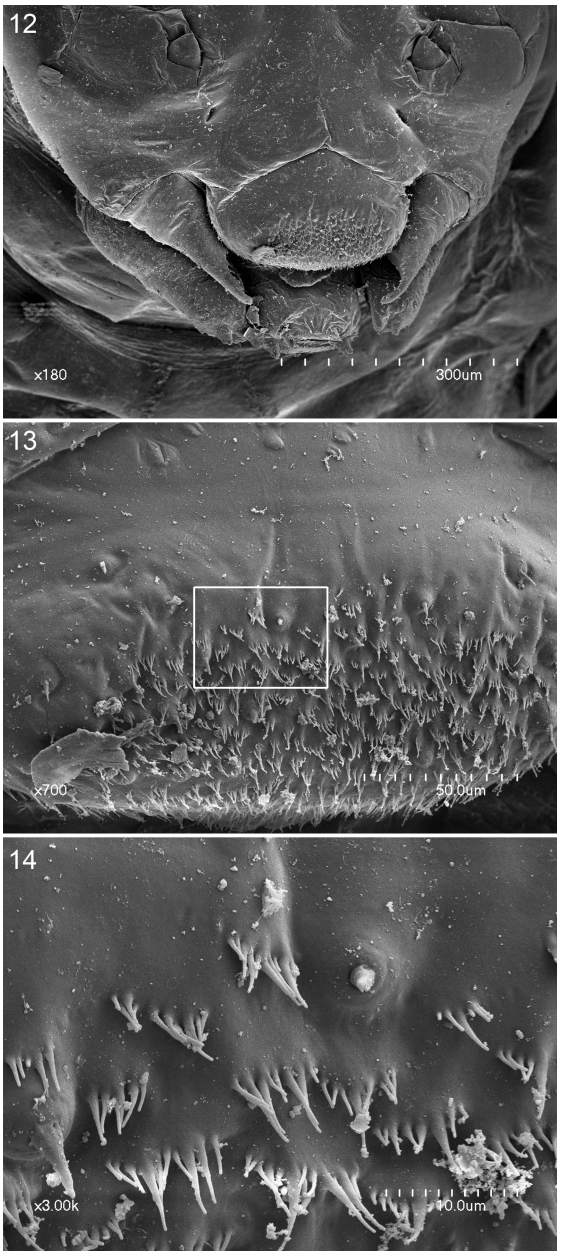


FIGURE 11. Microphotograph of right side of cleared head capsule showing antennal papilla (arrow) surrounded by membranous ring.

Preliminary Description of Mature Larvae of the Meliponini

Now that the last larval instar of *P.* "nan1" is known, it is perhaps worthwhile to characterize the known mature larvae of the Meliponini, even though all that have been studied are from the New World and comprise only about one-third of all genera worldwide. Previous studies on larvae were carried out by Michener, 1953; Lucas de Oliveira, 1960, 1965, 1968, 1970; and Rozen et al., 2019.

Although these papers are well illustrated, the reader should be aware that "dorsal views" of the head capsules are oriented differently in that all of those in the numerous publications by Lucas de Olivier present a more dorsal view than those in the publication by Michener (1953). As a result, the labial apex, salivary lips, and labial palpi tend to be seen in dorsal profile by Lucas de Oliveira (1968: fig. 2 E), contrasting with that by Michener (1953: fig. 256), in which these structures appear more frontal in appearance. Unlike the other two authors, J.G.R. here and elsewhere does not use "dorsal view," but instead takes a "frontal view," so that more of the labiomaxillary region as well as the rest of the head comes into a more face-on view. Thus, the pleurostomal ridges are perpendicular to the horizontal microscope stage. A true dorsal view of the head presumably might be called a top view, which is occasionally useful. Lateral views of corbiculate heads have been uniformly presented by all authors, as are views of entire larvae and mandibles.



FIGURES 12–14. **12.** SEM micrographs of lower part of head, frontal view, showing labrum, with lower, apical part densely spiculate. **13.** Close-up of labrum. **14.** Close-up of area identified by rectangle in figure 13, showing multipronged spicules.

Diagnosis: Because of paired, pointed dorsolateral tubercles on most abdominal segments as well as on the thoracic segments, mature larvae of the Meliponini can be distinguished from those of other bee taxa. Further, the slender, tapering mandibles contrast with the robust mandibles of other corbiculate larvae.

Description: Body size moderate (e.g., *Melipona*) to extremely small (e.g., *Plebeia*).

Head: Size relative to that of body tending to be small because robustness and shape of thoracic segments; front of head in lateral profile tending be approximately linear to slightly curved; head capsule in frontal view normally broad with vertex ranging from normally curved to bilobed; internal coronal ridge usually not evident, rarely slightly expressed near posterior margin of head capsule. Tentorial pits in normal positions. Parietal bands variably defined. Antennal prominences weakly projecting; antennal papilla projecting, sometimes nearly as far as basal diameter. Labrum broad but short, faintly bilobed when viewed from above or below; apex of labrum densely spiculate with multipronged spicules (figs. 12–14).

Mandible in outer or inner view broad at base, tapering quickly to more or less slender somewhat pigmented apex which in some cases is nearly parallel sided; adoral surface of apex with long apical concavity; dorsal edge of concavity usually bearing sharp spines; ventral edge with or without some spines; extreme apex sometimes slightly expanded before ending; apex curving in dorsal or ventral views.

Labiomaxillary region projecting as typical for cocoon spinning larvae; maxilla well defined, projecting nearly as far as labial apex, apically bearing distinct palpus, with cardo and stipes well defined; articulating arm of stipes, probably present but often hard to iden-

tify, perhaps because of a lack of strong pigmentation; prelabium distinct from postlabium and apically with projecting transverse salivary lips above well-defined palpi.

Body: Body vestiture consisting of very fine spicules restricted to dorsal body surface where its abundance varies greatly depending on taxon; in addition to spicules, dorsal integument with widely scattered, extremely fine setae that may serve as sensilla; ventral integument lacking spicules. Body form robust in that thoracic segments as large as, if not larger than, following segments; caudal annulets dorsally and laterally tending to be larger and more dominant and with more spicules than cephalic annulets; paired small dorsolateral tubercles occurring on thoracic caudal annulets and on more anterior abdominal caudal annulets. Spiracles tending to be large and always lacking strong ornamentation to atrial wall and primary tracheal opening.

Taxa Examined for Current Paper: *Melipona* (*Michmelia*) *fallax* Camargo and Pedro; *Melipona* (*Michmelia*) *trinitatis* Cockerell; *Nogueirapis mirandula*; *Partamona musarum* (Cockerell); *Plebeia* "nan1"; and *Tetragonisca angustula*.

Additional Taxa Described by Others: *Lestrimelitta ehrhardti* (Friese) (Lucas de Oliveira, 1968: figs. 1B, 3I); *Lestrimelitta limao* (Smith) (Lucas de Oliveira, 1968: figs. 1A, 3B); *Leurotrigona muelleri* (Friese) (as *Hypotrigona* (*Leurotrigona*) *muelleri* (Friese)) (Lucas de Oliveira, 1970: figs. 1, 3B); *M.* (*Melipona*) *quadrifasciata* Lepeletier (Michener, 1953: 1090); *Partamona peckolti* (Friese) (Michener, 1953: figs. 266–271, as *Trigona* (*Partamona*) *cupira* (Smith); and *Plebeia droryana* (Friese) (Lucas de Oliveira, 1965: figs. 1A, 34B).

DISCUSSION

As indicated above, the populations of these two nests when preserved included: (1) a large number of adults, mature larvae, and pupae, (2) an abundance of eggs in poor condition, and (3) the near lack of young and intermediate-stage larvae. Why should there be so many cells with mature larvae and pupae, yet so little evidence of immature larvae? With so many eggs in evidence, why were they so poorly preserved? This would seem to suggest that the recovered egg material was a long accumulation of infertile or dead eggs and empty chorions, and that the live adults, pupae, and larvae represented the next generation. Future studies will need to inquire if the colonies of this species are subject to some sort of seasonal cycle.

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