Nesting Biology and Immature Stages of the Panurgine Bee Genera Rhophitulus and Cephalurgus (Apoidea: Andrenidae: Protandrenini)

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Nesting biology and immature stages of the panurgine bee genera *Rhophitulus* and *Cephalurgus* (Apoidea: Andrenidae: Protandrenini)

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ABSTRACT

Herein is presented nesting information on the communal ground-nesting Argentinian bees *Rhophitulus xenopalpus* Ramos and *R. mimus* Ramos, which is compared with what is known concerning the closely related Brazilian bee *Cephalurgus anomalus* Moure and Lucas de Oliveira. The mature larvae of all three taxa are described, illustrated, and compared with one another and with those of other Protandrenini. While larvae of the three species share many similarities, those of *R. xenopalpus* and *R. mimus*, though each distinctive, are quite similar, and those of *C. anomalus* differ from the others in mandibular features and in dorsal body ornamentation. Male and female pupae of *R. xenopalpus* are also described.

INTRODUCTION

The purpose of this project was to advance our understanding of the nesting biology and immature stages of bees belonging to the panurgine genera *Rhophitulus* and *Cephalurgus,* a complex of small, obscure, mostly black, ground-nesting species restricted to South America (Michener, 2007). Presented in this report is information on the nesting biology of *Rhophitulus xenopalpus* Ramos and *R. mimus* Ramos, which is compared with the previously published biological account of *Cephalurgus anomalus* Moure and Lucas de Oliveira (Rozen, 1989). Although in the past considered only subgenerically distinct (Michener, 2007), *Rhophitulus* and *Cephalurgus* are now thought to be separate genera (Moure et al., 2007, 2012). Accordingly, their separate relationship is maintained here, and the two genera are treated together to be further compared.

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Additionally, available immature stages of the three taxa are here described, providing the first accounts of the immature stages of both genera. Specimens and biological information on *R. xenopalpus* and *R. mimus* were gathered on two field trips to Argentina, when I was accompanied by Arturo Roig-Alsina in 1989 and 1990. Kelli S. Ramos (2014) has recently named and published the descriptions of *R. xenopalpus* and *R. mimus* based in part on collections from those trips. Specimens of *C. anomalus* were collected in 1974 at Cosmópolis, São Paulo, Brazil, when I was accompanied by F.C. Thompson and J.S. Moure.

Larvae and pupae, all small-bodied forms, placed in Kahle's solution 25 and 40 years ago did not preserve well. When studied, they could not be cleaned beforehand without possibly causing further damage. As a result body surfaces in SEM micrographs are regrettably clouded by a certain amount of debris. Nonetheless, interesting anatomical features are revealed that both demonstrate relationships and raise questions that will require further studies of the genera. These questions are identified under Discussion of Larval Features following the larval descriptions.

Table 1 provides comparative statistics regarding nest measurements and egg size and attachment of the three species belonging to these genera.

BIOLOGICAL OBSERVATIONS ON *RHOPHITULUS XENOPALPUS*

Arturo Roig-Alsina and I found and examined a nesting site of *R. xenopalpus* on a pathway at 11 km north of El Cadillal, Tucumán Province, Argentina, on November 8 and 9, 1989, and returned again on December 3, 1989. I studied three nests grouped within an area having a radius of 8 cm on the first visit, and on the second visit I examined five more nests within an area about 35 cm in diameter. Other nests may also have been present nearby on both occasions. The sole larval food plant, *Heliotropium* sp. (Boraginaceae)[2], was broadly scattered and occurred within a meter of the nests on the first visit. Nesting data were gathered on both occasions; mature larvae and pupae were recovered mostly on the latter one.

Nest openings were along a gently sloping path bordered by shrubs and trees but exposed to the sun. The fine-grained soil was hard packed and moist. A single nest of the eight seemed to be occupied by one female, but all others were clearly communal. Of these, one nest was jointly used by more than eight females, and several were inhabited by four and five females each. Entrances did not exhibit tumuli, but such accumulations may have been dissipated by rain. Main burrows were open, descended vertically, and some divided, giving rise to obliquely descending branches. Open, more or less horizontal laterals, at most 10 mm long and about 1.5 mm in diameter, lead to open cells being constructed or provisioned; they were soil filled after cell closure. Thus, all closed cells were positioned near nest tunnels, so that in completed nests, cells were closely grouped. Although the lowest cell measured was 16 cm deep, others might have been deeper since we did not have time to explore beneath cells. They tilted to the rear from 20° to somewhat more than 45° from horizontal.

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[2] The plant genus of *Heliotropium* (Boraginaceae) is the unique host of other bees. It is the sole host of the three species of the Old World *Haetosmia* (Megachilidae: Osmiini) all species of which are characterized by labial palpi with long curved hairs that enable females to extract pollen from narrow tubular flowers (Gotlieb et al,, 2014). It seems likely that the modified labial palpi of *Rhophitulus xenopalpus* will be found to serve a similar function. Females of *Teratognatha modesta* Ogloblin with "deformed mouthparts" also collect pollen from *Heliotropium* (Rozen, 2011).

TABLE 1. Nest Statistics for Two Species of *Rhophitulus* and *Cephalurgus anomalus*. Numbers in parentheses refer to number of data when more than one.

Species	Burrow diameter (mm)	Cell depth (cm)	Cell length (mm)	Max. cell diameter (mm)	Provisions, length (mm)	Provisions height (mm)	Provisions width (mm)	Egg	Egg length (mm)	Max. egg diameter (mm)
R. xenopalpus	1.5	6–16	4.2–4.8 (4)	2.4–2.8 (5)	1.75–2.1 (3)	1.5–1.6 (3)	1.625–2.0 (3)	Posterior end only	1.15	0.38
R. mimus	3.5	20–40	7.0–8.0 (2)	4.0–4.2 (2)	3	2.4	?[b]	Posterior end only	1.3	0.38
Cephalurgus anomalus[a]	2.5–3.0	16–50	7.0–7.5 (6)	4.1–5.0 (6)	3.0–3.5 (10)	2.7–2.8 (9)	?[b]	Anterior and posterior ends	1.3–1.45 (4)	0.35–0.4 (4)

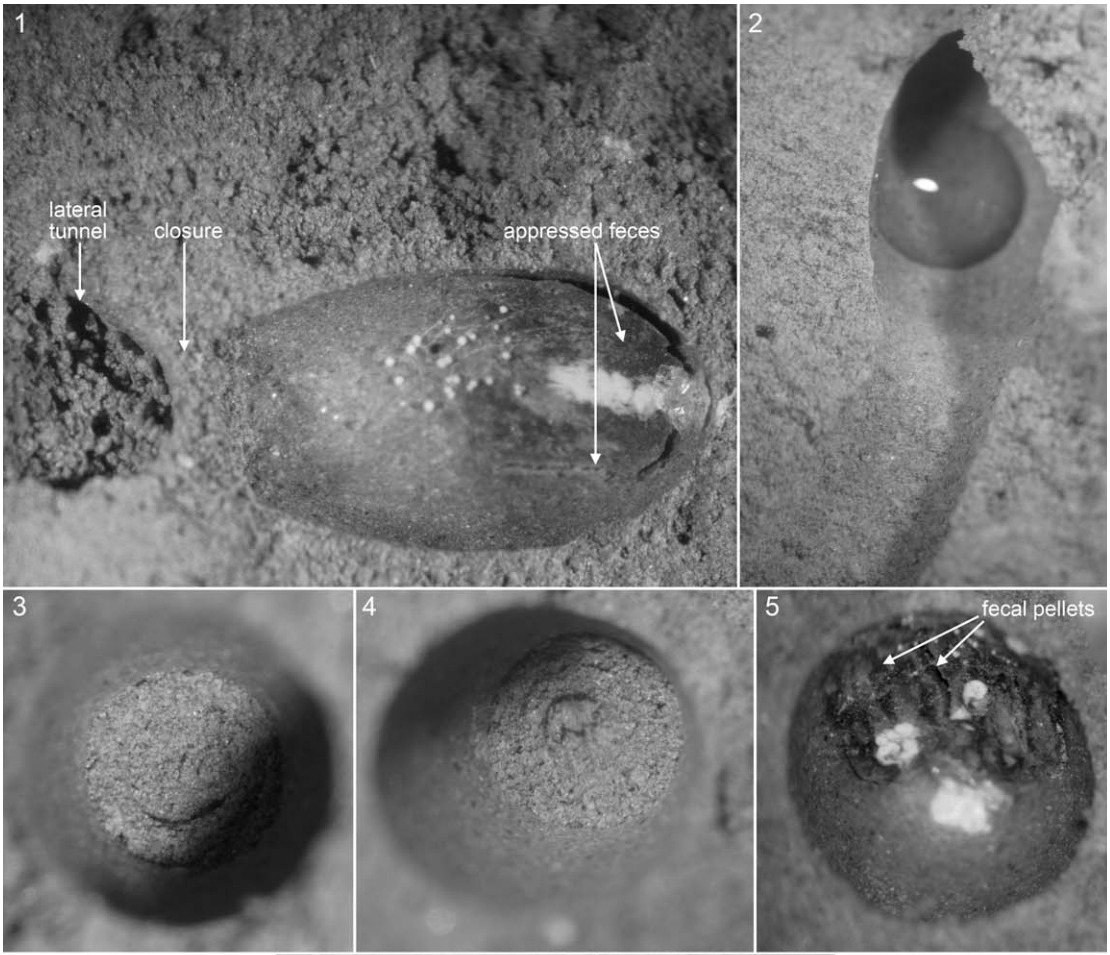
[a] Data for this species reported by Rozen (1989).
[b] Presumably same as length

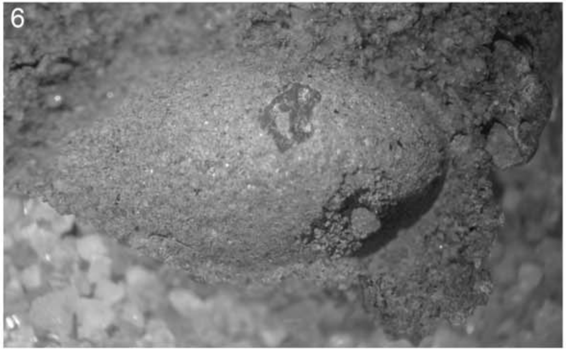
Cells were symmetrically oval (fig. 1) in that their maximum width was about halfway from front to rear and the contour of the front half of the cell was shaped like the rear half. The cell wall, of the same approximate color as the surrounding soil, was smooth with only a hint of shine. A water droplet applied by syringe beaded on the cell wall without being absorbed into its surface, indicating its highly water retardant nature (fig. 2). Inner surfaces of cell closures (figs. 3, 4) appeared porous (contrasting with the smooth cell surface) and consisted of 3–4 coils to the radius and were moderately concave.

Food masses appeared round at first glance, but on careful measurement three masses were slightly longer than wide and their height slightly less than their width, as follows (i.e., l-h-w): 2.1-1.6-2.0 mm; 1.75-1.5-1.625 mm; 1.875-1.5-1.8 mm. Provision masses did not readily disassemble when placed in water. Although no external coatings to the masses were detected, pollen grains on the surface and within masses tended to adhere inexplicably to one another.

A strongly curved egg was found on top of the provisions in the median sagittal plane of the cell. It was attached by its posterior end while its anterior end was elevated above the provisions and pointed toward the front of the cell. Its maximum diameter was just behind its rounded anterior end from which it tapered to a substantially smaller, rounded posterior end. The chorion was shiny translucent white.

Small larvae did not move from where they had been deposited as eggs, and half grown larvae were encountered in the same position after the front end of the provisions had been consumed. Postdefecating larvae rested on their dorsa while facing the cell closure. However, observations on last instars on their dorsa while completing the provisions were not made, so it is unknown whether they change direction in the cell as observed in Calliopsini (Rozen, 2013). As with all known Andrenidae, cocoons are not spun. Feces were deposited on the upper rear ceilings of cells (figs. 1, 5). Many immatures of all stages were found, indicating ongoing generations. No diapausing larvae were observed, although some active postdefecating larvae and pupae were seen. No cleptoparasites or other kinds of nest symbionts were encountered.





FIGURES 1– 5. Microphotographs of nest components of *Rhophitulus* aff. *xenopalpus*. **1.** Cell, viewed from below, showing symmetry of shape, soil-filled lateral, thickness of cell closure, and fecal material appressed to rear of top surface. **2.** Cell wall with water droplet testing absorption rate of hydrofuge surface. **3, 4.** Inner surfaces of cell closures showing spiral patterns. **5.** Posterior end of cell, frontal view, showing discrete fecal pellets attached to rear ceiling. FIGURE 6. Microphotograph of nest cell of *Rhophitulus mimus* demonstrating cell shape and faint sheen of lining.

BIOLOGICAL OBSERVATIONS ON *RHOPHITULUS MIMUS*

Brief notes were made concerning a nesting site of *R. mimus* found on March 21, 1990, at 18 km east of Cachi, Salta Province, Argentina. Many males and pollen-collecting females were observed on a yellow composite, but copulations were not seen. A number of nest openings were discovered along the lower surface of a low bank bordering a gully. Tumuli had accumulated along the downhill side of the entrances, which were open and 3.5 mm in diameter. Some burrows descended vertically, but many diverged from vertical because of numerous subterranean rocks. Although there were multiple cells to a nest, the number of females to a nest, if more than one, could not be ascertained with certainty because of the high density of nest entrances and because some tunnels seemed to anastomose.

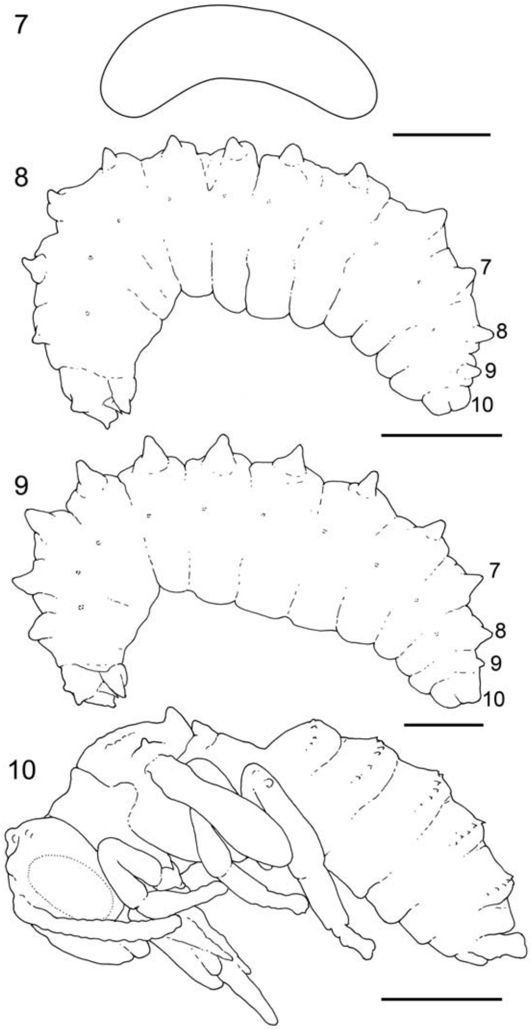
Cells, arranged singly and mostly tilted about 20° to the rear, were considerably larger than those of *R. xenopalpus* as shown in table 1, a range in size that is also reflected in adult body size. Cells appeared oval in lateral and top views (fig. 6), so that the widest point was probably close to halfway between the front and rear. Cell walls bore a distinct but only faintly reflective lining (fig. 6) that was water retardant when tested. One cell contained an oval mass of provisions, the front end of which had been partly consumed by an intermediate-size larva sitting on the top. Another cell contained a strongly curved egg attached to the top of a slightly flattened ball of provisions, the width of which was not measured though thought to be about the same as its length.

As also observed for *R. xenopalpus*, an egg of *R. mimus* (table 1) in the sagittal plane of the cell was attached by its posterior end while its front end pointed toward the cell closure. It had a smooth yellowish chorion. As in *R. xenopalpus*, its maximum diameter was just behind its anterior end, and it tapered its full length to a narrowly rounded rear.

Two females were dissected, and each had three ovarioles per ovary and one mature oocyte (fig. 7). One mature oocyte measured 1.33 mm long, and 0.40 mm in maximum diameter; the other was 1.45 mm long and 0.40 mm in maximum diameter; hence both oocytes were somewhat larger than the single deposited egg (table 1). When the length of each mature oocyte was divided by the distance between the outer rims of the tegulae of the female from which it was extracted, the following values of the egg index were determined: 0.73 and 0.75, yielding an average of 0.74. This value is almost at the boundary (i.e., 0.75) between "small" and "medium" categories of Iwata and Sakagami's (1966) classification of oocyte size relative to body size of the female.

BIOLOGICAL COMPARISONS

Rozen's (1989) account of the nests of the Brazilian *C. anomalus* enables a comparison of the nesting biologies of *R. xenopalpus*, *R. mimus*, and *C. anomalus*. Those features that seem to be more significant are boldfaced, as follows: ***Rhophitulus xenopalpus*** **and *C. anomalus* are communal, and the abundance of adults at the nest site of *R. mimus* suggests that it may also be communal; all three species are bi- or multivoltine.** Also, **all are ground nesting,** although, as indicated in table 1, *R. xenopalpus* may have nests that tend to be shallower than those of the other two. Both *R. xenopalpus* and *C. anomalus* nest in more or less level surfaces, but nests of *R. mimus* were at the base of a low embankment with some entrances also on the vertical surface of the bank. **Main tunnels of all remain open and tend to descend vertically.**



FIGURES 7–10. Diagrams of immatures of *Rhophitulus* and *Cephalurgus*, lateral views, anterior ends to the left. **7.** Mature oocytes of *Rhophitulus mimus*. Scale = 0.5 mm. **8.** Postdefecating larva of *Rhophitulus xenopalpus*. Scale = 1.0 mm. **9.** Postdefecating larva of *Cephalurgus anomalus*. Scale = 1.0 mm. **10.** Pupa of *Rhophitulus xenopalpus*. Scale = 1.0 mm.

Cells are arranged singly, not in linear series or clusters. Although **brood cells of all three species tend to be horizontal**, those of some *R. xenopalpus* tilt somewhat more than 45° to the rear. However, **all three species have distinct cell linings,** although those of *C. anomalus* were more reflective than any of the others (perhaps because the soil was finer). In all, **the lining was a distinct film,** not merely a water-retardant surface, as determined by cell linings preserved on museum specimens even now. **Provisions are formed as a slightly flattened, uncoated sphere.** Each species seemed to collect provisions from single plant species, but the plants belong to different families, as follows: *R. xenopalpus–Heliotropium* (Boraginaceae); *R. mimus*–yellow composite (Asteraceae); *C. anomalus–Sida* (Malvaceae). **Eggs (fig. 7) of the three species were slender, curved, and broadest at the anterior rounded end tapering to a narrower round posterior end.** The egg of *C. anomalus* was attached to the provisions by both its anterior and posterior ends whereas eggs of both *R. xenopalpus* and *R. mimus* were attached only by their rear ends. However, there is a possibility that eggs at first are attached only by their posterior ends but during hatching slowly bend downward eventually touching the provisions by their anterior ends. If this is the case, one must determine whether the egg of *C. anomalus* was close to hatching. Inner surfaces of cell closures consist of loosely compacted, moderately concave spirals of 3–4 coils to the radius. None of the species are attacked by cleptoparasites.

None of the features itemized above distinguishes *Rhophitulus* and *Cephalurgus* from other Protandrenini about which we have biological information. Indeed, such matters as the flattened provisions seem to be characteristic of the tribe (Rozen, 1967: table 2, all *Psaenythia* and *Pseudopanurgus* species which are now in the Protandrenini). Some of the less reported phenomena, such as communal nesting habits and multivoltinism, have recently also been reported for *Psaenythisca* (Ramos and Rozen, 2014).

MATURE LARVA AND PUPA OF *RHOPHITULUS* AND *CEPHALURGUS*

Methods and techniques used in preserving, storing, and examining immature stages of the species treated here are as described in Ramos and Rozen (2014).

Material Examined: *Rhophitulus xenopalpus* Ramos: 18 mature larvae, 7 male, 8 female pupae: Argentina: Tucumán Province: 11 km north of El Cadillal, December 3, 1989 (J.G. Rozen); 1 larva: Tucumán Province: 12 km north of El Cadillal. Coll. as young larvae, November 9, 1989, preserved as last instars Nov. 15, probably not mature (J.G. Rozen).

Rhophitulus mimus: 8 mostly predefecating larvae, 1 early instar: Argentina: Salta Province: 18 km east of Cachi, March 22, 1990 (J.G. Rozen, A. Roig).

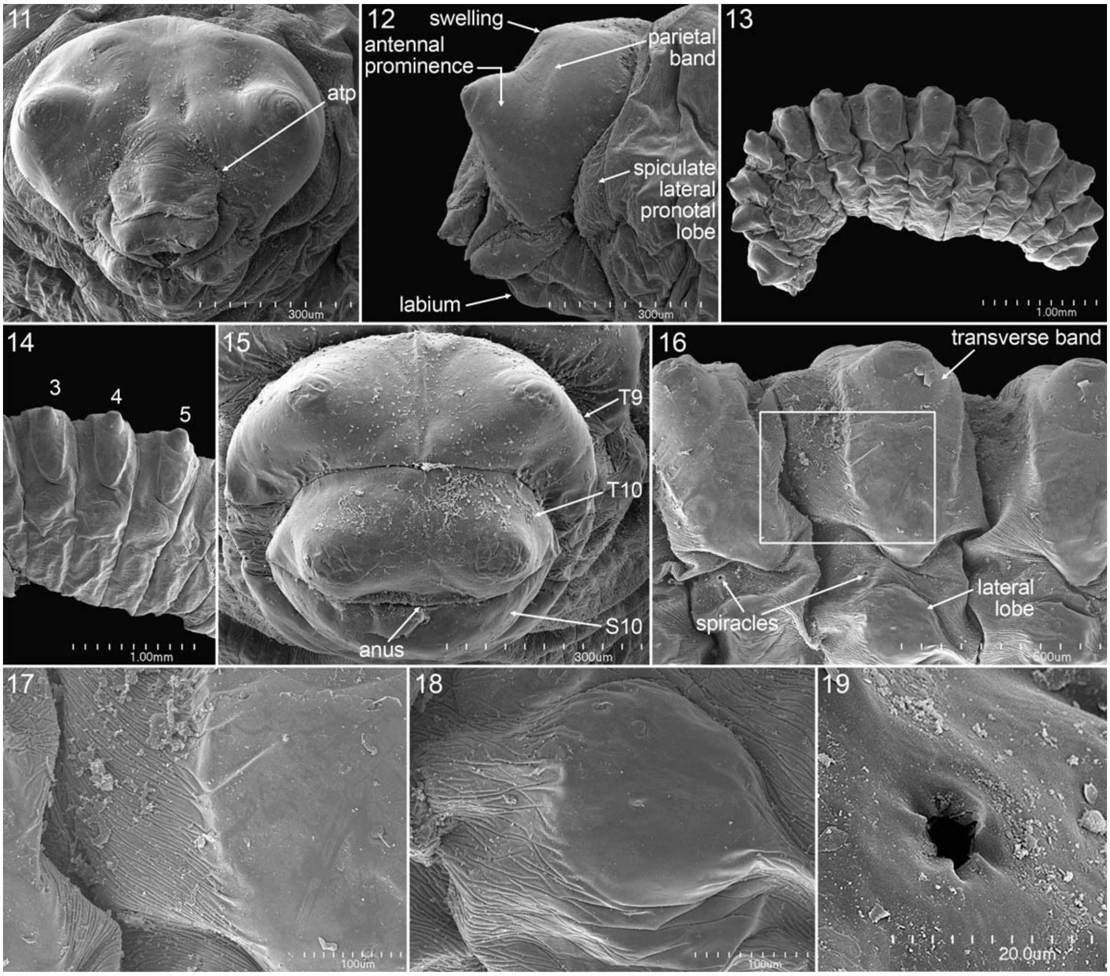
Cephalurgus anomalus Moure and Lucas de Oliveira: 1 postdefecating, 10 predefecating larvae, 1 pupa:[3] Brazil: São Paulo: Cosmópolis, I-27, 28-1974 (J.G. Rozen, F.C. Thompson).

Mature Larvae of Rhophitulus and Cephalurgus

The following description is based on mature larvae of *R. xenopalpus*, *R. mimus*, and *C. anomalus*. Comparisons with other Protandrenini are based on published descriptions of the immature stages of the following species (current names in boldface): ***Protandrena* (*P.*) *verbesinae* Timberlake** (Rozen, 1970, as *Psaenythia bicolor* Timberlake); ***Parasarus atacamensis* Ruz** (Ruz and Rozen, 1993**); *Protandrena* (*Pterosarus*) *boylei* (Cockerell)** (Rozen 1966, as *Pseudopanurgus boylei*); ***Protandrena* (*Pterosarus*) *occiduus* (Timberlake)** (Rozen, 1966, as *Pseudopanurgus* species A); ***Anthemurgus passiflorae* Roberston** (Neff and Rozen, 1995); ***Pseudopanurgus* (*P.*) *aethiops* (Cresson)** (Rozen, 1966); ***P.* (*P.*) *verticalis* Timberlake** (Rozen 1966, as *P.* species B); and ***Psaenythisca wagneri* (Vachal)** (Ramos and Rozen, 2014). The larva of *Neffapis longilingua* Ruz was originally described and placed in Protandrenini by Rozen and Ruz (1995), but the genus was subsequently moved to its own tribe (Ascher in Engel, 2005).

Diagnosis: Mature larvae of *Rhophitulus* and *Cephalurgus* are similar to those of most other Protandrenini in having a strongly projecting antennal prominence mounting a moderately large antennal papilla (figs. 12, 27), a strongly projecting maxilla with a large, conical palpus (figs. 12, 27), a recessed labial region contrasting with the enlarged maxilla (figs. 12, 27), and a mandible with a large subapical tooth along the dorsal apical mandibular edge (figs. 37–44). The robust body form and deeply recessed intersegmental lines are also characteristic of many, as are a pair of tuberclelike swellings (figs. 11, 12, 21) above and mesad of the antennae that in *C. anomalus* become tuberclelike (fig. 27), imparting in that species a distinct projection to the lateral profile of the upper part of the head.

One feature that seems to distinguish these two genera from all others is the terminal dorsal bilobed condition of abdominal segment 10 (figs. 15, 29) that appears to more or less duplicate the dorsal paired tubercles of the preceding segments. In these genera as well as some others (such as *Pseudopanurgus*), there is a pair of distinct dorsal tubercles on abdominal segment 9 (figs. 8, 9, 13, 28, 29) although absent in larvae of many other genera. Other protandrenine genera have abdominal segment 10 terminating as a simple curved surface, but at least some *Pseudopanurgus* have a single, median, upward-projecting, rounded tubercle. Another

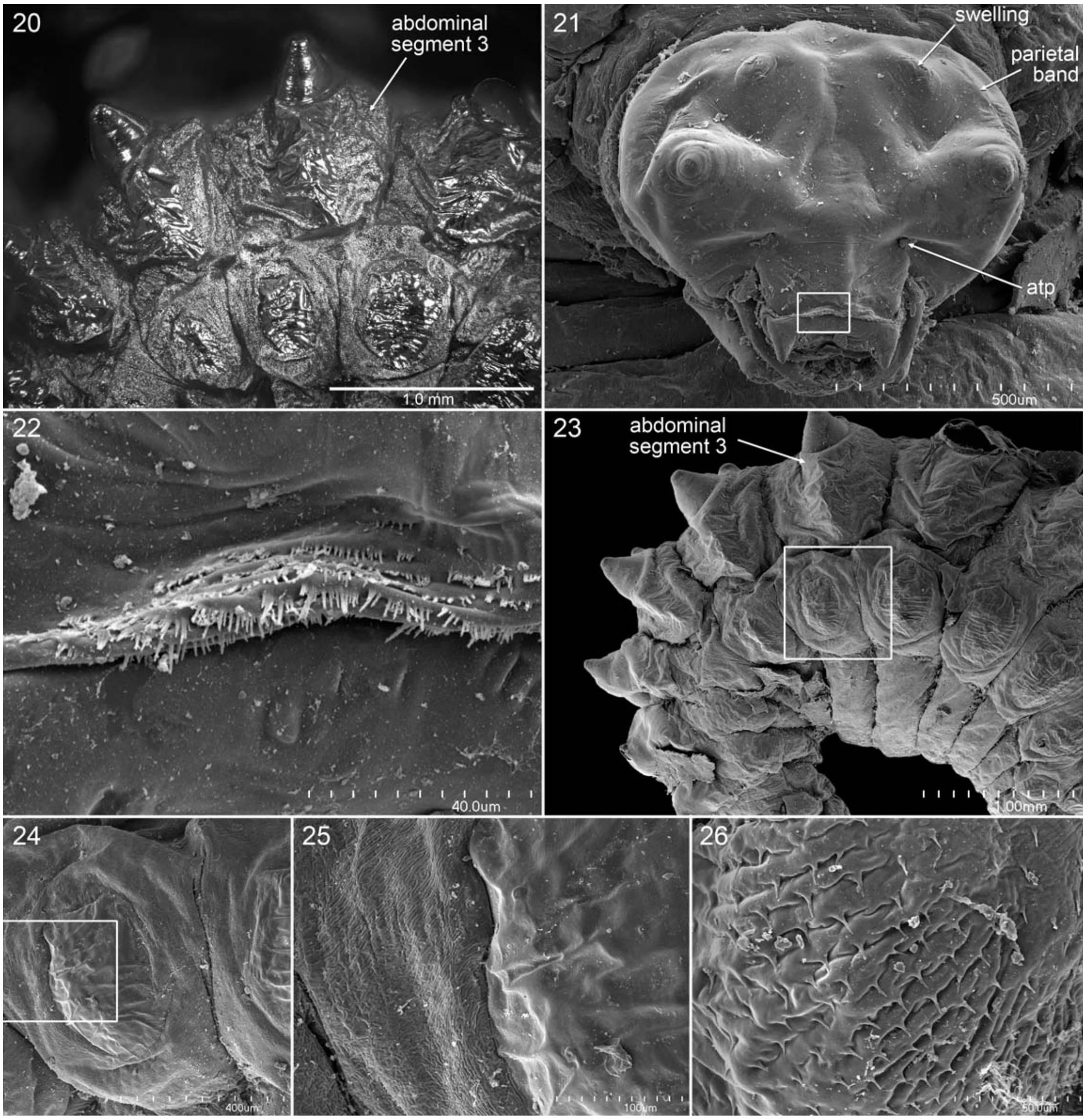


FIGURES 11–19. SEM micrographs of postdefecating larva of *Rhophitulus xenopalpus.* **11, 12.** Head, frontal and approximate lateral views, respectively; atp = anterior tentorial pit. **13.** Entire larva, lateral view. **14.** Abdominal segments 3–5 of another larva, lateral view, demonstrating pronounced dorsal sclerites mounting paired dorsal tubercle. **15.** Posterior end, showing paired low dorsal swelling on T10, posterior view. **16.** Abdominal segment 3, left side, demonstrating apical texture of paired dorsal tubercle and distinction between the integument of transverse sclerotic bands and wrinkled membranous integument separating bands of one segment from those of preceding and following segments. **17.** Close up of rectangle of figure 16 demonstrating texture of wrinkled membranous area and firmer smooth area. **18.** Close up of lateral lobe and surrounding area of abdominal lobe of abdominal segment 3. **19.** Spiracle of abdominal segment 8, demonstrating lack of raised atrial rim.

unusual larval feature of the three species treated here is the absence of an elevated atrial rim on all spiracles (figs. 19, 30).

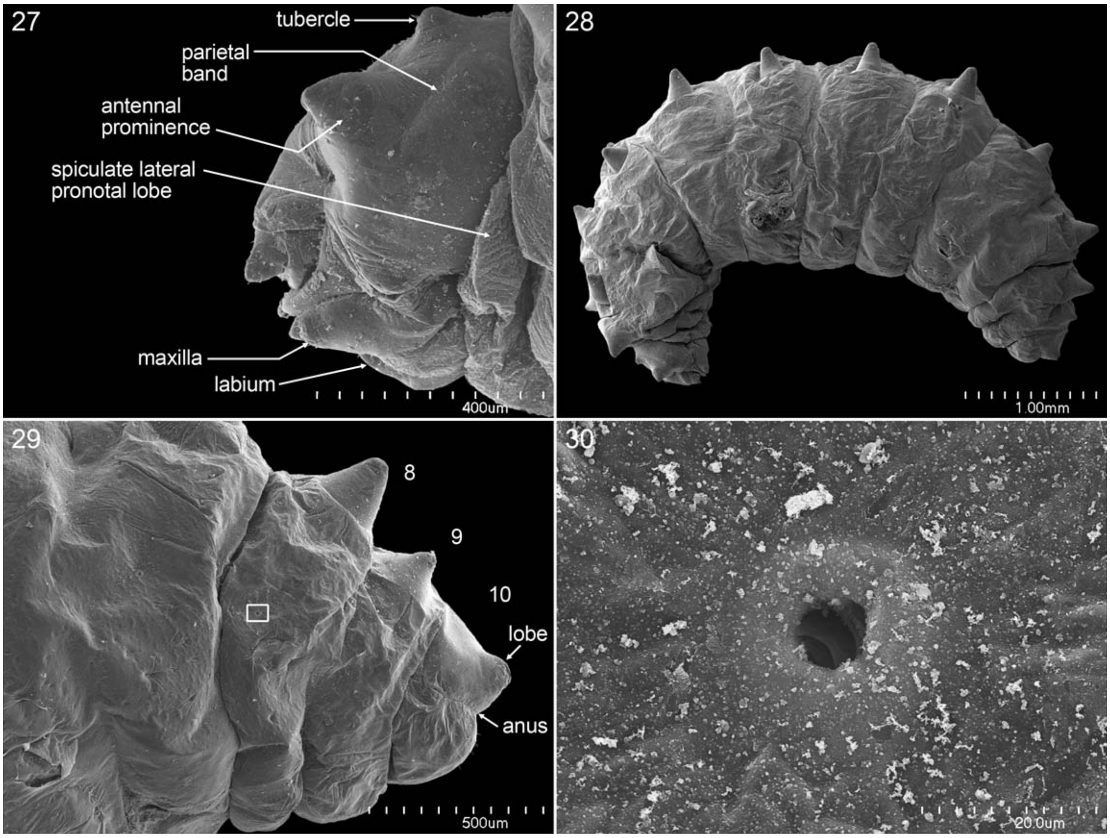
Description: **Head:** Integument with scattered, minute sensilla some of which are finely setiform; articulating membrane connecting lower clypeal margin and labrum with dense patch of transverse linear rows of minute sharp-pointed spicules, visible with SEM (fig. 22); dorsal surfaces of maxilla and upper part of hypopharynx densely spiculate; epipharynx with some spicules.

Head size (figs. 8, 9) of postdefecating larva moderate compared with body size (because predefecating larvae of all three species tend to have swollen abdomens, their heads and thoracic seg-



FIGURES 20–26. Macrophotograph and SEM micrographs of the predefecating larva of *Rhophitulus mimus*. **20.** Macrophotograph of abdominal segment 3 of larva coated with gold/palladium in preparation of SEM examination showing bright reflections of sclerotized parts and satiny sheen of minutely wrinkled membranous areas. **21–26.** SEM micrographs of same specimen: **21.** Head, frontal view; atp = anterior tentorial pit. **22.** Close-up of membrane identified by rectangle in figure 21, demonstrating linear series of fine spicules. **23.** Midsection of body of predefecating larva, lateral view identifying abdominal segment 3. **24**. Lateral lobe of abdominal segment 3. **25.** Close-up of rectangle in figure 24 demonstrating differences in texture of wavy surface texture of lobe and wrinkled integument anterior to it. **26.** Close-up of spiculate integument of lateral pronotal lobe.

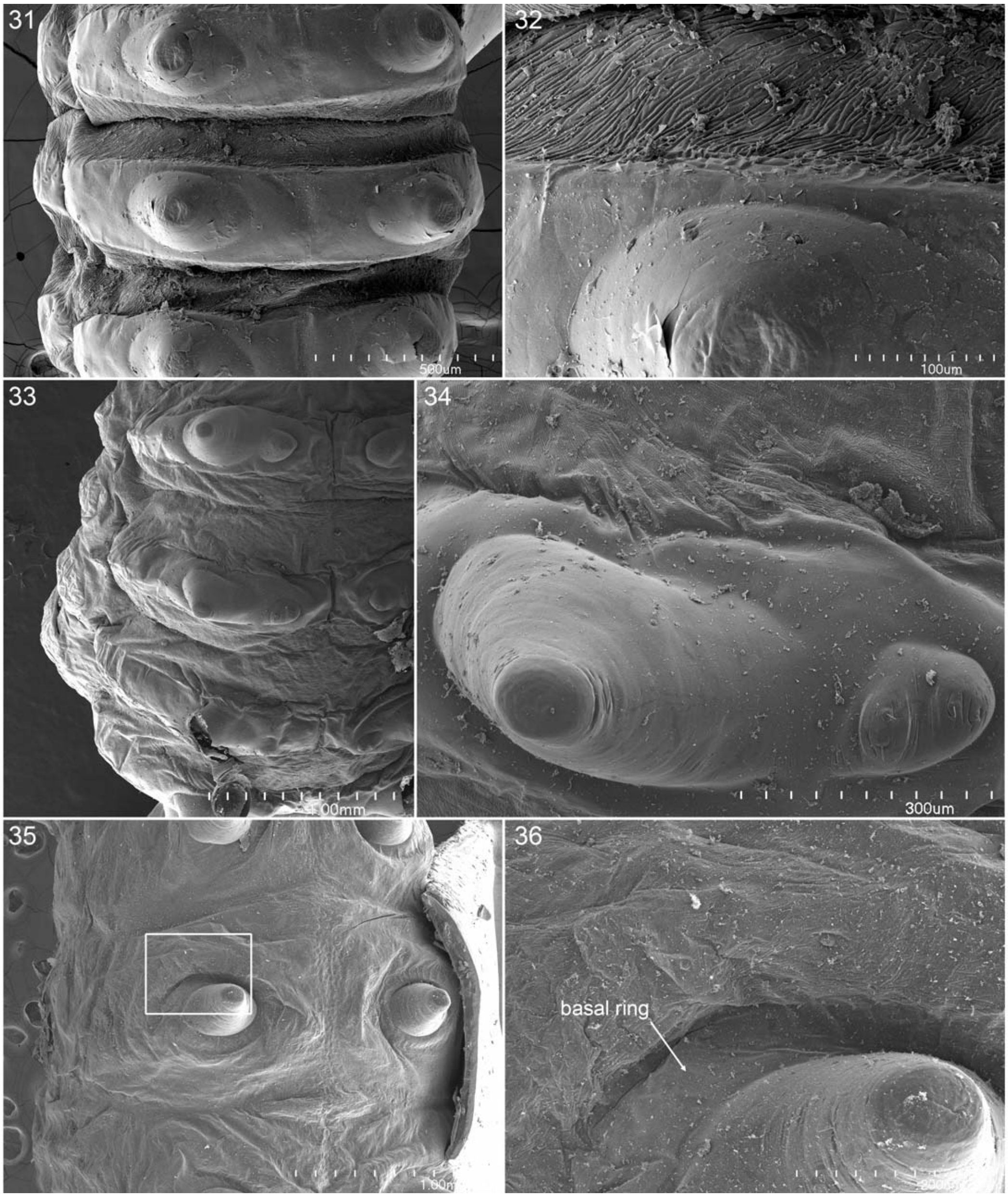
ments seem unusually small by comparison). Tentorium of predefecating larva complete, including dorsal arms, but not robust. Anterior tentorial pit close to anterior mandibular articulation in frontal view (figs. 11, 21); postoccipital, hypostomal, and pleurostomal ridges moderately well developed; dorsal ramus of hypostomal ridge absent; epistomal ridge between pits absent; median longitudinal



FIGURES 27–30. SEM micrographs of the predefecating larva of *Cephalurgus anomalus*. **27.** Head, lateral view. **28.** Entire larva, lateral view. **29.** Close-up of posterior end of abdomen. **30.** Close-up of spiracle of abdominal segment 8, identified by rectangle in figure 29.

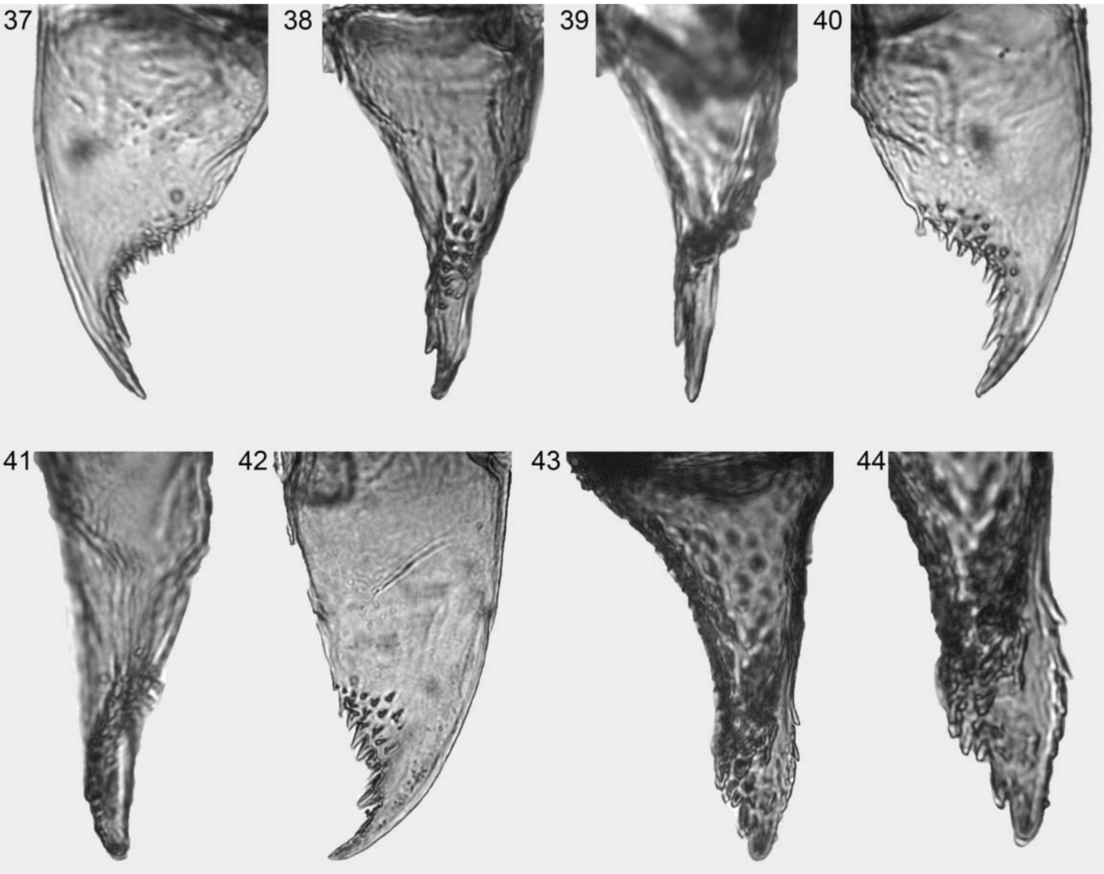
thickening of head capsule (coronal ridge) absent. Parietal bands faintly evident. Antennal prominence very strongly developed (figs. 12, 27), especially in *Cephalurgus anomalus*; antennal disc as seen on cleared specimen moderately small, its diameter distinctly less than distance from its outer rim to center of anterior tentorial pit in frontal view; antennal papilla conspicuous, unpigmented, strongly domelike to subconical, projecting about, or clearly more than, one-half basal diameter in profile view, bearing three to five sensilla (figs. 11, 12, 21, 27). Upper part of head in lateral profile (fig. 12) projecting, angled (in *C. anomalus*, fig. 27, upper part of head so modified that vertex appears to have paired, forward-projecting tubercles). With all three species (as well as with *Psaenythisca wagneri*) membrane connecting lower clypeal margin to base of labrum with numerous linear series of extremely fine spicules (fig. 22). Labrum projecting farther than clypeus in lateral view (figs. 12, 27), with pair of large sensilla-bearing tubercles.

Mandible elongate, in outer or inner views (figs. 38, 41, 43) tapering evenly to narrowly pointed apex; dorsal apical edge with large subapical tooth in addition to long row of smaller, sharply pointed teeth that broadens basally to become broad multidentate cusp (figs. 37–44); ventral apical mandibular edge smooth except for some conspicuous teeth in *Cephalurgus anomalus* (figs. 43, 44); dorsal mandibular surface sparsely to distinctly spiculate. Maxilla strongly projecting in lateral view (fig. 27), but labium recessed (figs. 12, 27); apex of maxilla projecting about as far as apex of labium



FIGURES 31–36. SEM micrographs with central row in figures 31, 33, and 35, each representing abdominal segment 3, dorsal views. **31.** *Rhophitulus xenopalpus* show transverse sclerotic band with paired dorsal tubercles. **32.** Same, close-up of integument on left side showing wrinkled texture of membrane and smooth texture of sclerite. **33.** *Rhophitulus mimus*, mostly left side, showing midline interruption of transverse tubercle and small rounded tubercle mesad of each paired tubercle. **34.** Same, close-up of tubercles on left side. **35.** *Cephalurgus anomalus*, showing basal sclerotic ring to each paired tubercle. **36.** Close-up of left tubercle and ring.

in lateral view; cardo evident as sclerite, articulating arm of stipital sclerite not evident, although hypopharyngeal groove extremely deep because of bulging hypopharynx above and bulging labial apex below; maxillary apex broad because of strong development along adoral side, with result that large, conical palpus arises from outer edge of maxillary apex. Labial pre- and postmentum scarcely



FIGURES 37–44. Microphotographs of cleared right mandibles of *Rhophitulus.* **37–40.** *Rhophitulus xenopalpus*, dorsal, inner, inner with apex in maximum profile, and ventral views, respectively. **41, 42.** *Rhophitulus mimus*, inner and ventral views, respectively. **43, 44.** *Cephalurgus anomalus*, inner view and close-up of apex, respectively.

distinguishable (premental sclerite not evident); labial apex only faintly bilobed, each lobe serving as base of small palpus. Labial lobes separated by small, median salivary lobe, around which is salivary opening. Hypopharynx protuberant, projecting forward about as far as labial apex in lateral view; hypopharyngeal groove strongly defined because of protuberant surrounding surfaces.

Body (figs. 8, 9, 13, 28): Integumental vestiture consisting of minute, widely distributed spicules and very fine setiform sensilla; only lateral pronotal lobes immediately posterior to posterior tentorial pits conspicuously spiculate with large sharply pointed spicules in all three species (figs. 12, 26); paired body tubercles lacking setae visible by stereoscope but with minute setiform sensilla visible with SEM (figs. 32, 34); these tubercles somewhat more sclerotized than elsewhere, and with extreme apices finely roughened (figs 15, 31, 32). Body form of predefecating larva robust; that of postdefecating larva obviously more linear; dorsal intrasegmental lines not evident; **paired dorsal tubercles present on all thoracic segments and abdominal segments 1 to 9 (figs. 8, 9, 13, 28); abdominal segment 10 apically bilobed (figs. 13, 29), these lobes presumably serial homologs of preceding paired dorsal body tubercles;** paired dorsal tubercles of metathorax and abdominal segments 1–6 tapering to narrowly rounded apex but varying in height according to species; abdominal segment

9 not produced ventrally (figs. 8, 9, 29); abdominal segment 10 positioned medially on segment 9 as seen in lateral view (figs. 8, 9, 13, 28, 29); anus apical on segment 10 in lateral view (figs. 8, 9, 29). Spiracles (figs. 19, 30) difficult to study because of small size, lack of pigmentation, and absence of atrial rims so that peritreme is continuous with body surface (although in some cases slightly bulging); on *R. xenopalpus* and *R. mimus* first pair of atrial openings on thorax moderately small and those of following segments decreasing in size so that those of abdominal segment 8 with diameter of paired atrial opening about one half of that of first thoracic pair; on *C. anomalus* opening apparently more uniform in size; **for all three species atrium without elevated rim,** globular; peritreme narrow but difficult to evaluate because of continuation with body surface; atrial wall smooth; primary tracheal opening with collar; subatrium with perhaps 12 chambers, externally tapering from body surface inward. Sex recognition characters unknown.

Discussion of Larval Features: Larval *C. anomalus* can immediately be distinguished from *R. xenopalpus* and *R. mimus* because of the dentate lower apical edge of its mandible (figs. 43, 44). Unlike those of *R. xenopalpus* and *C. anomalus*, the larva of *R. mimus* has a small rounded tubercle immediately mesad of the paired dorsal tubercle of the midbody segments (figs. 33, 34). Furthermore, *C. anomalus* can be distinguished from the other two because the slight paired swellings on their larval vertexes (figs. 11, 12, 21) are replaced by conspicuous paired tubercles on the vertex of *C. anomalus* (fig. 27).

The scanning micrographs of the larvae of two species of *Rhophitulus* and *C. anomalus* have enabled us to see unexpected features, some of which reinforce our perception of the relationships among these species. For example, the linear series of fine spicules on the membrane connecting the labrum to the lower end of the clypeus found in all three and also in *Psaenythisca* suggests a strong relationship of the three protandrenine genera (figs. 21, 22). Also the wrinkled, callouslike apices of the paired dorsal tubercles of the three species demonstrates their close relationship to one another, as does the lack of atrial rims on their spiracles.

At the same time, SEM studies have demonstrated some surprising differences regarding integumental features that were not apparent when examining larvae with stereomicroscope either before clearing or after clearing and staining. Illustrations of the mature larvae of *R. xenopalpus* (fig. 8) and *C. anomalus* (fig. 9) were made with stereomicroscope and camera lucida before SEM examination, and while giving a reasonable impression of the outline of the larvae, they overlook contours of surfaces probably because white, faintly translucent surfaces presented at different angles cannot easily be distinguished from one another, and fine texture differences of these white surfaces are obscured. Certainly figures 8 and 13, both of a postdefecating larval *R. xenopalpus*, are markedly different from one another.

SEM micrographs (figs. 13, 14, 16, 20, 23, 28, 31, 32, 35) show clearly that body integument is far more extensively sclerotized than previously thought. These sclerotized regions do not readily stain much more darkly than membranous areas, perhaps because the sclerotized areas may not be substantially thicker than membranous areas. However, they are more rigid and can impart shape to sclerites. Using larval abdominal segment 3 of *Rhophitulus xenopalpus* as an example (figs. 13, 14, 16), one sees that the paired dorsal tubercles rise from a curved smooth sclerotic band that stretches across the dorsal body surface and terminates on each side just before the spiracle (fig. 16). Similar bands are easily identified on most other body segments (figs. 13, 14). A somewhat similar band occurs on most body segments of *R. mimus* except here it is interrupted along the larva's midline, so each paired dorsal tubercle appears to rise from a separate transverse sclerite (fig. 33). Close-up micrographs show that the smooth texture of the sclerite contrasts with the finely wrinkled membrane surface (fig. 34).

Further exploration of body surface using SEM micrographs reveals that the lateral lobes of midbody segments are also sclerotized. On *R. xenopalpus* (figs. 16, 19) this was apparent only from SEM examination. However, in the case of *R. mimus,* simply coating the specimen with gold/palladium provided enough contrast between the shiny, smoother though nodular sclerotized surface and the duller satiny sheen of membrane by light reflection (i.e., macrophotography, fig. 20).

The presence of body sclerites in the case of *C. anomalus* is less understood. As revealed by SEM examination of a predefecating larva (figs. 28, 29, 35, 36), only the paired dorsal tubercles and a small ring around each of their bases seemed to be sclerotized. The single postdefecating specimen collected was cleared before the discovery of the sclerites on the other species.

Because the presence of body sclerites of these taxa were discovered before the study of *Psaenythisca* went to press (Ramos and Rozen, 2014), its pre- and postdefecating larvae were reexamined with an SEM and found to lack sclerotic surfaces of the lateral lobes, and the dorsal body tubercles were sclerotized and their bases was surrounded by basal rings (Ramos and Rozen, 2014: fig. 77). This would seem to be a surprising similarity with the condition found in the mature larva of *C. anomalus* (fig. 29), made more surprising by the fact that spiracular atria lack rims in known *Rhophitulus* and *Cephalurgus* but have distinct rims in *Psaenythisca wagneri.* Variability of this feature among Protandrenini needs analysis because it brings into question the phylogenetic relationships among the included taxa.

Although most descriptions presented above refer to abdominal segment 3 and to the other main body segments, the thoracic segments are different and have yet to be explored. Also yet to be determined is how these sclerites and paired dorsal tubercles on all body segments function in the life of these larvae?

Pupa of Rhophitulus

The following treatment is primarily of pupal *Rhophitulus xenopalpus* since there is but a single pupal specimen of *Cephalurgus anomalus* and none of *R. mimus.* The description excludes consideration of coxal and trochanteral tubercles, because to be accurate legs would need to be detached, thereby damaging the specimens for future study. The format follows that of Yager and Rozen (1966), and the terminology for head tubercles is that of Neff and Rozen (1995).

Diagnosis: Pupae of the following protandrenine species have been described earlier: *Protandrena verbesinae* (Rozen, 1970, as *Psaenythia bicolor*), *A. passiflorae* (Neff and Rozen, 1995), and *P. wagneri* (Ramos and Rozen, 2014). Although differing considerably in body size, they are quite similar structurally; all have the same arrangement of head tubercles.

Female Pupa

Figure 10

Description: **Head:** Pedicle with rounded tubercle on outer surface. Ventral surface of mandible without tubercle near base. Vertex and upper part of frons with paired median vertical tubercles just mesad of lateral ocellar tubercles; lateral vertical tubercles smaller than median vertical tubercles; median ocellus with small tubercle; upper frontal tubercle large, conspicuous, apparently bilobed; each side of frons with chain of more or less distinct tubercles extending from below lateral ocellus toward antennal socket; area between inner orbit and antennal socket verrucose; gena with small tubercles.

Mesosoma: Posterior margin of pronotum including lateral angles somewhat produced and verrucose. Lateral angles and posterior lobes of pronotum not produced; mesoscutum without tubercles, indistinctly verrucose; mesoscutellum with pair of dorsally projecting tubercles; axillae not swollen; metanotum swollen medially. Mesepisternum without tubercle. Tegula with small, distinct tubercle. Outer surface of forewing particularly at base uneven, but without distinct tubercle. Hind tibia close to base with moderately small, rounded tubercle on outer surface; tibia expanding toward apex (accommodating developing adult scopa and spurs); hind basitarsus with elongate tapering apical tubercle (accommodating developing adult setae).

Metasoma: Terga 1–5 each with subapical row of low, small, tubercles some of which bear small acute apex without spine. Terminal spine moderately short, apically rounded.

Male Pupa

Description: As described for female except metasomal terga 1–6 each with subapical row of small tubercles and T7 with a few apical tubercles. In addition, the hind tibia more slender and the hind basitarsus lacking apical tapering tubercle on outer surface. Male antenna considerably longer than that of female.

Remarks: As pointed out by Ramos and Rozen (2014) the combination of paired median and lateral vertical tubercles and paired upper frontal tubercles, in addition to ocellar tubercles exhibited now by representatives of four Protandrenini genera (*Anthemurgus*, *Cephalurgus*, *Psaenythisca*, and *Rhophitulus*), appears to be a tribal characteristic.

In addition to the pupae of *R. xenopalpus*, a single male pupa of *Cephalurgus anomalus* was available. Although substantially larger than those of *R. xenopalpus*, it differed little from them in pupal features, except its metasomal T7 lacked tubercles.

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