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First Observations on Nesting and Immatures of the Bee Genus *Ancyla* (Apoidea: Apidae: Apinae: Ancylini)

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ABSTRACT

Herein we present information on the nest architecture and nesting biology primarily of *Ancyla asiatica* Friese and, to a lesser extent, of *A. anatolica* Warncke, both found near Adana, Turkey. These two ground-nesting species visit Apiaceae for mating and larval provisions, with *A. asiatica* going to *Daucus carota* and *A. anatolica*, to *Eryngium*. The cocoon of *A. asiatica* is described in detail as are the mature oocytes of both species and the pre- and postdefecating larvae of *A. asiatica*. Each site was attacked by a separate, unnamed cleptoparasitic species of *Ammobates* (Nomadinae). The relationships of the Ancylini to other apine tribes are discussed based on their mature larvae, and a revised tribal key to mature larvae of nonparasitic, noncorbiculate Apinae is presented.

INTRODUCTION

Of all tribes of nonparasitic Apidae, the natural history of the Ancylini³ has been the least investigated. Until now, nothing has been recorded concerning the nests of any species,

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³ The spelling of tribe Ancylini Michener, 1944, has recently been emended to Ancylini to remove homonymy with Ancylini Rafinesque, 1815 (Mollusca, Gastropoda) (Engel et al., 2010: ICZN Ruling (Opinion 2246-Case 3461)).

and their immature stages have been completely unknown. We therefore offer the first accounts of the nesting ethology of two species, *Ancyla asiatica* Friese and *A. anatolica* Warncke, and describe the last larval instar of the former. Nesting sites of these species were found within a kilometer of one another near a large impoundment referred to by the name of the dam creating it, Seyhan Baraji, northeast of the city of Adana in southern Turkey. As noted below, the nesting cycles of the two were not synchronized.

In 2011 J.S. traveled to southern Turkey where he and his party discovered the two species nesting. In the first part of this paper we present information about the nests, the adult and larval activities associated with nesting sites, and the cleptoparasitic genus *Ammobates* (Nomadinae), species of which attack the sites. The second part of the paper describes the mature oocytes of *Ancyla asiatica* and *A. anatolica*, treats the ovarian statistics of the two species, and includes the description of the mature larva of *Ancyla asiatica*, the first for any member of the Ancylaini.

According to Michener (2007) the tribe contains only the two Old World genera *Ancyla* and *Tarsalia*, although Baker (1998) had previously considered *Tarsalia* a basal genus in the Eucerini. In the discussion of this paper, we explore the phylogenetic relations of *Ancyla* with other apine tribes on the basis of the data we have uncovered. We look forward to the discovery of *Tarsalia* nests.

J.S. and his party provided all field observation reported here. He later sent the immatures and nest samples that they had collected to J.G.R. for preparation and study. J.G.R. contributed the descriptions of larvae and oocytes, investigated the ovarian statistics, and analyzed details of cocoons and cells from the samples.

METHODS AND TERMINOLOGY

In preparing the larva, J.G.R. separated head from body, cleared both parts in an aqueous solution of sodium hydroxide, rinsed them in water, and then transferred them to 70%–75% ethanol where he stained them with Chlorazol Black E. This imparted a bluish hue to reveal otherwise undetectable, slightly sclerotized areas on postcephalic integument and to better define details of the head. The specimens were then washed in ethanol and submerged in glycerin on a well slide for examination, illustration, and storage. Larvae and oocytes to be examined with a Hitachi S-4700 scanning electron microscope (SEM) were critical-point dried, mounted on stubs, and then coated with gold/palladium. The cocoon did not require staining or critical-point drying for SEM study; its damaged lower end (resulting from larva removal) was excised with scissors to reveal the inner surface of the upper end, which was then mounted on a stub and coated.

In the description and discussion of the larva, the long axis of the mandible is assumed to be horizontal with the mandibular apex to the left, so that the upper mandibular surface is dorsal and the lower surface ventral.

BIOLOGY

Ancyla asiatica Friese

J.S. discovered and studied the nesting area of *Ancyla asiatica* near the edge of the impoundment created by the Seyhan Baraji dam near the village of Karaömerli in Adana Province (N37°07'2.43" E35°20'10.28") from June 22 to 24, 2011. During this visit he observed adult behavior and surveyed the area for nest entrances, marked some, and excavated the first nest to confirm the identity of the bee. Males were commonly seen either sitting on umbels of the host plant *Daucus carota* (Apiaceae⁴; fig. 3) or patrolling from one umbel to another, presumably looking for females. Females were less abundant on umbels but could occasionally be observed gathering provisions from them. No observations of mating were made nor was there evidence of males fighting one another. Both current and old nests were widely scattered in grassy areas of an abandoned cornfield that was used mostly by *Ancyla asiatica* but also served as nesting areas of *Eucera*, *Megachile*, and other bees (fig. 1). During the few days of observation, males of *A. asiatica* became active about two hours earlier in the morning than females, but exact times depended on temperature and other weather conditions. J.S. returned on July 9 and 10, 2011, to excavate nests previously marked; females were still active but less abundant.

The *Ancyla* nests were widely scattered. J.S. discovered and examined six nests of *A. asiatica*. Most occurred on flat or slightly sloping surfaces, but one was found on a 45° slope, an indication that slope is probably not a limiting factor for nesting in this species. The substrate was very hard, dried clay soil (as was also the case for *A. anatolica*). Most nest entrances and most nest-searching females were in partial shade, but nests were also found in full sun. They occurred on bare ground, such as field paths, field margins, and cleared cornfields.

When fresh, entrances were surrounded by tumuli (fig. 2); later, just a rounded entrance remained on the soil surface. Nest burrows were single tunnels extending almost vertically downward 7–10 cm, following a slightly sinuous path. At the bottom of the burrow cells were arranged end to end in simple linear series (figs. 4, 5) of up to three cells, with long axes tipped between 45°–90°, front end higher. Old cocoons recovered while digging recent nests suggest a maximum of four cells to a series.

Cell shapes and dimensions are easily interpreted from cocoons (fig. 7). Those preserved in fluid and containing mature larvae had a maximum diameter of 6.6 mm at about one-third of the way from the rear and a maximum length of 10.0–10.5 mm ($N = 2$). They obviously conformed to the shape of the cell because their outer surface contained soil particles from the cell wall. At the closure end, the cocoon is sharply truncated, with the plane of truncation slightly tilted from 90° along the long axis of the cell (fig. 6) in these two representatives.

⁴ In response to a reviewer's requesting information as to whether *Ancyla asiatica* is an oligoleg of *Daucus*, we dissected the midgut content of a larva that had not completed defecation. The entire sample retrieved consisted only of *Daucus* pollen. Such information supports (though does not prove) the bee's oligolectic status.



FIGURES 1–3. Nesting area of *Ancyloasiatica asiatica* near the edge of the impoundment created by Seyhan Baraji [dam] near the village of Karaömerli in Adana Province, Turkey. 1. Grassy nesting area of *A. asiatica*, *Eucera*, and *Megachile* with arrows pointing to positions of two nests of *A. asiatica* that were studied. 2. Close-up of nest entrance of tunnel of *A. asiatica*. 3. Male of *A. asiatica* on umbel of *Daucus carota*.

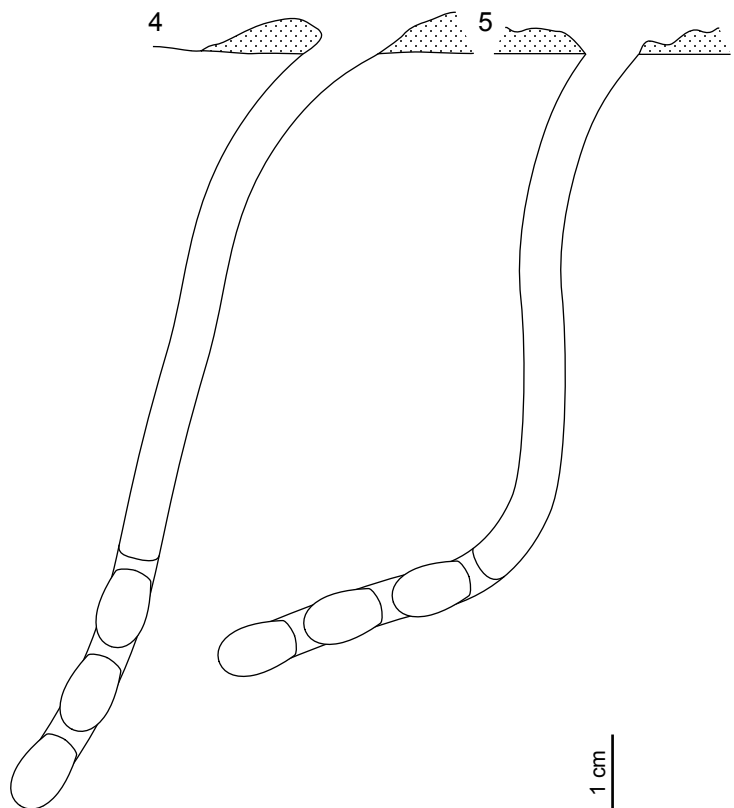
Cell walls before provisioning have satiny reflective surfaces with a faint, elongate ripple texture (figs. 8, 9) that parallels the long axis of the cell. This appearance is the result of a thin, clear, colorless coating that closely adheres to the surface but that can be peeled (arrows). Small stones can easily be observed through this coating and some larger pebbles actually intrude part-way into the cell. The coating extends to the cell mouth where it fades as it meets the somewhat rougher surface of the side tunnel. When tested with water droplets, water beads on the surface and is not absorbed by the cell wall, but a droplet is slowly absorbed on the side tunnel surface. Interestingly, a water droplet placed on the broken surface of substrate was immediately absorbed, suggesting that the burrow wall is somehow modified by the bee's manipulations. The source of the coating material on cell walls is unknown but obviously is applied by the female as part of cell construction and likely stabilizes cell humidity.

Ancyloasiatica asiatica larvae start spinning cocoons after consuming provisions but before onset of defecation. Cocoons available for study consisted of several preserved in fluid, one dry cocoon from which a larva had been extracted, and two cell fragments with cocoons undergoing early construction. From this limited material, we present first a preliminary description of the main part of the cocoon followed by a more detailed, though still provisional, understanding of the cell closure and the front end of the cocoon. In cocoons preserved in fluid, material that imparts

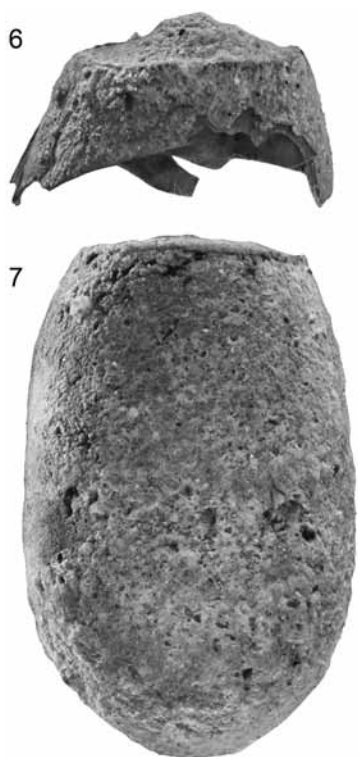
rigidity to the cocoon fabric was a semitransparent brownish, plasticlike outer layer almost certainly produced by the silk gland because one preserved larva had a strand of this distinctively colored material extruding from the salivary opening when it was preserved. The cocoon wall formed by this silk was nonfibrous when viewed with transmitted light, and fibers could not be detected along broken edges. This silk adhered to the earthen cell wall, and soil particles crusted the cocoon after it was extracted from the soil. After constructing the outer cocoon layer, the larva then applied fecal material to its inner surface up to, but not including, the front end. No silk fibers were detected in the feces, an indication that silk emission was suspended during defecation. Afterwards, silk production commenced again, this time to provide the inner lining to the cocoon. At the front end of the completed cocoon where there were no feces, a thin webbing of fine, pale fibers occurred between the outer and inner cocoon layers.

The cell closure consisted of a soil plug, the inner surface of which was a low-relief, slightly concave spiral on the inside, consisting of about four coils to the radius (figs. 10, 13). A unique attribute was that the entire disclike closure either projected slightly into the cell behind it or shrank slightly, so that at its periphery a gap existed between it and the front of the cell viewed from the inside (fig. 10). When the larva spins a cocoon, this gap is filled

with silk (figs. 13, 14); thus the completed cocoon exhibits a distinct elevated rim (figs. 6, 11, 12) when removed from the substrate, as viewed from outside. It is unclear how the female constructs such a closure, but its function may have been revealed in two cells in which larvae were apparently starting cocoon construction by depositing the outer brownish cocoon layer to the closure end. In figure 10, the circular crack identifying the gap between the spiral closure and cell front end is clearly visible except at the top where a thin sheet of some secretion (silk?) had been applied (arrow). In the other cell (fig. 13), more of the plasticlike outer cocoon layer had been deposited, as



FIGURES 4, 5. Diagrams of two nests of *Ancyra asiatica*, side views. Scale = 1.0 cm.



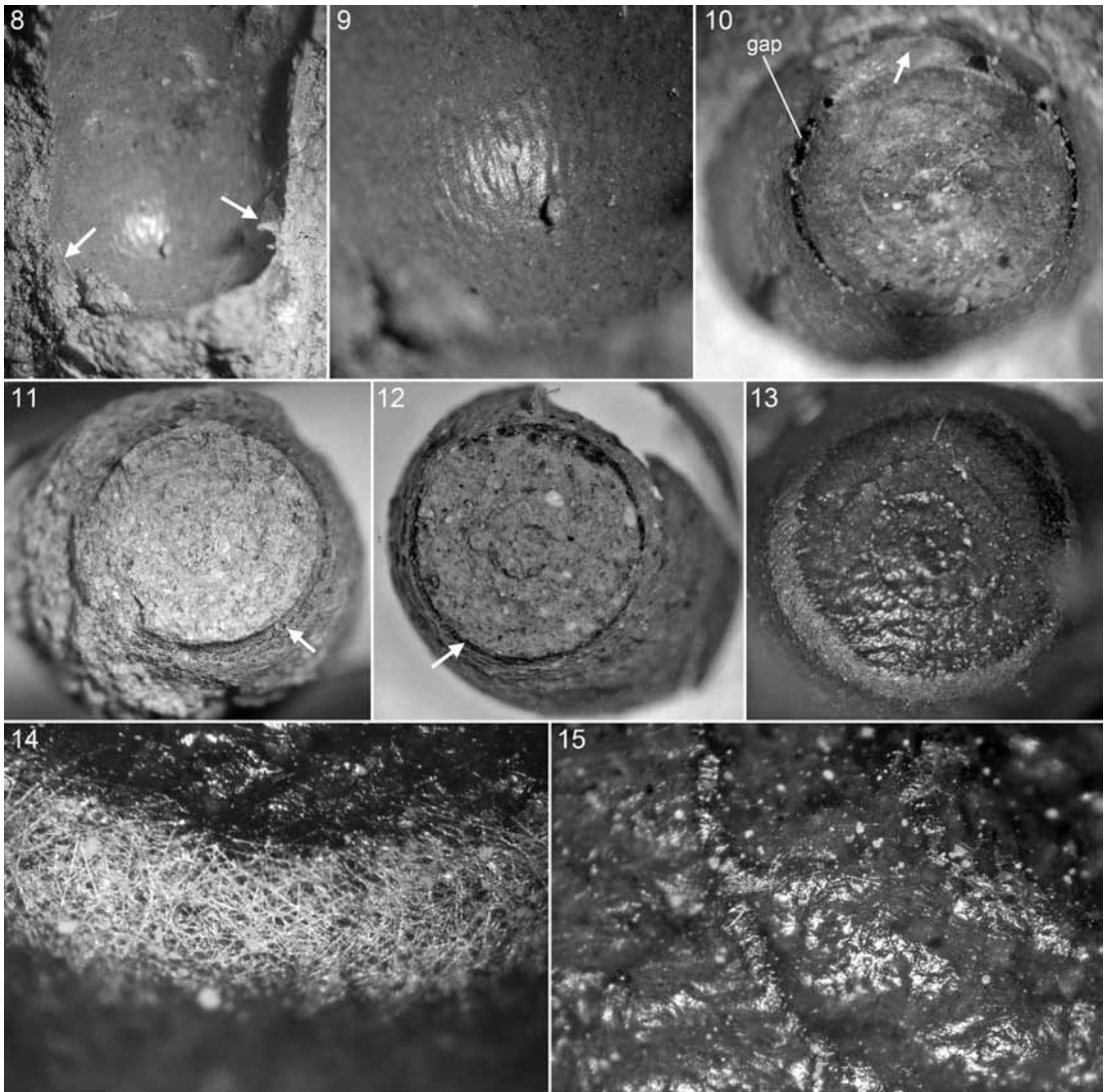
FIGURES 6, 7. Cocoons of *Ancyla asiatica*, both in lateral view. 6. Closure end of cocoon demonstrating slight convexity of cell closure. 7. Entire cocoon, except closure end partly destroyed, demonstrating cell shape.

indicated by the darker, shinier inner closure surface, and now a bright circular band of nearly white webbed fibers covered the crack. The crisscrossing fibers (fig. 14) are strongly reminiscent of cocoon fibers that are thought to exclude parasites from invading cocoons of many groups of bees while permitting air exchange between the outside and the cocoon atmosphere usually associated with the nipple end of cocoons (Rozen and Hall, 2011; Rozen, 2011a). Although initially we thought that this was an indication that gas exchange took place through the periphery of the closure, a close examination of the indented spirals of the closure (fig. 15) revealed shorter fibers there as well. Thus, any perceived indentation of the cell lining was probably treated with webbing.

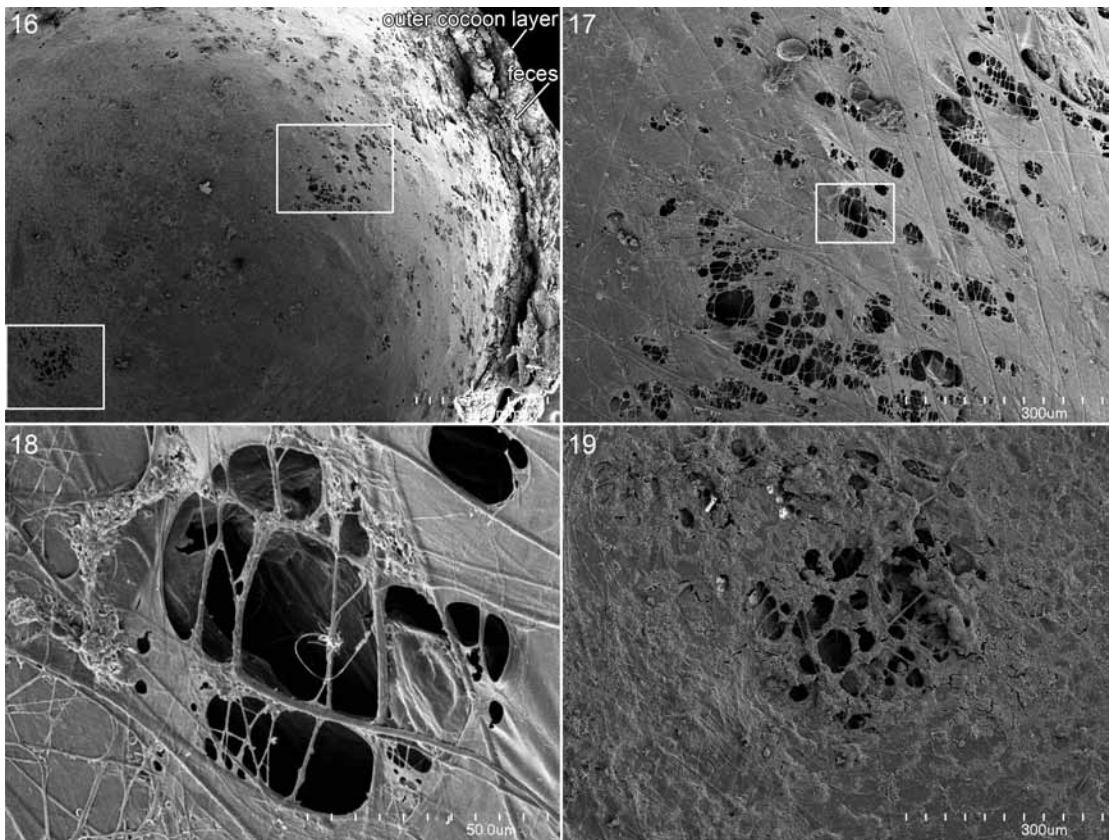
A completed cocoon that had been cut open to remove the larva had a pale, reflective, more or less clear, inner cocoon surface that appeared fibrous. SEM examination of the front inner surface (fig. 16) of this cocoon revealed a smooth surface with imbedded silk strands and random, widely scattered clusters of small and large openings. The thin surface that covers the silk strands (figs. 16–19) may also be silk or some other material that adheres to the strands. Before being prepared for SEM inspection, this material is transparent and colorless. We do not know whether the difference in color between silk of the outer layer of the cocoon and that of the inner layer resulted from the difference in thickness between the two, or whether the inner layer is derived in part from some other secretion.

The openings in the thin inner layer presumably allow for exchange of air through the cocoon. In many bee cocoons (e.g., *Anthophorula* (Apidae), Rozen, 2011a: figs. 58, 59; *Osmia* (Megachilidae), Rozen and Hall, 2011: figs. 27, 28) there is a single screened aperture centrally positioned on the inner cell lining behind the front end of the cocoon for air exchange. However, with cocoons of *Ancyla asiatica* air exchange seems to take place through the entire front end of the cocoon if not through the full length of the cocoon (we were unable to examine the cocoon fabric throughout the entire length of the cocoon).

Although air diffusion likely takes place through the apertures in the inner cocoon lining, we are uncertain how air is exchanged through the outer, rigid, plasticlike layer. The webbing of fine pale fibers between the two layers at the closure end could be interpreted as a screen, excluding parasites, suggesting that air penetrates either through or around the outer cocoon layer there (as demonstrated in Rozen and Hall, 2011). Future studies are required.



FIGURES 8–15. Microphotograph of cells of *Ancyła asiatica*. **8.** Lower end of cell before provisioning, with arrows pointing to transparent lining peeled from surface. **9.** Close-up of same showing satiny texture of surface. **10.** Inner view of closure end of cell showing gap around spiral closure. **11.** Exterior view of front end of cocoon with part of closure adhering to, and surrounded by, rim of cocoon (arrow). **12.** Same as figure 11 but with most of closure removed and rim more pronounced. **13.** Interior view of front end of cell showing spiral closure covered by dark cocoon material surrounded by band of white cocoon fibers covering gap between closure and front end of cocoon. **14.** Close-up of same showing fibers. **15.** Close-up center of closure showing white fibers between coils.



FIGURES 16–19. SEM micrographs of inner surface of front end of cocoon of *Ancyla asiatica*. **16.** Long shot of front end. **17.** Close-up of cluster identified by upper rectangle in figure 16. **18.** Close-up of openings identified by rectangle in figure 17. **19.** Another cluster identified by lower rectangle in figure 16, to same scale as figure 17.

Ancyla anatolica Warncke

On his return trip to the area on July 9 and 10, 2011, J.S. discovered an active, dense population of adult *Ancyla anatolica* at a location (N37°06'36.5" E35°20'15") somewhat less than 1 km away from the *A. asiatica* nesting area. This species foraged on the blue-flowered umbels of *Eryngium* (Apiaceae) plants, which were growing among sparse, tall grass in a picnic area. Males patrolled the *Eryngium* and also searched the ground, presumably for females. J.S. did not find nest entrances on the earth surface, but he observed numerous females entering long cracks in the hard, dry, fine-grained soil, strongly indicating that unlike *A. asiatica*, this species normally nests in cracks. Because he observed females disappearing into the fissures in different places, he concluded that the species is likely not a communal nester. Because of limited time and the hard nature of the soil, he was unable to precisely dissect nests, but from soil associated with two nests in cracks, he noted that cells occurred

at the depth of about 10 cm, they were in close proximity to one another, and their orientation was tilted between 45°–90°, as in *A. asiatica*. He learned that stored provisions were blue as was scopal pollen on female legs.

PARASITISM

After completing the fieldwork J.S. compared specimens of *Ammobates* (Nomadinae: Ammobatini) collected at the two nesting sites of *Ancyla asiatica* and *A. anatolica* with material from Warncke's collection in the Max Schwarz collection in Linz, Austria, and with specimens associated with *Ancyla holtzi* Friese that J.S. had collected on a previous trip to Turkey. He concluded that the nesting sites of the two species were attacked by separate species of *Ammobates*, both of which are unnamed but related to *Ammobates* (*Ammobates*) *biastoides* Friese. The two species differ significantly from each other in size, integumental sculpturing, and flight phenology. *Ammobates* females associated with *Ancyla asiatica* frequently entered nests and remained inside for about 60 seconds. No live immatures of *Ammobates* were recovered from nests of either species of *Ancyla*. However, J.G.R. found a cast head capsule (fig. 20) of a first instar cleptoparasite in a fragment of two cells in linear series near a presumed egg insertion hole, one of six in the fragment (fig. 21) of *Ancyla asiatica*. This was likely the head of *Ammobates* as evidenced by the egg insertion holes that are characteristic of this bee tribe (Rozen and Michener, 1968; Bohart, 1970; Rozen, 1969, 1986; Rozen and Özbek, 2003).

On two occasions J.S. noted that *Ancyla* females in their nests protect the nests as he excavated them: when threatened by straw probes, the females, while facing downward, curled their metasomas, thereby blocking the tunnel completely, and buzzed loudly. These actions presumably prevent *Ammobates* (and other threats) from entering brood cells.

DESCRIPTION OF IMMATURE STAGES

MATURE OOCYTES AND OVARIAN STATISTICS

Single preserved females of *Ancyla asiatica* and *A. anatolica* had four ovarioles per ovary, hence an ovarian formula of 4:4, typical for solitary, nonparasitic Apidae. Each species also had a single elongate, nearly white, and nearly parallel-sided mature oocyte that was strongly curved (fig. 24), with the following dimensions: *A. asiatica*, 2.15 mm long, 0.48 maximum diameter; *A. anatolica*, 1.98 mm long, 0.38 mm maximum diameter. Although the oocyte of *A. asiatica* was somewhat misshapen in the preservation process, that of *A. anatolica* (fig. 24) was slightly more narrowly rounded at its posterior end, which would likely have been the same for a normal one of *A. asiatica*. The egg index of *A. asiatica* was 0.62 whereas that of *A. anatolica* was 0.66, calculated by dividing the length of each oocyte by the intertegular distance of the female from which it came. Thus, both values fall well within the *small* category in the classification of egg/oocyte size relative to the size of the female (Iwata and Sakagami, 1966). SEM examina-

tion of the mature oocyte of *A. anatolica* revealed the micropyle to be a tight cluster of openings at the anterior tip (figs. 22, 23). Chorionic ridges, most strongly developed on the outcurved surface of the oocyte, radiate away from the micropyle before dwindling and disappearing. The granular texture of these lines as seen in figure 23 suggests that the chorion had not been fully deposited when the female was collected and preserved.

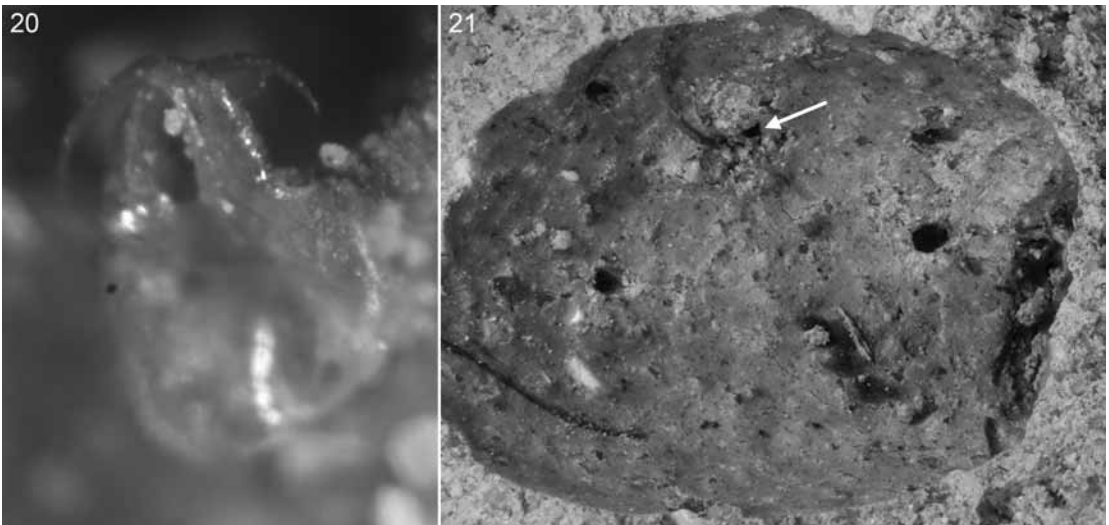
MATURE LARVAE OF *ANCYLA ASIATICA* FRIESE

Figures 25–45

In describing and discussing mandibular anatomy, the long axis of the right mandible is assumed to be horizontal, so that the upper (or top) surface is dorsal and the lower surface is ventral. In depicting inner views, the adductor apodeme base is carefully aligned over that of the abductor apodeme, and in dorsal and ventral views, bases of the two apodemes are positioned at the same level. The following refers to both the pre- and postdefecating larvae of the species.

DIAGNOSIS: In the following description, text in **boldface** refers to features that when considered collectively will distinguish the mature larva of this species from those of all other nonparasitic, noncorbiculate tribes of Apinae.

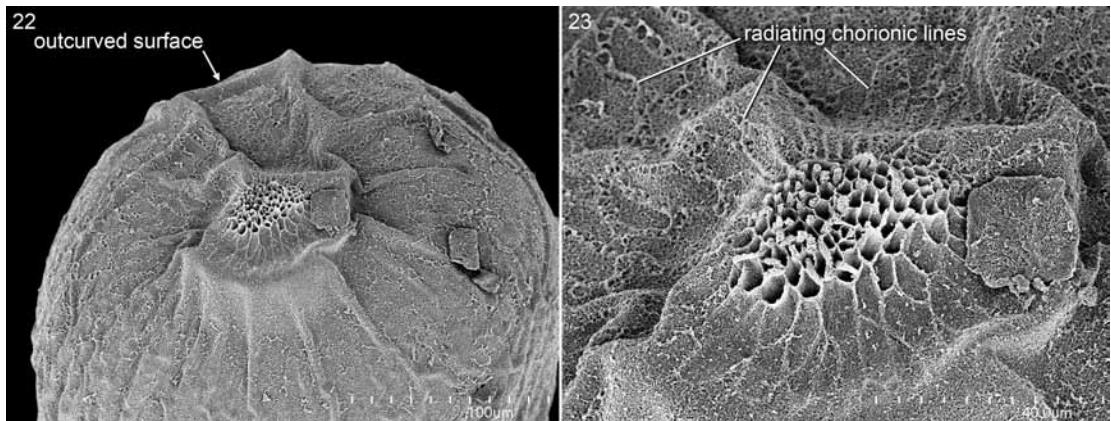
DESCRIPTION: Head: Integument of head capsule with scattered, minute sensilla; vertex finely spiculate (fig. 27); dorsoapical surface of maxilla densely spiculate (figs. 29, 32); epipharyngeal surface with patch of dense, strong spicules underneath each apicolateral swelling; labiomaxillary sensilla large, conspicuous (fig. 32); mandibular corium possibly finely spiculate. On postdefecating larva, mandibular apex darkly pigmented; pigmentation of mandibular



FIGURES 20, 21. Photomicrograph of cast head capsule of *Ammobates* at egg insertion hole, and one of two cells in fragment of two cells containing head capsule, showing five of the six egg insertion holes, respectively (arrow points to partly hidden fifth hole).

points of articulation with head capsule, cardo, articulating arm of stipes, and premental sclerite conspicuous; pigmentation of palpi and salivary lips faint but evident.

Head moderately small compared to large body (figs. 25, 26); width of foramen magnum compared to head width 0.78; tentorium moderately weakly developed for cocoon spinning larva. Center of anterior tentorial pit somewhat closer to anterior mandibular articulation than to outer ring of antenna in frontal view (fig. 27); posterior tentorial pit (i.e., junction point of postoccipital ridge, hypostomal ridge, and tentorial bridge) in normal position; epistomal ridge laterad of (below) anterior tentorial pit well developed, between pits scarcely evident; pleuros-



FIGURES 22, 23. SEM micrographs of anterior end of mature oocytes of *Ancyla anatolica*, and close-up of micropyle showing radiating chorionic ridges (not to be confused with radiating folds of chorion due to SEM preparation in figure 22), respectively.

tomal ridge broad near anterior and posterior mandibular articulations but narrowing and weakening between articulations; hypostomal ridge moderately developed; postoccipital ridge well developed; coronal ridge fading out considerably less than halfway from postoccipital ridge to level of antennae in frontal view (fig. 27); dorsomedial portion of postoccipital ridge straight or nearly so (not bending forward) as viewed from above; hypostomal ridge without distinct dorsal ramus. Parietal bands evident as integumental scars. Antennal prominence (figs. 28, 29, 30) almost nonexistent; parietal area above antenna strongly depressed (fig. 29, supra-antennal depression); antennal papilla tapering toward apex (fig. 31), somewhat cone shaped, about twice as long as basal diameter, bearing approximately three sensilla. Apex of labrum (figs. 27, 29) nearly straight, faintly bilobed; front surface of labrum bearing pair of low lobes; transverse labral sclerite absent.

Mandible as seen in inner or outer views (figs. 36, 38) robust at base, gradually tapering to single, broad, subtruncate, darkly pigmented apex; dorsal apical edge lacking teeth but faintly irregular as seen in dorsal or ventral views (figs. 35, 37, 40), curving mesad short distance from apex, forming strongly projecting cuspal area that then extends basad as ridge to form dorsal boundary of apical concavity (figs. 37, 39); this ridge then curving ventrally and finally apically to define ventral boundary of elongate apical concavity (fig.

39); this concavity actually consisting of two smooth concave surfaces, one apical to the other; these surfaces not separated by defined boundary, so that they are difficult to observe in certain views (fig. 39) but quite obvious in inner view (figs. 36, 41); dorsal surface of mandible with broad, shallow concavity, which rises sharply along inner mandibular edge forming ridge defining apical concavity immediately basad of cusp; surface here with spicules, some of which appear setiform (fig. 41); outer surface of mandible with three or four small, presumably sensilla-bearing tubercles (fig. 38). Labiomaxillary region small in frontal view compared with expanded head capsule (figs. 27, 29); maxilla apically rounded, bearing palpus apically (fig. 32); two small tubercles each bearing long sensilla positioned mesad of palpus possibly representing galea (fig. 32); cardo and stipes sclerotized; articulating arm of stipital sclerite long, conspicuous, darkly pigmented (figs. 33, 34); maxillary palpus well developed, parallel sided, with length about three times basal diameter. Labium clearly divided into small, narrow prementum and postmentum; premental sclerite (figs. 33, 34) conspicu-

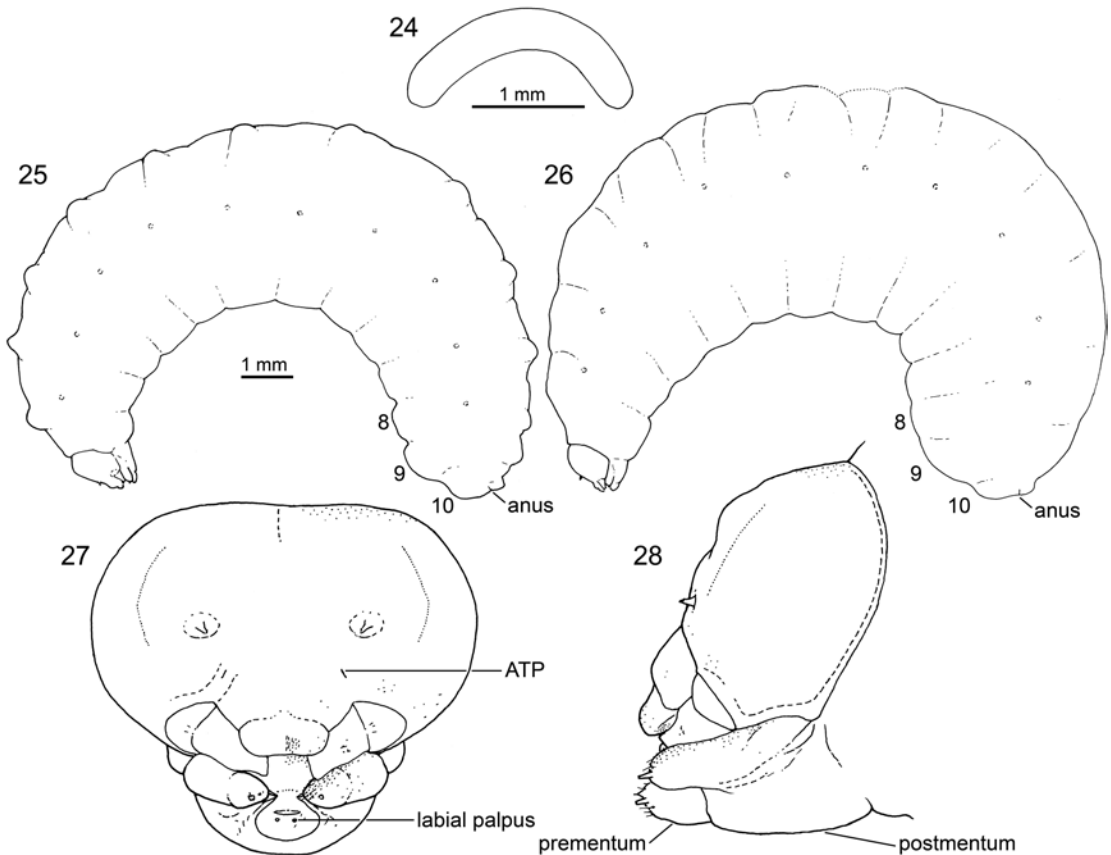
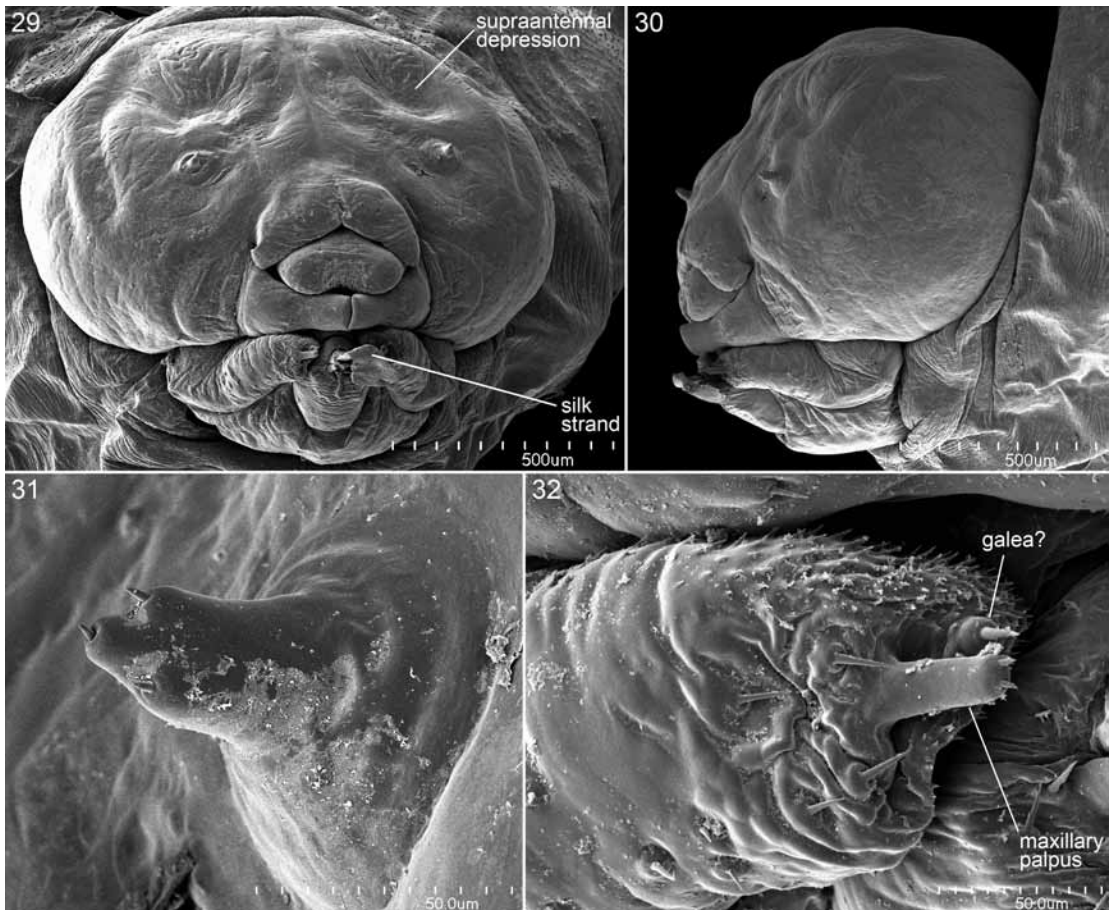


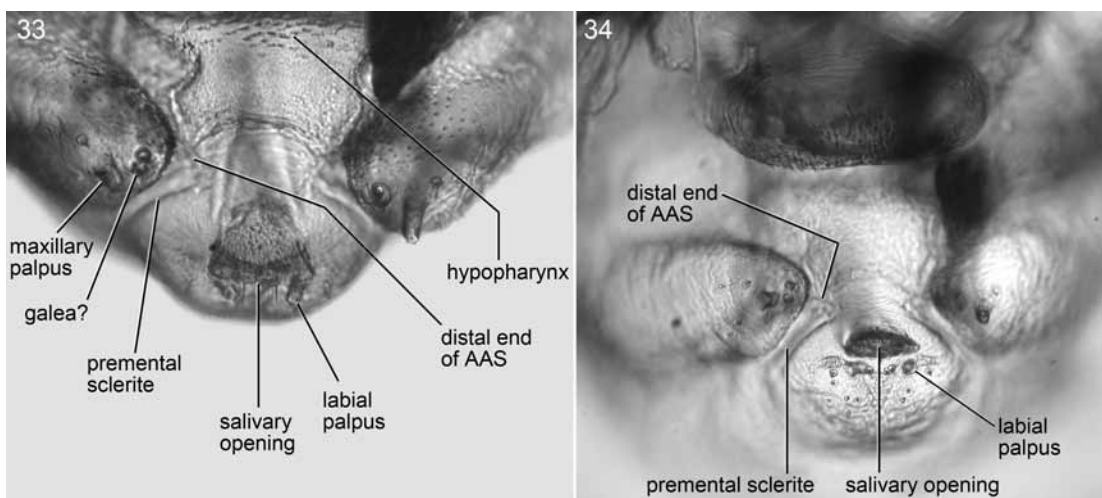
FIGURE 24. Diagram of mature oocyte of *Ancyla anatolica*, lateral view, anterior end on left. FIGURES 25–28. Diagrams of mature larvae of *Ancyla asiatica*. 25. Entire postdefecting larva, lateral view. 26. Entire predefecting larva, lateral view. 27, 28. Head, frontal view and lateral views, respectively. ATP = anterior tentorial pit. (Scale under fig. 25 also refers to fig. 26.)

ously pigmented, strongly sclerotized immediately laterad of apex of articulating arm of each stipital sclerite, but pigmentation and sclerotization fading out below level of salivary opening in frontal view (fig. 34); labial palpus about as long as maxillary palpus. Salivary opening on apex of prementum, with transverse, strongly projecting lips. Hypopharynx with paired, low spiculate lobes well behind apices of articulating arms of stipes; hypopharyngeal groove present, nonpigmented.

Body: Integument without general body setae, but most of integument regularly spiculate, with spicules better developed on dorsal body surfaces and fading out toward ventral body surfaces; abdominal segment 9 on cleared, stained larva with faintly sclerotized band stretching across dorsum of segment (figs. 42, 43, sclerites), and abdominal segment 10 with nearly all of dorsum faintly sclerotized in front of circumanal ridge (figs. 42, 43); this sclerotization forming band that extends down each side of body and gradually tapers before ending two-thirds of



FIGURES 29–32. SEM micrographs of mature larva of *Ancyla asiatica*. 29. Head, frontal view, with silk strand emerging from salivary opening. 30. Head, semilateral view. 31. Close-up of antenna, lateral view. 32. Close-up of right maxilla, lateral view.

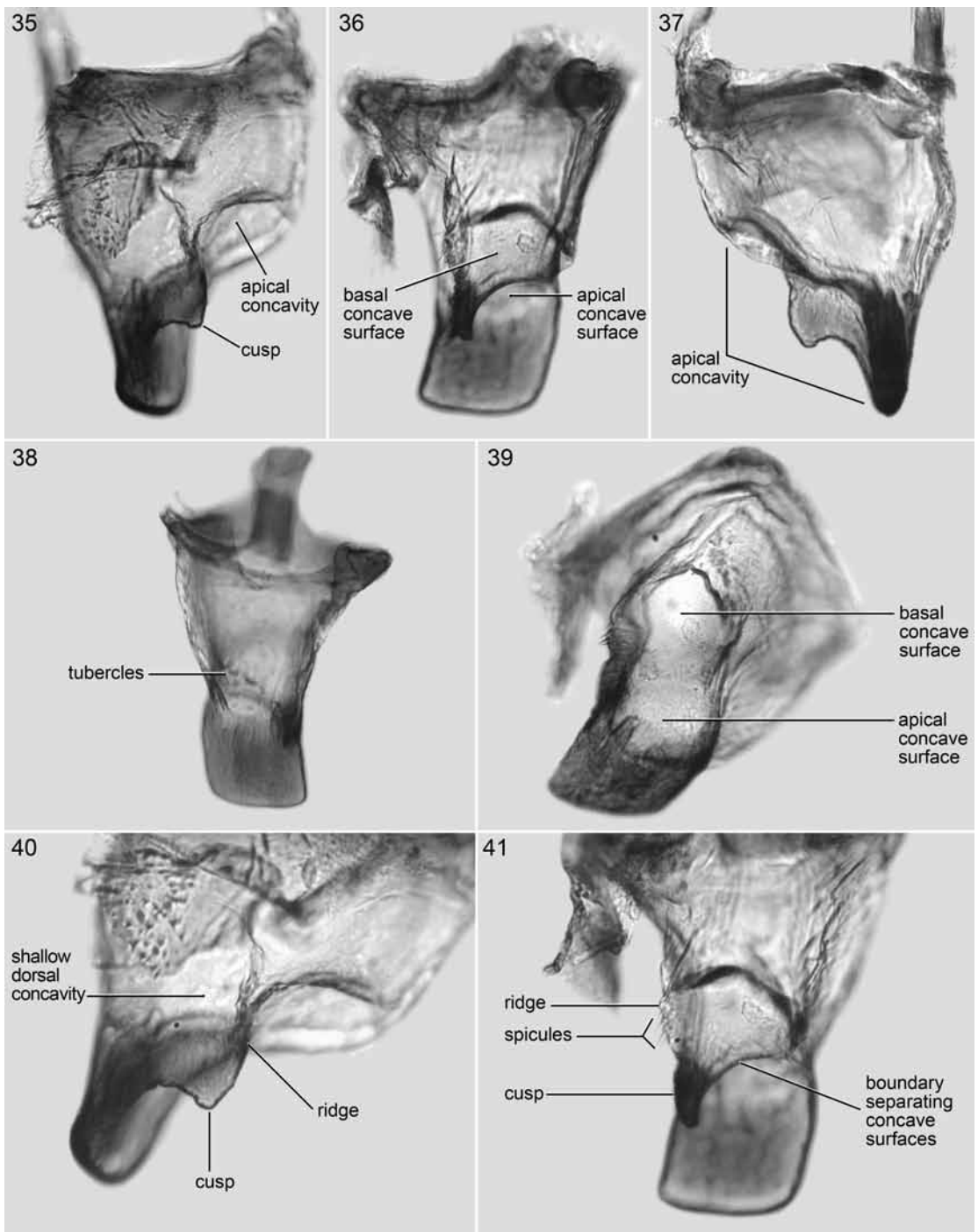


FIGURES 33, 34. Microphotograph of labiomaxillary region of cleared head of postdefecating larva of *Ancyla asiatica* with right mandible removed, dorsofrontal view and oblique frontal view, respectively, showing disappearance of heavily sclerotized premental sclerite below level of salivary opening. AAS = articulating arm of stipes.

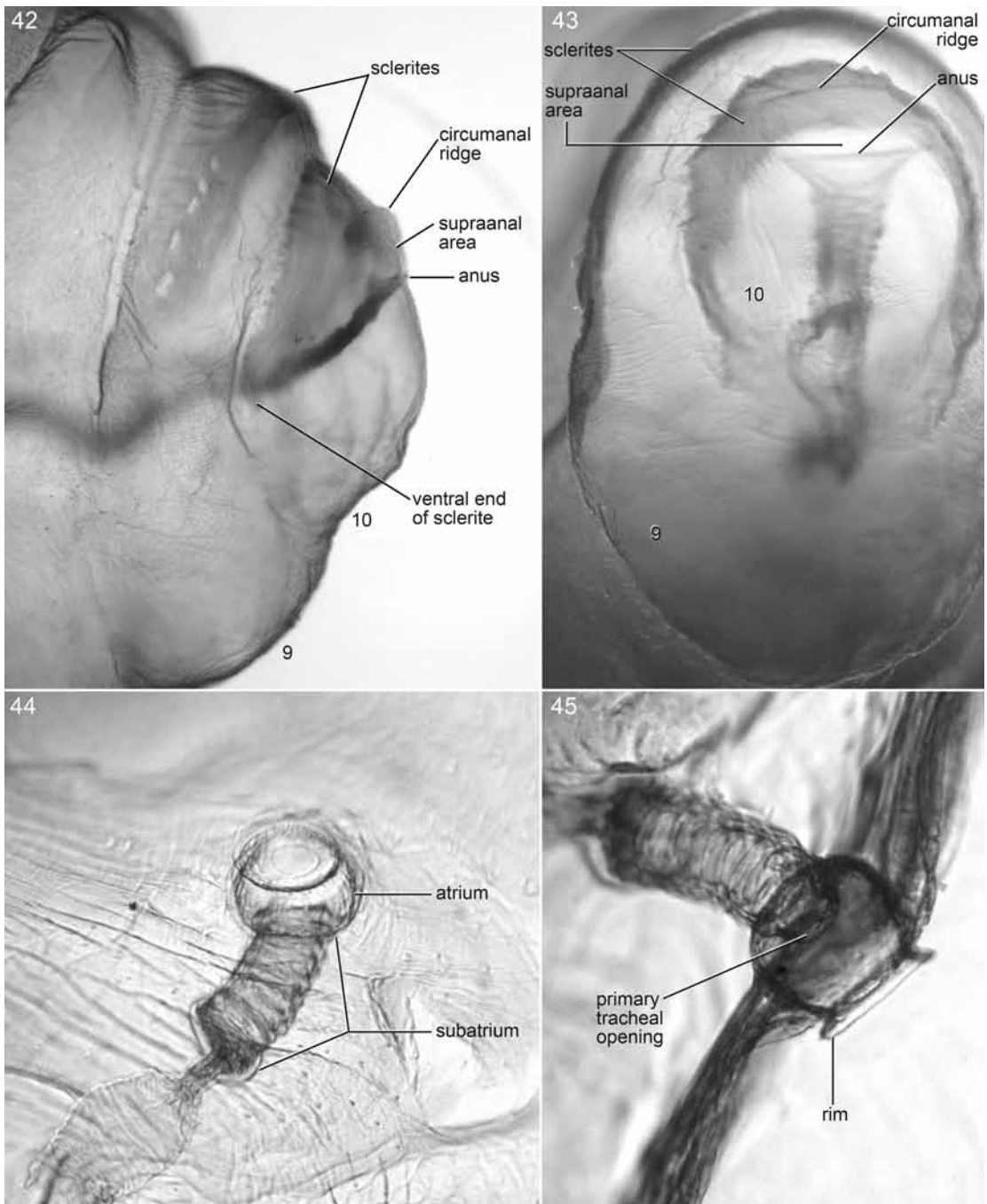
way to bottom of segment in lateral view. Body form of predefecating larva (fig. 26) robust; extent of expression of inter- and intrasegmental lines as well as of paired dorsal tubercles determined by amount of food ingested, but on fully fed larva (fig. 26) dorsal tubercles not visible and inter- and intrasegmental lines obscure; on postdefecating larva, dorsal cephalic and caudal annulations evident; paired dorsal tubercles evident but always low; abdominal segment 9 on pre- and postdefecating forms produced ventrally as seen in lateral view (figs. 25, 26), so that abdomen robust, tapering little posteriorly in lateral view; abdominal segment 10 positioned somewhat dorsally on 9, small, contrasting with robust preceding segments in lateral view (figs. 25, 26, 42); anus positioned close to dorsal surface on segment 10 (fig. 42); on postdefecating larvae, dorsal surface of segment 10 traversed by faint groove posteriorly, forming circumanal ridge, which extends dorsally from one side of anus to other; this ridge defining dorsal boundary of somewhat swollen, faintly verrucose supraanal area (figs 42, 43). Spiracles (figs. 44, 45) moderate and subequal in size throughout, not surrounded by sclerites, and not on tubercles; **peritreme with flared, elevated rim (fig. 45)**; atrium globose; atrial wall smooth, without ridges or spines, moderately thick; primary tracheal opening with collar; subatrium normal in length, consisting of about 10 chambers; subatrial chambers approximately equal in diameter from body surface inward. Male with single median transverse scar on apex of ventral protuberance of abdominal segment 9; female sex features unknown.

MATERIAL STUDIED: Four postdefecating larvae: Turkey: Adana Province: Seyhan Baraji [dam] near Karaömerli village (N37°07'2.43" E35°20'10.28") VI-22-VII-10-2011 (J. Straka), elev. 80 m. The site was about 12 km north of the center of Adana.

REMARKS: Although the mature larva of *Ancyla asiatica* has a truncate mandibular apex, a fourth or perhaps third instar of the same species from the nesting site had a bidentate



FIGURES 35–39. Microphotograph of cleared right mandible of postdefecating larva of *Ancylo asiatica*, 35. dorsal, 36. inner, 37. ventral, 38. outer, and 39. apical views, respectively. FIGURES 40, 41. Close-up of apical end of mandible of same showing details of shallow dorsal concavity and apical concavity, dorsal and inner views, respectively.



FIGURES 42, 43. Microphotographs of abdominal segments 9 and 10 of cleared postdefecating larva of *Ancyla asiatica*, lateral and posterior views, respectively. FIGURES 44, 45. Spiracle of same, showing atrium in oblique view and side view, respectively.

mandible, as did an even earlier instar of *A. anatolica*. In the earlier instar of *A. asiatica*, the ventral apical tooth was acutely narrowed but apically rounded and the larger dorsal tooth was apically truncate.

DISCUSSION

The mandibular morphology of the mature larva of *Ancyla asiatica* can be interpreted in light of several recent studies of mandibular morphology of other apine tribes, most specifically Exomalopsini (Rozen, 2011a) but also Emphorini (Rozen, 2011b). As indicated in the description above, the mandible ends in a single, broad, diagonally truncate apex (figs. 36, 41). The dorsal apical mandibular edge bears a conspicuous projection, which we interpret to be the cusp because of its position, even though neither the dorsal apical edge nor the cusp reveals distinct teeth that are obvious in the Exomalopsini (Rozen, 2011a) and some other bees. The dorsal surface of the mandible possesses a shallow concavity that no doubt functions in conjunction with the epipharyngeal surface above and bears spicules along the inner edge that would appear to interact with the epipharyngeal spicules, as discussed in the exomalopsine study (ibid.). The extremely long and doubly concave apical concavity is an interesting feature because of its length, shape, and well-defined boundaries.

Does the mature larva of *Ancyla asiatica* reveal information about the relationship of Ancyloini with other tribes among the nonparasitic and noncorbiculate Apinae? A recent molecular study by Cardinal et al. (2010) reports that the tribe (as represented by only *Ancyla*) is most closely related to the Eucerini. Roig-Alsina and Michener's (1993) anatomical study, based on only *Tarsalia*, indicated that the Ancyloini was a sister group of the Eucerini. However, the investigation of Silveira (1993b), which added *Ancyla* to the taxa and data employed by Roig-Alsina and Michener, indicated that such a relationship was unlikely. The postcephalic features of the two tribes are not dissimilar, but throughout the noncorbiculate, nonparasitic Apinae, these features show little variability, except for Tetrapedini.

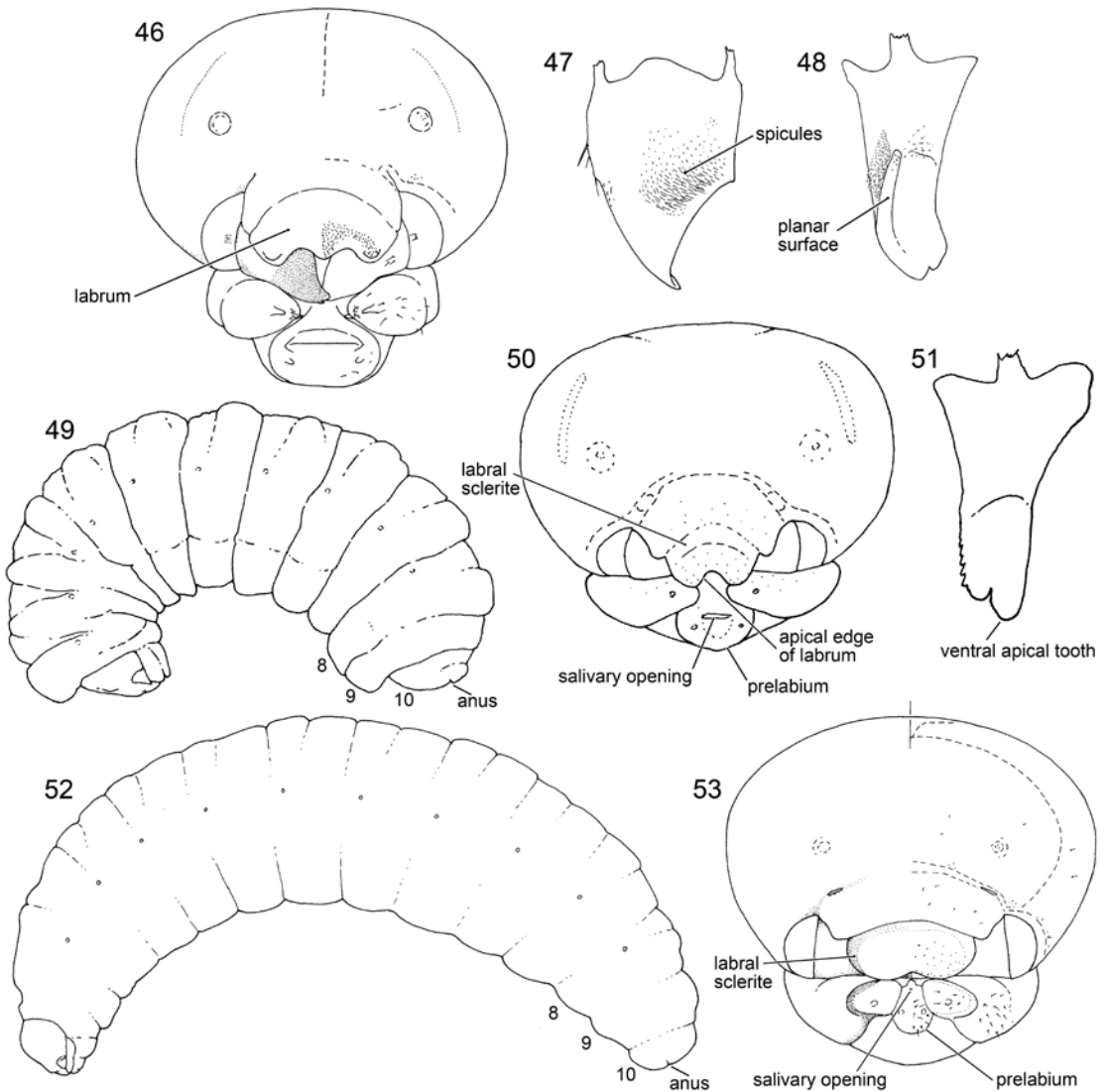
With respect to cephalic characters, there is surprisingly little congruity between *Ancyla asiatica* and the Eucerini. Major differences include the following: The labrum of *Ancyla asiatica* is narrow, slightly bilobed apically, whereas the labrum of Eucerini is large, unusually broad, and in most cases (except for *Tetraloniella*) distinctly trilobed. The mandible of *A. asiatica* bears a single, transversely truncate apical edge, whereas mandibles of all Eucerini end in two teeth that are apically pointed. In *A. asiatica* the mandibular cusp projects strongly in dorsal view as a lobe with a single though somewhat uneven edge. The eucerine cusp in inner view is generally elongate, consisting of a dorsal apical edge and a ventral edge that dorsally borders the apical concavity. The surface between the two edges is often planar, but in some genera (*Tetraloniella*, *Thygater*) it bears spines. (The uneven cuspal edge of *A. asiatica* may actually be a homolog of the spined surface of these two eucerine genera, as could be the case for the spined cuspal surface in many Exomalopsini and some Tapinotaspidini.) The dorsal mandibular surface of *A. asiatica* lacks spicules except for a few setiform ones immediately along the edge of the cusp, whereas the dorsal surface of the eucerine

mandible is extensively covered with setalike spicules. The articulating arm of the stipes, though present, is not pronounced, and the sclerotization of the premental sclerite is also not pronounced; in *A. asiatica* the articulating arm of the stipes is strongly sclerotized as are the lateral parts of the premental sclerite, though the ventral part is essentially absent. The small size of the labiomaxillary region compared with the head width in frontal view (figs. 27, 29) of *A. asiatica* is unique, though difficult to quantify, and is not exhibited by other tribes including the Eucerini in the subfamily (with the possible exception of Ctenoplectrini, Rozen, 2010: fig. 4). In summary there are few features of mature larvae that support a close relationship between *A. asiatica* and the Eucerini, and features that are shared are mostly plesiomorphic.

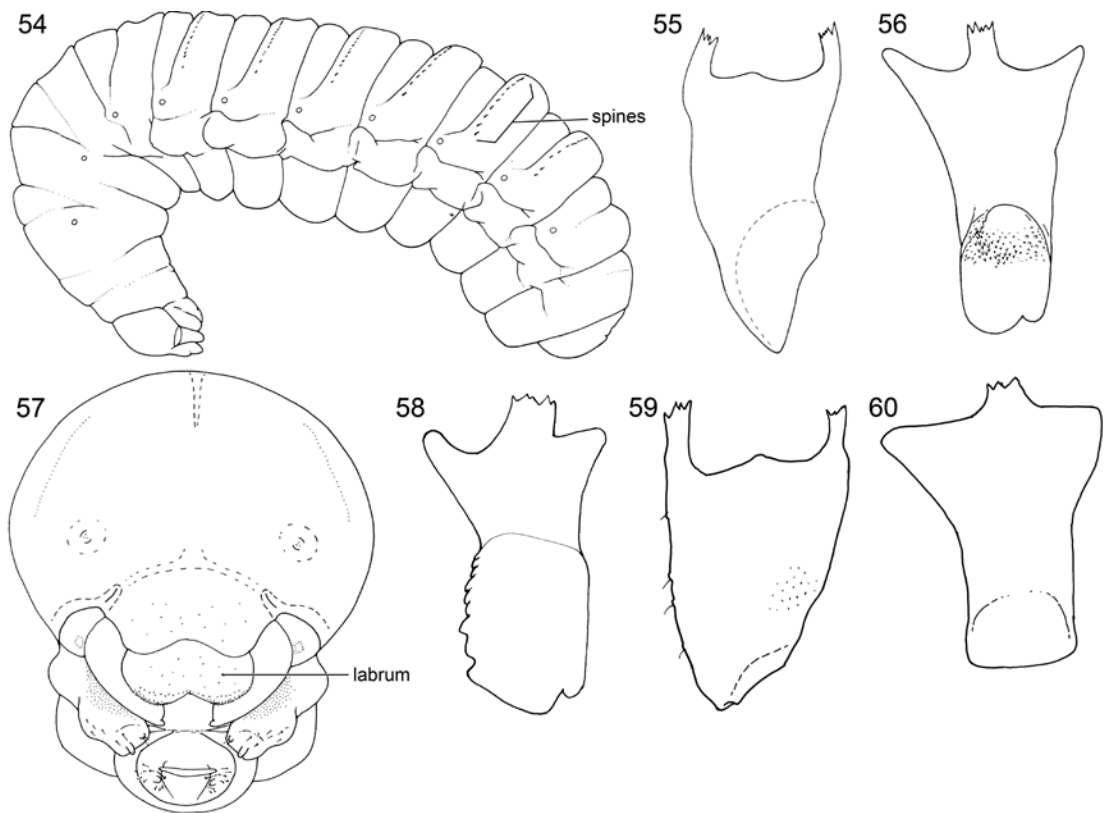
On the other hand, the larva of *A. asiatica* does not show strong affinities with any of the other apine tribes. For example, the mandibles of both *Ancyla asiatica* and many Anthophorini are simple (Rozen and Ding, 2012), that is, not bifid as in most other tribes, but anthophorines do not spin cocoons and therefore do not have larval features for doing so, and their maxillary apices are uniquely elongated beyond the palpal attachment, strongly contrasting with comparable features as found in *A. asiatica*.

One of the anonymous reviewers raised the interesting question whether nest architecture reveals a relationship of *Ancyla* with Eucerini in light of the findings by Cardinal et al. (2010). Although much has been written about nesting of many eucerine taxa (Michener, 2007, and references therein), our understanding of this diverse tribe is still fragmentary, but several features regarding nests are worth noting. In *Ancyla*, cells are arranged in linear series at the end of the main tunnel, whereas in Eucerini cells are arranged singly at the end of a soil-filled lateral. *Ancyla* cells are oriented at various angles, from horizontal to nearly vertical; those of most Eucerini are vertical, with one exception: *Canephorula apiformis* Friese (Michelette et al., 2000). With most Eucerini, larval defecation commences before cocoon production starts, so that much of the fecal mass is external at the top (front) end of the cocoon. However, larvae of both *Ancyla* and *C. apiformis* first construct a brownish outer cocoon layer with an apical circular screened area that presumably permits air exchange with the outside atmosphere. They then deposit fecal material over the entire inner surface of this layer except for the screened area. Subsequently they apply an inner woven layer, so that the entire cocoon except for the screened area is composed of fecal material sandwiched between two silken layers. Other features of the outer cocoon top of *C. apiformis* seem to have no counterpart either to other Eucerini or to *Ancyla*. On the other hand, the mature larva of *C. apiformis* is clearly that of Eucerini.

The same reviewer asked us to comment on the hypothesis that the unusually short mouthparts of adult *Ancyla* are adaptively linked to the shallow flowers of Apiaceae. As already stated, J.S. observed both species of *Ancyla* foraging on Apiaceae (*Daucus* and *Eryngium*) flowers, supporting the suggestion of Silveira (1993a) that the *Ancyla* depends on this plant family. We have not observed any visits of *Ancyla* on other plant families, nor have we found a different pollen grain among the provision remnants in brood cells. Thus, the short tongue of this “long-tongued” bee might well be an adaptation to shallow flowers of Apiaceae, but further observations on more *Ancyla* species are needed to confirm their narrow choices.



FIGURES 46–53. Diagrams of exemplars of mature larvae of tribes of Apinae. **46–48.** Eucerini (*Canephorula apiformis* Friese) head (frontal view), right mandible (dorsal and adoral surfaces), respectively, from Michelle et al. (2000). **49–51.** Ctenoplectrini (*Ctenoplectra cornuta* Gribodo) entire larva (lateral view), head (frontal view), and right mandible (inner view), respectively, from Rozen (2010). **52, 53.** Tetrapediini (*Tetrapedia diversipes* Klug) entire larva (lateral view), head (frontal view), respectively, from Alves-dos-Santos et al. (2002).



FIGURES 54–60. Diagrams of exemplars of mature larvae of Centridini and Anthophorini. **54–56.** Centridini (*Epicharis fasciata* Lepeletier and Serville) entire larva (lateral view) and right mandible (dorsal and adoral surfaces), respectively, from Rozen (1965). **57, 58.** Centridini (*Centris caesalpiniae* Cockerell), head (frontal view) and right mandible (adoral surface), respectively, from Rozen and Buchmann (1990). **59, 60.** Anthophorini (*Anthophora melanognatha occidentalis* Cockerell) right mandible (dorsal and adoral surfaces).

PRELIMINARY TRIBAL KEY TO MATURE LARVAE OF
NONPARASITIC, NONCORBICULATE APINAE, UPDATED

The following key has been modified from a previous version (Rozen, 2011a) to incorporate the addition of Ancyilaini (in the form of *Ancyla asiatica*) and new knowledge resulting from further study of larval Eucerini.

1. Labrum apically trilobed in frontal view (fig. 46) except in *Tetraloniella* and *Thygater* middle lobe essentially absent leaving two conspicuous, forward projecting lobes at corners of lower apical labral margin, separated from one another by distance 1.5 times basal diameter of lobe; dorsal mandibular surface with strong pattern of long, decumbent, setalike spicules (fig. 47); mandibular cusp present, usually with smooth elongate beveled surface lacking teeth or denticles, (fig. 48) but several taxa (*Tetraloniella*, *Thygater*) with surface ornamented with small spinelike tubercles.....Eucerini

- Labrum apically truncate (fig. 27), bilobed (figs. 53, 59), or curved, never trilobed in frontal view; dorsal mandibular surface not broadly covered with setalike spines or spicules but often with a few such spicules near inner edge (fig. 41); mandibular cusp usually present, with minutely irregular surface (fig. 40), teeth, and/or denticles.....2
- 2(1). Mandible apically simple (figs. 36, 38) *and* salivary opening with distinct lips, positioned close to labial palpi (figs. 27, 33, 34); mandibular cusp as seen in dorsal view a strongly projecting lobe, as in figs. 35, 40.....Ancylini
- Mandible apically bifid or simple but, if simple (some Anthophorini), then salivary opening without lips (larva noncocoon spinner), far removed from labial palpi (Rozen and Ding, 2012.: figs. 18, 28) and mandibular cusp not projecting in dorsal view.....3
- 3(2). Abdominal segment 10 with fine scattered setae, best seen on cleared specimen with compound microscope; mandible with 7 or more setae on outer surface except for Chilean *Toromelissa*. Emphorini.....4
- Abdominal segment 10 without setae except in Argentine *Eremapis parvula*, which has 3 setae or fewer on outer mandibular surface.....5
- 4(3). Entire mandibular apex rotated and flattened, bladelike so that dorsal edge directed adorally, forming very broad, ventrally directed, apical concavity (Rozen, 2011b: figs. 12, 21, 22); dorsal apical tooth elongate, gradually narrowing to acute point directed adorally (mandible appearing rapacious) (ibid.: figs. 12, 21, 22); ventral apical tooth greatly reduced (ibid: figs. 12, 21, 22).....Emphorini, subtribe Ancyloscelina
- Mandibular apex rotated less or not at all, not bladelike (Rozen, 2011b: figs. 23–34); dorsal tooth not sharply pointed, not appearing rapacious (fig. 69); ventral apical tooth not greatly reduced⁵.....Emphorini, subtribe Emphorina
- 5(3). Labral apex strongly bilobed in frontal view (figs. 50, 53); labrum with distinct (though not necessarily pigmented) basal sclerite (figs. 50, 53); ventral apical mandibular tooth at least slightly longer than dorsal tooth (fig. 51).....6
- Labral apex usually simple, curved, rarely faintly bilobed; labrum without basal sclerite; relative lengths of apical mandibular teeth variable.....7
- 6(5). Anus in middle of abdominal segment 10 in lateral view (fig. 52); prelabium unusually small (fig. 53); salivary opening a simple hole, not transverse or on projecting lips (fig. 53); labrum less strongly emarginated (fig. 53).....Tetrapediini
- Anus more or less dorsally situated on abdominal segment 10 (fig. 49); prelabium normal in size (fig. 50); salivary opening transverse, on projecting lips (fig. 50); labrum more strongly emarginated (fig. 50).....Ctenoplectrini
- 7(5). Mandible subapically constricted and usually expanding apicad of constriction before terminating in inner or outer views (Rozen, 2011a: figs. 6, 29, 30, 32, 33, 42), but if only slightly expanding (*Anthophorula uncicornis* González-Vaquero and Roig-Alsina, ibid.:

⁵ In *Diadasia enavata* (Cresson) (and perhaps in some other species in that genus) the ventral apical tooth appears missing (Michener, 1953: figs. 209, 210). Examination of a predefecating larva shows it clearly present, but in postdefecating forms it is worn away leaving the mandibular apex obliquely truncate, bearing a large, adorally directed apical concavity.

- fig. 31), then entire apex very narrow; ventral edge of oblique apical mandibular concavity not defined by ridge (ibid.: figs. 7, 12).....Exomalopsini
- Mandible ending in single tooth or in two teeth, neither constricted subapically nor widening before terminating, as seen in outer or inner views; apical cavity usually broad, well defined ventrally by sharp, often serrate edge.....8
- 8(7). Larva noncocoon spinning; salivary lips not projecting strongly or at all; labial and maxillary palpi short, no longer than basal diameter.....9
- Larva cocoon spinning; salivary lips strongly projecting, labial and maxillary palpi elongate, much longer than basal diameter.....10
- 9(8). Caudal annulets of most body segments with single transverse row of posteriorly directed, pigmented spines (fig. 54); maxillary apex not greatly elongate, only slightly bent mesad, never reaching head midline; mandible terminating with two distinct apical teeth (fig. 56).....Centridini, *Epicharis*
- Caudal annulets of all body segments without pigmented spines; maxillary apex extremely elongate, bent mesad, reaching or nearly reaching head midline; mandible usually terminating in single broad apex (fig. 60).....Anthophorini
- 10(8). Labrum normally narrow; mandible normally with broad, multispined cusp (Rozen, 2011a: fig. 4), except in *Monoeca* (ibid.: fig. 2), with cusp narrow at first glance, appearing composed of single row of spines but on closer inspection possibly consisting of two or more rows of spines.....Tapinotaspidini
- Labrum unusually wide (fig. 57); mandibular cusp seemingly absent, replaced by simple coarsely to finely serrate dorsal apical edge of mandible (fig. 58).....Centridini, *Centris*

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