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Nesting biology and immatures of the oligolectic bee *Trachusa larreae* (Apoidea: Megachilidae: Anthidiini)

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ABSTRACT

Herein we report on the nesting biology of ground-nesting *Trachusa* (*Heteranthidium*) *larreae* (Cockerell) from New Mexico and Arizona, an oligolege of creosote bush, *Larrea tridentata* (DC.) Coville (Zygophyllaceae). Nests are single, slanting, open burrows at the lower end of which are horizontal cells lined with resin collected from creosote bush, also the source of the orange, mealy-moist provisions. Eggs are placed on the surface of the provisions, and the first three instars remain in the same position as the eggs from which they hatched. The fourth instar separates its body from the provisions, and only the fifth (final larval) instar moves around the brood chamber while consuming remaining provisions and defecating prior to cocoon spinning. It is suggested that middorsal body tubercles and an integumental body vestiture of short setae and setiform spicules restricted to this instar are adaptations enabling the movement of the fifth instar not only of this species but possibly those of other Megachilinae, all of which have a body vestiture and presumably middorsal body tubercles.

The cocoon is spun after most of the feces are voided. Like cocoons of many other Megachilidae, it bears a pronounced nipple at its anterior end. From its construction as well as by comparison with cocoons of other bee taxa, the nipple seems to serve a number of functions: it enables exchange between the interior air of the cocoon and the external ambient air; it screens out parasites and predators from attacking the cocoon inhabitant; and it probably regulates cell humidity.

Eggs are briefly characterized, and three females each were found to have three ovarioles per ovary and to carry a single mature oocyte, which is classified as *medium* in Iwata and Sakagami's classification of egg/mature oocyte size relative to body size of female. The fifth instars (both pre- and postdefecating forms) are described and found similar to those of other Anthidiini.

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INTRODUCTION

The nesting biology of the handsome bee *Trachusa* (*Heteranthidium*) *larreae* (Cockerell) was treated in several earlier papers. MacSwain (1946) first described the distinctive nest architecture and resinous nesting material from a site consisting of "several dozen nests" near Loving, Eddy County, New Mexico; his nest data correspond well with those presented below. Fifty years later, Cane (1996) reported an extensive, presumably perennial, nesting site of this species at Silver Bell, Pima County, Arizona, near Tucson. He described in detail: cell linings and closures made from resin obtained from creosote bush, *Larrea tridentata* (DC.) Coville (Zygophyllaceae); chemical and physical makeup of this resin; the process of harvesting and transporting resin; and the rarity among bee species to obtain nest-lining material and larval food from a single source. Of special interest, he pointed out that reliance of this bee on creosote bush was a recent evolutionary event since fossil evidence indicates the presence of this originally South American plant genus in North America for only the last 20,000 years.

In the current study, we describe nests, confirming and adding new details to the observations of both MacSwain (1946) and Cane (1996). We also describe the provisions, aspects of larval behavior, egg deposition habits, anatomy of eggs and last stage larvae, and cocoon construction. Finally, we discuss which aspects of this bee's biology require further investigation.

For such a widely distributed genus, nesting ethology has been infrequently investigated. Aside from *Trachusa* (*Heteranthidium*) *larreae*, only two other species, each belonging to a different subgenus, have been studied. Michener (1941) presented a brief but concise account of *T.* (*Trachusomimus*) *perdita* Cockerell, and Friese (1923), Hachfeld (1926), and Westrich (1989) offered nest descriptions and other biological observations on the European *Trachusa* (*Trachusa*) *byssina* (Panzer), with the latter two works accompanied by helpful photographs.

OBSERVATIONS ON NESTING BIOLOGY

We found this species visiting a large stand of blooming creosote bush, *Larrea tridentata*, alongside the road at 5 mi east of Animas, Hidalgo County, New Mexico (N31° 56.868′ W108° 48.363′), on May 7, 2012. As usual, roadside stands of this plant tend to flourish due to rain runoff from the paved road. That day, H.G.H. identified two nest entrances about 0.5 m apart on unshaded horizontal ground between two creosote bushes (fig. 1), and next day we excavated the nests. A third nest (fig. 2) about 2 m from the first two was discovered a few days later and excavated on May 11, 2012. Nest entrances at this site were on a somewhat uneven surface strewn with small rocks, pebbles, fragments of macadam pavement, and broken dead twigs, although surface soil was clearly visible among the debris. Here and there were small plants, and additionally much of the area was covered with blooming *Eriogonum trichopes* Torr. (Polygonaceae) (fig. 1). With only basal leaves, stems cast little shadow on the site, though landing bees were slowed as they avoided encounters with plant stems. As these were the only nests found in five days, this site was far less populated than that described by Cane (1996) despite the fact that adult bees were numerous on host bushes.

In addition to these three nests, J.G.R. found a second site of at least four active nests approximately 200 meters to the west along the same side of the road and associated with the same stand of creosote bush on May 12, 2012. Because the substrate was somewhat different and nest contents younger, these nests are treated after the first three.

First Site

The substrate at the first site was covered by ca. 6 cm of dry, unconsolidated soil containing assorted small pebbles as well as finer soil. Beneath was a layer of consolidated soil, which at lower depths showed darkish hints of moisture. Where the first two nests occurred, the soil at lower depths had small rocks, whereas this soil at the third nest contained much larger inclusions. Despite the ground vegetation, plant roots did not impact either our excavations or those of the bees.

Each of the three entrances bore a moderate amount of dry tumulus on the side opposite the shallowly descending tunnel, as also observed at nests in the second site. Main tunnels, about 5–6 mm in diameter and unbranching, were open, though pebbles constricted passageways here and there. Tunnel walls were unlined, and those in softer upper soil occasionally collapsed as we excavated, but in lower, consolidated soil, tunnel walls were firmer. Main tunnels were open though occasionally clogged with loose soil because we used aspirators to blow away excavated soil as we carefully uncovered tunnels with penknives. The main tunnel of the first nest (fig. 3) descended at an angle less than 45° from horizontal about halfway through the unconsolidated top layer, reversed direction, and at about the same inclination, extended for a length greater than its upper part. At its low end it turned sharply downward and immediately enlarged into a chamber from which a cluster of three horizontal cells (figs. 3, 4), connected side by side, radiated into the soil at a depth of 8 cm below surface. The dimensions of this chamber (termed entrance chamber to the cells) were uncertain, but the horizontal cluster of connected cell entrances along one side extended somewhat more than 1.5 cm as seen from above (fig. 4) or from the front (fig. 5). MacSwain (1946) stated that the entrance chamber was "13 mm at its greatest diameter" at a depth of 19 cm, considerably deeper than 8–10 cm recorded by Cane (1996) and herein.

The main tunnel of the second nest descended at an angle of approximately 25°–30° from horizontal without turning, and just as it entered the lower zone of consolidated soil it turned abruptly and opened into the entrance chamber to the cells. This chamber also bore a horizontal radiating cluster of 3 cells, but a single unconnected horizontal cell faced them from the other side of the chamber, and yet another horizontal free cell of uncertain position was close to the cluster, all at a depth of about 8 cm (figs. 6, 7).

The tunnel of the third nest had a more sinuous path because of larger subterranean rocks. At its terminus, at about the same level as the other two, an open space of slightly larger diameter than the tunnel occurred, at the end of which was stored a single sphere about 3.5 mm in diameter of soft, malleable resin, no doubt material intended for cell wall construction. When squeezed between fingers, the resin had a distinct greenish hue, far more apparent than observed in hardened cell walls.

As also reported by Cane (1996), cell walls are constructed from resin incorporating soil. H.G.H. noticed one female rapidly chewing the terminal green branches of *Larrea*, which, as noted by Cane (1996), is the source of the resin. The fact that we did not notice females carrying resin spheres to the nests as did Cane may indicate that we were there too early in the nesting season and/or the population size of the bee was too small.

External cell wall surfaces are partly covered by soil particles, large and small (figs. 5, 6), some protruding from the resin. Uniform in general shape, cells as defined by their exterior surfaces are 16–18 mm long ($N = 8$) with a maximum diameter near the base of 8 mm ($N = 3$). Between the maximum diameter and the front end, they gradually narrow to 6 mm outside diameter ($N = 3$) within several mm from the front end, beyond which cells flare slightly to nearly 7 mm ($N = 8$) at the entrance. Inside diameters of the necks of two open cells were 4.5 and 4.7 mm (thus sufficiently large for a female to insert her head but only questionably large enough for the rear of her mesosoma). All cells have approximately the same external shape, but measurements of the diameters of maximum widths and necks were difficult to record because cells and cell clusters are imbedded in soil. After a female deposits her egg, she constructs the resinous cell closure at the anterior end of the neck, so the terminal flare becomes the rim of the closed cell (figs. 5, 7). In each case the rim edge is uneven, and the top part of the rim tends to extend farther forward than the lower part (fig. 8). The exterior surface of each closure is a smooth concave surface often deflecting slightly downward because of the slight jutting of the upper rim.

Cells in clusters radiate because the largest diameter of each is toward the rear; thus, long axes of cells radiate outward when viewed from above (figs. 4, 6) (or below). The fact that all cells are surrounded by soil indicates that they are built into cavities made in the soil. We have as yet no information as to how these bees go about building their cell walls and cell clusters and manipulating the soft resin, although future studies of cells under construction will certainly provide answers. Cell closures are obviously the last step in cell construction, as is the case for all bees with closed cells. It is unknown whether the common entrance chamber to cell clusters is constructed before the first cells are built, or it results simply from the construction of one cell next to another and all radiate from the same general spot. There is no indication that soil in the vicinity of cells has been compressed, treated with secretions, or modified in any other way by the female to protect the cell or its contents; the resinous cell wall seems to afford whatever protection is necessary.

The outer surface of the resinous cell wall (e.g., fig. 6) is coated with fine soil and pebbles of various sizes, all of which cling to the surface, providing the cell with a rough, uneven external surface that is so hard that it cannot be easily penetrated with sharp forceps. While soil and pebbles give the surface a dull tannish hue (figs. 6, 8), the resin is a darker gray with a faintly greenish hue and is highly reflective under high magnification when seen on broken edges (fig. 9). Such edges also reveal that soil, small pebbles, and plant fibers are incorporated into the resin and are not merely surface adherents. It is surprising that the resin, which is malleable at first, solidifies after being mixed with soil into a material that seems almost as hard and unyielding as baked pottery. In contrast to the very rough outer surface of the cell wall, the inner surface is far more uniform in texture and similar to, but shinier than, the outer surface of the cell closure.

Three closed cells of this site were opened a few days after collection. This was accomplished by circumcising a narrow trough in the hard resin-soil mixture just behind the neck at the closure end accompanied with occasional slight perforations into the cell lumen, so that the closure end broke away from the rest of the structure. The inner surface of each closure (fig. 10) lacked any regular pattern, such as a spiral found on inner surfaces of most bee cells. It consisted of highly irregular, shiny, brownish material that appeared unconsolidated but was actually quite solid when tested with forceps. Its reflective quality suggested wetness, though in actuality it was dry. The minimum thickness of the hard cell wall in that region, as well as throughout the cell, is slightly less than 1.0 mm, although somewhat thicker regions occur due to the uneven nature of the external cell surface.

Provisions in each cell at both sites were orange, mealy-moist, with a reflective front surface, and filled the cell's rear end. The provisions in cells containing small larvae or eggs occupied a surprising volume, as they filled about three-fourths of the cell's length. Despite the horizontal cell orientation and rather soft nature of the provisions, the provision surfaces were nearly vertical, i.e., perpendicular to the long axis of the cell.

The first cell opened (fig. 14) contained a feeding larva, about 3 mm long, its venter lying on the surface of the provisions, with its head about 1 mm from the cell wall and its posterior end about 0.5 mm from the opposite cell wall, and a depression in the surface near the larva's head end (indicating that food had been ingested, as corroborated by the orange tinge of the larva's midsection). Almost certainly the larva, perhaps a third instar, had not moved from the position of the egg (later observed in cells of the second site), for no cast skins or chorion was visible on the surface of the provisions and the surface was unblemished by tracks of a crawling larva. This larva became a fifth instar with a short but conspicuous body vestiture of setae and setiform spicules by May 14, 2012. As discussed below, such vestiture characterizes fifth instars of all Megachilinae.

The second cell contained a smaller larva (fig. 13), perhaps second instar, ca. 2.5 mm long. Its rear end was somewhat less than 0.5 mm from the cell wall, and its head reached the approximate center of the provisions, which had slumped slightly, so that the lower half of the surface was angled from the vertical top half. In other respects, the cell contents were as described for the first cell.

The third cell contained a fourth instar (fig. 15), longer than the cell diameter. Its body had pulled away from its broad attachment to the substrate. The surface of the provisions had developed a marked depression at the larva's front end and appeared drier (less reflective). This larva molted to the fifth instar on May 12, 2012, at which time short, abundant body setae and setiform spicules of similar length were visible (fig. 16). A day later the larva had reoriented itself so that it rested against the provisions with its dorsum against the cell wall and its right side against the provisions while it turned its head toward the provisions to feed. It started defecation though still obviously feeding. At this time the middorsal tubercles of abdominal segments 1–5 as well as those of the meso- and metathorax (figs. 17, 18) were pronounced. The body of the larva moved in relation to the inner cell surface while slowly curling and uncurling its body in the process of defecating. As discussed in the introduction to the Fifth Instar, below, we think these observations may at least partly explain how the last stage larva is able to com-

plete feeding on the provisions and move around the cell as it spins its cocoon. As explained below, most feces are discharged before cocoon spinning, but the final discharge of pale, fine-grained material occurs after the entire cocoon, except its nippled end, is complete.

Second Site

After the three cells of the first site were described, above, the second site, consisting of four nests, was discovered on May 12, 2012. A single cell not associated with any nest was accidentally encountered as well. These nests were within an area 5 m in diameter, also on a mostly horizontal surface as in the case of the first site, but the ground surface here was mostly free of assorted pebbles, though covered in places with decumbent, often dead vegetation (figs. 20, 21). The soil beneath tended to be more uniformly consolidated and with fewer rock inclusions than at the first site. One nest entrance was at the edge of a small declivity on open ground, but others were partly hidden under decumbent, mostly dead vegetation, so that tumuli were difficult to identify without clearing the brush. The four entire nests excavated had one or two cells at depths ranging 8–9 cm below surface with open, unbranched burrows descending more or less sinuously, usually at an angle of less than 45° from horizontal, except one descended to a depth of 8 cm within a horizontal distance of 4 cm from its entrance. Just before reaching cell level, main burrows curved downward, enlarged to about 10 cm in diameter, thus forming the entrance chamber to the cells. While all cells were horizontal, only two of them in one nest were connected palmately; two nests each had two independent (i.e., not connected) cells. The fourth nest consisted of a single cell containing a malleable resin sphere, which, when pulled apart, was semitransparent and tannish without a greenish tinge and did not give the odor of creosote bush that Cane (1996) found, perhaps an indication that these qualities are gradually lost before hardening. All these nests were incomplete. All cells and cell provisions were as described for the first site, but the inhabitants were younger, consisting of one perhaps second instar, two eggs, and one cavity in which the cell wall had yet to be constructed. A third egg was recovered from the single cell not associated with any nest. Two eggs were deposited lengthwise on the surfaces of their provisions (fig. 11); the other was angled with one end buried in the provisions (fig. 12). Because we had scouted the area intermittently since May 7, these findings suggest that our field observation may have been early and maximum nesting activity had not yet been attained when we first started. All eggs were preserved, and the small larva later died from unknown causes.

Upon returning from the field trip resulting in the above observations, J.G.R. discovered that in 1988 he had found four or five nests of *Trachusa larreae* at 6 mi NE of Portal, Cochise County, AZ, clustered within 1 m^2; one nest consisted of a slanting open tunnel 6 mm in diameter descending to a depth of 7 cm at the end of which were two horizontal cells, all data consistent with the current study. A single postdefecating larva collected from one nest is included in the description, below.

DEFECATION AND COCOON STRUCTURE AND CONSTRUCTION

Three closed cells from a single nest from the first site were maintained in a natural horizontal position and opened at the end of May 2012. Two produced mature postdefecating

larvae in cocoons, and the other contained a diapausing meloid larva, the only nest predator/parasite associated with the site. Observation of other developing fifth instars from the first site also contributed to our understanding defecation and cocoons. The fact that all larvae maintained alive from this study entered diapause indicates that this bee has only one generation a year, synchronized with the spring blooming of its host plant, as was reported by Hurd and Linsley (1975). This fact is of some interest because *Larrea tridentata* may have a second blooming period depending on the distribution of summer rains. The only oligoleges (two species of *Perdita* [Andrenidae]) to visit this plant typically have two annual generations (Hurd and Linsley, 1975), although, as pointed out by one anonymous reviewer, others, such as *Hesperapis larreae* Cockerell, may occasionally have a second.

Almost all of the meconial mass is voided before silk production commences. It is a tannish material incorporating pollen exines. The greatest amount of this material is applied to the first 5 mm of the wall at the front end of the cell in the form of elongate pellets (figs. 23, 27) that later will surround the nipple end of the cocoon near the front of the cell. Elsewhere, elongate streaks of tan fecal material paralleling the long axis of the cell are thinly pressed against all other parts of the cell wall. After cocoon construction they become attached to the outer surface of the cocoon fabric (fig. 22), but nowhere are silk fibers incorporated in any of these feces.

However, we know from examining cocoons that the exine-bearing material is not the last anal deposit. The front end of the cocoon possesses the final anal discharge consisting of pale, faintly yellowish white, fine-grained material lacking evidence of pollen exines, which is extruded after most of the cocoon has been constructed (figs. 23, 26) and, after the cocoon is completed, is between two layers of silk at the front end of the cell, as explained below. Because of its color we suspect that it may represent excretion from the Malpighian tubules.

The completed cocoon is a semitransparent silken oval chamber approximately 13 mm long with a maximum diameter of 6.0 mm. At its front end is a centrally located nipple, an external, rounded projection with a basal diameter of 2.0 mm and projecting 0.7–1.0 mm beyond the front curvature of the cocoon (figs. 24, 25). Adhering to the outer surface of the cocoon (figs. 22, 34) are elongate flattened streaks of tan fecal material, as mentioned above. The front end of the cocoon gradually narrows because of the feces that are applied there (fig. 27). Feces on the wall at the front leave a narrowing, median, open passageway about 2 mm in diameter between the lumen of the cell and cell closure that accommodates the nipple that is spun into it as the final step in cocoon construction.

The silk fabric of the cocoon, except at the front end, consists of a single, moderately thick laminate sheet, the outer surface of which has the luster of tissue paper (fig. 34). The rear 3 mm of the inner surface is transparent and quite reflective (fig. 35, rear part of cocoon), showing traces of fibrous structure only where small pebbles projected into the lumen, but most of the fabric of the midsection on the cocoon (fig. 35, right side of partial cocoon) reveals an inner gauzelike covering of white fibers reducing transparency and extending to the front of the cocoon (not shown in fig. 35), where the fabric loses its pliable texture and becomes stiffer and leathery. Under SEM examination the rear 3 mm of the cocoon fabric (fig. 42) takes on a reflective, finely pitted texture, which under high magnification (fig. 43) is seen to be created by a dimpled, sheetlike

covering (fused silk or some other substance) with embedded fibers and fine, scattered round holes. The midsection of the cocoon (figs. 39, 42) is also covered by sheetlike material, which is smooth, not dimpled, and which bears apertures irregular in shape, size, and distribution (figs. 40, 41). Through these openings can be seen a strongly fibrous subsurface. We currently have no explanation for the difference in structure, construction, or function between these two surfaces.

On the inner surface of the cocoon, the nipple (figs. 36–38) is evidenced only by a circular screen 2.0 mm in diameter composed of pale silk fibers continuous with the inner cocoon surface. Silk surrounding the screen is smooth and covers the fine-grained, yellowish white material of the final anal discharge, which had been deposited on the inner surface of the cocoon surrounding the passageway into which the nipple was later constructed. Only here is the cocoon fabric composed of two thin layers of silk separated by a thin layer of the pale discharge. Unlike the thin flexible cocoon fabric elsewhere, the double layer fabric sandwiching the pale discharge is leathery. Thus, when constructing the cocoon, the larva first deposits fecal material, then spins the entire wall of the cocoon, positions itself to deposit the pale discharge surrounding the passage into which the nipple will be constructed, and then repositions itself to start weaving the nipple. From our observations obtained by watching two larvae constructing nipples in cells from which we had removed the front ends, a larva spends considerable time reinforcing the entrance to the passageway with layers of silk (fig. 28), thus eventually forming the sides of the nipple. The larva then starts constructing a network (fig. 29) that eventually forms the outer dome of the nipple; thereafter it spins a dense cushion of fibers that fills the nipple (figs. 29–33); and lastly it forms the inner screen of the nipple (fig. 36). We do not know whether the inner surface of pale discharge is covered with silk before the nipple is constructed or it is deposited when the inner screen is constructed. It might be partly applied beforehand and completed at the end. In the construction of the nipple, silk of the outer dome when at first extruded from the salivary lips is clear and colorless and when strands are applied over one another, the fabric appears white (figs. 29, 32). Over a 12 hr period, the strands gradually turn reddish (figs 32, 33) without any indication that any substance has been added. Thus is constructed the nipple, forming a filter permitting air exchange between cocoon lumen and outside atmosphere and excluding parasites from entry.

DEVELOPMENT RATES

Of the three larvae recovered from cells of the first nest site, opened shortly after our excavation, all became fifth instars. The youngest was preserved on May 17, 2012, so we could compare its anatomy as an example of a predefecating larva (fig. 52). The other two were maintained alive in the rear part of their cells, and having completed defecation, they were constructing the front ends of their cocoons on May 24, 2012. The younger of the two larvae had become quiescent by May 29, whereas the older one was completely encased in its cocoon and could only be estimated to have entered diapause three to four days earlier. The estimated period of larval activity from egg eclosion to entering diapause at the end of cocoon construction is 21–24 days.

FIGURE 1. H.G.H. pointing to approximate position of first nest of *Trachusa larreae* discovered in area covered with blooming *Eriogonum trichopes*; note pollen plant *Larrea tridentata* to his right. FIGURE 2. First site of nests studied, with first two nests excavated in middle foreground and third nest in background being examined by H.G.H.

EGGS AND OVARIAN STATISTICS

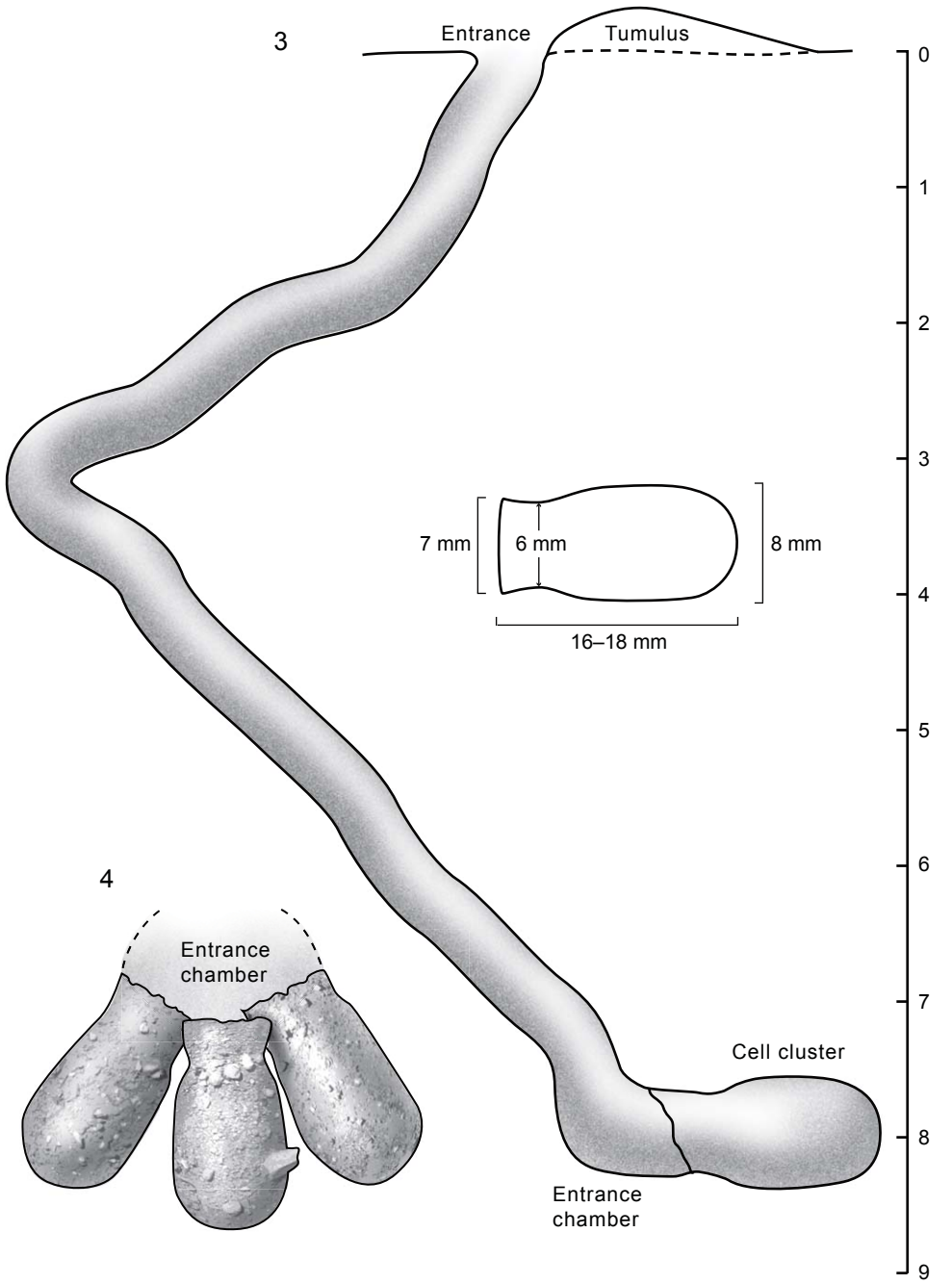
Three eggs of *Trachusa larreae* were uncovered from nests collected on May 12, 2012. All were elongate, slightly curved, moderately shiny, and 1.0 mm in maximum diameter at a point between 2/3 and 3/4 the total egg length. From that point both front and back tapered gradually to rounded ends, as in figure 11. One egg had its posterior end embedded in the provision while the front end projected into the cell lumen (fig. 12); the other two eggs lay lengthwise on the surface of the provision (fig. 11). The length of the embedded egg could not be measured; the other two eggs had lengths of 2.7 and 3.5 mm, a surprisingly large range. Preservation was inadequate to observe chorionic ornamentation and micropyle.

Three adult females preserved in Kahle's solution each had 3 ovarioles per ovary, an intertegular distance (i.e., distance between outer rims of tegulae) of 4.0 mm, and one mature oocyte. Mature oocytes were 2.8, 3.0, and 3.3 mm long and 0.825, 0.85, and 0.95 mm in maximum diameter. Dividing the average length (E) by the intertegular distance (M) yields an egg index of 0.76, placing the species at the low end of the *medium* category of Iwata and Sakagami's (1966) classification of bee eggs based on size of egg relative to body size of the female, i.e., E/M = egg index.

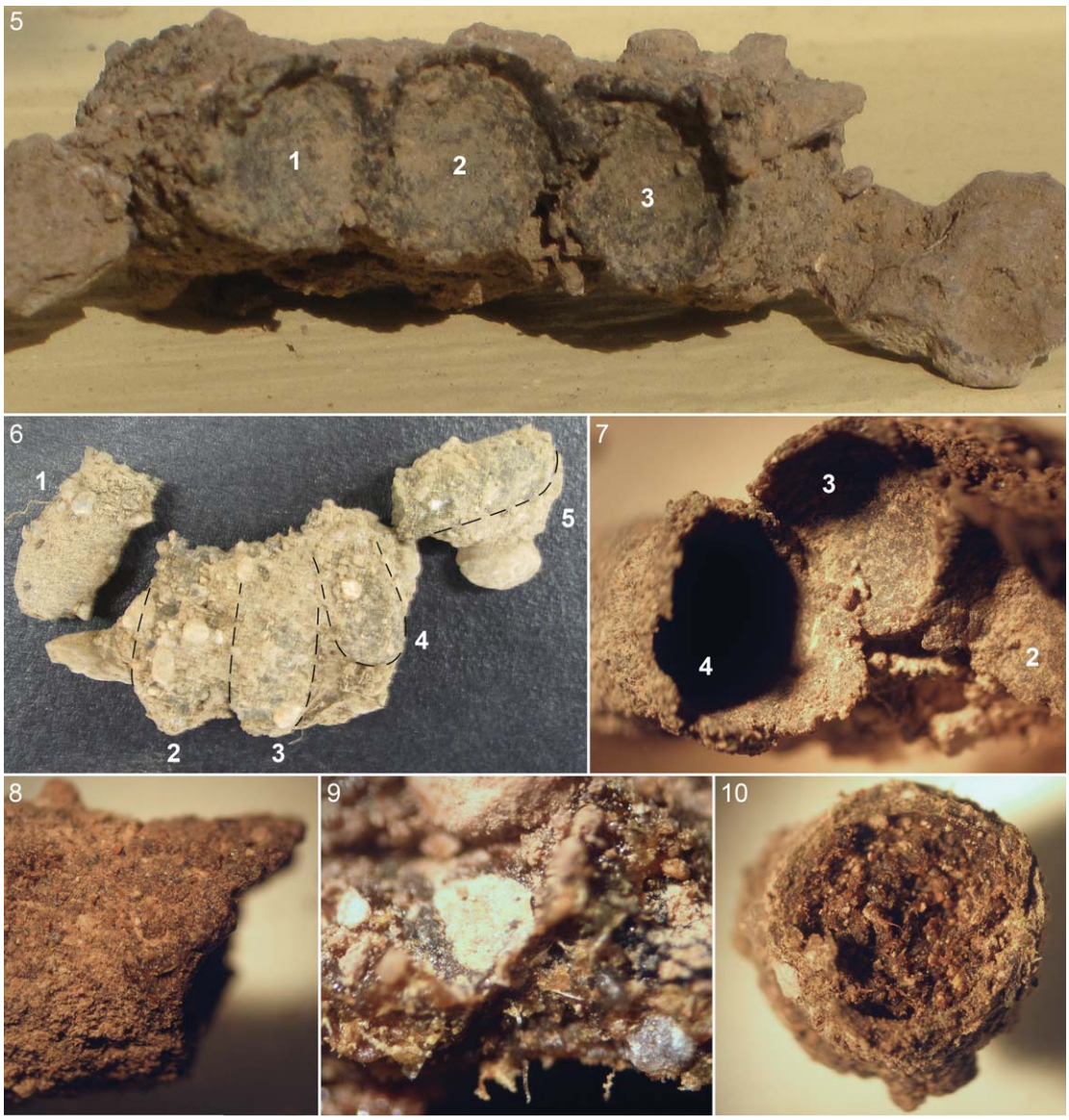
FIFTH INSTAR

Figures 8, 19, 44–65

A noteworthy feature of fifth instars of Megachilinae is their body vestiture. It has widely been referred to as setose (e.g., Michener, 1953; Rozen, 1966; Rozen and Özbek, 2004). However, Rozen (1987) pointed out that the body covering of *Hoplostelis bilineolata* (Spinola) was composed of setiform spicules rather than setae as he had originally stated (Rozen, 1966). He also noted that a larval *Megachile* had a vestiture of mixed setae and setiform spicules. An SEM



FIGURES 3, 4. Diagrammatic representation of first nest excavated with approximate dimensions of cell indicated. **3.** Entire nest (with only one cell represented), lateral view, with scale (mm) on right referring to nest depth. **4.** Radiating cell cluster, top view.



FIGURES 5–10. Microphotographs of nests of first site. **5.** Connected cell closures from first nest discovered, frontal view. **6.** Five cells from second nest, with position of cells in cluster outlined, top view, single cells not positioned. **7.** Front ends of cluster of three radiating cells from second nest, frontal view; note cell 4 still open. **8.** Front end of cell, lateral view, showing pronounced projection of upper rim. **9.** Close-up of broken edge of cell wall showing dark, highly reflective resin, imbedded, fractured pale small pebbles, and scattered plant fibers. **10.** Cell closure, inner view.

examination of *Trachusa larreae* sheds further light on this situation. Using the dorsal surface of the caudal annulet of abdominal segment 3 as an exemplar, one can detect spicules (figs. 44, 45, 48) and two types of sensilla: a stouter one arising from a donut-shaped alveolus (fig. 46) and a long slender one with a bulbous base (fig. 47). However, when viewing the dorsolateral

FIGURES 11–19. Microphotographs of immature stages of *Trachusa larreae.* **11.** Egg lying on surface of provisions. **12.** Egg inserted in provisions. **13.** Probable second instar. **14.** Probable third instar. **15.** Fourth instar. **16.** Early fifth instar, same individual as figure 15, photographed a day later. **17.** Same individual as figure 15, photographed a day later, after reorienting, lying on side against provisions while feeding. **18.** Live early fifth instar demonstrating middorsal body tubercles and body vestiture of stubble of short setae and long spicules, lateral view. **19.** Same, except ventral view, demonstrating pronounced lateral abdominal lobes and absence of same on thorax.

part of the prothorax (fig. 51), distinction between setiform spicules and setae becomes problematic, as is also the case when looking at lateral lobes of abdominal segment 8 (figs. 49, 50).

We think that the body vestiture of setae and setiform spicules of the fifth stage larva may be adaptive: providing the larva with traction and perhaps proprioception for moving around

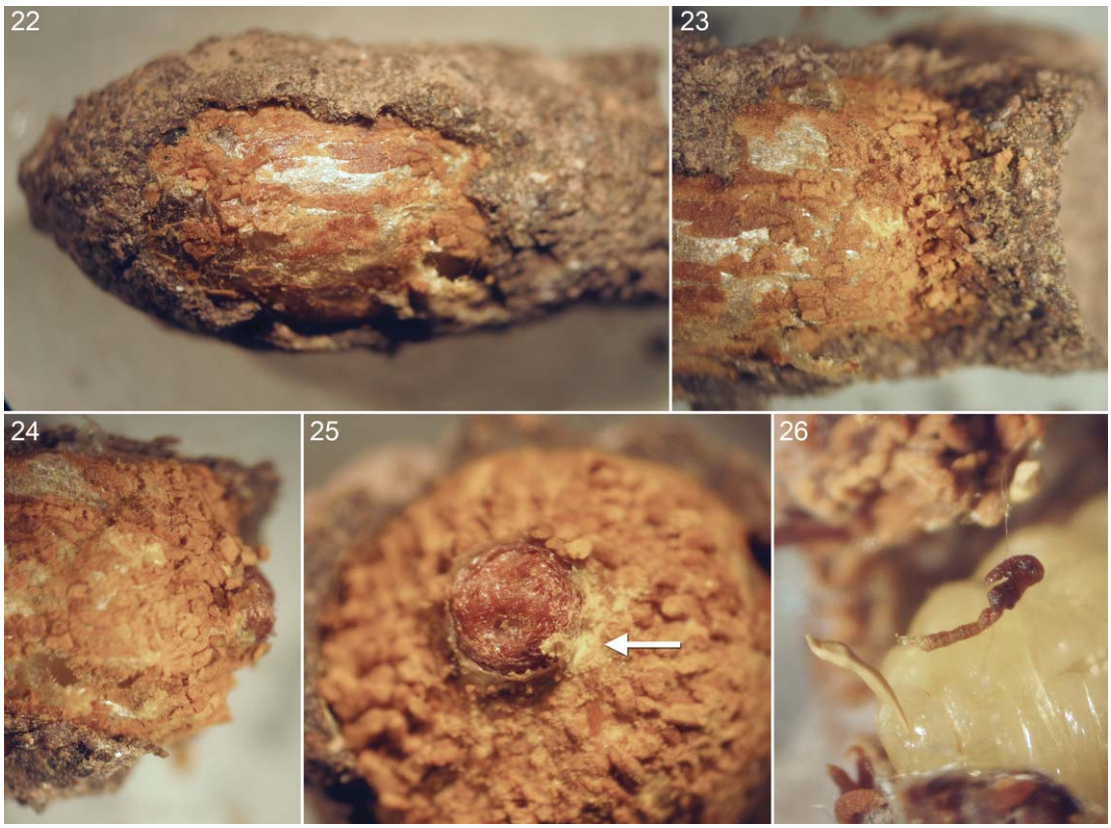
FIGURES 20, 21. Photographs of nest entrances at second site, with coins used to identify tumuli (arrows).

the cell for feeding and cocoon spinning in the case of *Trachusa larreae* and other solitary and cleptoparasitic megachilines.

As stated in the biology section, we also think that middorsal body tubercles may be associated with and facilitate body movement in *Trachusa larreae* and possibly other megachilines. At first we thought they might somehow pull the front part of the body forward, but when we discovered that the apices of the tubercles lacked setae and spicules, we suspect that they suppress the setose/spiculate surface of the integument elsewhere, so this rough surface does not contact the cell wall. Hence, the front part of the body can move with agility during feeding and cocoon spinning. The very pronounced lateral, setose/spiculate lobes, particularly of the first few abdominal segments, may stabilize the rear of the body while the front part moves about. There is a suggestion that, as the larva slowly curls and straightens its body, forward motion of the body might be provided as the spiculate/setose posterior dorsum on the abdomen pushes against the cell wall. With this in mind, new observations may explain body motion in greater detail.

The middorsal tubercles are most pronounced at the beginning of the instar and seemingly become reduced in prominence as the larva feeds, so that in postdefecating forms they hardly seem to exist. However, as we can see in figure 65, though greatly reduced in elevation, their wrinkled surface clearly persists. Pronounced middorsal tubercles are probably a reliable indication that a megachiline larva is a last instar and that it is in the early stages of that stadium, e.g., *Coelioxys* (Rozen and Kamel, 2007: fig. 54), *Stelis* (Rozen and Hall, 2011: figs. 85, 86).

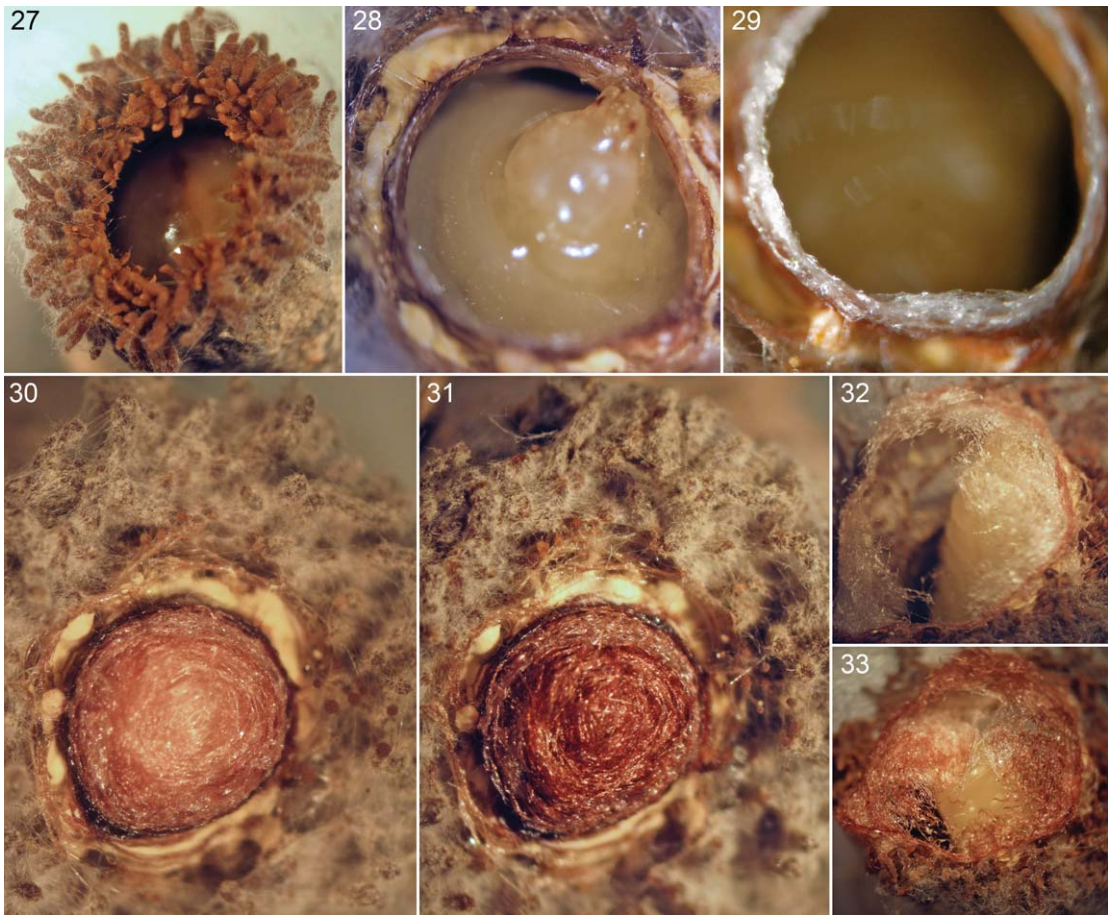
Diagnosis: The only larva of this genus described before was that of *Trachusa* (*Trachusomimus*) *perdita* Cockerell (Michener, 1953). We reexamined the specimens used by Michener and found them nearly identical to those of *T. larreae* except body setae and setiform spicules are more abundant with the lateral lobes of abdominal segment 8 bearing perhaps 40–50 points



FIGURES 22–26. Microphotographs of fecal deposition of larval *Trachusa larreae.* **22.** Cell with wall partly removed showing cocoon with fecal streaks partly covering fabric. **23.** Front end of same cell with more wall removed, showing fecal pellet deposited behind cell closure. **24.** Front end of cocoon with cell closure entirely removed as well as many fecal pellets to reveal nipple centered on front end. **25.** Another view of nipple end; note trace of pale yellow discharge (arrow). **26.** Close-up of rear of mature larva as it discharges pale, fine-grained anal material.

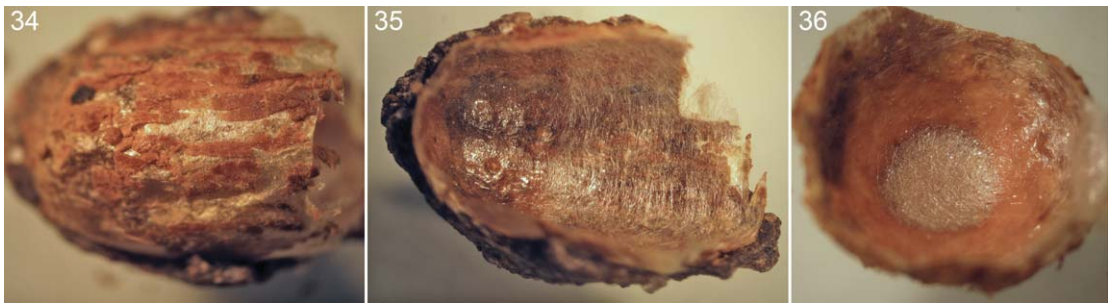
(i.e., setae and spicules combined), about twice as many as those of *T. larreae*. Additionally, almost the entire atrial wall of *T. perdita* is finely spiculate, and its subatria tend to be shorter than those of *T. larreae*.

Because of its obliquely truncate mandibular apex, postdefecating *Trachusa* larvae (represented by these two species) can be distinguished from those of other Anthidiini whose larvae are known, since almost all others have apically bidentate mandibles including one or more species in the following genera: *Anthidiellum*, *Anthidium*, and *Dianthidium* (Michener, 1953); *Hoplostelis* (Rozen, 1966); *Rhodanthidium* (Micheli, 1935); and certain but not all *Stelis* (Rozen and Kamel, 2007; Rozen and Hall, 2011). However, some species of *Stelis*, such as *S. lateralis* Cresson (Michener, 1953), have mandibles that end in a single apical point, but it is an elongate apically curved, gradually tapering, acute point, unlike the broad, subtruncate mandibular apex of *Trachusa* (figs. 54–56).



FIGURES 27–33. Microphotographs of two cells from which closure had been removed before defecation had started. **27.** With the cell maintained in a vertical position in the laboratory during defecation, the larva still applied most feces to the anterior end of the cell resulting in this display. It demonstrates that the stimulus to the larva as to where to place feces is obviously not gravitational in nature and suggests that cell shape and/or texture of the inside of the cell closure (despite the fact the closure had been removed earlier) might be the stimulus (as had been suggested by Cooper, 1957). **28.** Same cell, 3 days later, larva now adding abundant silk forming base of nipple; note outer cocoon layer, layer of pale anal discharge, and thick base of nipple. **29.** Same cell, 2 hr later, showing white silk of outer dome. **30.** Same cell, one day later, dome of nipple now complete with color mostly changed. **31.** Same, four day later, final coloring; note conspicuous display of fine-grained, pale-yellow discharge surrounding nipple. **32.** On another cell (cell opening misshaped) dome partly constructed, still white. **33.** Same cell, 12 hr later, fibers now reddish.

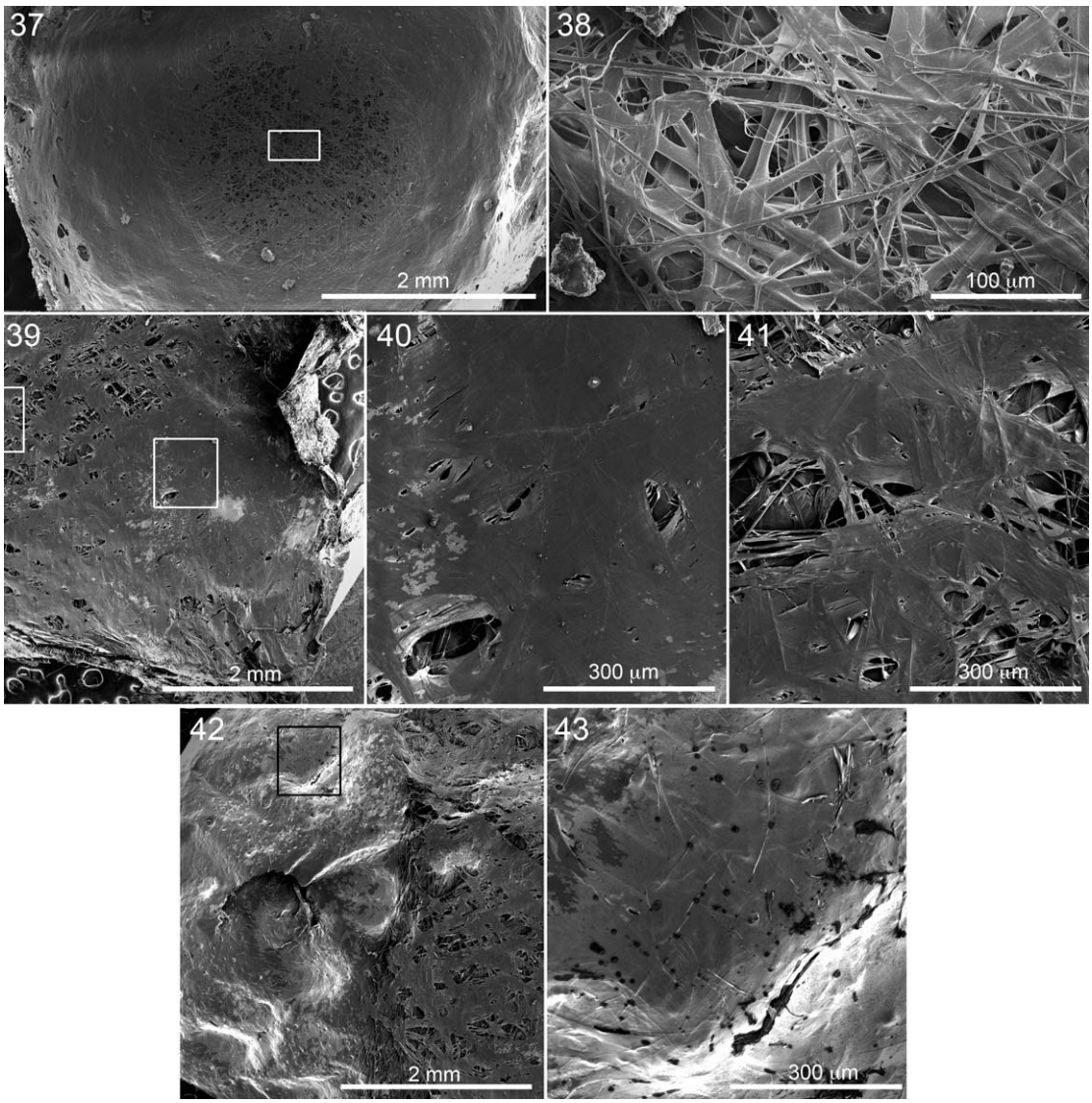
DESCRIPTION: **Head** (figs. 60, 61): Setae moderately long, basally stout, tapering, moderately abundant. Following areas moderately to faintly pigmented: mandibles, especially at apices and points of articulation; internal head ridges at articulation with mandibles; dorsal surface of premental sclerite between articulating points with articulating arms of stipites; distal end of cardo, all palpi. Fine spiculation restricted to dorsal surface of maxilla and lateral lobes of hypopharynx. Area immediately above hypostomal ridge and just behind posterior mandibular articulation not produced as downward-directed tubercle as present in many



FIGURES 34–36. Microphotographs of cocoon of *Trachusa larreae.* **34.** Outer surface with front end removed, side view. **35.** Inner surface, side view, showing reflective part of rear on left and fibrous part of midsection on right. **36.** Inner surface of nipple end, showing outer ring of part of fibrous midsection surrounding smooth, nonfibrous fabric covering fine-grained anal discharge that in turn surrounds pale filter in center.

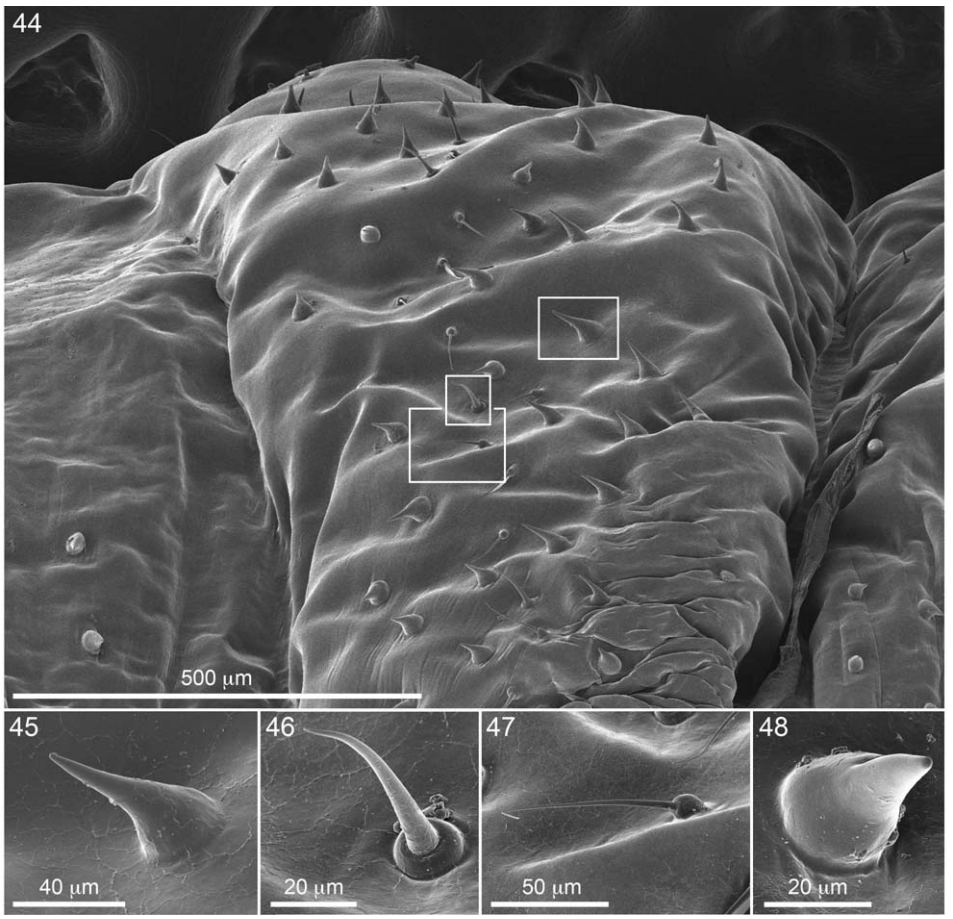
Coelioxys (Rozen and Kamel, 2007: fig. 47). Coronal ridge absent; postoccipital ridge moderately developed; as seen from above, ridge gradually curving forward toward median line; hypostomal ridge well developed; dorsal ramus scarcely developed; anterior tentorial pit distinctly closer to anterior mandibular articulation than to basal ring of antenna; epistomal ridge present only laterad of (below) anterior tentorial pits; tentorium moderately robust including dorsal arms. Parietal bands evident. Maximum diameter of basal ring of antenna about equal to distance from ring to center of anterior tentorial pit; antennal papilla (fig. 63) slender, tapering apically, somewhat longer than basal diameter, bearing about three sensilla (fig. 63). Lower margin of clypeus strongly angled upward at midline (figs. 60, 61), so that at midpoint margin nearly at level of anterior tentorial pits. Labral sclerite transverse, unpigmented, with lower margin extending beyond apical band of sensilla; apical labral margin broadly emarginate (fig. 31).

Mandible (figs. 54–57) moderately robust; that of postdefecating larva transversely truncate with longest surface acutely pointed, apical edge slightly uneven; dorsal apical edge faintly crenulate; mandible of young predefecating larva also transversely truncate, but apical edge with numerous uneven teeth and dorsal apical edge also with numerous sharply pointed teeth; apical concavity moderately well developed; mandible possibly derived from apically bidentate mandible with ventral tooth longer than dorsal tooth; outer surface of mandible with single large seta arising from tubercle (fig. 61). Cardo and stipital rod sclerotized but not extensively pigmented; articulating arm of stipes evident; maxillary apex directed mesad far beyond insertion of maxillary palpus, so the palpus is subapical in position; maxillary palpus slender, length about twice basal diameter. Labium clearly divided into prementum and postmentum; apex normally wide (fig. 61); premental sclerite weakly sclerotized, most evident dorsally on postdefecating larva because of pigmentation there; labial palpus slender, length about three times basal diameter, distinctly longer than maxillary palpus. Salivary lips projecting, transverse, width about equal to distance between bases of labial palpi; inner surface of at least lower lip, visible only after specimen subjected to critical-point drying process, with numerous parallel, raised ridges extending outward (fig. 62). Hypopharynx consisting of two separated lateral lobes that are spiculate.



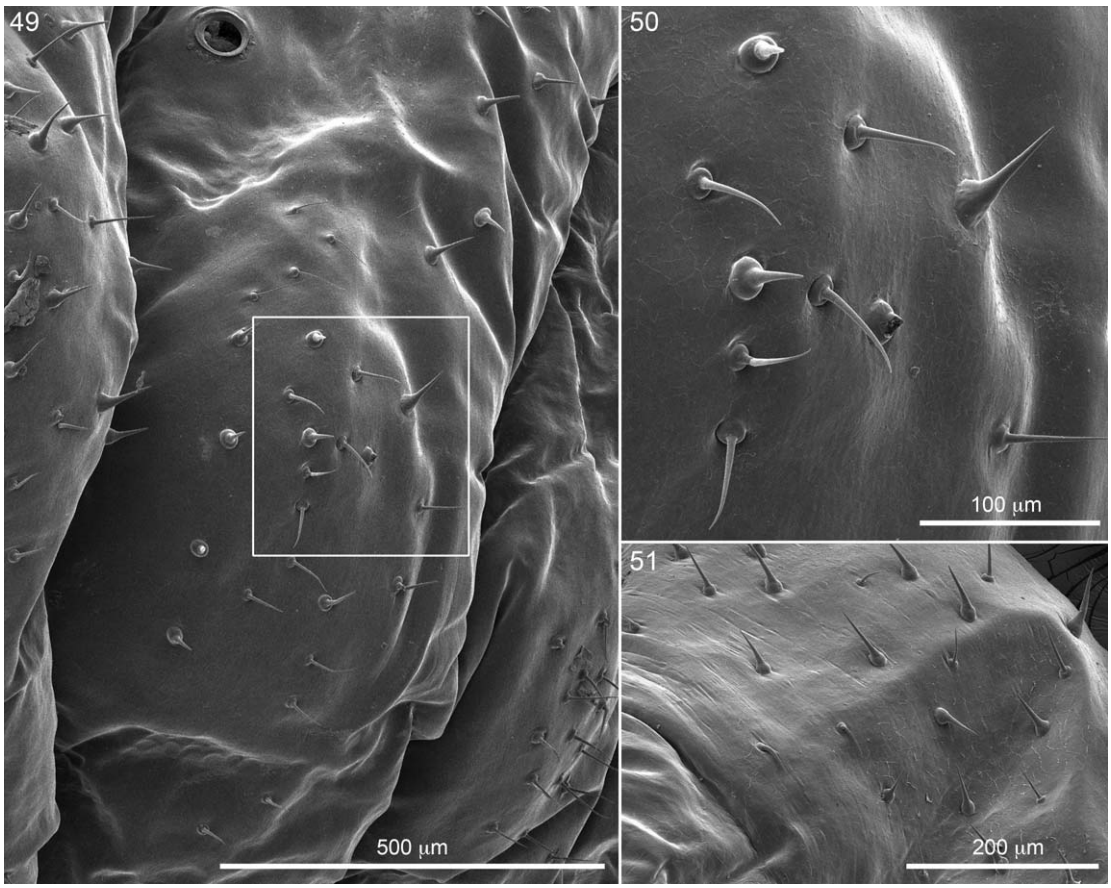
FIGURES 37–43. SEM micrographs of internal surface of cocoon of *Trachusa larreae*. **37.** Front end showing filter of nipple surrounded by nonfenestrated cocoon fabric covering fine-grained anal discharge. **38.** Close-up of rectangle in previous figure. **39.** Front end of sidewall showing numerous fenestrations as well many areas without fenestrations. **40.** Close-up of central rectangle in previous figure. **41.** Close-up of part of left, partly shown rectangle in figure 39. **42.** Rear end of sidewall showing texture of highly transparent rearmost area (at left) and gauzelike semitransparent anterior area (at right). **43.** Close-up of rectangle in previous figure.

Body (figs. 44–53): Body vestiture consisting of short setae mixed with spicules, discussed in the introduction to Fifth Instar, above; lateral swelling of abdominal segment 8 with approximately 25 setae and setiform spicules combined (figs. 49, 50); middorsal tubercles without setae/spicules apically on both pre- and postdefecating forms. Body form of postdefecating larva robust (fig. 53); although intersegmental lines not deeply incised on predefecating larva,



FIGURES 44–48. SEM micrographs of dorsal part of caudal annulet of abdominal segment 3 with cephalic annulet to the left, lateral view, showing close-ups of vestiture. **44.** Dorsal part of caudal annulet, with top rectangle referring to figure 45, middle to figure 46, and lowest to figure 47. **45.** Spicule. **46, 47.** Different kinds of setae. **48.** Spicule on cephalic annulet.

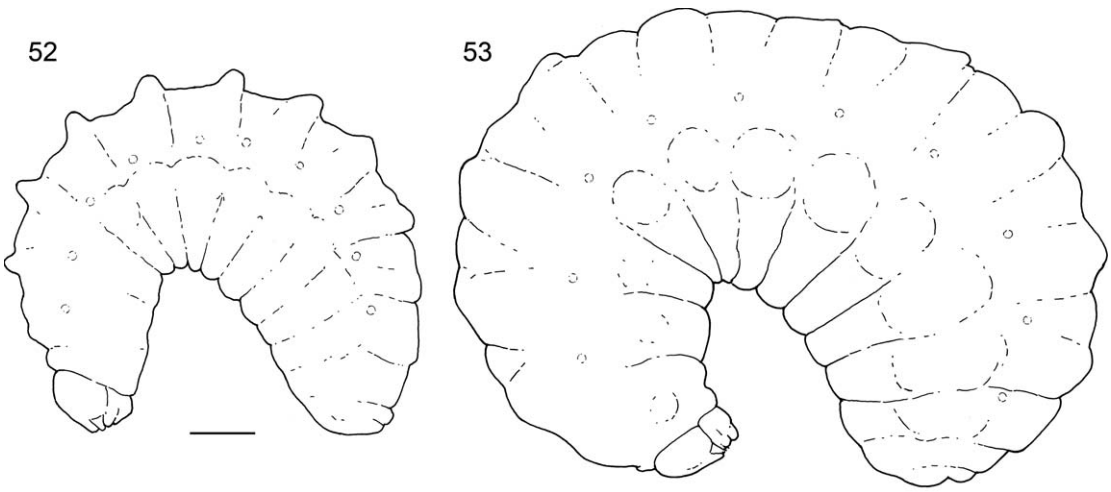
their position evident because of projection of caudal annulets compared with recessed cephalic annulets (fig. 52); intrasegmental lines well developed on postdefecating form; paired dorsal body tubercles absent; on postdefecating larva middorsal body tubercles weakly evident on thoracic segment 3 (fig. 65) and abdominal segments 1–5 (except those of midbody apparently artificially compressed by larva lying on dorsum), decreasing in size posteriorly; on predefecating larva middorsal body tubercles pronounced on meso- and metathorax and on abdominal segments 1–5 but decreasing in size from the front of the abdomen until disappearing toward the rear (figs. 18, 52); pleural thoracic swelling (presumably allowing appendage development) evident but less pronounced and more ventral than lateral abdominal lobes; these swellings moderately pronounced on abdominal segments 1–9 (fig. 19); abdominal segment 10 attached to approximate middle of segment 9; anus positioned toward top of segment 10. Spiracles (figs. 58, 59, 64) well sclerotized, unpigmented, subequal in diameter; atrium globular with width



FIGURES 49–50. **49.** SEM micrographs abdominal segment 8, lateral view, showing spiracle above and lateral lobe with its vestiture. **50.** Close-up of middle part of lateral lobe; note presence of elongate setiform spicule and setae of three morphologies. FIGURE 51. SEM micrograph of dorsolateral surface of prothorax of larva, viewed somewhat in front, with head (not visible) toward lower left, showing variety of vestiture.

about equal to depth, projecting considerably above body wall, with rim; peritreme narrow, so that diameter of atrial opening three to four times peritreme radial width; atrial inner surface immediately beneath peritreme with rows of small spicules (figs. 59, 64) concentric with primary tracheal opening; these rows and spicules tending to fade out inward from body surface, so that most of atrial wall nearly featureless; primary tracheal opening with collar; subatrium with about 15 chambers; externally, subatrium (fig. 59) tapering only slightly from body surface inward. Sex-specific characters unknown.

Material Examined: One postdefecating larva: AZ: Cochise Co.: 6 mi NE Portal, V-17-1988 (J.G. Rozen). One postdefecating larva: NM: Hidalgo Co.: 5 mi E Animas, collected as 2.5 mm long larva V-8-2012, preserved as quiescent, post–cocoon-spinning, postdefecating larva, V-29-2012 (J.G. Rozen, H.G. Hall); one postdefecating larva, same data except from unopened cell until V-29-2012.

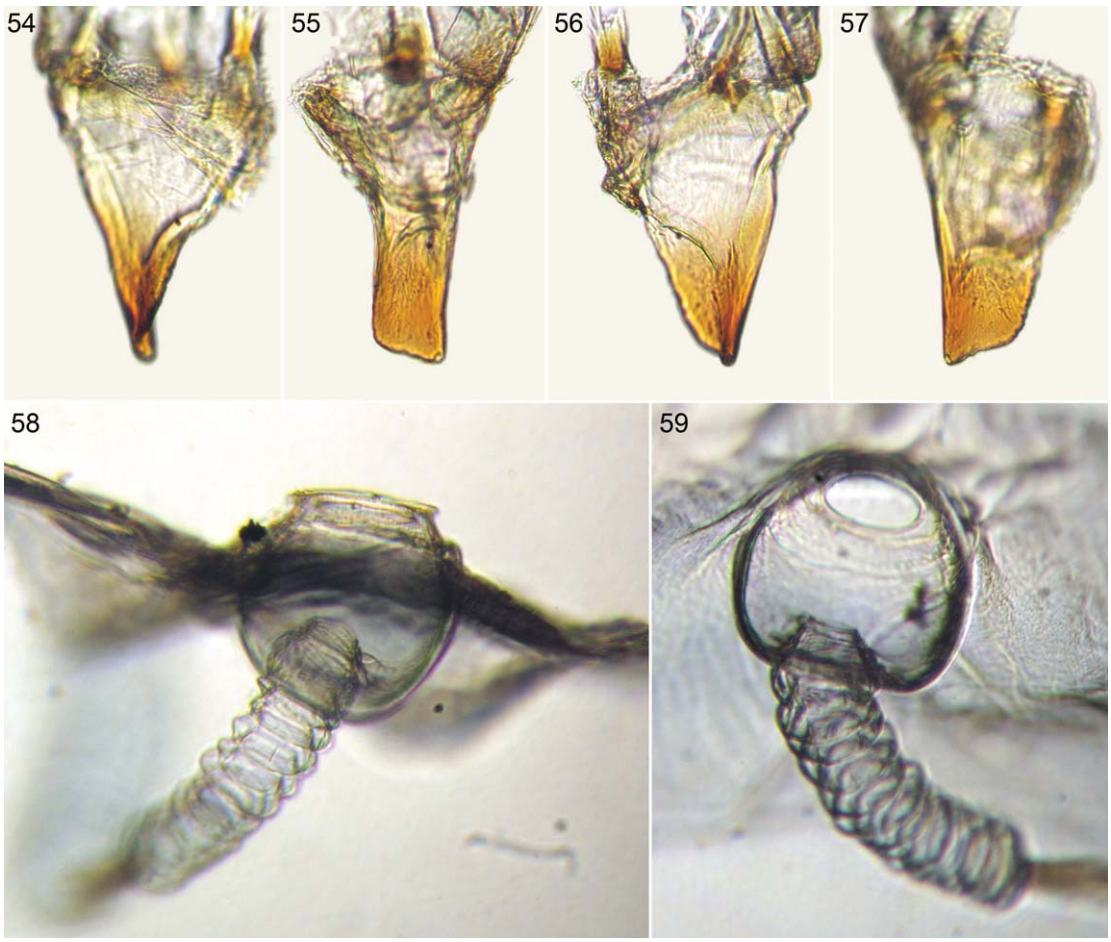


FIGURES 52, 53. Diagrams of fifth instars, lateral view, without vestiture, to same scale (= 1.0 mm), showing pre- and postdefecating morphologies, respectively.

DISCUSSION AND CONCLUSIONS

The three species of *Trachusa* studied to date share certain biological features but differ in others (Friese, 1923; Hachfeld, 1926; Michener, 1941; Westrich, 1989; current study). All are ground nesting, with burrow entrances lacking turrets. Burrows descend as open passageways at various angles, at the lower end of which are attached brood cells. However, cells of *T. larreae* are constructed side by side, radiating horizontally from a common point and often physically connected. Those of *T. byssina* and *T. perdita* are customarily connected end to end in linear series. Cell walls of all three species employ resin that is sticky at first but hardens over time. The outer surface of the walls of *T. byssina* and *T. perdita* consists of elongate leaf snips rolled perpendicularly to the long axis of the cell, but cell walls of *T. larreae* are constructed solely of resin with some sand inclusions and totally lacking any leafy material. No doubt other differences will be found within the genus when more species are compared.

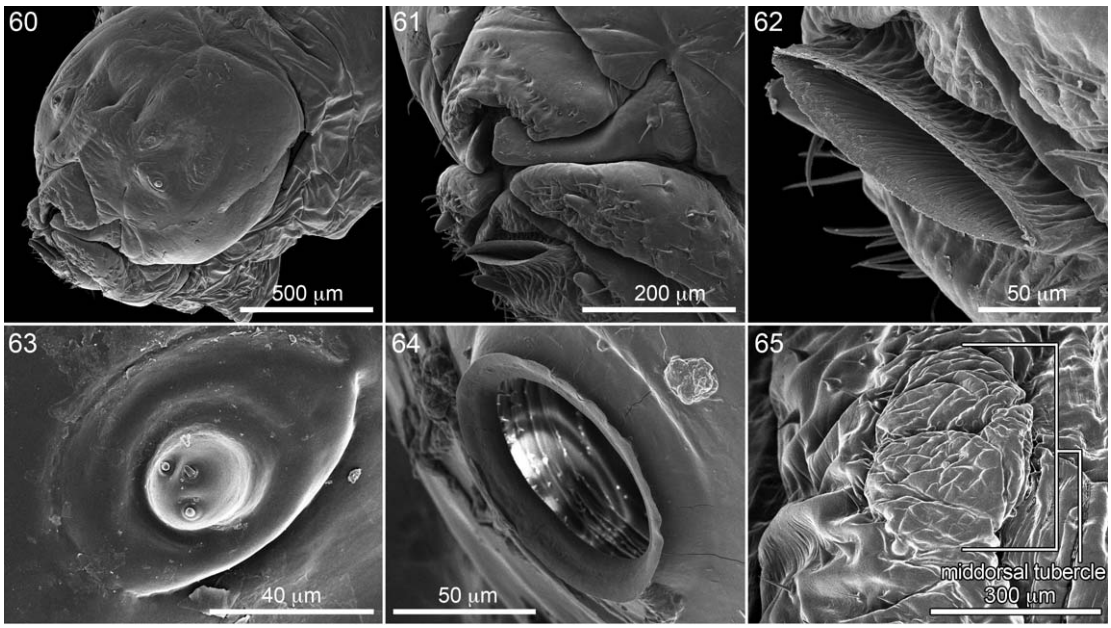
It has gradually become apparent that young cleptoparasitic megachilid larvae are seemingly unable to move from the position where their eggs have been deposited (Baker, 1971; Rozen and Kamel, 2008; Rozen et al., 2011), and observations on *Trachusa larreae* recorded here indicate that this is also true for at least some nonparasitic megachilids. We also suggest that body vestiture consisting of short setae intermixed with spicules of similar length in conjunction with the middorsal tubercle of young fifth instar megachilids probably assist the fifth instar in moving to finish feeding on provisions and cocoon spinning, although exactly how this is accomplished has yet to be explained. It has also been known for some time that only fifth instar megachilids have abundant body vestiture. This coincidence of behavioral and anatomical features strongly suggests they are functionally related, but the hypothesis needs further scrutiny for confirmation.



FIGURES 54–57. Microphotograph views of right mandible: **54.** dorsal; **55.** inner; **56.** ventral; and **57.** outer. FIGURES 58–59. Microphotographs of spiracles of cleared postdefecating larva: **58.** side view; and **59.** oblique outer view.

We report here that the pale yellow anal discharge of larval *Trachusa larreae* is deposited well after the exine-bearing feces have been deposited and suggest that it may come from the Malpighian tubules. This could easily be investigated by preserving and dissecting larvae collected at appropriate intervals during defecation and cocoon spinning to check the contents of their tubules. If this suggestion proves correct, how universal is this phenomenon among bee taxa? Why does the larva spin the entire cocoon body, stop cocoon spinning, excrete the pale material, and then resume spinning to produce the nipple? Does the three-layered anterior end of the cocoon offer a selective advantage for survival?

How does the female manipulate soft resin and apply it as the cell wall? When it hardens the cell wall obviously protects against invasion of the cell by potential predators and parasites, but does it help protect the cell contents from desiccation (or flooding)? If it serves as a water vapor barrier, how does air exchange take place between inside and outside of the cell?



FIGURES 60–65. SEM micrographs of postdefecating larva of *Trachusa larreae.* **60.** Head (with rear left parietal partly torn), dorsolateral view, viewed from somewhat in front. **61.** Mouthparts, frontolateral view. **62.** Salivary lips, showing parallel ridges of lower lip, frontolateral view. **63.** Antenna, close-up. **64.** Spiracle, abdominal segment 8. **65.** Abdominal segment 3, dorsolateral view of caudal annulet showing flattened surface of middorsal tubercle.

Recent studies of cocoons of *Osmia chalybea* Smith and *Stelis ater* Mitchell (Rozen and Hall, 2011: Rozen et al., 2011) have demonstrated a reasonable understanding of their structure relative to their presumed function of protecting the offspring and its food while allowing air exchange. The resinous cell wall of *Trachusa larreae* probably functions more than cocoon fabric to exclude parasites and predators from entering the cocoon, but we still need to understand how air exchange is possible. We suspect that the cocoon nipple is the conduit as is the case with *Osmia* and *Stelis.* Because the *Trachusa* nipple lies next to the inner surface of the cell closure, the cell closure must be porous despite its smooth outer surface. This needs confirmation.

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