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Nest Site Selection and Nesting Behavior of the Bee *Lithurgopsis apicalis* (Megachilidae: Lithurginae)

JEROME G. ROZEN, JR.,[1] AND H. GLENN HALL[2]

ABSTRACT

This paper reports on the biology of *Lithurgopsis apicalis* (Cresson) that were found excavating nests in the dead and dying flower/seed stalks of *Agave* in southern Arizona. Females normally gain entry to the soft inner tissue of the stalk by seeking out naturally occurring longitudinal cracks in the hard outer surface of the stalk. Once inside they chew branching tunnels through the soft plant tissue, at the end of which are one or more extremely elongate brood cells. The cells were normally found to contain one or more eggs, each in a small empty pocket entirely within the provisions of soft pollen, which completely filled the cell. The attachment of the egg to the provisions is described, as is the egg itself.

The first four larval instars remain attached to the provisions while the elongate fifth (final larval) instar is free from the provisions and starts defecating while still eating the food, which gradually intermixes with fecal pellets. Toward the end of defecation, larvae start spinning strands of silk to form cocoons. After finishing spinning, larvae enter diapause, becoming quiescent over a period of more than a week. However, when in diapause, they still react to touch by curling and uncurling their bodies unlike totally quiescent diapausing larvae of most bees. Cocoon structure and function are described.

Throughout the paper, aspects of nesting biology of this species are compared with those of other lithurgines. New details concerning the cocoon of *Trichothurgus dubius* (Sichel) are presented, and ovarian statistics for *Lithurgopsis apicalis* are appended.

[1] Division of Invertebrate Zoology, American Museum of Natural History.
[2] Department of Entomology and Nematology, University of Florida, Gainesville, FL.

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INTRODUCTION

Lithurgopsis apicalis (Cresson) is a large, conspicuous bee commonly found visiting flowers of the cactus *Opuntia* in the spring throughout most of the western half of the United States as well as the northern states of Mexico (Snelling, 1983; Ascher and Pickering, 2013). Parker and Potter (1973) first described the nesting biology of this species based on discovering it nesting in cottonwood (*Populus*) in Utah. Its mature larva, collected from that site, was described by Rozen (1973) in a paper dealing with other mature lithurgine larvae. The purpose of the present paper is to offer new information regarding the nesting requirements and behavior of this species and to provide descriptions of its egg, first larval instar, and early stage nesting biology. This investigation resulted from finding nests in several areas in Cochise County, Arizona, in May 2013. Much of the study therefore was carried out that month. However, the second author (H.G.H.) returned to the site in August of that year and collected a larger number of nests, then all quiescent, which were dissected in early September to provide further details especially on fecal placement, cocoons, and late-stage nesting biology.

Michener (2007) cites a number or references pertaining to the biology of other species then in subgenera *Lithurgus* and *Lithurgopsis* as well as in other genera in the subfamily. The same year Moure and Melo (2007) first recognized *Lithurgopsis* at the generic level, a practice followed by Gonzalez et al. (2013a). The taxa referenced include: *Lithurgus atratiformis* Cockerell (Houston, 1971); *L. atratus* Smith (Lieftinck,1939, and as *L. huberi* in Camillo et al., 1983, 1994, but see Gonzalez et al., 2013b, regarding the status of this taxon); *L. collaris* Smith (Kitamura et al., 2001); *L. chrysurus* Fonscolombe (Rust et al., 2004); *L. cornutus* (Fabricius) (as *L. fuscipennis* Lep. in Malyshev, 1930); *L. tibialis* Morawitz (Cros, 1939); *Lithurgopsis apicalis* (Cresson) (Parker and Potter, 1973); *Lithurgopsis gibbosa* Smith (Brach, 1978); *Microthurgus corumbae* (Cockerell) (Garófalo et al., 1992, and as *L. corumbae* in Garófalo et al., 1981). These references were briefly summarized by Rozen (2013). Several other papers, include: Hanna and Maeta, 2007 (on *L. collaris*); Rozen, 2013 (on *L. chrysurus*); Rozen, 1973 (on *Trichothurgus dubius* (Sichel)); and Sarzetti et al., 2012 (on *T. bolitophilus* Durante and Roig-Alsina).

METHODS AND TERMINOLOGY

Field methods for finding adults and immatures of *Lithurgopsis apicalis* were straightforward after we discovered, as described below under Field Activities and Observations, that the bee nested in the dead flower and seed stalks of *Agave palmeri* Engelm. (Asparagaceae). From literature accounts we knew that its larval food plant was *Opuntia*, which is noteworthy because its pollen grains are unusually large. We correctly predicted that, where these two plant species cooccurred, we would find nests in stalks.

Habitat photographs were taken with a Sony Cyber-shot DSC-HX1 and a Canon PowerShot A2300, 16.0 megapixels. The latter camera, handheld, was used to take microphotographs in the laboratory as well as in the field through one lens of a Leitz Wetzlar stereomicroscope. Laboratory microphotographs using a Carl Zeiss compound microscope were also taken with the Canon PowerShot.

With most larvae studied, the head was removed from the body, and both parts were cleared by boiling in an aqueous solution of sodium hydroxide, transferred to 75% ethanol, stained with Chlorazol Black E, and placed in glycerin on well slides for study and eventual storage. The first instar, however, was punctured with a pin and placed in warm lactic acid for about 24 hrs for clearing before study. Shed exoskeletons were also placed in warm lactic acid for 4 hr and then placed in ethanol and lightly stained with Chlorazol Black E before being examined in glycerin on well slides, in attempts to recognize the numbers of, and differences between, larval instars.

Larval specimens to be examined with an Hitachi S-5700 scanning electron microscope were first critical-point dried and then coated with gold/palladium; cocoon sections were simply mounted on stubs and coated without being dried.

Specimen records in the AMNH Division of Invertebrate Zoology database (Schuh et al., 2010) were obtained using Arthropod Easy Capture (2013) software.

There has been an inconsistent use of terminology dealing with nest components in the Lithurginae because, among a good many of its taxa, more than a single egg is deposited in the provisions that occupy a single enclosure. For the purpose of this paper, a **cell** is a single enclosure defined by the tunnel walls and by partitions made of a substrate (i.e., wood chips, wood fiber, wood dust, dung) at both ends or at the front and far end of the tunnel. Thus a cell may contain one or more than one individual (egg, larva, or cocoon). A **branch** of a nest implies that the entrance tunnel (or gallery) divides. A branch may contain one or more cells arranged in a linear series, each defined by a partition in front[3] and either by a partition in back or by the far end of the tunnel. Two cocoons in linear series are not in separate cells unless separated by a partition of wood chips. Separation by accumulated feces alone implies a single enclosure (cell) for a common food mass, no matter how many individuals feed on it.

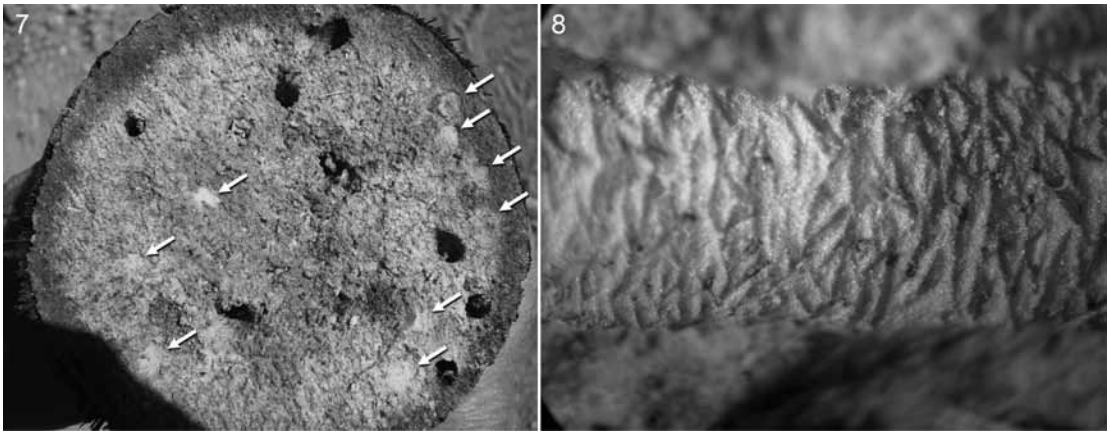
FIELD ACTIVITIES AND OBSERVATIONS

On May 17, 2013, we discovered a cluster of plants of *Agave palmeri* along the north side of the road leading westward from the town of Dos Cabezas, Cochise County, Arizona (fig. 1). Some of these plants had apparently bloomed the previous year, and a few of these had had their flowering stalks sawed off approximately 1 m above ground, leaving truncated, upright stalk bases surrounded below by the radiating cluster of drying spiny leaves. In each of two truncated bases, two open nest tunnels penetrated the soft inner tissue at the cut end. Descriptions of the first nest uncovered and others that were subsequently found are presented in Description of Nests, below.

This discovery and our understanding of the nesting biology of other species in the genus strongly suggested that *Lithurgopsis apicalis* requires a nesting substrate of soft, dead, plant tissue, such as afforded by the inner part of *Agave* stalks. At the same time, the presence of the nesting tunnels penetrating only the truncated stalks suggested that these bees are incapable of nest construction through the hard, tough outer wood of these stalks. Thus unanswered was the question where could similar soft plant tissue be found in such a thorny, sclerotic landscape

[3] A partition at the front of cell that is the first in the branch may contain not only substrate material (e.g., wood chips) but also miscellaneous debris associated nests closure.

FIGURE 1. Road leading from Dos Cabezas, Cochise County, Arizona, where nests of *Lithurgopsis apicalis* were first discovered in the decapitated stalks of *Agave palmeri.* FIGURES 2–6. Study area 8 mi north of Portal, Cochise Co., Arizona. **2.** Landscape; note presence of *Agave palmeri* and *Opuntia.* **3.** Female *Lithurgopsis apicalis* in flower of food plant *Opuntia.* **4.** Base of *Agave* plant with rosette of basal leaves, which once dry fortifies bee nests from predations by vertebrates. **5.** H.G.H. removing leaves from another *Agave*. **6.** Crack (arrow) in *Agave* stalk that allows females of *Lithurgopsis apicalis* access for nesting.



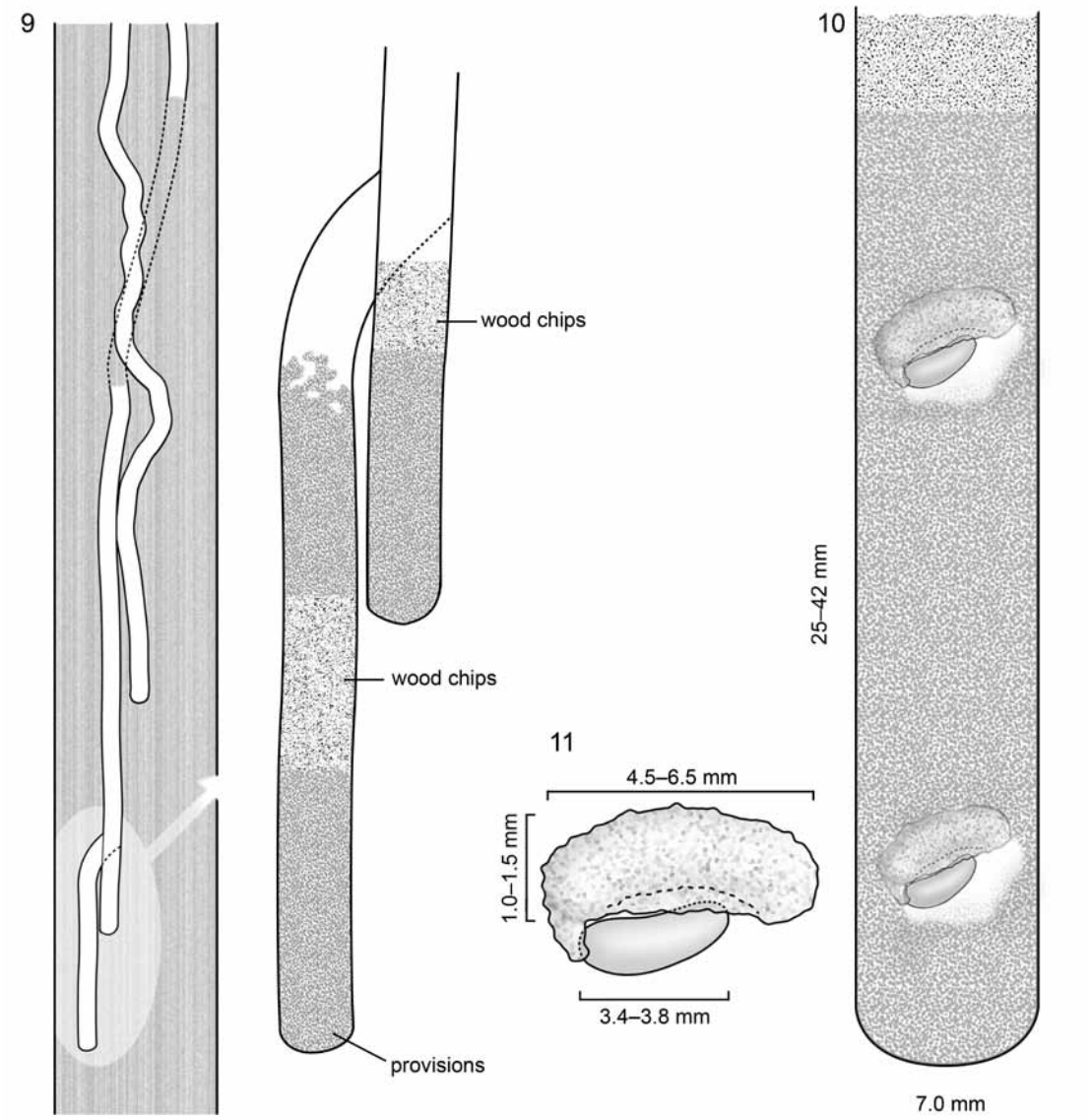
FIGURES 7, 8. Nests of *Lithurgopsis apicalis*. **7.** Cross section of *Agave* stalk where multiple nests were first found, showing pollen-filled cells (arrows), open tunnels, and tunnels filled with wood chips (gray). **8.** Close-up of burrow wall through fine-grained plant tissue showing characteristic transverse patterning created by the female's mandibles during excavation.

(fig. 2). Dead cottonwood trees, already known to be an appropriate nesting substrate (Parker and Potter, 1973), were not in sight.

The following day we selected an area strewn with *Opuntia* and *Agave* plants as a study site at 8 miles north of Portal, Cochise County, AZ, (N32°0′25″ W109°11′03″) (fig. 2) near the Southwestern Research Station. There we selected *Agave* plants from the previous year and removed their upper parts, leaving the standing stumps, thus simulating the nest-bearing *Agave* plants at Dos Cabezas. We planned to return in several months to study the nests that we hoped would have been constructed by these bees. The site was filled with many *Agave* plants, both dead and new, intermixed with abundant flowering stands of *Opuntia*. Fortuitously, the following morning we learned that it had an abundant population of adult *Lithurgopsis apicalis* as well (fig. 3). That day we began to remove the upper stalks of such plants. As we approached perhaps the sixth *Agave* plant, H.G.H. observed two bees flying from an elongate crack running along its stalk, probably resulting from drying of the plant tissue and the stress of its slightly leaning posture.[4] This is normal deterioration of flower stalks as evidenced by numerous cracked old stalks lying about the desert landscape. We immediately set to work; after removing much of the spiny basal leaves of the plant, we sawed through the lower base of the stalk, and observed that we had with a single slice cut through approximately eight pollen-filled cells and an additional 13 open and closed tunnels (fig. 7) in that one stalk. We had discovered a secret nesting location of *L. apicalis* in this region of the Southwest: the soft inner plant tissue of *Agave palmeri*, which is exposed if and when plant stalks split in the process of decaying following blooming and seed production.[5] Confirmation came through discovery of similar pollen-filled

[4] On May 22 H.G.H. made a similar observation at Ft. Bowie, Cochise Co., resulting in the discovery of another active nest.

[5] Interestingly, J.G. Rozen and R.J. McGinley had retrieved several cocoons, smaller than those of *Lithurgopsis apicalis*, presumably of *Lithurgopsis echinocacti*, from an *Agave* plant 9 mi east of Douglas, Cochise Co., AZ, August 29, 1977 (collection of AMNH), indicating that this plant genus hosts more than one lithurgine species.



FIGURES 9–11. Diagrams of nest found near Dos Cabezas, Cochise County, Arizona. **9.** Entire nest, side view containing two burrows, with close-up of section containing three cells, one of which had not been closed. **10.** Single cell containing two eggs, each associated with small open space ("egg chamber") and with disc of firmer provisions. **11.** Close-up of disc and egg.

cells in stalks of other individual dead or dying *Agave* plants during the following few days. Further, vacated cocoons from old nests of *L. apicalis* were also recovered, as are described below. Clearly, if the distribution of *L. apicalis* is found to extend far beyond that of *Agave palmeri*, other *Agave* species might also be used.

Description of Nests: The first nest discovered provided an understanding of general nest architecture in *Agave* stalks, i.e., this nest consisted of a long descending open main tunnel

that branched at the lower end giving rise to a tunnel consisting of a single, elongate, vertical brood cell. The other branch led to two similarly elongate cells arranged end to end (i.e., in linear series), which paralleled the cell of the other branch (fig. 9). Tunnels (fig. 8) and cells are dug by the nesting female with her apically tridentate mandibles, the central tooth of which is longest. In the first nest (fig. 9), the two cells in linear series were separated by 25 mm of moderately consolidated wood chips,[6] and the single cell was closed with 8–9 mm of similar wood chips. These chips presumably result from burrow and cell construction, although toward the end of nesting, they may be produced to fill in access to cells throughout the lower nest passageways. We were unable to detect reuse of nests by subsequent generations, as reported by Parker and Potter (1973).

There may be a selective advantage for cells to be positioned near bases of drying spiny leaves at the base of *Agave* stalks, which are an effective barrier against attack by small mammals. If this is true, then the length of the entrance gallery may be determined by the position of the entrance slit in the *Agave* stalk relative to the basal fortress.

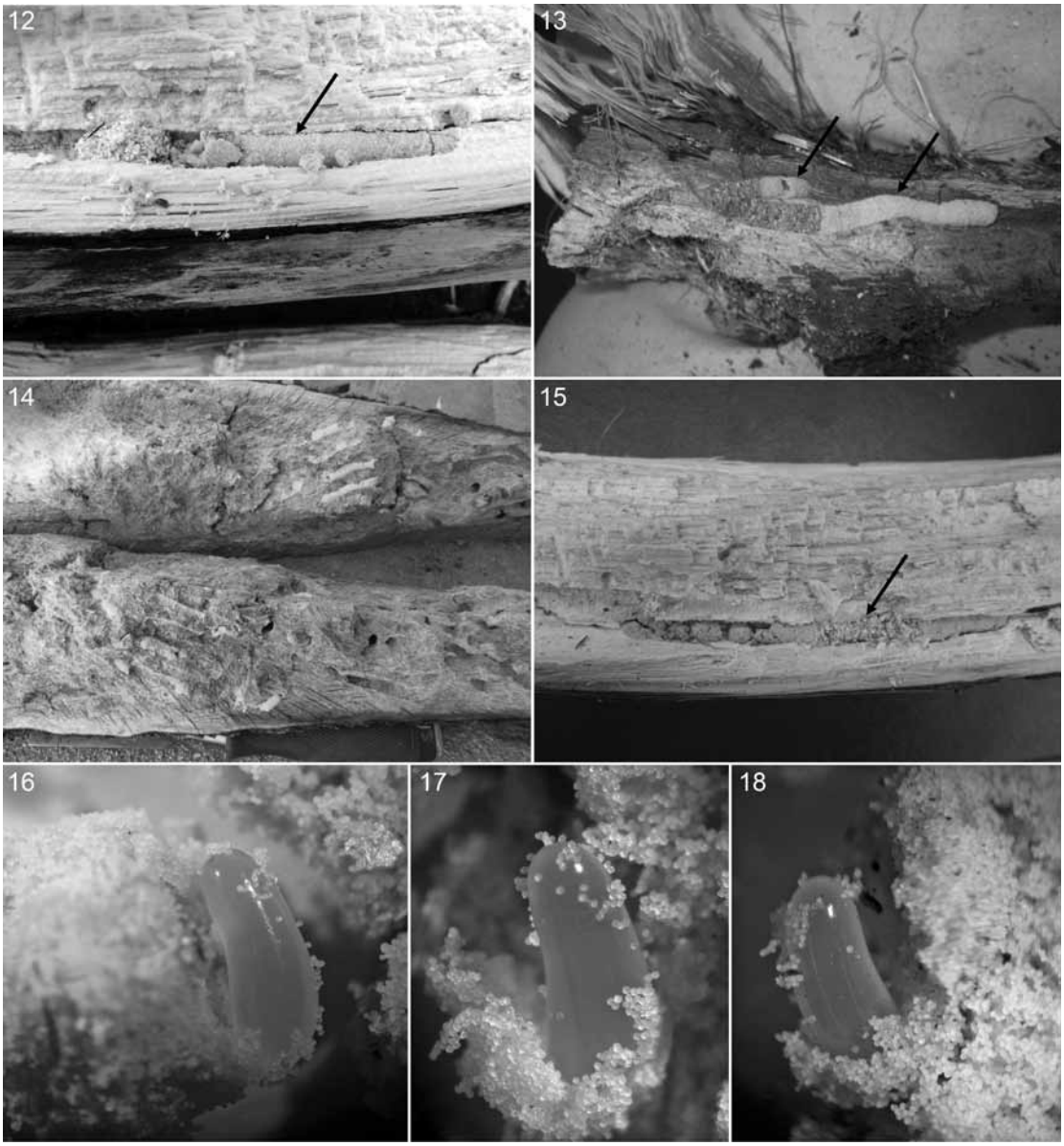
Main nest tunnels are defined here as the tunnel leading from the outside opening to where it first branches. The main tunnels may be long, as in figure 9, or relatively short. Beyond the point of branching, they can no longer be distinguished since branches were identical to their main tunnels in every respect. Each branch may lead to a single cell, a linear series of cells, or simply end. Branches (and their cells) tended to run in parallel with one another, particularly if the wood was sound, but a certain amount of cell bending occurred particularly where the plant tissue was decaying.

Cells of all nests encountered were remarkably elongate compared with their diameters, ranging in length from 25 to 42 mm ($N = 5$)[7] and a nearly constant diameter of about 7 mm ($N = 5$). Although all cells uncovered were completely filled with soft (i.e., loosely packed) pollen, none showed any indication of a special cell lining or had walls smoother than those of tunnels leading to them, as found in most bee cells. Thus, cells seemed to be simply the ends of burrows. We detected no nectar used to form partitions and nest closures as did Rust et al. (2004), but, on the other hand, we did not observe any construction of partitions or closures.

Provisions: Each cell was completely filled with light (in density), remarkably loosely packed, large, yellow *Opuntia* pollen grains (figs. 12, 13, 15) that were slightly sticky but only faintly moist, unlike the firm or sticky provisions in cells of many other bees. Hence, there were no shaped food masses or large empty spaces in cells, although eggs were surrounded by a small amount of more or less open space that other authors (e.g., Malyshev, 1930) have called egg chambers with respect to other lithurgine species. This open space in the case of *Lithurgopsis apicalis* does not appear to be as well defined and large as depicted for *Lithurgus cornutus* (as *L. fuscipennis* in Malyshev, 1930: figs. 2–4). The entire cell content was a cylinder

[6] Partitions between cells in linear series in nests studied subsequently suggest that a 25 mm partition was unusually long; others much shorter were observed.

[7] After that range was recorded, we discovered a cell that was slightly longer than 50 mm. Although we found at most two eggs in cells, we wonder whether cells of this length might hold more, particularly after learning that Gutbier (1916) reported as many as three eggs in cells of *Lithurgus cornutus* and that Garófalo et al. (1981) counted as many as six immatures in a cell of *Microthurgus corumbae.*



FIGURES 12–14. Single cell provisions of *Lithurgopsis apicalis* from various nests. FIGURE 15.Two cells in linear series. FIGURES 16–18. Eggs of *Lithurgopsis apicalis* attached to disc, and same egg with attachment to disc now broken, and another egg and disc, respectively.

of provisions shaped by the circular cell wall, the flat closure at the top end, and the round rear of the cell wall at the lower end (in the case of a single cell). When viewed from the outside, the cylinder of pollen grains seemed to be homogeneous throughout. It was not until these cylinders were carefully examined that we realized that this was not so, as discussed below under Oviposition Behavior. As larvae developed and defecated, there was no hint of mold or other indications of deterioration of the food supply despite accumulation of fecal pellets intermixed with pollen.

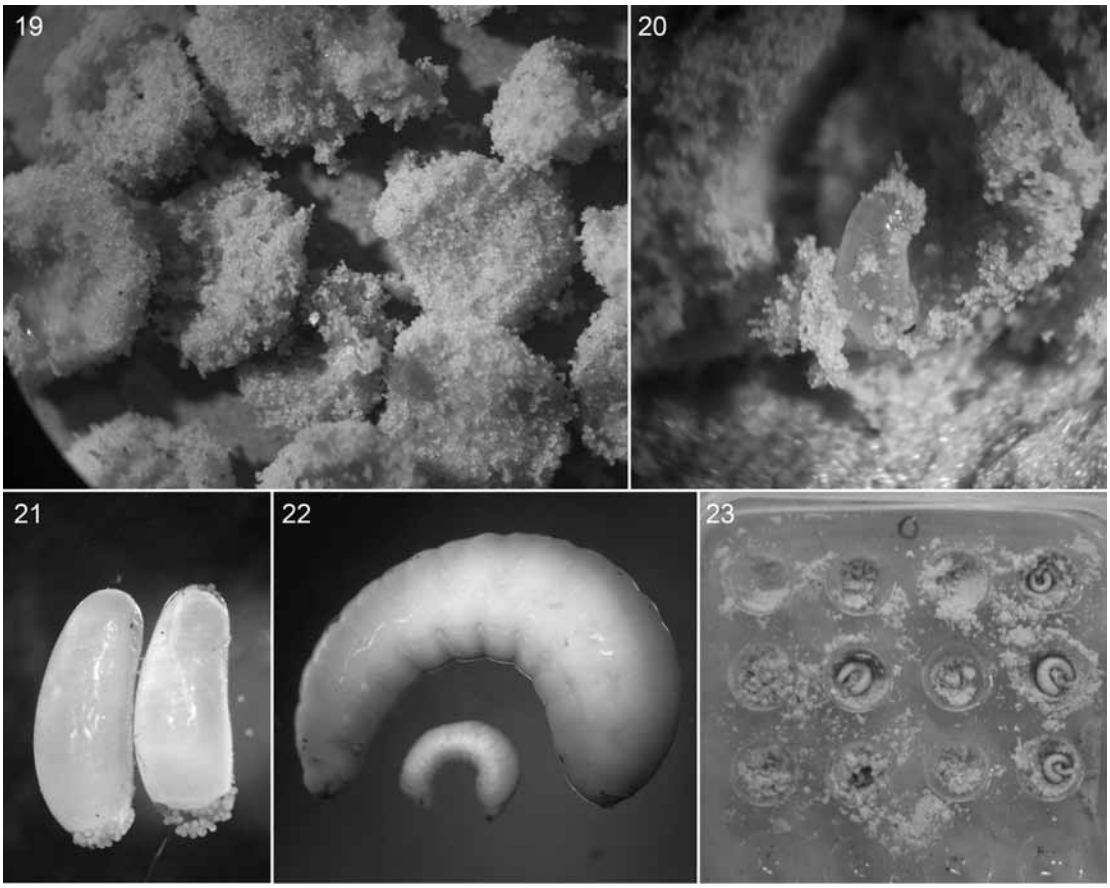
Several papers have mentioned pollen robbing behavior by females of *Lithurgus*: *L. collaris* (Hannan and Maeta, 2007) and *L. atratus* (as *L. huberi* in Camillo et al., 1994). Although we saw no evidence of this with respect to *Lithurgopsis apicalis,* our observations were perhaps too brief to identify such behavior.

Oviposition Behavior: Some cells clearly accommodated two individual eggs, and at first none seemed to have had a larger number, although later casual observations suggested that occasionally there was a larger number. Many cells were found with only one inhabitant, and others seemed to contain none. The reason for this variation was not understood at first, but with additional observations, we concluded that females normally deposit two eggs to a cell. One is deposited roughly 10 mm from the posterior (lower) end and the other about the same distance behind the anterior (upper) end of the cell. Clearly some of these eggs failed to hatch, leading to our oversight in recognizing this at first. Although the cylinder of provisions that seemed to completely fill the cell lumen appeared to be composed of fluffy, loose, almost dry pollen, each actually contained imbedded in it two thick discs, which were concave and slightly more defined on the posterior (lower) surfaces than on the upper surfaces. Discs (figs. 11, 16–19) were composed of slightly more consolidated (firmer) and apparently moister pollen than the surrounding pollen. When more disc shaped, they ranged from about 4.5–6.5 mm in diameter, but sometimes they were more scoop shaped with their side-to-side width somewhat less than their length.[8] Thickness in all cases was 1.0–1.5 mm.

The concave surface of the disc faces the posterior end of the cell, with part of the disc's perimeter projecting more toward the posterior. The projection is the platform onto which the broad, curved, rear end of the egg is firmly attached (figs. 11, 16–18) with the egg's ventral surface facing the posterior surface of the disc. The pocket of more or less open space (i.e., egg chamber) that surrounds the egg is defined by the concave posterior surface of the disc, the platform to which the egg is attached, and the wall of loose pollen that surrounds it elsewhere. The egg lies close to the posterior surface of the disc and curves slightly toward it but does not contact it (figs. 11, 16, 18). Because discs, composed of yellow pollen, are only slightly more consolidated than the surrounding pollen, and because they lack sharp, well-defined outlines, except for the concave posterior surface and are buried in the provisions, they are easily overlooked. Later, presumably after the egg hatches, the inner surface of the concavity gradually becomes harder, stiffer, and sometimes reflective. It is more easily identified, and the concavity becomes more spacious. Young larvae face (fig. 20) the disc and probably eat pollen from its posterior surface as well as from the surrounding supply. The egg chamber becomes the larval chamber as the larva feeds.

Observers of other lithurgines made reference to these discs with two apparent exceptions. In Houston's (1971) study of *Lithurgus atratiformis,* he uncovered a single egg and noted: "The lower end of the pollen mass which curved over the egg appeared to be formed of a separate specially moulded piece." From the picture presented, that piece appears to be a large projection, here called the *egg platform*. The egg of this species was found with no provisions beneath it at the end of the tunnel, as is also true for some *L. atratus* (as *L. huberi*) according to Camillo

[8] The so-called disc and scoop are essentially the same structure; a disc with lateral edge bend slightly toward one another becomes a scoop particularly when the egg platform projects toward the rear of the cell (fig. 11).



FIGURES 19–23. Microphotographs of nest components of *Lithurgopsis apicalis*. **19.** Assorted discs showing variation primarily in views of posterior surfaces from numerous cells. **20.** Young larva, probably first instar, facing posterior surface of disc, but not yet feeding. **21.** Two preserved eggs, side view. **22.** Newly emerged fifth instar compared with fully grown fifth instar. **23.** Plastic rearing dish containing five live larvae, which permitted us to observe feeding and cocoon-spinning activities throughout the study period.

et al. (1883). The other apparent exception is the study of *L. chrysurus* by Rust et al. (2004: 273) wherein the authors describe two layers of pollen provisions. It seems likely that the moister layer will be found to relate (be homologous) to the disc and platform discussed here.

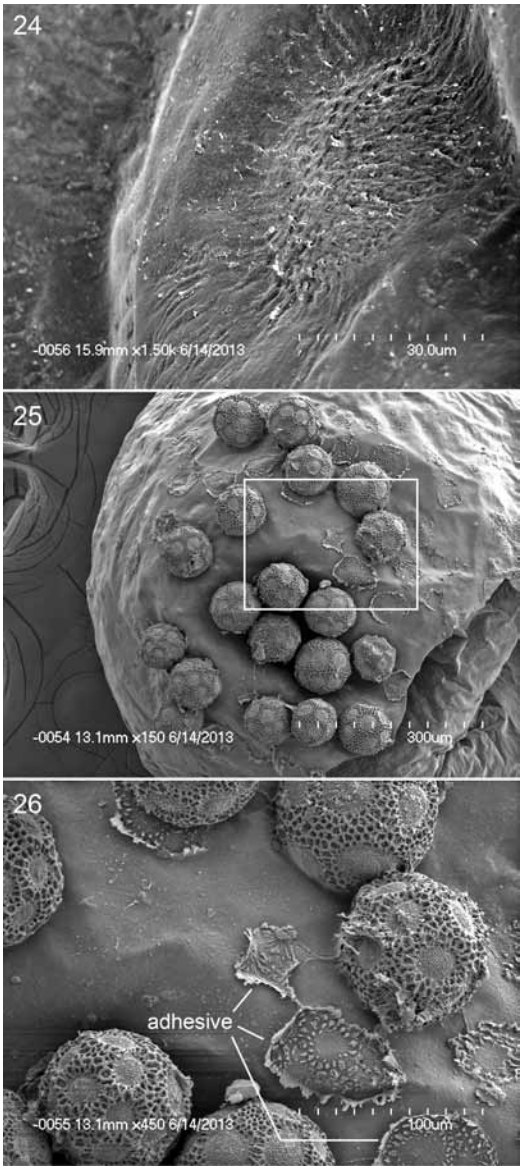
Many eggs, recovered from provisions before we recognized the existence of discs, were attached at their posterior ends to scraps of more consolidated pollen (figs. 17, 21). Almost certainly this situation is an indication that the rest of the disc (or scoop) had broken away from the projection, which is the scrap to which they were still attached. How and when such a disc is made is a matter for future investigation. It may merely be a byproduct of oviposition, or the female may manufacture it beforehand, since it seems required for proper egg orientation and development.

We have no direct evidence as to whether eggs are deposited after a cell is fully supplied with provisions, as generally occurs with solitary bees, or whether eggs are deposited as provisions are added. However, the latter is more probable since this is known for both *Lithurgus atratus* (as *L.*

huberi in Camillo et al., 1983) and *Microthurgus corumbae* (as *L. corumbae* in Garófalo et al., 1981), and because how would such a large female back into such a narrow cell filled with pollen?

Eggs appeared to have been deposited at more or less right angles to the long axis of the cell, thus in an approximately horizontal position, since cells parallel the long axis of the *Agave* stalk in many, though certainly not all, cases.

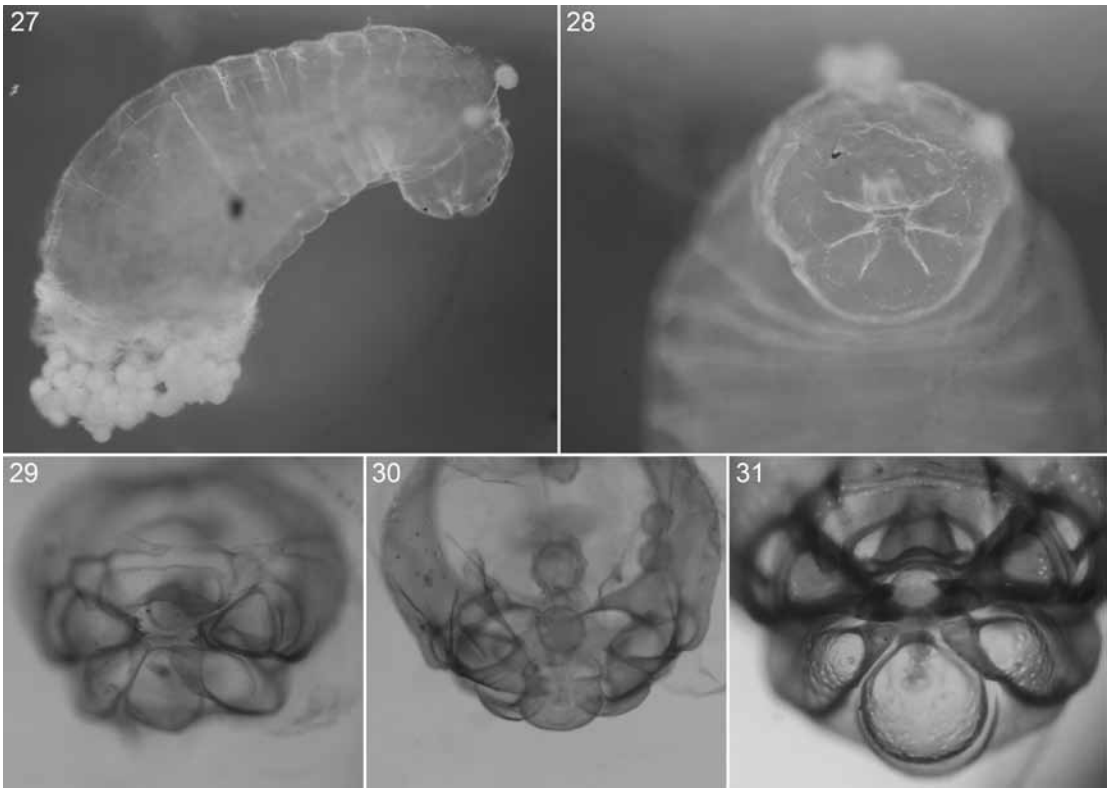
Description of Egg: The numerous eggs of *Lithurgopsis apicalis* encountered were essentially uniform in size and shape. They are shaped as depicted (fig. 21) with a length of 3.6–3.8 mm, (mean 3.7 mm, $N = 4$) and maximum width of 1.5 mm ($N = 4$) in the middle of its posterior half. The posterior end is broadly rounded, while the thinner front end is more narrowly rounded. The long axis is moderately curved (fig. 21). Translucent white in color, the chorion is clear and shiny, and the micropyle is not visible with a stereoscope. With almost all eggs, the posterior end is covered with pollen grains that are attached to it and are the remnants of the firm attachment to the disc. Under SEM examination a small cluster of pores at the anterior end is the micropyle from which there is a radiating chorionic pattern (fig. 24); elsewhere the chorion is smooth. However, at the posterior end (fig. 25) evidence shows that the attachment of the egg to the pollen grains is effected by an adhesive substance of unknown origin, visible where pollen grains have detached (fig. 26).



FIGURES 24–26. SEM micrographs of eggs of *Lithurgopsis apicalis.* **24.** Micropyle at anterior end. **25.** Pollen grains attached to posterior end. **26.** Close-up of rectangle in figure 25, showing evidence of adhesive attaching pollen grains to chorion.

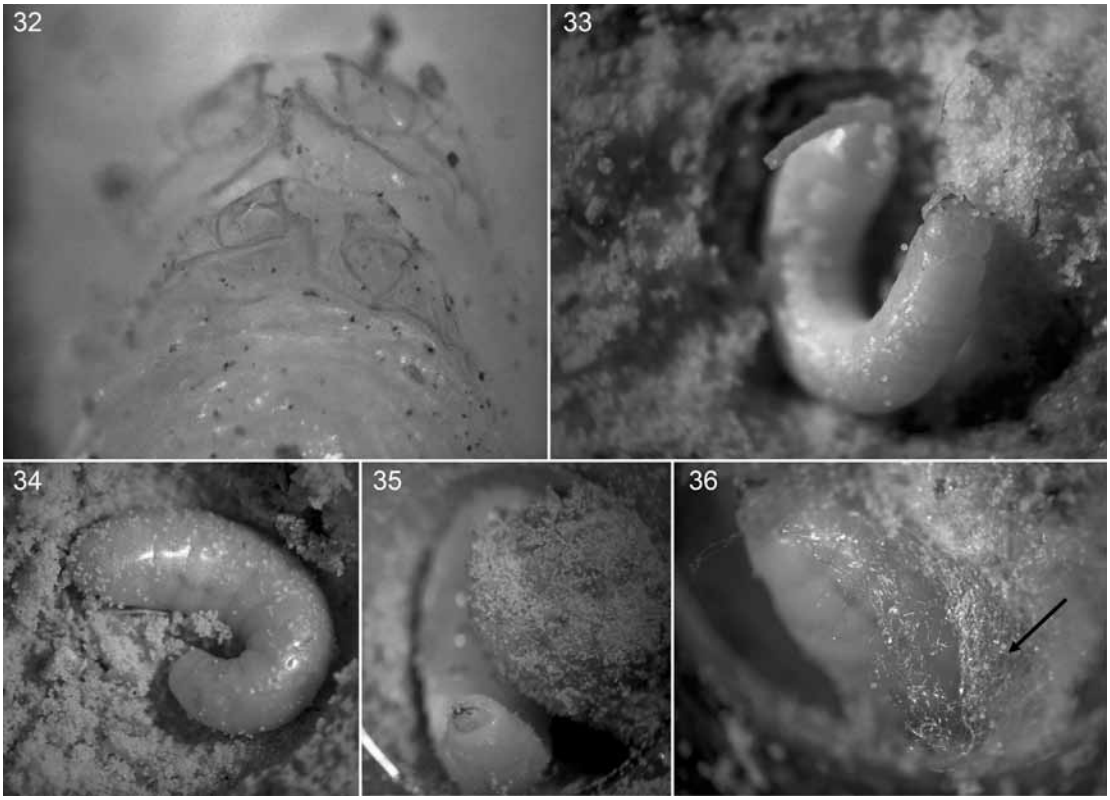
When the larva hatches it remains attached to the rear end of the chorion, while the rest of the chorion appears to fold and encircle the lower part of the larva (fig. 27). In turn the chorion remains attached to the projection on the disc, resulting in the feeding larva adhering to the disc (fig. 20). Because a fourth instar was still attached in the substrate, we surmise that detachment occurs during or at the end of the fourth larval stadium.

Larval Development and Feeding: We assumed that this species, like other bees that have been carefully studied, undergoes five larval stadia, i.e., has five larval instars. Last larval



FIGURES 27–31. Microphotographs of cleared larvae of *Lithurgopsis apicalis*. **27.** Entire first instar, lateral view, with cast chorion and pollen grains adhering to posterior end. **28.** Head of same, frontal view. **29.** Head capsule of third/fourth instar, frontal view, showing pollen grain being ingested. **30.** Same but more dorsal view showing pollen grains being ingested sequentially into esophagus. **31.** Lower part of head of early fifth instar; note pollen grain being ingested behind mandibular apices.

instars when newly molted were extremely small (fig. 22) but could be easily identified because they immediately started to defecate and their projecting salivary lips could be recognized. Distinguishing between younger instars was a problem because there was little difference in sizes. However, like other megachilids that have been studied (Rozen and Kamel, 2007), the larva of this species also carried its cast, flattened exoskeletons appressed, one next to the other, to the undersurface of its abdomen in sequence, with that of the first instar most ventral and most posterior. Most (though not all) early instars of *Lithurgopsis apicalis* bore two cast larval exoskeletons (i.e., skins) on their venter as detected by flattened, sclerotized head capsules (fig. 32). We assumed that these skins represented the second and third instars and that the fourth instar was carrying them. The only certain first instar (recognized since it contained no pollen in its intestine) that we had recovered bore no sclerotized areas on the head capsule (fig. 28). We assumed, therefore, that the cast first instar exoskeleton was invisibly represented among the numerous folds of cast exoskeletons attached to the fourth instar. This assumption proved correct when the exoskeleton of a first instar was identified attached to the body of a third instar, because the substantially smaller spiracles of the first instar could easily be separated from the much larger spiracles of the second instar on their respective exoskeletons.



FIGURES 32–36. Larvae of *Lithurgopsis apicalis*. **32.** Cast head capsules of second and third instars attached to venter of fourth instar. **33.** Early defecating fifth instar, showing slender body shape. **34.** Intermediate-aged fifth instar demonstrating more tapered body shape. **35.** Spinning fifth instar with fibrous cushion of feces and pollen intermeshed with silk. **36.** Silken network that has been partly covered by thin film of clear silk (identified by arrow).

The first instar, without pollen in its gut and lacking sclerotization of its head capsule and mouthparts, was in other respects quite complete (figs. 27, 28) and in general form, much like subsequent instars. Although body vestiture was not visible, such internal features as the complete posterior tentorial bridge and anterior arms of the tentorium were. Facial features including mandibles, maxillae, bilobed hypopharynx, and body segmentation including separation of cephalic and caudal annulets were clearly indicated, as well. We think that the first instar probably does not feed on pollen but emerges from the egg and shortly thereafter molts and transforms to the second instar, which commences to feed actively on the provisions. Further studies are needed to confirm the details of the transformation.

All young larvae up to and including fourth instars were found in the chamber posterior to the more consolidated pollen disc (or scoop) where they had been deposited as eggs. These as well as slightly larger fifth instars, like all eggs, were completely hidden within the food cylinder when viewed externally. Larval chambers of early fifth instars had become larger due to larval feeding. Soon thereafter a hole in the side of the cylinder of provisions appeared.

Fifth instars defecated by releasing an elongate continuous strand of feces that, shortly after being voided, tended to break into cylindrical though somewhat flattened pellets, at first more

orange than the surrounding pollen. Fecal pellets of older fifth instars appeared more similar in color to the pollen. As fifth instars fed and grew, their bodies became extremely elongate and linear, presumably an adaptation permitting the larva to reach for the surrounding food, the surface of which receded as the larva fed.

Larvae, collected in the field, were subsequently reared in hemispherical depressions in a plastic insertion to a petri dish (fig. 23). The depressions were about 12 mm in diameter. Each depression was first filled about halfway with provisions, a larva was placed on the top surface, and more provisions were added to the depression more or less filling it. Although completely covered at first by the provisions, the activities of the larvae soon pushed the provisions aside, so that feeding actions and later cocoon spinning by the larva could be viewed and documented with a microscope.

Feeding small fifth instars repeatedly bent and straightened their long slender bodies and could propel themselves forward short distances by extending the anterior part of their body and apparently pulling their midbody forward, so that their posterior body segments were dragged along. The precise mechanisms as to how this is accomplished needs yet to be fully explained. No doubt the dorsal body surface plays a significant role, as this is the surface that comes in contact with the pollen substrate that surrounds the larva. However, this instar does not have middorsal body tubercles that seem to play a role in larval ambulation of early fifth instars of other Megachilinae (Rozen and Hall, 2012).

As the fifth instar feeds, it grows rapidly and the elongate body swells more posteriorly, so that in lateral outline it evenly tapers from back to front; abdominal segments 7 and 8 are about twice the diameter of the prothorax (fig. 34). The body is often curled, so that the head and terminal body segments are normally close together, and the dorsal body surface is the outside surface. While the rear of the body tends to be stationary in the pollen, the anterior of the body, while elevated, twists and turns agilely in various directions as larval mandibles rapidly open and close (e.g., 9 times per 5 sec period), ingesting pollen from the walls of the chamber. Attempts to move the entire curved body appear to be accomplished by contraction and expansion of the dorsal surface of the curved body against the provisions. The ventral body surface is not involved.

Fifth instars discharged feces into the provisions that surround the larva, so that fecal pellets were scattered throughout the food supply in the rearing dish as larvae matured. While feeding they seemed to be able to discern between pollen grains and fecal pellets, perhaps on the basis of size since fecal pellets were about 0.25 mm in diameter whereas pollen grains were 0.1 mm in diameters. They were observed ingesting only pollen. However, feces appeared to have a more adhesive surface than did pollen grains, so other differences may be involved with pollen recognition. That said, from laboratory observations larvae seem quite capable of detecting and selecting single large pollen grains from a mixture of fecal pellets and loose pollen grains. Pollen grains are then ingested one by one.

By June 6, 2013, larvae collected on May 19 were starting to produce silk strands, which over the following five days tended to bind feces and pollen grains into soft, loose cushions of material that apparently were not affixed to the plastic rearing dish. A week after first being detected, the mass of feces and pollen held together by strands of silk had grown in two of the rearing depressions. It seems likely they may be involved with forming partitions between two

cocoons in a single cell, as mentioned by Gutbier (1916) with respect to *Lithurgus cornutus* (as *L. fuscipennis* Lep.).

Freshly produced silk is colorless but appears white when spun as a large mass, as happened in the artificial rearing dishes. However, over a 10-day period, it becomes somewhat pinkish, though still far paler than the brown color of the vacated cocoon. The cocoons spun were obviously misshapen because the depression in the rearing dish did not conform to the shape of a cell. A month after first silk production, silk in the rearing dish had gradually deepened in color to a medium brown.

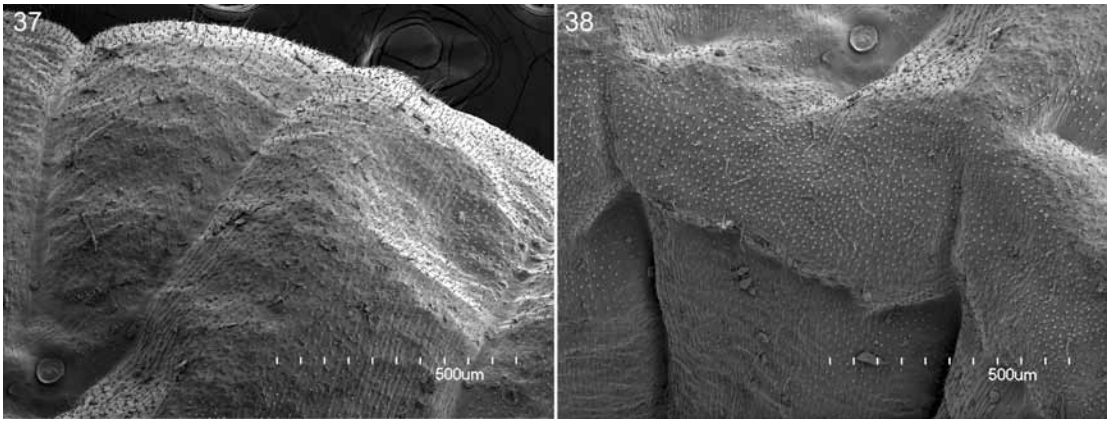
By June 21, 2013, two of the three surviving larvae had become quiescent (the third was still slowly moving); active spinning had ceased a few days earlier. This suggests that cocoon production requires somewhat less than two weeks and is followed immediately by diapause, which in turn implies that the species is univoltine.[9] Rozen (2013) noted that after defecation and before cocoon production, some bee species produce an anal discharge of whitish to yellowish, fine-grain material. This material may be secretion/excretion from the Malpighian tubules; a few white fecal-size pellets were detected in the case of only one larva.

Examination of Completed Nests: In August H.G.H. returned to the site to sample completed nests over a period of four days. He retrieved nests that had been constructed in *Agave* stalks from which we had removed the upper stalks in May as well as a good many newly discovered nests. These were temporarily stored in the laboratory at the Southwestern Research Station (SWRS) and then shipped to the AMNH where they were studied in early September. Approximately 33 cocoons containing live diapausing larvae were recovered, all with the larval head at the front of the cocoon with more than half of the larvae moving by bending and unbending with some rapidity while being extracted from their cocoon. A few larvae not only bent and straightened their bodies but also rotated the distal part of their abdomens providing a jumping action caused by a sudden shift in position when on an open surface. Hence, larvae of this species are only in partial diapause while overwintering, just as was the case for the three larvae collected in May. Although body motion was obvious, no overwintering larva moved its mandibles, and no cells were found with larvae still feeding. The adaptive function of body motion on part of an otherwise diapausing larva is not understood.

From nests collected in August and examined in September, we observed the material surrounding cocoons consisted in most cases only of abundant fecal pellets. However, with several accumulations of feces, we observed much fresh pollen intermixed, indicating that the intermixing of feces with superfluous pollen observed in the rearing dish in May was a normal phenomenon if a cell contained more provisions than required by the inhabitants.

Description of Mature Larva: The mature larva of *Lithurgopsis apicalis* was described by Rozen (1973) and compared with those of *Lithurgus atratiformis*, *Lithurgopsis echinocacti* (Cockerell), and *Trichothurgus dubius*. Recently SEM micrographs of its head and abdominal segment 8 were published (Rozen, 2013: figs. 30–33). Here we place on record SEM micro-

[9] Through the years, adult specimens of *Lithurgopsis apicalis* have been collected in Cochise County, Arizona, from April 30 to September 3 (Schuh et al., 2010). This broad range would seem to suggest that the species is not strictly univoltine, despite the fact that larvae collected in May went into partial diapause (as defined herein) in late June.



FIGURES 37, 38. SEM micrographs of defecating fifth instar of *Lithurgopsis apicalis*, lateral view, showing spicules and fine setae on dorsal surface of abdominal segment 3. **37.** Dorsal surface and **38.** lateral lobe. Specimens used for SEM examination were from Nebraska, Keith Co., Cedar Point Biological Station, July 9, 1987 (J.G. Rozen), from nests in a dead cottonwood snag, preserved as last stage larvae (collection of AMNH). They were compared with and found to be identical to the Parker and Potter material and to those reared from the current study.

graphs of lateral views showing the distribution of spicules and setae on abdominal segment 3 (figs. 37, 38) of *L. apicalis*. Rozen and Hall (2012) and Rozen (2013) have suggested that the setose and spiculate surfaces of the body as well as the presence or absence of middorsal tubercles on body segments may be functionally related to body movement in last larval instars of Megachilidae. We note that the fifth instar of this species does not have prominent middorsal body tubercles at any time. Furthermore, the spicules are small, extremely abundant, and uniformly spaced, and the setae are thin and therefore appear rather obscure. The larva's activity involves little whole body movement and apparently never on its venter, which appears to remain out of contact with the surrounding pollen. Thus the adaptive role of body vestiture remains obscure, although perhaps will be revealed when observations are conducted on cocoon spinning larvae in their brood cells.

Cocoon Structure: During the May 2013 investigations, two cocoons containing a dead pupa and adult provided our first understanding of cocoon structure that was then enhanced by the abundant cocoons containing live larvae recovered in August. The cocoon of *Lithurgopsis apicalis*, both externally and internally, closely resembles that of *Lithurgus chrysurus* described by Rozen (2013), although on close examination several differences are apparent. External shapes vary individually in both (compare those of *L. apicalis* with one another and with those of *L. chrysurus*, Rozen, 2013: fig. 7). Those of *L. apicalis* are slightly larger, length 12.0 –16.5 mm, and 6.0–7.2 mm in maximum diameter (N = 10), compared with 10.0–12.5 mm and 5.5–6.0 mm for *L. chrysurus* (N = 10). The front surface of both is flat to slightly bowed outward (although the outward bulging in the case of *L. apicalis* is often exaggerated by accumulation of feces adhering to the cocoon front, fig. 39), and the rear end of each is rounded in lateral view, although in some cases also appears extended and exaggerated because of more external feces. With both species, the front end of the cocoon forms a rim that tends to affix the cocoon to the burrow wall. This attachment in *L. chrysurus* seems firmer than that of *L. apicalis*.

FIGURES 39–41. Microphotographs of cocoons of *Lithurgopsis apicalis*. **39.** Sample of cocoons most of which had been opened to remove larvae, showing variation in color, texture, and external shape. **40.** Longitudinal section of front of one side of front end of cocoon, showing outer layer of dark silk between which thin line of white fine-grained layer of discharge is sandwiched by inner thick layer of dark silk. **41.** Longitudinal section of posterior end of cocoon showing multiple layers of silk fibers.

The fronts of the cocoons of *Lithurgus chrysurus* and *Lithurgopsis apicalis* form a strong solid silk barrier against parasites and predators that might attempt to attack from the front. It attaches to the tunnel wall by its rim. In both species, the larva first spins an outer coating of silk, then deposits against that a whitish (possibly anal) discharge of fine-grained material, and then spins the silken inner lining to the front of the cocoon, resulting in the firm barrier with a shiny, dark reddish brown inner surface. In the cocoons of *L. apicalis*, the quantity of the white material seems considerably less abundant and conspicuous than in the cocoons of *L. chrysurus* and, as a result, cocoon fronts of *L. chrysurus* tend to be more opaque. The source of the white material is unknown, but since it appears well after the exine-laden feces have been voided, we wonder whether it may come from the Malpighian tubules, as suggest by a recent study of *Trachusa larreae* (Cockerell) (Rozen and Hall, 2012).

The cocoon walls of *Lithurgus chrysurus* and *Lithurgopsis apicalis* taper slightly before forming the rounded, posterior end of the cocoon. Externally, cocoon fabric appears to be reddish brown to pale or even dark brown where visible among the scattered fecal pellets that cover a good deal of the fabric, which tends to be semitransparent in transmitted light. Externally the fabric surface is moderately dull. However, with both species the internal surface of the cocoon is shiny brown because of a smooth internal layer of silk. The rear end of the cocoons is where the greatest structural difference between the two species appears. In the case of *L. chrysurus* the inner layer tends to disappear close to the posterior end of the cocoon, presumably permitting an exchange of gas between the inside of the cocoon and the outside.

With that species the most posterior point on the cocoon consists of a single layer of silk (Rozen, 2013). However, with *L. apicalis* the posterior end of the cocoon becomes a tangled web of multiple layers of fibers (figs. 41–45) that provide a cushion that presumably allows exchange of air and simultaneously screens out possible parasites.

While watching one of the cocoon-spinning larvae on June 14, 2013, we noticed that it had spun a thin, mostly transparent network of fibers near the large opaque cushion composed of feces and pollen held together with silk. The larva actively stretched silk fibers across open spaces from one point to another. After doing this for a short time, it switched behavior and rapidly stroked the ventral surface of its head forward and backward against the network of fibers, releasing a thin clear window of semiliquid silk from its salivary opening. Almost immediately the silk solidified between the fibers into a thin transparent film. After a short time, the larva returned to adding fibers to the network. Later, it again released more semiliquid silk adding to the film. The windowlike deposits were highly reflective (fig. 36). This is compelling evidence that the thin, clear material covering fibers on the inner surface of cocoons of this and other Megachilidae is silk and not a composite material, suggested as a possibility by Rozen and Hall (2011). No liquid was observed being voided from the larva's anus, which would have been expected if the clear material were from the Malpighian tubules.

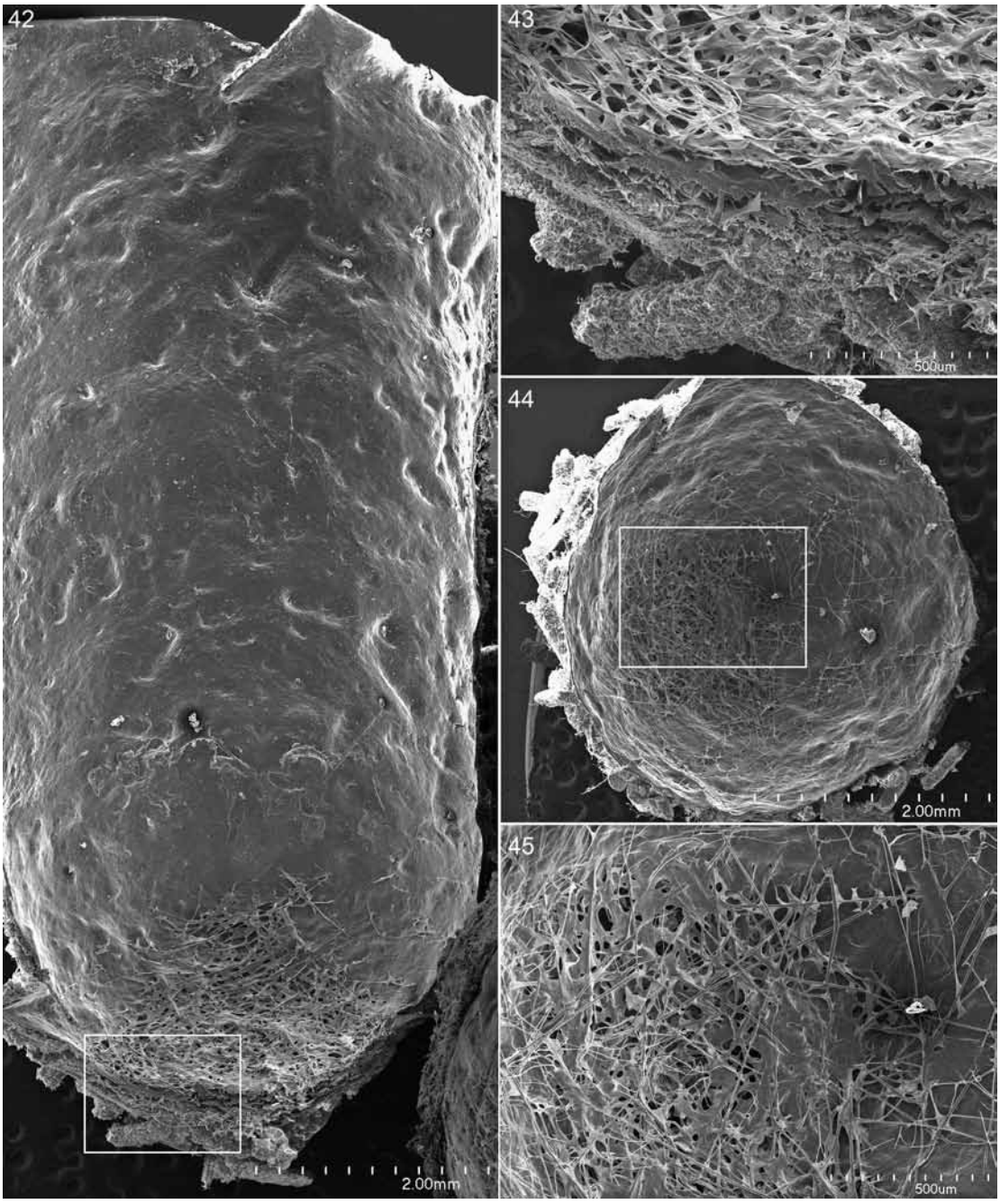
Parasitism and Predation: No parasites of any sort were found associated with the nests of *Lithurgopsis apicalis* in May, and ants were not noticed to be a problem. However, from nests gathered in August, two kinds of parasites were also encountered: many fly larvae, presumably Bombyliidae, and a single Leucospidae wasp pupa, tentatively identified as *Leucospis affinis* Say, that had consumed a mature larva of *L. apicalis* after the bee had spun its cocoon.

Parker and Potter (1973) found larvae and pupae of *Anthrax cintalapa* Cole (Bombyliidae), which parasitized 41.5% of the overwintering cells of the bee. The mite *Chaetodactylus lithurgi* Klimov and O'Connor was reported as phoretic on this bee as well as several other species of *Lithurgus* (Klimov and O'Connor, 2004). Hannan and Maeta (2007) commented that the abundant mites associated with provisions and feces in nests of *L. collaris* did not harm larvae.

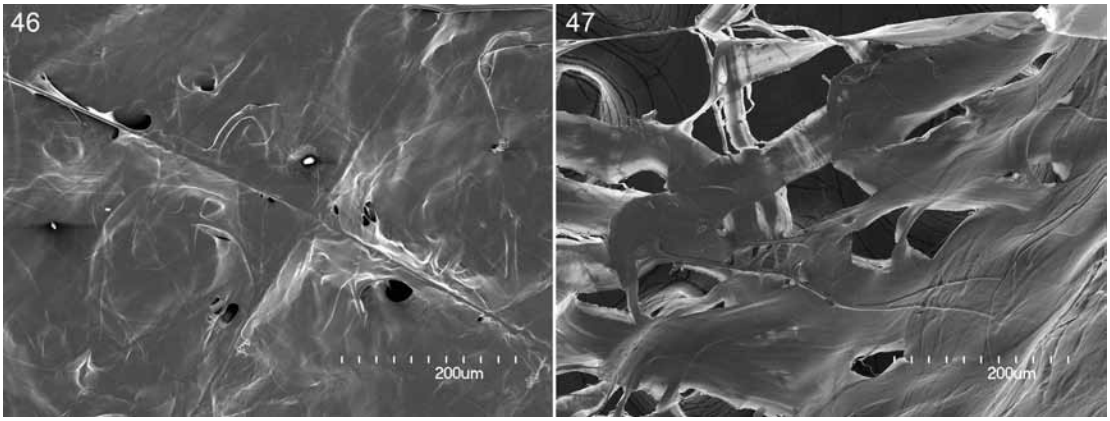
DISCUSSION

Over the last century, a general understanding of the nesting biology of Lithurginae has gradually grown. Although most often considered as a wood-nesting group, it seems clear that the wood must be dead, and reasonably soft, often due to weathering, age, and deterioration. Lithurgines have also been found nesting in dung (Michener, 2007; Sarzetti et al., 2012) and weathered fiberboard-backed shingles (Roberts, 1978). Thus, the subfamily should be characterized as requiring a nesting substrate composed of such porous cellulose materials, which enable small to moderate-sized females to excavate tunnels and allow air exchange between outside ambient air and the brood cell.

Nest tunnels are often characterized as having rough interior surfaces (as seen here, fig. 8) (exception: *Trichothurgus bolitophilus* has smooth tunnels, Sarzetti et al., 2012) and approximately uniform in diameter throughout a nest. Cell diameters and wall textures match those of tunnels



FIGURES 42–45. SEM micrographs of inner surface of cocoons of *Lithurgopsis apicalis*. **42.** Longitudinal section of cocoon, front removed, showing long shiny inner surface of wall and modified apical tip showing cushion of multiple layers of silk fibers. **43.** Close-up of surface of multiple layers identified by rectangle in figure 42. **44.** Posterior end of another cocoon, inner view, showing cushion of multiple layers of silk fibers, somewhat off center. **45.** Close-up of cushion of multiple layers of silk fibers identified by rectangle in figure 44.



FIGURES 46, 47. SEM macrographs of thin film of cocoon fabric spun on June 44, 2013, as identified in photomicrograph, figure 36, **46.** showing small apertures that presumably would have been closed with more applications of silk, and **47.** edge of same, showing texture of silk that is extruded, respectively. These macrographs plus observations made at the time strongly suggest that the inner surface of cocoons is composed solely of silk; see text for further explanation.

leading to them, i.e., they are mere extensions of tunnels. No cells have been found with evidence of a special lining added by the female, either of her own secretions or of materials imported from outside of the nest. Cell partitions and closure are made from the substrate.

We propose that nest tunnels of lithurgines generally divide, giving rise to branches that are similar to the entrance tunnel, which itself is variable in length, perhaps depending on the amount or quality of substrate present at the point of entry. Branching allows access to areas of the substrate suitable for nesting without the female bee having to look for another point of entry. The lengths of the branch are in part dictated by the boundaries of the substrate, by characteristics of the substrate, and, of course, by how many cells of what lengths are built into the end of a branch.

Some workers have noticed an empty branch near the entrance to a nest and have variously referred to it as a "chamber" (Garófalo et al., 1981) or as "an empty, blind, secondary tunnel" (Sarzetti et al., 2012). Rozen (2013) found evidence in the case of *Lithurgus chrysurus* that an empty branch may be the source of closure material for the nest or for a branch before construction of another, as suggested earlier by Hannan and Maeta (2007) for *L. collaris*. This hypothesis needs further testing, but still seems valid. Cells (as defined in Methods and Terminology) often vary greatly in length usually within even a single nest.

Most lithurgines may deposit more than one egg per cell ("cell" as defined under Methods and Terminology), the only apparent exception is that of *Lithurgus atratus* (as *L. huberi* in Camillo et al., 1983, 1994).

Pictures of cocoons of *Lithurgopsis apicalis* (Parker and Potter, 1973: figs. 5, 6), *Lithurgus atratus* (as *L. huberi* in Camillo et al., 1994: fig. 2), *L. atratiformis* (Houston, 1971: plate I-C), *L. chrysurus* (Rozen, 2013: fig. 7), and *L. collaris* (Kitamura et al., 2001: fig. 2) did not differ significantly from ours (fig. 39). Future studies of *Lithurgus* and *Lithurgopsis* may confirm that the broad, flat to somewhat domed cocoon front is tightly bound to the burrow wall and serves to block parasites; a band of apertures near the rear of the cocoon permit air exchange.

However, the cocoon of *Trichothurgus dubius*, pictured and briefly described by Rozen (1973: fig. 22) appears quite different externally. Three specimens were retrieved from a cactus in 1971 from which newly emerged adults had been removed and held in the collection of the AMNH since then. From these specimens, a more complete description is given as follows, based on the uncertain assumption that the more pointed end is the front end of the cocoon. In shape, the rear of the cocoon is broadly rounded while the front end gradually narrows, as shown (Rozen, 1973: fig 22). The fabric consists of two appressed layers, which can be easily separated. The exterior surface is completely, thickly, and roughly coated with a mixture of truncated, slightly flattened, cylindrical fecal pellets, presumably plant tissue, and silk, all of which is mostly opaque. The inner layer is thin, mostly semitransparent, faintly fibrous, and its inner surface is a smooth, highly reflective brown. Toward the front end of all three cocoons, there are internal blotches of whitish, fine-grained material covering part of the internal cocoon surface as well as the circular filter areas, which are about 3 mm in diameter and consist of coarsely woven silk strands. Because of its fibrous nature, the filter is less transparent and in cross section is somewhat thicker than the inner cocoon layer elsewhere. The filter is presumed to allow air exchange between inside the cocoon and external ambient air. The outer layer of the cocoon overriding the filter may or may not be slightly thicker than it is elsewhere. How and where air is exchanged through the outer cocoon layer is unknown, but likely the entire surface is permeable. The external features of the cocoon of *T. bolitophilus* are shown by Sarzetti et al. (2012: fig. 11), but structural details are not given.

Nest reuse has been cited as a common phenomenon among lithurgines (Parker and Potter, 1973; Garófalo et al., 1992; Camillo et al., 1994; Hannan and Maeta, 2007). However, in most (but not all, see Camillo et al., 1994) only the main gallery is reused. Branches are constructed anew; old branches are rarely cleaned out and reused. Recent observations of *Lithurgus chrysurus* (Rozen, unpublished data, 2013) revealed that this species showed particular interest in gaining access to soft plant tissue by using natural occurring cracks and woodpecker damage in solid pinewood, thereby immediately accessing soft springwood. This suggests the common driving force is to gain access to soft wood via any method, be it natural cracks (splits in *Agave* stalks), woodpecker damage, bee-made old nest openings, or human-generated routes (sawed off *Agave* stalks).

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APPENDIX

Ovarian Statistics Of *Lithurgopsis apicalis* (Cresson)

Because ovarian statistics have not been reported for any Lithurginae, the availability of females collected during this study provided an opportunity to do so. Two females were dissected, and each was found to have three ovarioles per ovary, i.e., an ovarian formula of 3:3, the normal formula for Megachilidae. One of the females seemed to have a mature oocyte 2.8 mm long and an intertegular distance of 4.9 mm, yielding an egg index of 0.57, which in Iwata and Sakagami's (1966) classification of egg size relative to body size is **"small."** The other female appeared to bear no mature oocytes.

However, the length of the mature oocyte of 2.8 mm compared with the actual egg lengths of 3.6–3.8 mm, i.e., 3.7 ($N = 4$) seems unreasonable. Therefore, the following is based on the average length of 4 eggs, 3.7 mm, divided by the mean intertegular distance of 7 females collected from that locality, i.e., 4.83 mm, giving an egg index of 0.77, i.e., **"medium"** in the classification of Iwata and Sakagmi (1966). This is probably a more reasonable analysis.

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