

Larval development and nesting biology of the adventive wood-nesting bee *Lithurgus (L.) chrysurus* Fonscolombe (Hymenoptera: Megachilidae: Lithurgini)

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ABSTRACT

Announced herein is the discovery of a nesting site of *Lithurgus (Lithurgus) chrysurus* Fonscolombe (Megachilidae: Megachilinae: Lithurgini), an adventive solitary bee of Western Palearctic origin that had first been detected in North America in the mid-1970's and thought to have become extinct locally since then. The occurrence of the new nesting site in eastern Pennsylvania, close to the point of first discovery in New Jersey, suggests its persistent but low presence over the past 35 years. Several previous papers offered insight into nests of this species, which bores into dead wood to nest, and described its larva and pupa. The current investigation reveals new information concerning nest architecture, nest closure, its cocoon structure and functions, and anatomy of its mature larva. Its larva is compared with that of *L. (Lithurgopsis) apicalis* Cresson (a congener in another subgenus) and with that of *Trachusa larreae* (Cockerell) with consideration of how body vestiture and anatomy function to permit mature megachilid larvae to move in the confines of their brood cells.

INTRODUCTION

In a recent study on the biology of *Trachusa larreae* (Cockerell) (Megachilidae: Anthidiini), Rozen and Hall (2012) propose that body-surface vestiture (combination of setae and elongate spicules) and certain body tubercles may enable the last larval instar to move about in the brood chamber. Earlier instars of this species do not have body vestiture and are incapable of

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moving from where their eggs were deposited. This correlation suggests that vestiture functionally may enable larval movement. Since “hairy” mature larvae are characteristic of most Megachilidae, this hypothesis can and should be tested broadly within the family. The recovery of the Western Palearctic *Lithurgus (Lithurgus) chrysurus* Fonscolombe (Lithurgini) at Lehigh Gap, in Slatington, Lehigh Co., Pennsylvania (PA), may provide such an opportunity.

Adult specimens of this bee were collected in a bowl-trap survey in 2007 conducted by the Naturalists Club of the Lehigh Gap Nature Center (LGNC) under the direction of then visiting scientist Anita M. Collins, working in collaboration with USGS.² Because this area is close to where the species was originally reported nesting in the New World (i.e., Phillipsburg, Warren Co., New Jersey (NJ) [Roberts, 1978]), its current presence reveals that the bee must have existed in low numbers in the region during the intervening years despite predictions that it might have become extinct in North America (Michener, 2007). In 2012, a homeowner in Palmerton, Carbon Co., PA, after hearing a talk by Collins on bees and thereby becoming curious about them, brought four specimens of an unknown species nesting in her wood pile (fireplace logs) at her home to a meeting of the Naturalists Club (Anita M. Collins, personal commun.: Oct. 14, 2012). Thus was the discovery of the current nesting site of *Lithurgus chrysurus* in North America.

The purposes of this paper are to increase our knowledge of the nesting biology of this species (and thereby broaden our understanding of the nesting behavior of the tribe) and to augment anatomical details of its mature (i.e., last stage) larva so that, when earlier larval stages are recovered and their behaviors observed, more immediate comparisons among stages can be rendered. It is hoped that this information will shed light on mechanisms and patterns of body movements within the brood chamber during development. A more thorough understanding of the nesting behavior may also provide insight as to why *Lithurgus chrysurus* has not become a serious pest in North America over that past 35 years, although this was an expressed concern at the time of first discovery (Roberts, 1978).

Earlier studies on the biology and immature stages of this species have been carried out. Roberts (1978) presented considerable information about the foraging and flight behavior, nesting sites and nest architecture, and behavior of immatures, all acquired at Phillipsburg. He found an abundance of them nesting in a limited area in the composition wood-fiber backing of asphalt siding of a porch and also in the softwood beams of the roof, wall studs, and porch railing. He described and illustrated the mature larva and female pupa. Later, Rust et al. (2004) reported on the nesting biology of this species from Forcalquier, Alpes de Haute Provence, France, including data concerning pollen analysis, cocoon structure, and egg placement. Friese (1923) mentioned that *Stelis “aterrima”* was associated with a nest of *L. chrysurus* in Algeria, but the correct identity of the cleptoparasite is uncertain, as is the nature of the association.

PREVIOUS STUDIES OF LITHURGINI

In addition, others have presented similar information on related taxa that completes our current understanding of the natural history of the Lithurgini. One of the earliest observations

² The bee was identified as *Lithurgus chrysurus* by Sam Droege in 2008 (Sam Droege, e-mail: Jan. 9, 2013)

on nesting of *Lithurgus* was that of Giffard (1922) on male swarming followed by an emergence of adults of both *L. (Lithurgus) scabrosus* (Smith) (as *L. albofimbriatus*) and *Xylocopa sonorina* Smith (as *X. varipuncta*) sharing an old fencepost used as a nesting site in Hawaii. Claude-Joseph (1926) briefly described the larva and nesting biology of *Trichothurgus dubius* (Sichel) (as *Lithurgus dubius* P. Herbst) from Chile. Malyshev (1930) over several years of study provided a very complete account of *L. (Lithurgus) cornutus* (Fabricius) (as *L. fuscipennis* Lep. and *L. fuscipennis* Fonsc.), including: structure of nests in old wood (noting lack of partitions between adjoining cells in linear series), provisioning, egg deposition within the provisions, single generation per year, and diapausing larvae in brown cocoons. Lieftinck (1939) presented an account of *L. (Lithurgus) atratus* Smith in Java (Indonesia) and associated the cleptoparasitic bee *Euaspis polynesia* Vachal (as *Parevaspis abdominalis* F. Smith) with it. Cros (1939) presented considerable nesting information concerning the univoltine *L. (Lithurgus) tibialis* Morawitz in a dead poplar in Algeria. Houston (1971), writing about *L. (Lithurgus) atratiformis* Cockerell from Queensland, Australia, pointed out that this species' nesting in decayed wood is typical for the genus, as is its nest architecture. According to Parker and Potter (1973) cell partitions of *L. (Lithurgopsis) apicalis* Cresson were made with scrapings of wood, and the nests were in a weathered cottonwood log. The same year Rozen (1973) described immatures of other Lithurgini and included nesting information on *Trichothurgus dubius*, as had Claude-Joseph (1926) earlier. The single Japanese species, *L. (Lithurgus) collaris* Smith, is said to burrow into weathered cypress wood, and the same burrow is "used for a long time" during which the burrow elongates, according to Iwata (1976); he added that the burrow has a series of branches, each with a series of cells. Brach (1978) discovered numerous nests of *L. (Lithurgopsis) gibbosus* Smith in a weathered softwood post in Florida and induced some females to nest in trap nests made from fir, thereby contributing to our understanding of various aspects of nesting biology of this univoltine species. With respect to the Brazilian *Microthurge corumbae* (Cockerell) (as *L. corumbae*), Garófalo et al. (1981) found that it had two generations per year with the second one undergoing diapause, and nest cells were not separated by partitions. Camillo et al. (1983) presented preliminary observations on nesting of *L. (Lithurgus) atratus* Smith (as *L. huberi*) and compared them with what was known about other taxa. Mello and Garófalo (1986), again studying *M. corumbae*, analyzed the differences in the cocoons of the two generations and attempted to explain the adaptive significance of the differences. Mello et al. (1987) found that *L. atratus* (as *L. huberi*) had a single generation per year with a cocoon similar in composition to that of *M. corumbae*, and Camillo et al. (1994) emphasized nest reuse and robbing behavior of *L. atratus* (as *L. huberi*). Kitamura et al. (2001) described the abandon nests of *L. (L.) collaris*, and Hannan and Maeta (2007) documented active nests of the same species. Most recently, Sarzetti et al. (2012) interpreted the nests of *Trichothurgus bolithophilus* Durante and Roig-Alsina recovered from horse dung pads in Argentina.

Thus, scattered worldwide records indicate that the Lithurgini can be characterized as a group of wood-nesting megachilids that usually select old, dead wood (rarely dung) as nesting sites in which they excavate branching galleries with terminal cells of the same diameter as the gallery arranged either singly or in short linear series end to end. Partitions between cells are



FIGURES 1–4. Photographs of nesting site and nests of *Lithurgus chrysurus* in horizontally stacked firewood in Palmerton, Carbon County, PA. 1. Plastic cover removed to show wood. 2. Cover in place, while bees nested there. Photographs courtesy of Dan Kunkle. 3. Entrances of two nest on surface of log. 4. Another log split lengthwise, showing numerous branches and main tunnels of old, vacated nests, demonstrating cell series following wood grain.

present in some cases and, when so, are made of unconsolidated wood fibers. Otherwise, the provision mass of one cell adjoins the mass of the previous cell. Eggs are deposited toward the rear end of their cells after some early provisions have been deposited, although most provisions are subsequently added in front of the egg, thereby creating an egg chamber. Consequently, while provisioning a single cell, a female interrupts foraging for ovipositing. Cocoons vary from tan to dark reddish brown on the inner surface. Cocoons are abundantly surrounded by truncate, cylindrical fecal pellets. There is a suggestion (Roberts, 1978) supported by Hannan and Maeta (2007) that feces are extruded as an elongate coil that is later broken into short truncate rods, as also described for *Stelis* (Rozen and Hall, 2011). Some species have two generations annually, but most are univoltine.

METHODS

Nests were recovered from logs that had already been collected from a horizontally stacked wood pile containing cut and sometimes split logs, ca 15–18 in. (38–46 mm) long, in Palmer-



FIGURES 5–10. Microphotographs of nests of *Lithurgus chrysurus*. 5. Nest branch discovered on October 03, 2012, showing three cocoon-bearing cells in linear series with first cell containing mummified inhabitant removed and one cocoon partly opened revealing diapausing larva. 6. Close-up of cocoon with diapausing larva; note flecks of white discharge (arrows) on front end, possibly from Malpighian tubules, characteristic of fresh cocoons. 7. Six cocoons removed from nests demonstrating variability in color and shape because of superficial coverings of fecal material and cell wall fragments. 8. Cocoon from nest discovered October 26, 2012, faintly revealing live larva therein, deposition of fecal material both in front of and behind cocoon fabric, and abundant white discharge at anterior end. 9. Close-up of front end of another cocoon showing rim, which has been detached from tunnel wall. 10. Entrance of complete nest discovered October 26, 2012, with dash line showing path of main tunnel that had been emptied of fill, and pale closure plugging nest entrance in hole.

ton, Carbon Co., PA, where they had originally been stored for burning (fig. 1). Some of the logs were hardwood, probably maple or beech, but many were so weathered that the kind of wood was unidentifiable. The wood pile had been partly sheltered under a plastic covering (fig. 2). A team from LGNC photographed the pile (figs. 1, 2) and selected logs exhibiting evidence of nests (saw dust, holes) and stored them elsewhere for rearing and future study. I examined them on October 03, 2012, and again on October 26, 2012, at the LGNC, at which times the more likely logs were split with an ax, hatchet, penknife, and chisels. As the logs were dissected, discovered nests were examined with a stereoscope, and then promising sections were removed to the laboratory at the American Museum of Natural History (AMNH) for further study.

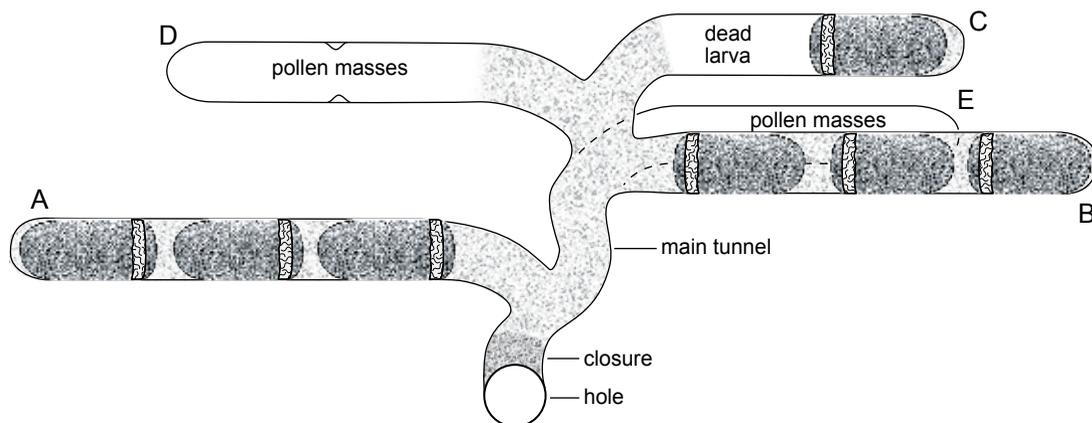


FIGURE 11. Diagrammatic representation of complete nest of *Lithurgus chrysurus* found on October 26, 2012; feces and cell partitions not depicted.

Larval descriptions are based on specimens both from this nesting site and on those that had been preserved in 1977 when I visited the Phillipsburg, NJ, site. Larvae were examined with a scanning electron microscope (SEM) after they had been critical point dried and coated with gold/palladium. Descriptions are also based on cleared specimens where the preserved larva is first examined and illustrated, and then head and body are separated and cleared by boiling in an aqueous solution of sodium hydroxide. After being washed in water, the two parts are transferred to 70%–75% ethanol, stained with Chlorazol Black E, washed in ethanol, and submerged in glycerin on a well slide for study and storage. All specimens used in this study are in the collection of the AMNH.

In descriptions and discussions of mandibles, below, the long mandibular axis is assumed to be horizontal, the upper surface is dorsal, and the lower surface ventral.

NESTING BIOLOGY OF *LITHURGUS CHRYSURUS*

The following observations were made on two site visits to LGNC, as indicated above. The first, on October 03, 2012, yielded a branch of a single nest containing live larvae as well as fragments of old nests. All larvae were encased in cocoons arranged in a linear series, head to toe (figs. 5, 11). Another cell immediately in front of the series enclosed a mummified individual, indicating that there had been four cells in the series. As is characteristic of all nests examined, three live larvae were oriented with anterior ends pointing toward the entrance tunnel, and the axis direction of the series followed the wood grain direction, as had also been noted by Roberts (1978) with larvae nesting in wood. The second visit, on October 26, 2012, produced a number of partial nests with live larvae and, importantly, a single complete and closed nest, which was in a weathered log approximately 10 cm in diameter. The information presented below is derived from these active nests as well as from numerous nest fragments from previous generations, all from about eight logs.

Nest entrance holes (fig. 3) were circular when at right angles with the log surface, 5–6 mm ($N = 5$) in diameter, and open as far as can be viewed externally. However, in the case of the complete nest discovered on October 26, 2012 (figs. 10, 11), the main tunnel of that nest branched

from just inside the entrance hole and was closed with compacted wood chips, so that the closure appeared to be merely the smooth side of the entrance hole (fig. 10). I suspect that this (open hole extending into wood, with sealed nest entrance attached to one side) may be a normal way that nest entrances of this species are constructed; see Discussion. With this nest, the main tunnel beyond this closure was filled with moderately unconsolidated wood chips for its entire length (a contradiction to Roberts, 1978; again, see Discussion). It angled in various directions across grain and gave rise to five branches (fig. 11 A–E), each of which consisted of cells arranged end to end, so that their long axes paralleled the wood grain. As a consequence, all branches containing cells not only in this nest but also in the entire log were oriented in parallel (figs. 4, 11), assuming a straight grain. This orientation evidently is in response to the wood grain (probably ease of chewing through soft spring wood), because branches in cell series in the uniformly fibrous composition from the Phillipsburg infestation were oriented randomly, as pointed out and illustrated by Roberts (1978: figs. 1, 2), as also seems true of *Trichothurgus bolithophilus* nesting in homogeneous horse dung. Examination of all other current and older nests examined at LGNC clearly substantiated that all cells and cell series are oriented to follow grain. Interesting results of this are: (1) if there is more than one nest in a straight log, all cell series are in parallel; (2) because all cell series of a nest are in parallel, the main tunnel, by traversing the grain (the hard summer wood), allows cells in a nest to exist in more than one tree ring; (3) because the logs were stacked horizontally (fig. 1), nest branches were also horizontal.

Burrow walls and cell walls are indistinguishable in diameter and have the same roughened surface texture resulting from the female chewing out the tunnel with her tridentate mandibles. There was no indication of a special cell lining. The fill of the completed main tunnel almost certainly is produced as the female creates the next branch. This raises the interesting questions: whence the fill that occupies the distance between the branch closest to the closure and the nest closure itself? might the open hole outside the nest closure be the source of the fill and of the material for the closure? See Discussion for possible answers.

Within all nests, cocoons in a series are arranged with their front ends pointing toward the main tunnel and larvae within invariably rest with their heads directed toward the front of the cocoons. Branches occasionally attach to a main tunnel at the same point, so that their cells are pointing in opposite directions.

In the case of the complete nest studied, three of the five branches contained cocoons holding live, diapausing larvae. Of these branches, two each held three cells in linear series, and the other contained a single live larva, in front of which was a cell containing the remnants of another individual. Other nests not infrequently contained four cells in series. The other two branches contained only moldy pollen masses indicating each had contained two or three cells in series, but immatures had failed to survive for unknown reasons. Thus, assuming that the live larvae would have reached adulthood, approximately half the progeny of the nesting female would have survived.

Most megachilids nesting in preformed tunnels (such as found in trap nests) construct cells that are defined by partitions made from material brought into the nest from outside. Rarely does more than one bee develop in a cell, although *Megachile (Sayapis) polycaris* Say is known to have two individuals develop in a cell (Krombein, 1967). With *Lithurgini*, partitions, when

present, are obviously made from wood chips or fibers, which are in some cases difficult to distinguish from fecal material because of their unconsolidated condition and perhaps because of manipulations of the larva in preparation of cocoon production. As noted by Roberts (1978), *Lithurgus chrysurus* does not always construct partitions between cells. In the case of the complete nest studied from LGNC, the position of the large mass of fecal material was variably placed in front of the cocoon, both in front of and behind the cocoon, or sometimes only around the rear $\frac{3}{4}$ of the cocoon. Hence, boundaries of cells are difficult to define, and similarly, cell lengths vary considerably (as also indicated by Rust et al., 2004). Numbers of cells in a series are easily counted if each cell has or had a cocoon, since the firm attachment (described next paragraph) of the front rim of the cocoon marks the wood with a dark cocoon color that persists well after the cocoon has been removed either by emerging adults or in the course of study.

Cocoons were first examined with a stereoscope and then with an SEM in an attempt to understand their structure and function. When first retrieved from their nests, they lack uniformity in shape and color because of variability of fecal material and shreds of cell wall adhering to them (fig. 7). However, exclusive of these superficial external coverings, they are quite uniform. Outside dimensions are 5.5–6.0 mm in maximum diameter and 10.0–12.5 mm in length ($N = 10$). The cocoon fabric of *Lithurgus chrysurus* is dark reddish brown (fig. 8). In shape, the thick, leathery-textured front end is slightly curved but, in side view, the silhouette of the front end tends to be more truncate than the rear. Its rim (fig. 9) firmly attaches to the burrow wall, so that the front end obviously seals off possible access by parasites to the rear part of the cocoon. The attachment seems to be an adhesive that bonds the rim to the burrow wall, so that when the cocoon is pulled from the burrow wall with forceps dark traces are left behind, while the rest of the cocoon easily separates from the wall, leaving no traces on the wall.

The tough leathery texture of the cocoon front apparently results from the application of more cocoon material to the inner layer of the fabric. On all cocoons containing live larvae at LGNC, external flecks of white material (figs. 6, 8) contrast with the dark surface of the cocoon rim suggesting that they might represent secretion/excreta from the Malpighian tubules as mentioned by Rozen and Hall (2012). However, such flecks were not found on any of seven cocoons from Phillipsburg, NJ, in 1977 from which larvae were extracted and examined for this study, questionably an artifact of long preservation of a degradable material. The presence of this white material combined with the roughened texture of the external surface of the front end suggests the need to investigate cocoon construction while in progress. Elsewhere, the cocoon fabric is softer and more pliable; just behind the rim of the cocoon, the cocoon closely fits the burrow wall, but, further back, fecal pellets tend to surround the cocoon, separating it from the burrow wall.

The cocoon fabric (including that of the front end) is semitransparent (except where fecal material adheres) and reflective on the inner surface. Although it appears to consist of a single layer because of its thinness, it is actually composed of two similar layers, both tan in color and both embedded with crisscrossing strands of silk, except at the rear end of the cocoon, as discussed below. Two layers of tan silk fabric closely applied to one another account for the darker brown color of the completed cocoon. Their adhesion appears to result from intertwining of individual fibers on adjoining surfaces. These two layers are quite strong and can be separated by pulling the sheets apart

with two pairs of forceps. There are fine pale strands of curled loose silk fibers on the external surface of the cocoon, but they do not substantially obscure the brown surface. The outer surface adheres closely to the tunnel wall but does not bond with it except at the rim, and, when pulled away, fecal fragments often continue to adhere to it in places. The reflective inner surface is somewhat uneven due to these external fragments and because of the embedded fibers.

The inner surface of the cocoon was scanned by SEM (figs. 12, 13, 15) and found to be composed of a single thin sheet of material bearing impressions of silken fibers, indicating that the sheet had been applied to the silken fibers, although the source of the sheet material may itself be fused silk or some other substance, such as Malpighian tubule material. However, a band of circular to oval apertures of various sizes rings the cocoon almost at its rear end (figs. 13, 15). These openings are reminiscent of those found associated with the “nipple” on other bee cocoons, where it has been proven that the openings enable air exchange between the cocoon lumen and the outside atmosphere, while the rest of the nipple screens out parasites and predators (Rozen and Hall, 2011; Rozen et al., 2011). The assumption is that bee cocoons function to protect their inhabitants from predators/parasites and to control cell humidity, while assuring air exchange.

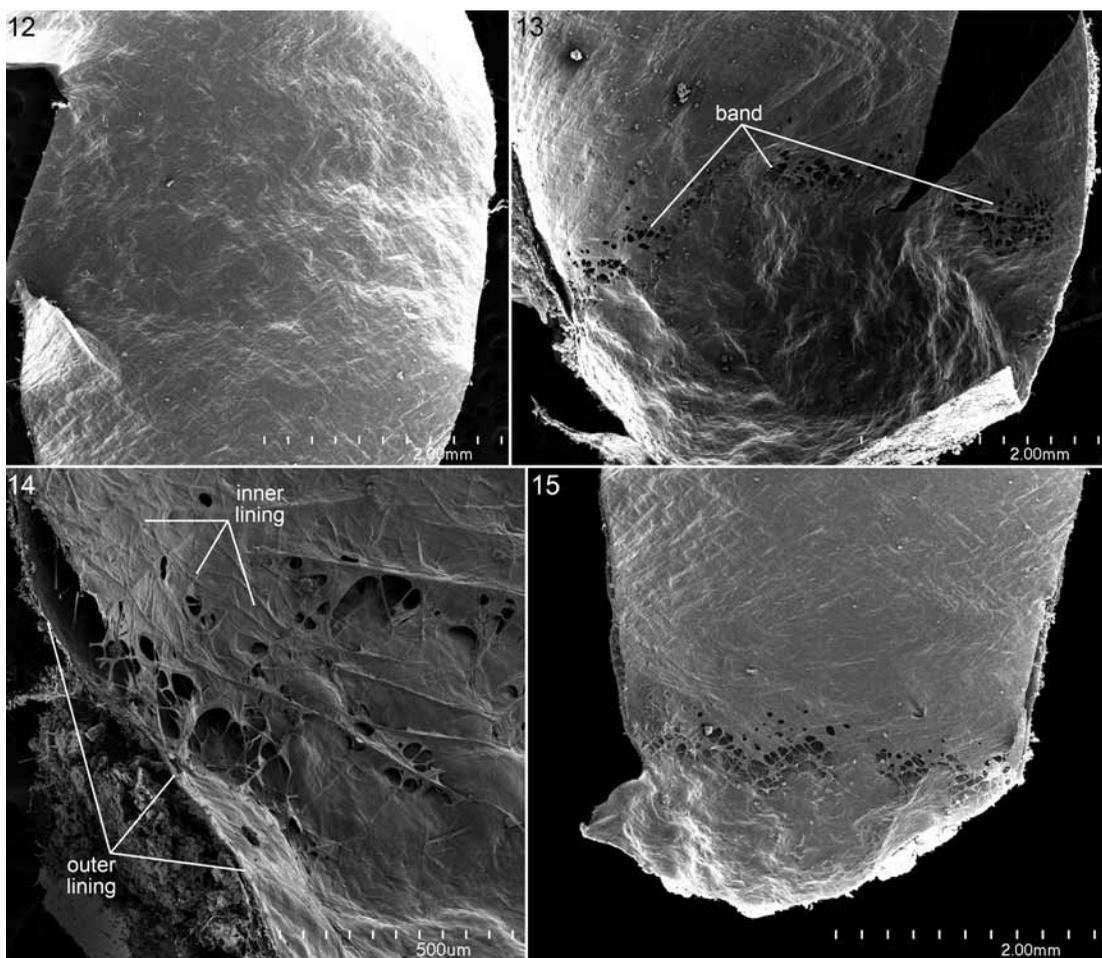
When stereoscopically examining the inner surface of the rear part of the cocoon with a broad light source, one can see that apertures are created by darkish silken strands of the inner lining of the cocoon attaching to the outer lining; they are the rear terminus of the inner lining. Behind the band, the cocoon tip is composed of a single layer (figs. 13–15).

With *Lithurgus chrysurus*, the front end of the cocoon blocks access to natural enemies and controls humidity with its water-tight inner lining. Although it seems likely that air exchange between ambient air and the cocoon lumen must pass through the band of holes at the rear of the cocoon, does the air diffuse through the outer cocoon lining? SEM micrographs of the outer surface of the cocoon (figs. 18, 19) reveal a surface that in places consists of an open network of fibers and elsewhere a mostly sealed surface, suggesting that visual inspection alone will not provide an answer. Thus, the route of air exchange through the cocoon fabric still needs to be critically tested. Most lithurgines seem to nest only in old, dead, often weathered wood, and perhaps wood in this condition provides air passage.

In the cell series collected on October 03, 2012, a deposit of firmly packed wood particles occurred at the front end of the series where the tunnel started to turn down. The deposit blocked an older series of moldy, vacated cells running in the opposite direction from the four cells containing live larvae, but also followed the grain. This suggests that the entrance tunnel had been used (and possibly constructed by) a female of the previous generation and then reused by a female of the next generation that had blocked the old branch with wood chips, as reported by Camillo et al. (1994) for *L. atratus* (as *L. huberi*). Of interest, with both species, only the short entrance tunnel is reused, not branches containing cells.

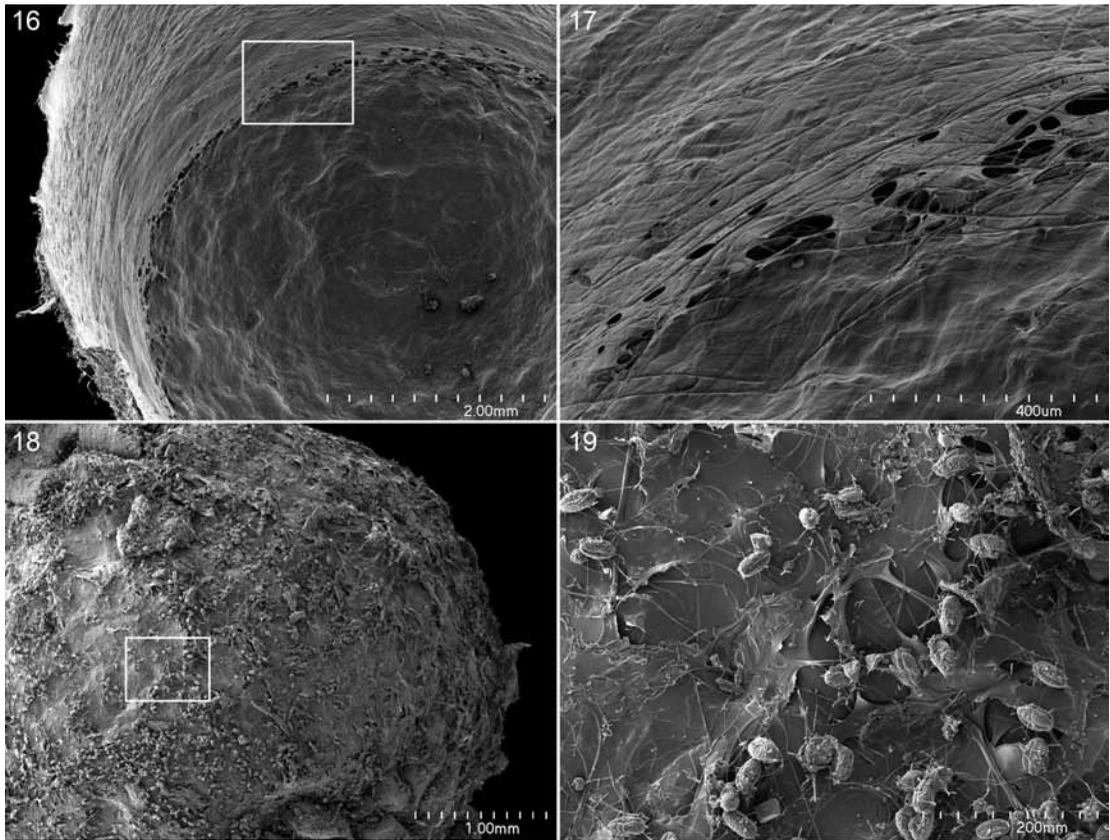
POSTDEFECATING LARVA OF *LITHURGUS CHRYSURUS*

The last stage larva of *Lithurgus chrysurus* requires redescription here because many new features, especially relating to body vestiture, can now be considered, owing to advances in



FIGURES 12–15. SEM micrographs of inner surface of cocoons of *Lithurgus chrysurus* cut lengthwise; figures 12, 13, 15 to same scale; all cocoons oriented with anterior ends up. 12. Anterior part of cocoon wall demonstrating inner surface without apertures. 13. Rear of cocoon showing band of apertures thought to permit air exchange between cocoon lumen and outside air; note double layer of fabric on each side anterior to apertures. 14. Close-up of another cocoon showing terminus of inner cocoon lining with outer layer. 15. Same cocoon as figure 13, other side, showing rest of band of openings; note double layer of fabric on each side anterior to apertures.

scanning electronic microscopy. Larvae used in this study were preserved August 11, 1977, and came from Phillipsburg, Warren Co., NJ, from the infestation where this species was first reported in the New World. This material was augmented by currently retrieved larvae from weathered wood from the outdoor wood pile stored in Palmerton, Carbon Co., PA. A number of infested logs were removed to the Lehigh Gap Nature Center for study, and nests were dissected and larvae collected on October 03, 2012, and October 26, 2012. These fresh larvae, however, proved to be unsatisfactory for SEM examination because, even after preparation, their bodies contained large quantities of oil that also coated the integument and obscured their fine external structures.



FIGURES 16, 17. SEM micrographs of rear end of another cocoon viewed from front showing: 16. band of apertures circumscribing rear end and 17. close-up of apertures identified by rectangle in figure 16. FIGURES 18, 19. SEM micrographs of the rear part of outer surface of a cocoon of *Lithurgus chrysurus*, showing a variety of textures but without evidence as to whether various openings allow gas diffusion between cell lumen and outside air: 18. Rear of cocoon. 19. Close-up of surface texture indicated by rectangles in figure 18.

The mature larva of *Lithurgus (Lithurgopsis) apicalis* Cresson is compared in the following Diagnosis and Description with that of *L. chrysurus* in an attempt to explore difference between these two species, which belong to different subgenera. Although it had been previously described by Rozen (1973) based on material from Utah (Uintah Co.: near Vernal, late summer 1972; F.D. Parker), the specimens of *L. apicalis* studied here came from Nebraska (Keith Co.: Cedar Point Biological Station, N41.24490° W101.67910°, VII-09-1987; J.G. Rozen) in a dead cottonwood snag.

LITHURGUS (LITHURGUS) CHRYSURUS FONSCOLOMBE

Figures 20–26, 34–40

Diagnosis: The mature larvae of the few lithurgine taxa available for study suggest that their larvae exhibit little variation. Mandibular shape may be the most distinctive feature for recognizing *Lithurgus chrysurus*. Its elongate ventral apical tooth (fig. 24), especially when compared

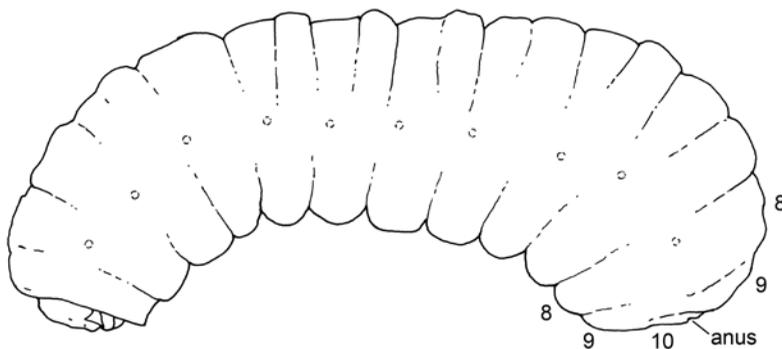


FIGURE 20. Diagram of live postdefecating larva of *Lithurgus chrysurus*, lateral view.

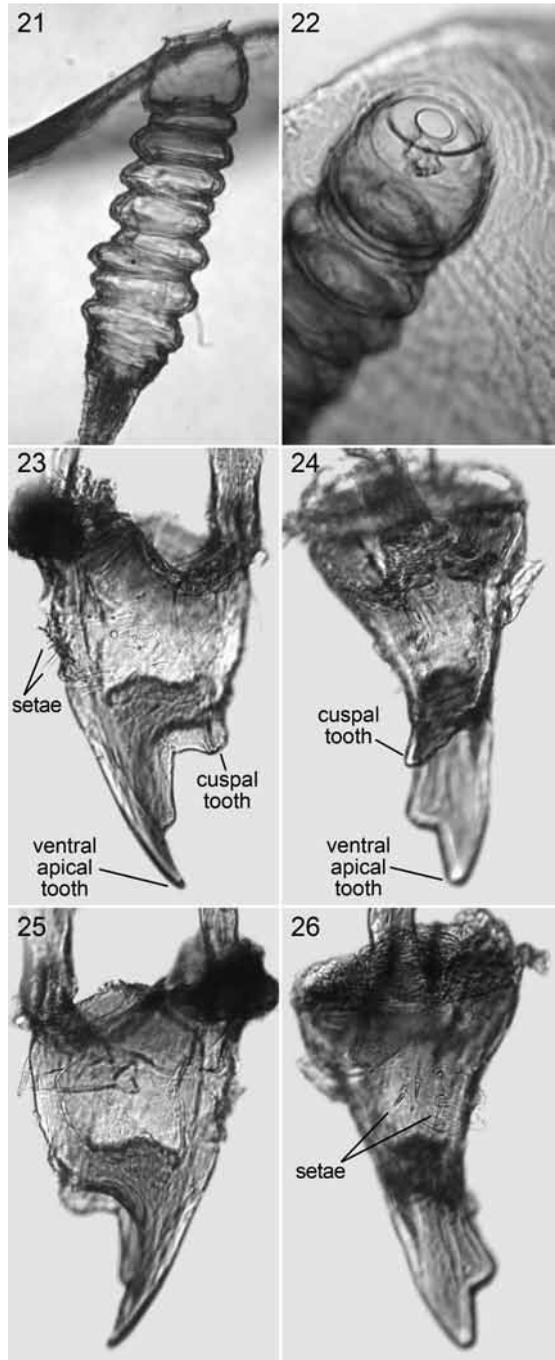
with the short, subtruncate dorsal tooth sets the species apart from *L. (Lithurgus) atratiformis*, in which the dorsal tooth extends nearly as far as the ventral one (Rozen, 1973: figs. 5–7). Subatrial width and length apparently are narrower and shorter (Rozen, 1973: fig. 5), respectively, in *L. atratiformis* than those of *L. chrysurus* (figs. 21, 22). The dorsal tooth of *L. (Lithurgopsis) apicalis* is nearly as long as the ventral one (figs. 27–29) and its subatrium is short, with about four chambers. These two species can also be distinguished because the antennal papilla of *L. chrysurus* (fig. 35) compared with that of *L. apicalis* (fig. 31) is far more pronounced. In *Trichothurgus dubius* (Rozen, 1973: figs. 19–21), depending on orientation, the dorsal tooth can be considered slightly longer than the ventral one; its spiracles are unknown. As typical of bee larvae in general, the reader should remember that mandibular wear can alter the shape and length particularly of apical teeth.

Although Rozen (1973) commented on the integumental rugosity of the parietals of *Lithurgus apicalis* in the description of its postdefecating larva, the degree of this wrinkling now can be demonstrated by an SEM micrograph (fig. 30). Similar wrinkling is also obvious on *L. chrysurus* (fig. 34).

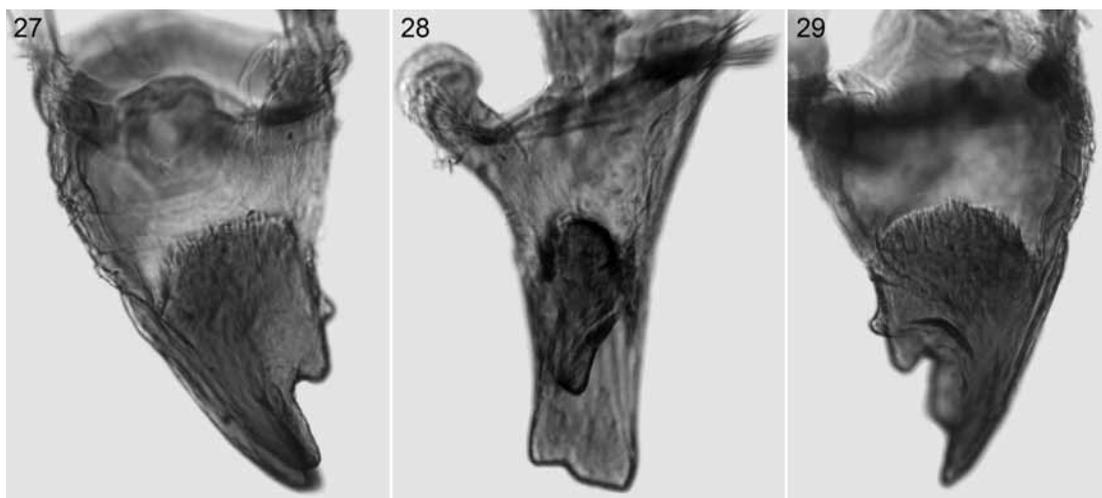
Description: Head (figs. 20, 23–26, 34): Setae of head capsule moderately long, basally stout, tapering, moderately abundant; setae of mouthparts considerably longer, abundant; parietal integument remarkably roughened (though on cleared specimens unevenness less apparent). In postdefecating posture: head capsule deeply recessed into prothorax (figs. 20, 34) (also true for *Lithurgus apicalis*, fig. 30), faintly pigmented, with mandibles and points of attachment on pleurostomal ridge, labrum in general, and rest of mouthparts moderately pigmented except salivary lips, mandibular apices, labral sclerite, all palpi, and maxillary apices darkly pigmented; on cleared larva following areas moderately pigmented: mandibles, especially at apices, and all other mouthparts including anterior end of esophagus, pleurostomal ridge, and surfaces of labrum and clypeus; internal head ridges at articulation with mandibles. Much of integument profoundly, irregularly rugose and pitted (fig. 34) (also true for *Lithurgus apicalis*, fig. 30), so that spiculation pattern difficult to separate from rest of surface. Area immediately above hypostomal ridge and just behind posterior mandibular articulation not produced as downward-directed tubercle as present in many *Coelioxys* (Rozen and Kamel, 2007: fig. 47). Coronal ridge weakly expressed internally; postoccipital ridge moderately developed; as seen from

above, ridge strongly curving forward, so that two sides form distinct median angle (as also characteristic of *Lithurgus apicalis*); hypostomal ridge well developed; dorsal ramus strongly developed, extending to postoccipital ridge; anterior tentorial pit midway between anterior mandibular articulation and basal ring of antenna in frontal view, deeply recessed; epistomal ridge present only laterad of (below) anterior tentorial pits and deeply recessed; tentorium moderately robust, dorsal arms short. Parietal bands strongly expressed. Maximum diameter of basal ring of antenna small, shorter than distance from ring to center of anterior tentorial pit; antennal papilla (fig. 35) very small, about as long as basal diameter, bearing approximately four sensilla (fig. 35), but considerably longer than that of *L. apicalis* (fig. 31). Lower margin of clypeus curving upward moderately at midline (fig. 30). Labral sclerite transverse, unpigmented, with lower margin extending beyond apical band of sensilla; apical labral margin broadly emarginated (fig. 30).

Mandible (figs. 23–26) moderately robust, with two apical teeth; ventral tooth much longer than dorsal one, acutely tapering to rounded apex; dorsal tooth subtruncate, recognizable as apical tooth primarily by homology with related taxa (Rozen, 1973: figs. 6, 10, 20); apical concavity smooth, well defined, with dorsal edge apically produced into toothlike projection (formerly called “adoral tooth” [Rozen, 1973], but appropriately termed “cuspal” tooth) as seen in inner view (fig. 24); dorsal surface bending upward in inner view (fig. 24); dorsal surface with spicules; outer surface irregularly sculptured at base, with approximately four conspicuous setae (fig. 26). Cardio and stipital rod sclerotized but not extensively pigmented;



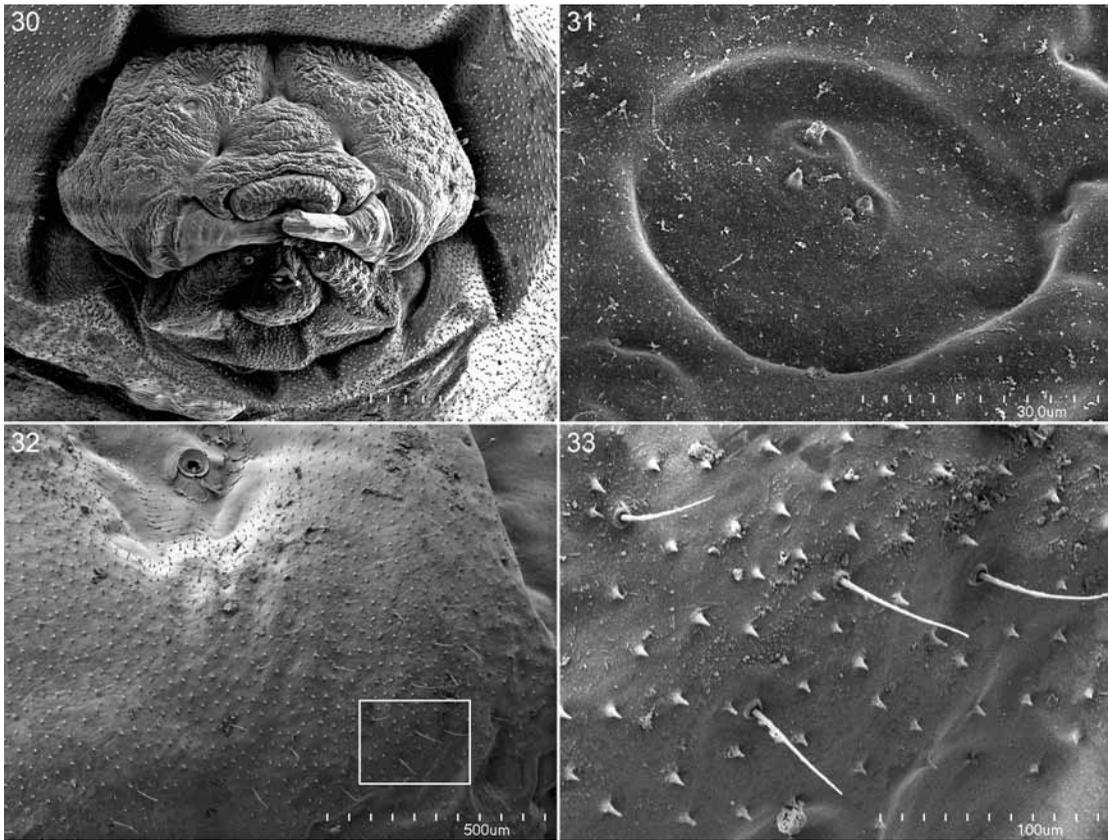
FIGURES 21–26. Microphotographs of cleared post-defecating larva of *Lithurgus chrysurus*. 21, 22. Spiracle, side and oblique views, respectively. FIGURES 23–26. Right mandible: 23. dorsal, 24. inner, 25. ventral, and 26. outer views.



FIGURES 27–29. Microphotographs of right mandible of cleared postdefecating larva of *Lithurgus apicalis*: 27. dorsal, 28. inner, and 29. ventral views.

articulating arm of stipes darkly pigmented; maxillary apex directed mesad far beyond insertion of maxillary palpus, so that palpus subapical in position; maxillary palpus slender, tapering, pigmented, length somewhat more than twice basal diameter. Labium clearly divided into prementum and postmentum; labial apex narrow, so that in frontal view, distance between palpal bases clearly less than distance from level of bases to top of labium; premental sclerite moderately weakly sclerotized; labial palpus slender, pigmented, tapering, length about twice basal diameter. Salivary lips projecting, transverse, but width less than distance between bases of labial palpi; inner surface of salivary lips unknown. Hypopharynx bilobed with surface deeply, finely roughened, perhaps spiculate.

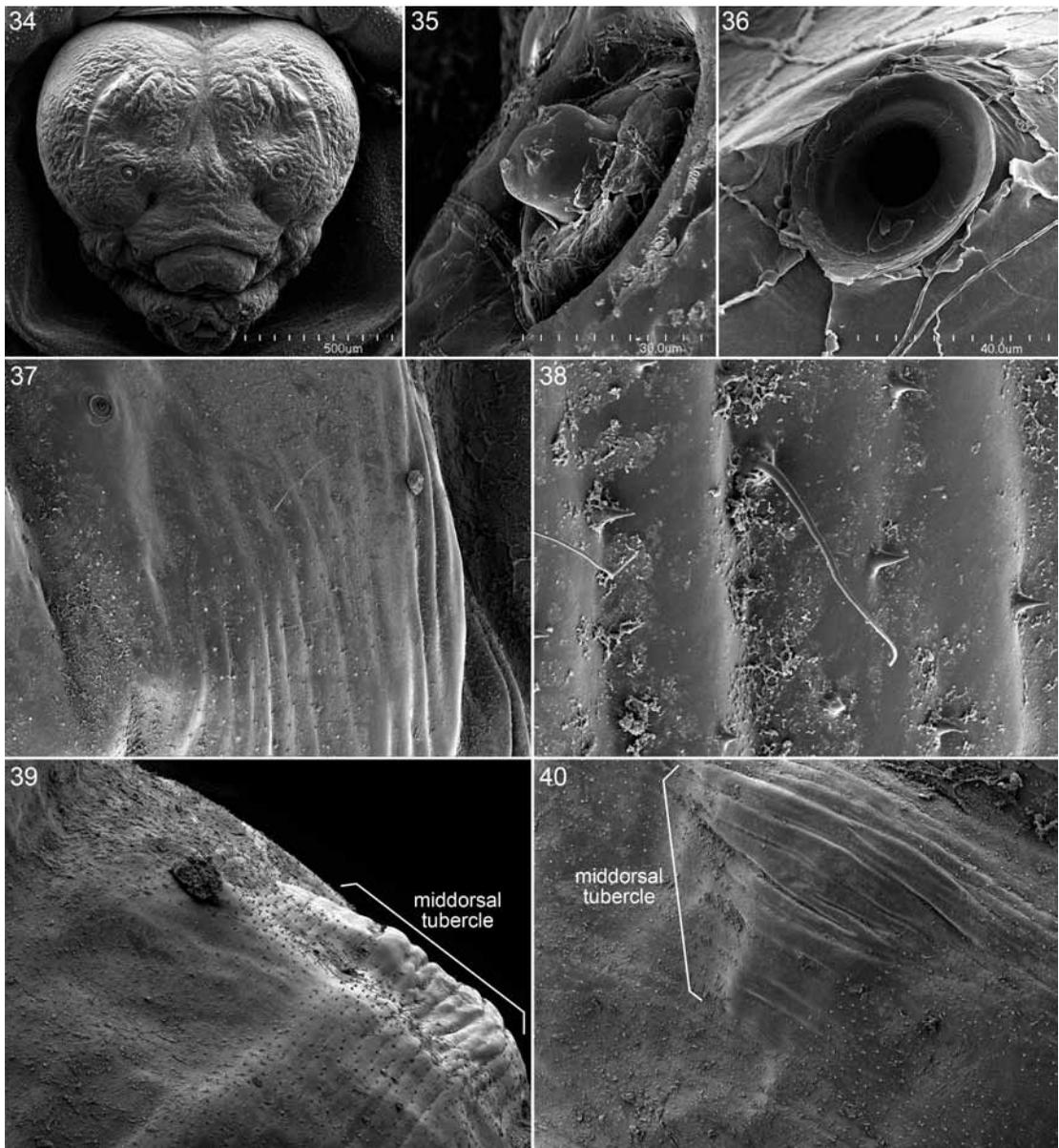
Body (figs. 20–22, 34, 36–40): Body vestiture consisting of slender setae and generally regularly spaced spicules that are much shorter than setae (figs. 37, 38) (also true for *Lithurgus apicalis*, figs. 32, 33); in general, spicules covering most of body, densest ventrally and also on thorax behind head; lateral swelling of abdominal segment 8 (defined as area below level of spiracle to bottom of swelling in lateral view) (fig. 33) with approximately 15 setae and very fine spicules; dorsal median spiculation on abdominal segments absent, almost certainly corresponding to apices of middorsal tubercles of earlier growth stages of last larval instar (figs. 39, 40). Body form (fig. 20) of postdefecating larva elongate, moderately robust with abdominal segments 6–8 markedly enlarged in lateral and dorsal/ventral views relative to preceding segments; abdominal segments 9 and 10 compressed from behind in lateral view (fig. 20), perhaps resulting from confinement at cocoon rear; intrasegmental lines moderately developed on postdefecating form; paired dorsal body tubercles not evident; middorsal body tubercles at most faintly evident as swelling (fig. 39) on postdefecating form, but often represented by wrinkled integument (fig. 40); lateral body swelling below spiracles not pronounced; abdominal segment 10 attached to approximate middle of segment 9; anus positioned toward top of segment 10;



FIGURES 30–33. SEM micrographs of postdefecating larva of *Lithurgus apicalis*. 30. Head, frontal view from below. 31. Antenna, showing weakly produced papilla. 32. Lateral lobe of abdominal segment 8 (area below and posterior to spiracle) showing uniform distribution of uniformly small spicules and scanty distribution of fine, long setae. 33. Close-up of area identified by rectangle in figure 32.

abdominal segment 10 with more or less distinct horizontal ridge above anus. Spiracles (figs. 21, 22, 30) well sclerotized, unpigmented, subequal in diameter; atrium globular with widest diameter near base tapering toward peritreme, hence pear shaped; atrium extending beyond body wall with funnel-shaped rim; diameter of atrial opening only slightly larger than peritreme radial (fig. 30); atrial inner surface smooth; primary tracheal opening with collar; subatrium with about 10 chambers; externally, subatrium (fig. 21) more or less parallel sided, unusually wide, with some chambers even somewhat wider than external maximum diameter of atrium. Male with small, transverse, median, integumental scar ventrally on posterior part of abdominal segment 9; female sex identification characters unknown.

Material Examined: Nine postdefecating larvae: NJ: Warren County: Phillipsburg, VIII-11-1977 (J.G. Rozen, R.B. Roberts, E.J. Rajotte); 22 postdefecating larvae: PA: Carbon County: Palmerton, (N40°48.700' W75°35.870'), X-03 & 26-2012 (J.G. Rozen, at Lehigh Gap Nature Center).



FIGURES 34–40. SEM micrographs of postdefecating larva of *Lithurgus chrysurus*. 34. Head, direct frontal view. 35. Antenna, showing well-defined papilla. 36. Spiracle. 37. Most of lateral lobe of abdominal segment 8 (area below and posterior to spiracle) showing uniform distribution of uniformly small spicules and scanty distribution of fine, long setae. 38. Close-up of same. 39. Dorsum of anterior abdominal segment, showing remnant of middorsal tubercle, lacking spicules, approximate lateral view. 40. Dorsum of another anterior abdominal segment, showing wrinkled integument remnant of middorsal tubercle and surrounding spiculate areas, dorsal view.

DISCUSSION

Roberts (1978: 738) stated in reference to *Lithurgus chrysurus* that “the main tunnel and nest entrance are never filled or sealed off” whereas the single complete nest described here was sealed with a closure and the main tunnel was completely filled with loose wood chips (sawdust) (fig. 11). This discrepancy may be explained by the fact that my observations were made in October, by which time adults were no longer flying, whereas Roberts’ studies were carried out during the height of adult activity, June–August, presumably before nest closures had been made. However, the reports by Garófalo et al. (1981), Camillo et al. (1983), Hannan and Maeta (2007), and Sarzetti et al. (2012) combined with the current study suggest an additional hypothesis: “empty, blind secondary tunnels,” “blind tunnels near the entrances,” and “chambers” are the apertures left behind after the female excavates closure material then used to fill passageways to cells and cell series, just as certain ground-nesting bees gather soil to close galleries leading to cells. The “plug” in the case of *Trichothurgus bolitophilus* is the closure itself (Sarzetti et al., 2012: fig. 11). One test of this hypothesis is to find out whether there is a plug of more compacted wood chips at the proximal end of galleries filled with “wood dust” in the case of wood-nesting species. Sequential observations of a nest closing might also be a test.

The above interpretation of the nest of *Lithurgus chrysurus* seems consistent with Rust’s et al. (2004) description of nests of the same species, although those authors were apparently unable to follow a single nest and did not consider the passageways cut through the sclerotic summer wood that “linked” “horizontal burrows” (i.e., branches) to be the main burrow.

Both Roberts (1970) and Rust et al. (2004) considered the possibility that *Lithurgus chrysurus* might be regarded a destructive species because it tunnels through wood to nest. As pointed out in the introduction, above, it has not proven to be so during the last 35 years while in the United States. It is an oligolectic species, specializing on the genus *Centaurea* (Asteraceae), also adventive in North America, but this plant grows abundantly in and far beyond the adventive range of the bee, so that sufficient larval food is not likely a factor restricting population growth of the bee in the United States. Similarly, dead, weathered wood, the nesting substrate of the bee, is abundantly available in the eastern United States. However, the bee may require a certain stage of decomposition of the nesting substrate material; not all dead wood may be suitable. The nesting site in Phillipsburg was dense and active with numerous larvae and adults, but quite restricted in distribution, as Roberts (1978) did not find any bees nesting in contiguous buildings even those of similar structure. According to Anita Collins (personal commun., October 14, 2012) the bee has also been found in northern New Jersey and eastern Pennsylvania as far west as Centre County. The nesting site in France (Rust et al., 2004) was merely in a pine beam of a veranda roof and consisted of numerous old burrows and three active nests. Its location was within the natural range of the species, where it seems not to be a pest problem either (Westrich, 1989). So far, we are left without an understanding of why this species is so limited in its spread into the United States, but we also note that other introduced species (e.g., *Anthidium manicatum* (Linnaeus)) have been introduced into North America, and at first have shown little range expansion, only to then undergo dramatic expansion (Strange et al., 2011).

Although mature larvae of *Lithurgus* (*Lithurgus*) *chrysurus* can be distinguished from those of *L. (Lithurgopsis) apicalis* by features presented in the Diagnosis above, studies of additional species are required before we can determine whether such differences are subgenus specific. However, the similarity of the mature larvae of these two species is impressive: integumental rugosity of parietals; middorsal angling of postoccipital ridge; overall body shape; body more or less covered with small spicules and with scattered, very fine, elongate setae. Although *L. apicalis* has more abundant spicules, the uniform shape of the spicules does not vary between the two taxa, and all setae appear to be of one morphology. This contrasts with the vestiture of *Trachusa larreae* (Cockerell) (Megachilidae: Anthidiini) (Rozen and Hall, 2012: figs. 44–51) in which the spicules are far larger and vary somewhat in shape, and setae are far more diverse and conspicuous. It is not understood exactly how any of these structures are used or how differences in shape or size affect their use, but Rozen and Hall (2012) postulated that spicules of the fifth instar of *T. larreae* may help it move about in the brood chamber. Now that these features have been identified and examined in quiescent postdefecating *L. chrysurus*, their use in active fifth instars can be scrutinized and interpreted.

Whereas the vestiture of these two lithurgine taxa differs from that of *Trachusa larreae*, the absence of vestiture on the middorsal tubercles of at least *L. chrysurus* (but probably also *L. apicalis*) is like that of *T. larreae*, suggesting that these tubercles function in the same way in both species. Rozen and Hall (2012) hypothesized that the middorsal tubercles of *T. larreae* may separate the surrounding spiculate integument from the cell wall, so that the anterior part of the body can easily slide on the smooth surfaces of their summits without abrading against the cell wall while the last larval instar completes feeding before cocoon construction.

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