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Nesting Biology of Diphaglossine Bees (Hymenoptera, Colletidae)

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CONTENTS

ABSTRACT

New data on the nesting biology of the following taxa of bees belonging to the Diphaglossinae are presented: *Ptiloglossa arizonensis, fulvopilosa, jonesi, guinnae, Crawfordapis luctuosa,* and *Policana albopilosa.* This information is summarized and analyzed in relation to literature and manuscript accounts on these and other taxa throughout the entire geographic range of the subfamily. Specific subjects dealt with are habitat preference; nest site requirements; social organization; nest structure, including cell orientation, shape, and size; provisioning; oviposition; development; cocoon

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construction and fecal deposition; daily adult activity; seasonal activity; and the relationship of cleptoparasitic bees (Epeolini) to diphaglossines. A formal, synoptic overview of the biological features of this subfamily is presented. Finally, the phylogenetic relationships of the subfamily to other colletids are briefly discussed in light of nesting biology, as well as the possible relationships between the Diphaglossinae and the Oxaeidae and Stenotritidae.

INTRODUCTION

This paper summarizes and analyzes existing information on the nest ecology and architecture, nesting behavior, including cocoon construction, nest site associates, and other aspects of the biology of the Diphaglossinae, a tropical and semitropical group of large-bodied, New World, ground-nesting bees.

The initial impetus for the study was the discovery of the nests of *Ptiloglossa arizonensis* in Portal, Arizona, in August 1982. The site yielded data that then permitted an interpretation of notes I had made on the nests of *Ptiloglossa fulvopilosa* years before on Trinidad. Dr. Mont A. Cazier, Curator Emeritus, American Museum of Natural History, immediately offered his field notes, photographs, and specimens of *Ptiloglossa jonesi,* nests of which he had excavated in the early 1960s in Portal. Dr. Radclyffe B. Roberts's informative paper (1971) on *Ptiloglossa guinnae* as well as cocoons he lent me afforded an even broader understanding of the biology of the genus. Dr. Gard W. Otis and his associates as well as Dr. David W. Roubik and Dr. Charles D. Michener kindly contributed their manuscripts and specimens of the cocoons of *Crawfordapis luctuosa* so that comparisons of that species could be made with *Ptiloglossa.* In the National Museum of Natural History, Smithsonian Institution, Washington, I discovered the cells and cocoons of *Policana albopilosa* excavated by Claude-Joseph in Chile in the early 1920s; I borrowed them through the courtesy of Dr. Karl V. Krombein. Because of the cooperation of so many people, it was possible to consider the nesting biology of the subfamily as a whole.

ACKNOWLEDGMENTS

I am grateful to the persons mentioned above for the loan of specimens, manuscripts, and other helpful contributions to the study. I also extend my appreciation to Mr. Vincent Roth, Resident Director of the Southwestern Research Station, for his personal hospitality and cooperation during the studies on *Ptiloglossa arizonensis.* Dr. Frederick D. Bennett (West Indian Station, Commonwealth Institute of Biological Control, Curepe, Trinidad, West Indies) assisted me in locating and excavating nests of *Ptiloglossa fulvopilosa* and lent specimens from his collection so that their identification could be determined. Dr. Philip F. Torchio (ARS Bee Biology & Systematics Laboratory, Utah State University, Logan) kindly permitted me to read his manuscript (referred to elsewhere in this study) on the nesting biology of *Hylaeus bisinuatus.*

Mr. Ian Stupakoff helped in numerous ways in the laboratory study at the American Museum of Natural History and is responsible for the scanning electron microscope photographs of the bee cocoons and cells presented here. Mr. Stupakoff's participation was supported by the Undergraduate/Graduate Research Program, which is funded by the Greenwall Foundation. I thank Ms. Deborah Hickman, who painstakingly prepared the manuscript for publication.

The Southwestern Research Station provided laboratory space and living accommodations for the initial phase of the study of the biology of *Ptiloglossa arizonensis.*

The following specialists have kindly read and contributed valuable advice on the manuscript: Dr. Radclyffe B. Roberts, Department of Entomology and Economic Zoology, Cook College, Rutgers, the State University of New Jersey; Dr. Charles D. Michener, Department of Entomology, the University of Kansas, Lawrence.

TECHNIQUES

Most of the procedures used in the present study are familiar to bee workers who exca-

vate ground-nesting species, and therefore I discuss here only two matters pertaining to technique.

(1) Even though these bees are large and their nest components correspondingly large and easy to observe, I consistently used a high quality stereoscopic microscope to examine cells, cell linings, food contents, cocoons, and behavior of various immatures of *Ptiloglossa arizonensis* as soon as they were excavated. Considerable detailed information would have been lost without immediate examination of material.

(2) Many features of the cells of bees are taxon-specific. These include not only the dimensions and proportions, composition and thickness of cell linings, cell walls, and types of closures, but also shape. Differences in shape may be obvious (e.g., the shape of the cell tops of *Ptiloglossa* compared with that of *Policana*) or subtle. When subtle, they are difficult to analyze as cavities. In recognition of this problem Stupakoff investigated ways of casting cells so that their shape as a solid could be more accurately studied, measured, and preserved for future comparisons. After consultation with the Department of Vertebrate Paleontology of the American Museum, he tested the use of latex and Smooth-on®, substances that have been employed for casting fossils, but found that they took too long to dry and could not be used in the field where the soil was wet. These substances also deteriorate after about five years of storage. Several brands of plaster were also tested: Gladstone® was very hard, but usually produced tiny air bubbles. Although somewhat softer, Speedrock® proved to be the most satisfactory in that it showed few air bubbles and took approximately an hour to harden. Diagrams of the shape of cells of *Ptiloglossa arizonensis* were made from camera lucida illustrations of casts prepared with Speedrock®. The powdered material was mixed with water on a 1 to 2 basis and a darkening pigment was added to better reveal details on the surface of the cast. The pigmented plaster was added to the water and stirred slowly so as to avoid air bubbles. The liquid mixture was tapped on a hard surface to remove additional air bubbles, and transferred to the cell lumen with a medicine dropper. After an hour the cell wall was broken down in water leaving the hard cast intact. Noncritical parts of the cast, as for example the pouring area, can be flattened with a file and collection data can be printed on the smooth surface. These casts are preserved in the collections of the American Museum of Natural History as are samples of adult bees, bee cells, and cocoons.

ORIGINAL OBSERVATIONS

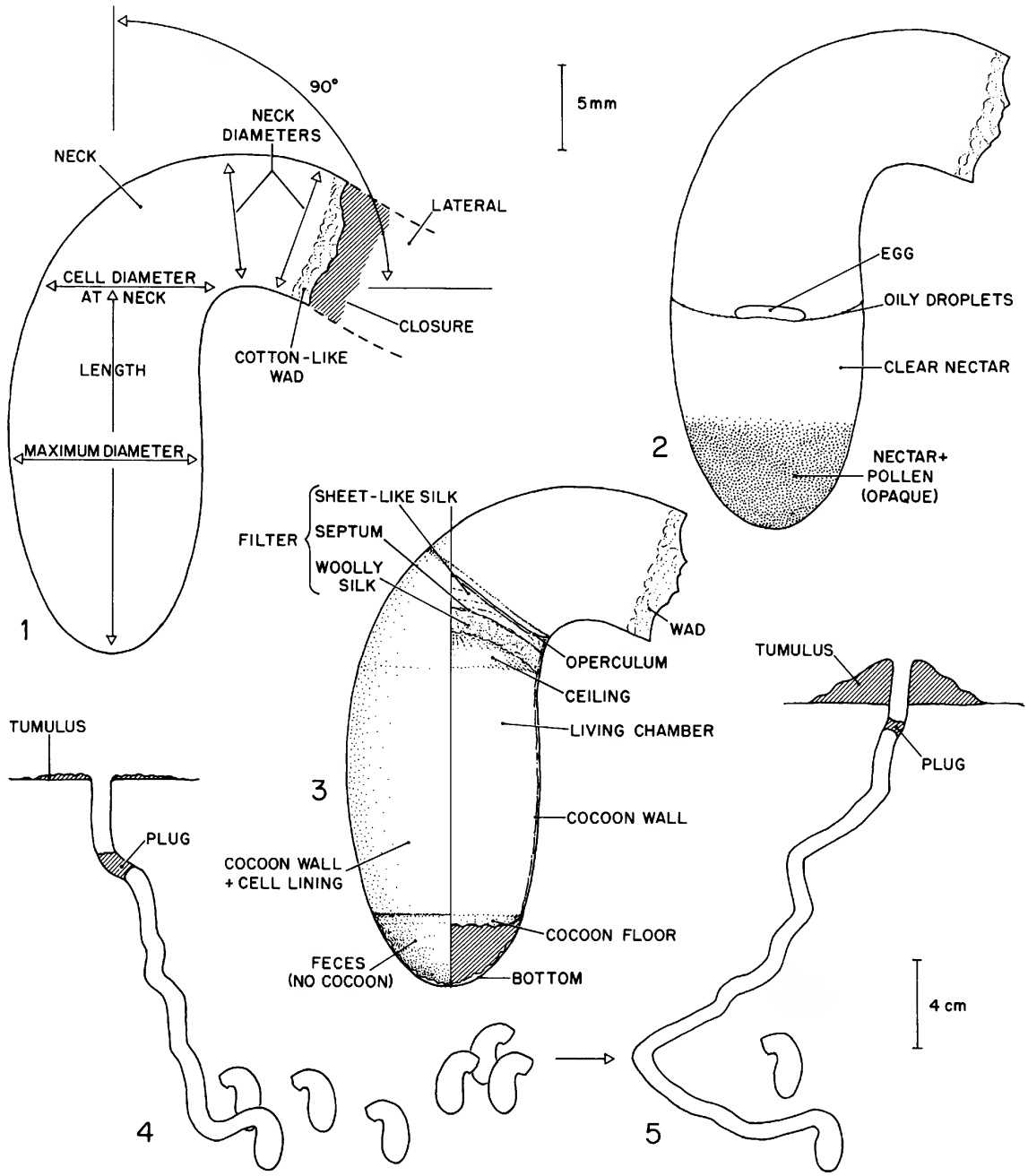
Ptiloglossa arizonensis Timberlake

LOCALITY: This species was first observed flying at approximately 5 A.M. Mountain Standard Time, on August 10, 1982, by Vincent Roth outside a window of his house in Portal, Arizona. Attracted by their loud buzzing at that early hour, he collected four females. After I noticed them later in the collection of the Southwestern Research Station, he directed me to the site on August 29. I found several nests during the daytime, began observations on August 31, and continued them through September 6, 1982. Nine nests were discovered where Roth had first seen the adults, and a larger concentration of more than 50 nests was centered approximately 2 m. away on the property of James F. Richards.[2]

DESCRIPTION OF SITE: Both nest groups occupied a terrace about 2 m. above Cave Creek, an intermittent stream, and were subjected to flooding under only the most extreme storm conditions. An open riparian forest canopy consisting primarily of sycamore and juniper permitted sunlight to reach the site during much of the day, and the sky overhead was visible from all burrow entrances except for a few at the edge of a large rock. Both aggregations (fig. 17) were in nearly horizontal ground, with a moderately dense covering of tall grass at the Roth site and with a sparse covering of grass but a thick accumulation of dried leaves at the Richards site. Most of the nest entrances were hidden by this dense ground cover.

The soil contained numerous, mostly water-rounded stones varying greatly from the size

[2] The site was revisited on a number of days between August 14 and September 2, 1983, after the manuscript had been submitted for publication. New information discovered in 1983 is added as footnotes.



FIGS. 1–5. *Ptiloglossa arizonensis.* 1. Cell, cell closure and lateral tunnel, side view. 2. Cell, egg, and provisions, side view. 3. Cell, cocoon and feces, right side, cut away. 4. Nest 1, August 31, 1982, side view. 5. Nest 6, September 2, 1982, side view. Scales refer to 1–3 and 4, 5, respectively. Drawing of cell made with camera lucida from cast of cell.

of a pebble to large rocks 25 cm. in diameter. The abundance of these stones may have accounted for the meandering tracks of the main burrows, the varying diameters of the burrows, and perhaps the rather unpredictable arrangement of brood cells. Between rocks, the soil was moist, very dark, and heavy, often containing organic material and numerous

earthworms as well as smaller rocks. Many small roots and a number of large ones were also encountered. The soil was easily compacted and contained a great deal of heavy, very fine clay that formed the walls of the cells, as discussed below.

The nine nests on Roth's property were scattered within an area of 1 m. in diameter, and some entrances were only 5 cm. apart, so that their tumuli were contiguous. The large nest concentration on the Richards property stretched over a triangular area 2.5 by 2.5 by 1.5 m. I did not determine the exact number of nests here because I did not wish to disturb the site by removing the ground cover. At least 12 nests were scattered in an area of one-quarter of a square meter.

DESCRIPTION OF NESTS: I carefully excavated eight nests (figs. 4, 5) on the Roth site to reveal overall nest structure, and quickly dug more than five nests clumped on the Richards site for information on food contents, immature stages, parasites, cocoons, and cell structure.

Almost all nest entrances were centered in copious dark tumuli. Tumuli ranged in diameter from 6 to 8 cm. when not close to one another, and varied in height from less than 0.5 to 2.5 cm. When close to others, tumuli were often much higher, one being 4.5 cm. Composed of claylike soil, they became consolidated when allowed to dry, even though they appeared to consist of loose fragments.

Active nest entrances had a diameter of 8 mm. (five measurements), but, because of twisting and turning, the tunnels below, circular in cross section, varied in diameter from place to place from 8 to 10 mm. Presumably the variation was created by the female's need to find a path around stones and roots. The moist tunnel and tumulus walls often bore the outline and striae of the female's pygidial plate. These demonstrated that the pygidium was used to compact the claylike soil of the wall. Burrow walls were moderately rough in contrast to the very even surfaces of the cells. When tested with water droplets the burrow wall, though not truly waterproof, slowly absorbed water. This slow absorption rate (discussed below) probably results from the fine claylike soil rather than from a secretion applied by the female.

Main tunnels (figs. 4, 5) initially descended nearly vertically. Although the entrance was open in almost every case, nests containing adult females were plugged with soil 2.5 to 6 cm. (10 measurements) below the surface, at least when the female was in the nest (i.e., during most of the day). Three of these plugs ranged in length from 3 to 20 mm. They tended to be flat and compacted below, and loose and unconsolidated above. The female, below the plug, apparently constructed it by tamping loose soil upward, probably with her clypeus, but possibly with her pygidium.

The overall nest configuration of *Ptiloglossa arizonensis* was never completely revealed, but the plan was clearly a main tunnel descending downward in a twisting fashion through the soil and around the roots and stones (figs. 4, 5), and cells randomly clustered around the burrow within a radius of approximately 4 to 15 cm. It was impossible to determine where and in what directions closed lateral tunnels branched from the main burrow. Open lateral tunnels approaching open cells rose approximately 1 cm. over a distance of 2 to 3 cm. before bending sharply downward at the cell neck into the vertical cell chamber. All laterals were filled after cell closure.

The more than 30 cells both of current and previous generations excavated at the Roth site varied in depth from 11 to 36 cm. Cells in a single nest gave the impression of being nearly equal in depth, perhaps because females took advantage of pockets of soil large enough to accommodate cells. The order of cell construction was not revealed, but clearly the older cells were not the lowest in the nest, nor the newest ones highest, as is the case with *Colletes*. Cells seemed to be not only coequal in depth, but also grouped into small units of two or three cells near one another. An understanding of cell arrangement will be gained when nests are found removed from one another so that the confusion of overlapping nest elements is avoided, and when cells are found in a substrate with fewer stones and other inclusions.

The cells (fig. 1) of *Ptiloglossa arizonensis* were unusual among bee cells in that the top (neck) of the cell, which was continuous with the rising lateral, bent downward more than 90 degrees so that the central axis of the main part of the cell was vertical. The lower end

was narrowly rounded (as opposed to broadly rounded) so that the maximum diameter was approximately halfway between the bottom point and the floor of the neck. Cells measured as in figure 1 varied from 20.0 to 23.0 mm. (12 measurements in length) and from 10.0 to 11.5 mm. (10 measurements) in maximum diameter. The diameter of the cell at the level of the floor of the neck was 9 mm. in one case and the diameter of the neck at various places varied from 7.4 to 8.0 mm. (five measurements). In a few cases, the entrance neck seemed slightly wider than high; in other cases it seemed circular in cross section.

The cell wall was very smooth and fine-grained with little coarse material projecting into the cell lumen. The even nature of the wall, its waxy texture, and the fact that it was retardant to water droplets suggested that the female impregnated it with a waxy secretion before she lined it. However, no waxy coat could be detected, either with solvents or by heating, and I finally determined that the clay in the soil gave the appearance and texture to the cell wall. Pieces of moist soil when rubbed with the rounded surface of a pair of forceps developed the same smooth, even surface retardant to water that was characteristic of the unlined cell wall. When heated over a Bunsen burner, cell walls became permanently hard like fired clay. Hence, I concluded that the female, after roughing out the cell, smoothed it, perhaps by tamping with her pygidial plate, thus imparting smoothness to the surface. Several cells showed pygidial imprints, particularly near the closure.

After smoothing the wall, the female applied a lining of nearly clear, nonfibrous, cellophane-like material (figs. 1, 15) over the entire inner surface, including the neck, as far as the position of the future closure. This lining consisted of at least three similar layers, appressed so closely that their individual nature could be distinguished only when the lining was torn with forceps. One layer revealed along the torn edge could then be peeled from the next. Together these layers adhered like one to the cell wall, there being no space between layers or between the lining and the wall itself, as is the case with some *Colletes*. This lining, impervious to water, was sufficiently thick and strong so that it could be carefully removed intact from the cell when the cell was softened by being submerged in water. There can be little doubt that the lining and claylike wall protect the contents of the cell from acquiring water or desiccating, as the case may be.

After foraging and egg deposition, the female closed the cell. All closures were marked by a thin, circular wad (fig. 1) of various thicknesses of cotton-like material that was usually stained amberish on the inside (cell side) and that was loosely attached to the end of the cell lining. Although the female may have provided this fibrous material before she constructed the closure of soil, this material may possibly also have been mold hyphae that grew from the closure and neck. Whatever its origin, it was present in all cells and clearly allowed air passage. Immediately in front of this circular, fluffy wafer was the cell closure proper. In five or six cases the closure appeared as a vague, coarse spiral of two or three rings to the radius, with the outermost ring the most consolidated. The inner area was infirmly attached to the outer ring and had vague fenestrations that extended to the somewhat denser fill of the lateral tunnel. The closure was more or less concave on the inside.

PROVISIONING: Linsley (1962) and Linsley and Cazier (1963, 1970) have discussed the floral relationships of this species and of other members of the Caupolicanini. The observation site for Linsley and Cazier (1970) was less than a kilometer from the nesting site that I studied.[3]

Provisions in the cells containing eggs or early instars (fig. 2) were a watery fluid, nearly clear on the top half and grading into a lower bottom half consisting mostly of yellow pollen that made the fluid opaque. All such cells also had oily droplets floating on the surface of the liquid.

Foraging activities are reported in the sec-

[3] Numerous males and females of *Ptiloglossa arizonensis* were discovered visiting *Agave Palmeri* with *Apis mellifera* and *Bombus* until 6:30 in the morning from Portal to Paradise in 1983. This plant, generally considered to be bat-pollinated, has sufficient nectar early in the morning to be visited by these bees as well as by moths. It may be the source of the copious nectar in the *Ptiloglossa* cells.

tion on Daily Adult Activity, but method of food transport is of interest here. Pollen was carried in a dry state, primarily on the scopa. The scopa in this genus was identified on pollen-laden females and consisted of hairs that tended to be very plumose; patches of the hairs occurred on the hind tibiae and femora, on the ventrolateral faces of the first metasomal tergum, and on the posterior side of each hind trochanter. On fully laden females, pollen also adhered to adjoining body areas, and the dorsum of the metasoma was ordinarily pollen-dusted so as to appear paler than that of nonpollen foraging females. Nectar was carried in impressive quantities in the greatly expandable crop in the anterior part of the metasoma.

DEVELOPMENT: Whitish, dull translucent eggs (fig. 2) floated on the surface film of the liquid provisions, probably because of the hydrofuge nature of the microscopically papillate chorion. Elongate and slightly curved, six eggs measured 3.9 to 4.6 mm. long and 0.90 to 1.05 mm. in maximum diameter. The anterior and posterior ends were equally rounded so that they could be distinguished from one another only after the embryo was observed. In each of four eggs the ventral side of the embryo was on or near the convex, dorsal surface of the egg. The embryo was either on its back (two cases) or 45 degrees from being on its back (two cases). Torchio (MS) has recently discussed embryo orientation in colletid bees.

One cell contained two *Ptiloglossa* eggs.[4] I assumed this to be an abnormality, for other cells seemed to contain single eggs and no cell was found with two larvae. However, because it was late in the season, relatively few cells with eggs were encountered; most cells housed large larvae. On the final day of the field study the last cell opened contained one very young larva of *Ptiloglossa* swimming on its side next to its cast chorion, and a *Ptiloglossa* egg floated nearby. This cell, then, had also contained two eggs of *Ptiloglossa*. The larva, which turned out to be a second instar, as discussed below, moved its head as do larger feeding larvae and seized the egg when it happened to touch it. It bit it a number of times with its apically pigmented, elongated, sharply pointed mandibles. The egg, though not punctured, was dented. Two cases of double oviposition suggest that this may not be an uncommon phenomenon. In yet another cell an intermediate stage *Ptiloglossa* larva (perhaps third instar) was feeding on its side, as described below, and the head capsule of a dead first instar *Triepeolus* was partly submerged in the provisions. These facts raise the interesting possibility that *Ptiloglossa* larvae are able to defend themselves against the larvae of parasitic bees such as *Triepeolus* and that the selection strategy favoring this type of behavior involves sibling combat resulting from double oviposition. Such a situation would be the first recorded case of defense against parasite eggs or larvae by the host first instars. Subsequent *Ptiloglossa* instars also had apically slender and darkly pigmented and sclerotized mandibles, so that somewhat older larvae may also be able to defend themselves. Young larvae of certain other solitary, ground-nesting bees have enlarged, pigmented mandibles (e.g., *Protoxaea,* Rozen, 1965b), suggesting that this may not be a unique phenomenon.

Young and intermediate *Ptiloglossa arizonensis* larvae were aquatic in that they floated in the nearly clear, upper layer of the provisions, with most of their bodies submerged, just as do the larvae of *Ptiloglossa jonesi* (fig. 7). When this stage larva was not in motion, all spiracles on the upper side of the body and surrounding integument were exposed to the air, but when it dipped its head downward, the anterior spiracles were submerged.

Feeding apparently took place as young and intermediate larvae very actively moved the submerged anterior part of the body dorsally and ventrally, that is, parallel to but under the surface of the provisions. Larvae occasionally ducked their heads into the thicker pollen-bearing stratum of the provisions and appeared to stir pollen upward into the clearer stratum so that with the further swinging of the anterior part of the body dorsally and ventrally, the mouthparts encountered the suspended pollen grains. The unusual attenuate larval thorax of *Ptiloglossa* (fig. 7) may be a modification for ducking into the pollen

[4] In 1983, seven cells with eggs were excavated and one of these contained two eggs.

layer of the provisions. The sharp-pointed mandibles of these larvae seem ill-suited for ingesting the provisions, and brief observations did not demonstrate their usage in feeding. Further detailed observations on feeding mechanisms as well as mode of ecdysis are needed.

At least five larval instars were identified on the basis of anatomical features and there was a suggestion of yet one more. The first stadium occurred apparently entirely in the chorion, because a cast skin was removed from the chorion found in the cell containing an active second instar and another *Ptiloglossa* egg (see above). The skin clearly showed body segmentation, spiracles with attached tracheae and denticulate mandibles. I noticed a similar situation with *Svastra* (Rozen, 1964), and referred to the cast skin as an "embryonic cuticle." Changes in the anatomy of instars most noticeably involve lengthening of palpi and the salivary spout, forward projection of the labiomaxillary region, and increase in head width. The changes in these structures from one instar to the next were not gradual but progressed in discrete "jumps" so that, for example, the palpi of the last instar were elongate, those of the penultimate larva somewhat short, and those of the antipenultimate even shorter. Body size increased in proportion to food ingestion. Additional specimens are required in order to document fully the number of larval instars and the differences between them.

There was no indication that the rate of development of the immature stages was abnormal in relation to other bees. As the nesting site was excavated rather late in the flying season, one would expect to find many large predefecating and postdefecating larvae and comparatively few early instars, which indeed was the case. This contrasts with the very slow development of such bees as *Colletes thoracicus.*

Food consumption depleted all provisions except for the oily material discussed elsewhere, and afterward the now very large larva almost completely filled the cell because of the massive amount of liquid in its soft body. At the end of feeding, larvae presumably had their heads at the bottom of the cell and the abdomen folded back on itself so that the anus was somewhat higher than the head. The larva then reoriented so that its head extended upward, and started cocoon construction.

The cocoon is complicated, and the sequence of its construction was pieced together from observation of cocoons being spun as well as of cells of previous generations. The larva, reaching up toward the neck of the cell, first spun a nearly flat disk (fig. 3) of salivary silk, the cocoon operculum. When completed, this appeared as an even, rigid, slightly concave reddish amber plastic-like surface with numerous small but distinct round holes (figs. 13, 24, 25) suggesting a sieve. This disk adhered to the lining near the top of the cell and was neither horizontal nor vertical, but rather uniformly angled toward the cell closure in all cases. In early construction, it appeared as a white, very fine translucent gauzelike material composed of fine silk. The larva moved its head back and forth over the fine netting and applied with its projecting labial region strand after strand of this viscous silk. These strands coalesced to such an extent that they fused, leaving only the circular holes in the completed disk. The silk was extruded from the peculiar spoutlike salivary opening of the larva, and the downcurved palpi as well as the tip of the spout rubbed over the surface of the fabric as the spinning proceeded. The taxonomically distinctive features of *Ptiloglossa* mouthparts (McGinley, 1981) thus seem correlated with the method of cocoon construction.

One larva traversed the closure 11 times in one minute when applying silk to the disk. Each traverse consisted of several short lines, in slightly different directions. Ten opercula, essentially circular, ranged in diameter from 7.5 to 9.0 mm. After completing the disk, the larva then began cocoon wall construction immediately below the operculum and apparently concomitantly spun a woolly, filterlike cushion of considerable thickness (fig. 3) immediately below the operculum. This filter consisted of an upper part of very open, sheetlike pale silk more or less parallel to the operculum but actually attaching to the central part of the operculum. No oil adhered to the fibers nor were nematodes encountered here. Separating the upper part of the filter from a denser mass of woolly material below was a thin septum of tightly woven parch-

ment-like silk. Strands of the dense woolly material below it were heavily coated with oil so that amberish oily deposits could be seen clearly when the filter was examined upside down under a stereoscopic microscope with transmitted light (fig. 12). Numerous nematodes also adhered to these strands. How the lower part of the filter became impregnated with oil and infested with nematodes while the upper remained free is not understood. The lower surface of the completed filter, that is the chamber ceiling, was dome-shaped and its plane horizontal.

A thin oily film covered the fibers of the operculum as well as the inner walls of the completed cocoon. The larva then extended the parchment-like cocoon wall downward. The cocoon wall and the clear cell lining formed a single sheet and were difficult if not impossible to separate. The combined cocoon and lining were semiopaque and pale amberish except near the operculum where they were somewhat darker. The cocoon (figs. 3, 20) did not extend to the bottom of the cell, but rather stopped at a line 3 to 4 mm. from the bottom. After the larva finished the cocoon wall, it destroyed most of the transparent lining at the bottom 3 to 4 mm. of the cell, leaving only flecks of the lining intact. The cell wall there was peppered in every case by fine incisions, indicating that the larva used the tips of its mandibles to scrape or peck away at the lining. The larva then placed its anal region over the bottom of the cell and discharged the entire yellow meconial mass into this area that bore no cocoon and only flecks of cell lining. Subsequently, the larva lowered its head and laid in a slightly concave floor of thick, dark, parchment-like silk that appeared continuous in structure with the cocoon wall. Sealed from its meconial mass, the larva then completed its activities and was now much smaller than the predefecating form. The destruction of the lining of the lower end of the cell presumably permitted water from the feces to drain into the soil.

All live postdefecating larvae examined five months after I excavated were still capable of moving slowly. Hence, there seems to be no true diapause. At the end of May 1983, an adult male and female emerged from the last remaining live immatures taken to the American Museum of Natural History. Three surviving *Triepeolus* larvae from the site metamorphosed at about the same time, but three more had not yet done so by the start of June.

DAILY CYCLE OF ACTIVITY: Observations on daily adult activity were made on August 31, September 1, and September 3, 1982, when astronomical sunrise ranged from 5:53 to 5:55 A.M., Mountain Standard Time. The eastern horizon was faintly light at 5 A.M. and bees could be seen at 5:30. All three days were mostly clear. On August 31 preliminary observations indicated the bees became active around 5 A.M., came and went frequently for about an hour, and then gradually reduced their frequency of departures and arrivals, and were almost inactive at about 7:10 A.M. However, females occasionally arrived and departed even as late as 9:45 when I revisited the site.

On September 1, starting at 4:45 A.M., I carried out further observations using a red electric light at first. Unfortunately, the light caused some buzzing and piping (short bursts of vibrations at a higher pitch than buzzing) in the leaves on the nest surface, and several females flew to it. This suggested that the red light disturbed their behavior, and the procedure was discontinued. I heard the first female depart at 5:06 A.M. and a total of 32 females left between then and 5:11. Fifteen more flew away between 5:11 and 5:16, and an additional 13, between 5:16 and 5:21. The first female was heard to return at 5:11 and by 5:21 so many females were returning and leaving that further aural monitoring was impossible. At 5:31 the same morning one or more females, whether coming or going, was buzzing at the site more than 75 percent of the time.

In order to obtain a sense of daily activity, I started at 5:45 A.M. to monitor by sight the number of returning females over a 10-minute period, as follows:

5:45–5:55 A.M.	16 females
6:15–6:25	19 females
6:45–6:55	10 females
7:15–7:25	4 females

These figures quantify the casual observations of August 31. Together with the aural observations before the bees could be seen, the data coincide closely with the temporal

distribution and the relative abundance of bees on the pollen plant as recorded by Linsley and Cazier (1970, table 1).

On September 3, I arrived at the site shortly before 5 A.M. The first signs of bee activity, as before, were piping and buzzing sounds among the dead leaves. The first female departed at 5:04. Seven females were captured at random as they returned, all before 5:30; none carried pollen, and when three of them were later dissected, all had crops filled to capacity with clear liquid, presumably nectar, as discussed below. The remaining four females, although not dissected, had wet faces when removed from the cyanide jar, indicating that they too had been replete with nectar.

Of five more returning females captured at random between 5:50 and 6:00 A.M., three had scopae abundantly packed with pollen, and two possessed only small quantities of pollen. One dissected bearer had a crop full of nectar and the other females had wet faces, indicating full crops.

A last sample of eight returning females, gathered between 6:50 and 7:00, showed three females with only limited pollen and the others without pollen. Three had wet faces, and one with a dry face, when dissected, had a full crop.

These observations suggested that large quantities of nectar but no pollen were gathered on the first short foraging run of the morning. Pollen and nectar were collected on later trips, and still later forays were primarily for nectar, either because the pollen supply was running low or nectar was the main requirement.

After the capture of females on September 3, an open cell in one nest contained only clear liquid. If this cell belonged to one of the captured females, its discovery indicated that cells were first supplied with nectar and then with pollen, which later sank to the bottom, forming the opaque yellow stratum of the provisions.

Of five females whose crops were examined, all had large quantities of clear nectar that contained only a very small quantity of pollen. When punctured, the crops of all discharged liquid miscible with, but somewhat heavier than, water. This is presumed to be nectar. Crops of two of these females also discharged droplets of oil-like material that floated to the surface in the dissecting dish. The quantity was small by comparison with the impressively large quantity of nectar. This material may be the same as that observed on the surface of the stored provisions in the brood cells. However, the nature, source, function, and relationships of all oily substances associated with the adult crops, larval body surfaces, cocoons, and provisions should be carefully scrutinized in the future.

Because crops of dissected females contained almost no pollen and therefore suggested that adults do not require pollen for food, I hypothesized that females captured from the nest after discharging nectar into cells would have depleted crops.However, two females recovered from their nests in mid-afternoon had crops about half-full with nectar. Further, the nectar was so filled with pollen that it appeared cloudy. As there was no general exodus of females from their nests after 7 A.M. until these two were captured, the cloudy contents of the crops suggest that females may obtain food in the form of both nectar and pollen from still-open cells provisioned earlier. Torchio (MS) reported such a situation for *Hylaeus bisinuatus* Forster.

Because my excavations took place over three or four days during daylight hours, it is clear that females did not generally depart on foraging trips during the main part of the day. Unfortunately, observations were not carried out during the evening when *Ptiloglossa jonesi* occasionally forages for nectar (Linsley and Cazier, 1970).[5]

No males were observed at the nesting site, indicating either that mating takes place elsewhere or that males had already ceased their seasonal activity. All females collected had worn wings, as did the females of *Triepeolus* collected at the nests.[6]

NEST ASSOCIATES: The cells of this species

[5] In 1983 the site was briefly visited several times in the evening between 5:30 and 7:00 P.M. and was inactive.

[6] On August 14, 1983, the site was observed between 5:40 and 6:30 A.M. Although this was earlier in the season than the previous year's study, only a single male was found for brief periods at the site, hovering and chasing females. No copulations were seen. Most of the mating activity apparently takes place at the flowers and perhaps along the flight path.

of *Ptiloglossa* were heavily infested with nematodes, probably representing a new genus in the superfamily Aphelenchoidea.[7] They seemed to be most abundant in cells where the bee larva had died, but there were scarcely any cells without a moderately heavy infestation. Nematodes could be seen on the sides of the cells, swimming through the provisions where larvae were young, and crawling over the surface of mature larvae. Bodies of nematodes were visible on cocoon opercula (figs. 14, 24). The impact of mature nematodes on the cell contents is unclear. Suzanne W. T. Batra examined two female *Ptiloglossa* and found "no nematodes in the Dufour's glands, guts or elsewhere" (*in litt.*).

Several large adult *Triepeolus* belonging to an undescribed species were collected flying over the nesting site, and numerous larvae of various stages as well as eggs were recovered from cells. This species is the same as the one Cazier (personal commun.) found infesting cells of *Ptiloglossa jonesi* and whose mature larva was described by Rozen (1966) as *Triepeolus* species b. All eggs were inserted through the cell lining into the cell wall, approximately at right angles to the cell wall, so that the anterior end of the egg was approximately flush with the cell lining. That end was circular, nearly flat, and surrounded by a narrow rimlike flange. The seven eggs or egg chorions discovered ranged from 8 to 15 mm. from the bottom of the cell. One egg of *Triepeolus* measured 2.4 mm. long, its anterior end was 0.7 mm. in diameter exclusive of the flange, and it tapered from there to the narrow posterior end. No cell had more than one *Triepeolus* egg.

First instars (fig. 23) with long, slender mandibles, pigmented head capsule, elongate labral tubercules, and very thin, elongate maxillary palpi will be described in a subsequent paper. Most of the body segments had a pair of tapering, elongate tubercules extending outward at right angles to the body, and the apical abdominal segment possessed a pair of eversible pygopod-like structures. The lateral body tubercules apparently assist in stabilizing the larva floating on the surface of the provisions as revealed by photographs of this species in cells of *Ptiloglossa jonesi* (fig. 23). The only live first instar of the *Triepeolus* in the nests of *P. arizonensis* was encountered on the side of the cell.[8] When I tipped the cell so as to make the provisions flow toward it, it retreated up the wall, seemingly to avoid the liquid. This larva was very active. Its dorsal surface constantly, rapidly quivered. It held its head, which also quivered, away from the cell wall and its mandibles were partly spread. When touched with a pin, the head swung up and down as it tried to seize the pin. Whereas the head and anterior body were able to leave the wall, the posterior body with the lateral extensions could not be elevated, suggesting that these lateral extensions, in addition to serving in floating, may be stabilizing devices to hold the larva *in situ* on the cell wall as it battles with the *Ptiloglossa*.

Last stage larvae of six *Triepeolus* were all found standing rigidly on end in the vertical cells with the anterior end curved forward, most of the abdomen straight, and the anal area resting on the cell bottom (fig. 6). All were in complete diapause and the fecal material, discharged as an opaque yellow semiliquid, was smeared irregularly over most of the cell wall. The greatest quantity of feces appeared to be heaped above the head of the hibernating larva so as to restrict, although in most cases not completely close off, the passage to the cell entrance. As is characteristic of other Nomadinae, this larva did not spin a cocoon, and in contrast to the larva of *Ptiloglossa* the cell lining was not destroyed. Hence, how the great quantity of liquid from the cell provisions was removed is not understood in the case of the cuckoo bee. The yellow integument of the larva, rigid and with pronounced dark spiracular atria and tubercules, contrasted with the flabby, whitish larvae of the host whose spiracles, though large, were unpigmented and therefore inconspicuous. As discussed above three of the post-defecating *Triepeolus* larvae brought to the American Museum metamorphosed by late May 1983, more or less synchronously with several host larvae.

[7] Kindly identified by Dr. William R. Nickle, Nematology Laboratory, Plant Protection Institute, United States Department of Agriculture, Beltsville, Maryland.

[8] Numerous other first instars were seen floating on provisions in 1983.

Ptiloglossa fulvopilosa Cameron[9]

LOCALITY AND DESCRIPTION OF SITE: Observations on the nesting habits and ontogeny of this species were made during 1964 and 1965 at three different localities on Trinidad, the West Indies. Dr. Frederick D. Bennett, Commonwealth Institute of Biological Control, Curepe, Trinidad, ably collaborated in gathering of data presented below.

The first area, in Nariva Swamp, on the eastern side of Trinidad, consisted of a few burrows. Three entrances, surrounded by tumuli and open on the surface, were situated under a trunk of a fallen tree in a clearing. The trunk was about 0.7 m. in diameter and 20 cm. above the ground. A single burrow was discovered in the earthen floor of a nearby hut.

The second nesting site was under a house in Curepe (fig. 18), where excavations were made on September 19, 1964, and again on February 6, 1965. Although about 10 entrances were observed in an area of 4 sq. m. on the latter date, all the bees were dead, presumably because the burrows had been thought to be those of the parasol ant *Atta,* and had therefore been treated with an insecticide.

The third nesting site, at Verdant Vale in Arima Valley, was discovered on February 7, 1965, at which time 25 to 30 active nests were seen in an area of 2 by 2.5 m. under a house (fig. 19). This site was in the approximate position of the one mentioned by Vesey-FitzGerald (1939) in his discussions of the genus. Both the second and third sites had been active for more than a generation, as evidenced by vacated cells.

All three areas were similar in that they were sheltered from the rain and sunlight did not reach them, except perhaps late in the afternoon. All occupied gently sloping ground that lacked vegetation. The one at Verdant Vale had an oblique half-meter rise on the ground surface, along which many but not all the nest entrances were found. Although Trinidad is tropical, the microenvironment of the sites was nearly xeric because of the shelter provided by the houses and the fallen tree. The soil in each site was moderately easily excavated and contained some moisture; few hard inclusions were encountered. The soil, at least at Verdant Vale, was very pale, decomposed schistlike material, in contrast to the very dark, claylike soil associated with *P. arizonensis.*

At the second and third sites the burrow openings were irregularly spaced, some being separated by only 4 or 5 cm.

DESCRIPTION OF NESTS: Although most observations were made at Verdant Vale, those from the other sites agreed. These observations were incomplete and therefore difficult to interpret at the time. The notes on *P. fulvopilosa* as well as the nest components stored at the American Museum now can be interpreted more meaningfully because of the more extensive investigations on *P. arizonensis.*

Each nest consisted of a nearly vertical, slightly meandering main tunnel between 9 and 10 mm. in diameter and numerous singly arranged vertical cells ranging from 2 to 6 cm. from the main tunnel. Over most of its surface, the main burrow seemed to have a "plastered" wall of soil of variable thickness but averaging approximately 1 mm. thick. Whether it was constructed from soil prepared elsewhere or merely the result of tamping by the female is unknown, but it apparently did not have a counterpart in the nest

[9] The correct name for this species is in doubt. Vesey-FitzGerald (1939) and McGinley (1981) referred to it as *fulvopilosa*; Moure (personal commun.) believes that it is *lucernarum* Cockerell. I compared a long series of males and females from a number of localities from the north range of Trinidad, all of which represent a single species. Males from this species differed from a male Panamanian specimen of *fulvopilosa* described by Michener (1954) in that the pale hairs of the thorax and first metasomal tergum were consistently more infuscated than those of the Panamanian specimen. Further, the narrow, apical, pale bands of terga 2, 3, and 4 were somewhat more conspicuous than those of the Panamanian individual. Other aspects of the external anatomy were remarkably similar in the two samples. Trinidadian females were compared with a single Panamanian female of the *lucernarum* also referred to by Michener (1954). All Trinidadian females possessed narrow but distinct apical bands on terga 2, 3, and 4, whereas the Panamanian female lacked such bands. Furthermore, the ocellocular distance was distinctly shorter than the ocellar diameter in Trinidadian females, whereas in the Panamanian specimen it was somewhat greater. Hence, whether the Trinidadian population represents *fulvopilosa, lucernarum,* or yet another species cannot be resolved until a taxonomic revision of the genus is worked out, or at least until types are examined. Because *fulvopilosa* has been applied to the Trinidadian species in print in the past, it is used here.

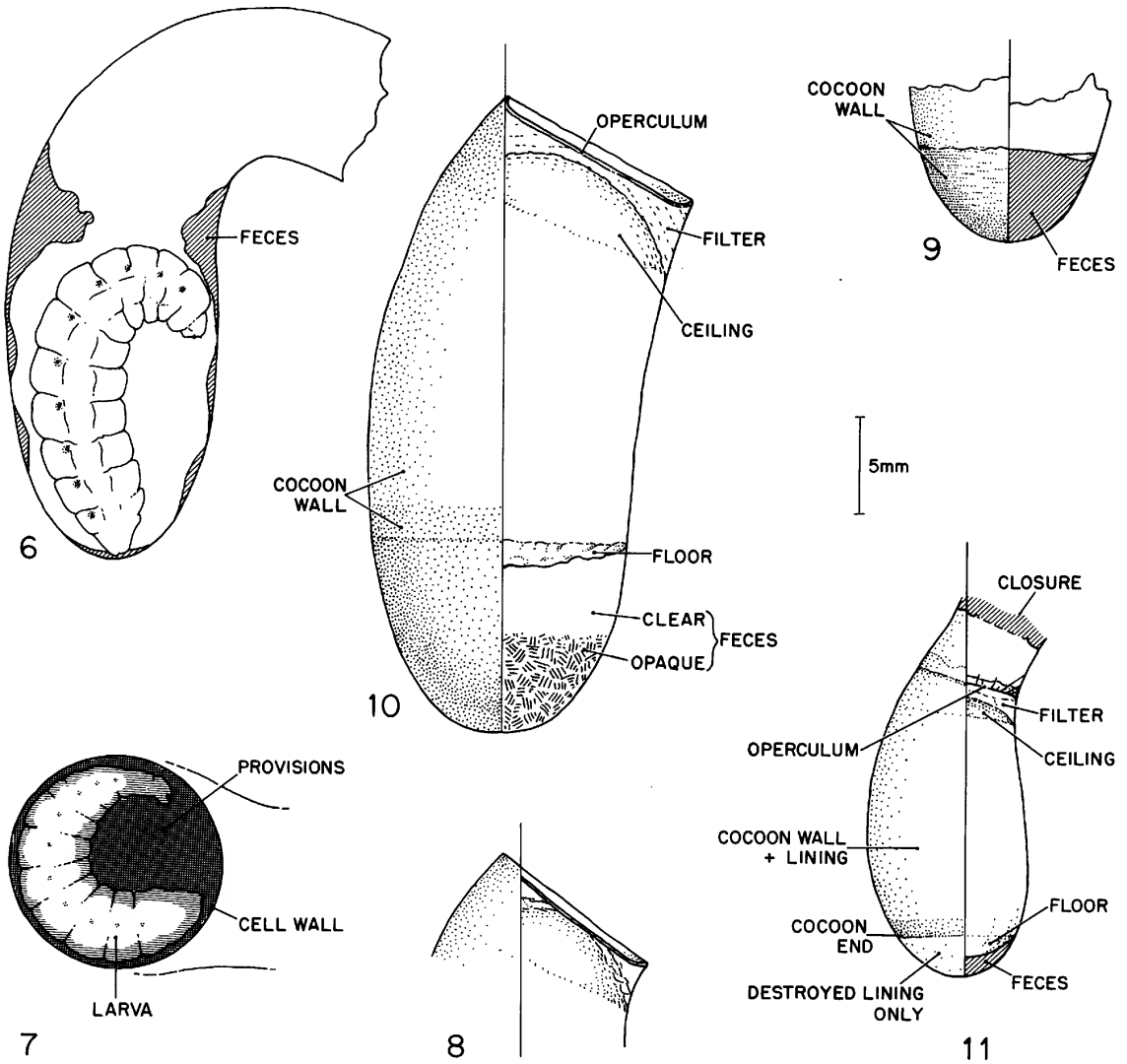
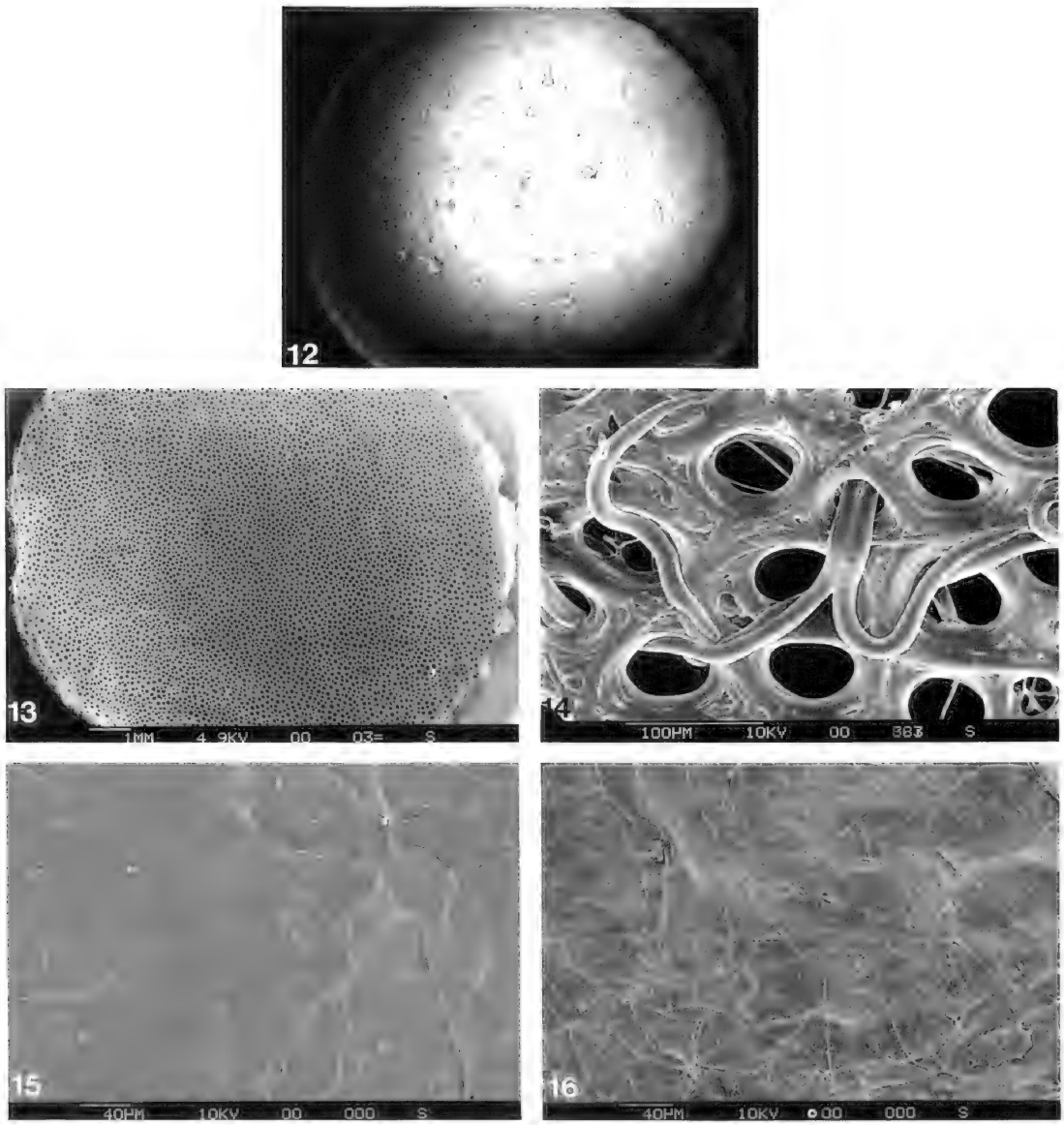


FIG. 6. *Triepeolus,* postdefecating larva in cell of *Ptiloglossa arizonensis,* side view.
FIG. 7. *Ptiloglossa jonesi,* intermediate larva, partly submerged in provisions, showing part of body above surface in white, top view of cell.
FIG. 8. *Ptiloglossa fulvopilosa* cocoon, top part, right side cut away.
FIG. 9. *Ptiloglossa guinnae,* bottom of cocoon and fecal chamber, right side cut away.
FIG. 10. *Crawfordapis luctuosa,* cocoon, right side cut away.
FIG. 11. *Policana albopilosa,* cell and cocoon, right side cut away.
Scale refers to figures 6–11.

of *P. arizonensis.* The entrance of fresh burrows was marked by a conspicuous tumulus (fig. 19) of loose soil 3 to 4 cm. high and 7 to 8 cm. in diameter on a flat surface. The tumulus soil next to the entrance was moist, consolidated, and continuous with the burrow wall. About half of the burrow entrances at Verdant Vale were plugged, either at or just below ground level, with a septum of soil which in two cases measured 4 and 8 mm. thick. Below the plug, the actively worked burrows were open and approximately 28 to 30 cm. deep, although one nest extended down 42 cm. Each cell was connected to the main burrow by a lateral that usually rose about 3 cm. as it neared the cell. This side tunnel, about 9 to 10 mm. in diameter, had a plastered coating, at least in most places



FIGS. 12–16. *Ptiloglossa arizonensis.* 12. Photograph of cocoon filter area taken with light transmitted through the filter as seen from below. Note oily droplets. Magnification approximately 6×. 13. Scanning electron micrograph of cocoon operculum, seen from above. Magnification approximately 8×. 14. Scanning electron micrograph of operculum, showing several nematodes. Magnification approximately 190×. 15. Scanning electron micrograph of cell lining attached to cell wall as seen from inside of cell, showing nonfibrous nature of lining. Magnification approximately 150×. 16. Scanning electron micrograph of cocoon wall, adhering to cell lining and cell wall taken from inside the cell, showing fibrous nature of cocoon fabric. Magnification approximately 150×.

and, like the main tunnel, it was smooth with some irregularities.

The cells, similar in shape and size to those of *P. arizonensis,* were vertical and possessed a distinct neck that angled downward toward the lateral. They were 11.5 to 13.0 mm. in maximum diameter (four measurements) and 22.0 to 23.0 mm. in length measured from

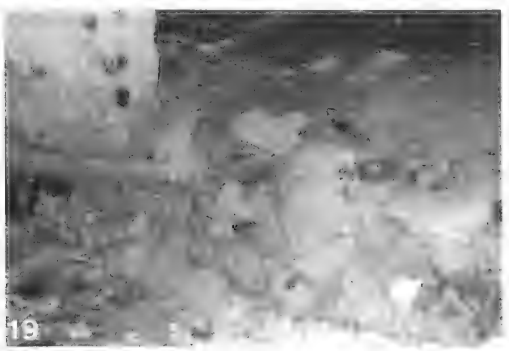


FIG. 17. *Ptiloglossa arizonensis,* view of nesting site in tall grass on both sides of barbed wire fence, Portal, Arizona.

FIGS. 18, 19. *Ptiloglossa fulvopilosa.* 18. View of nest area under house, Curepe, Trinidad, being examined by Dr. Frederick D. Bennett. 19. View of nesting area under house in Verdant Vale, Arima Valley, Trinidad, showing abundant, dry tumuli, some on sloping surfaces.

the bottom to the level of the floor of the neck (three measurements). The diameter of the neck was approximately 8.0 mm. Neck floors in several cases formed a sharper angle with the cell wall below than did those of *P. arizonensis.* All the cell bottoms were narrowly rounded. Unlike those of *P. arizonensis,* cells possessed a plastered wall that varied in thickness roughly from 1 to 4 mm. The thickest places seemed to be both around the neck and at the cell bottom. The wall surface was coated with a semitransparent, extremely smooth, waterproof lining that was perhaps thinner than but in other ways similar to the lining in *P. arizonensis.* This lining extended into the end of the neck as in *P. arizonensis.* The somewhat obscure spiral cell closure consisted of several rings of slightly consolidated soil in front of which the side tunnel was filled with soil. Immediately inside several closures in the American Museum collection were small wads of whitish or grayish fibrous material, either hyphae or female secretions, as in *P. arizonensis.* The order of cell construction was apparently not sequential, for older larvae were found both above and below younger ones.

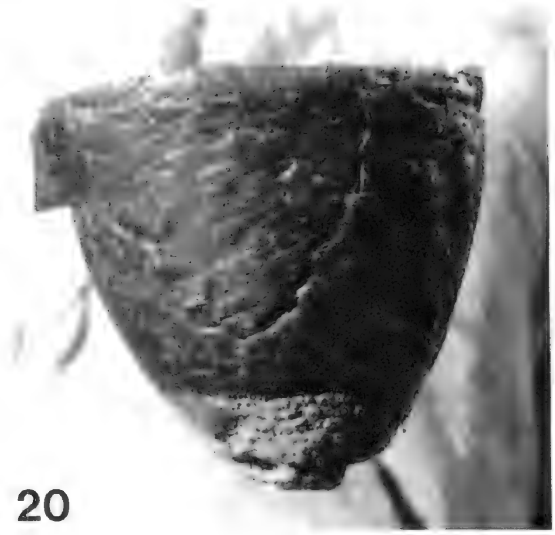


FIG. 20. *Ptiloglossa arizonensis,* bottom of vacated cocoon, side view, showing dark cocoon wall and cell lining above and pale dried fecal material at very bottom. Magnification approximately 4×.

PROVISIONING: The liquid provisions filled a little less than half the cell. Semitransparent on the surface, they were less fluid and more opaque at the bottom because of the yellowish pollen. The mixture was stringy and appeared to have the consistency of a broken hen's egg, though not so yellow in color. In this respect they differed from the fluid, nonstringy stores of *P. arizonensis* but agreed with

the "ropey slime" in the cells of *P. guinnae* (Roberts, 1971).

The elongate white egg, 4.7 mm. long (two measurements), floated on the surface of the provisions. As is the case with *P. arizonensis,* young larvae fed on their sides while partly submerged in the provisions.

After all provisions were consumed, the larvae closed the cells with a medium brown cocoon operculum (figs. 26, 27) just below the elbow. Circular and 9.8–10.0 mm. in diameter (three measurements), the operculum slanted toward the cell closure. Its upper surface was flat to slightly concave, dull, fine-textured, and semiopaque. Paler than the cocoon operculum of *P. arizonensis,* its texture was more fibrous and parchment-like, and the holes were more numerous and much smaller.[10] The filter beneath the operculum was restricted primarily to the periphery of the operculum (fig. 8). The lower surface of the filter, though fenestrated, was somewhat more consolidated than the "dome" of *P. arizonensis* and was actually composed of several sheets of fibrous material.

The tan cocoon wall was appressed to the cell lining so as to form a single sheet of semi-transparent, thin, parchment-like material. The outline of the larva could be easily seen. This material extended down to the level 4 to 5 mm. from the bottom of the cell. Below that level, the fibers were no longer fine and multidirectional, but rather were now thick, primarily parallel to one another, and grouped into bands, much as in *Ptiloglossa guinnae* (fig. 9). These bands were concentric with the bottom point of the cell. Now the material had a "rice-paper" effect, and not only looked like strapping tape, but also tore along lines of weakness between the bands. This banded material extended down 2 to 3 mm. and then gradually returned to a parchment-like fabric, with strands becoming multidirectional at the very bottom of the cell.

[10] These small holes were most numerous around the periphery where the ceiling of the living chamber was not appressed to the operculum. In addition to these fine holes, two of the three preserved opercula possessed a few (maximum eight) much larger holes (three or four times the diameter of the smaller holes) scattered in the periphery.

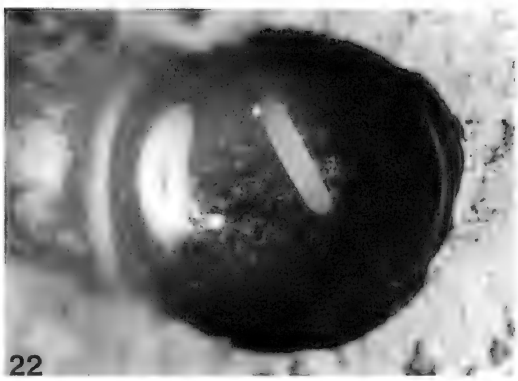
The banded fabric and the very bottom of the cocoon adhered closely to the cell lining just as did the cocoon material above. However, the lower 3 to 4 mm. of the cell lining had been perforated, presumably by the mandibles of the larva before the silk had been applied. These fine incisions penetrated into the soil and were often in linear sequence and other times randomly arranged. A drop of water placed on the inside of the banded fabric slowly penetrated the combined cocoon and cell lining. This indicated that the fabric here was not waterproof and that the puncture marks made in the lining, though not destroying the lining, permitted seepage of water from the fecal material into the soil.

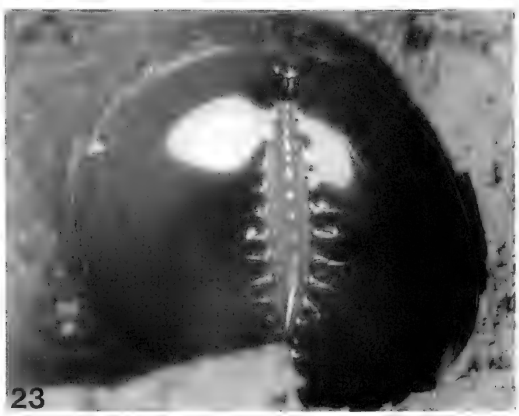
After the lower section of the cocoon had been produced, the larvae deposited the meconial mass at the very bottom of the cell and then spun a floor of parchment-like silk over the feces as was characteristic of *P. arizonensis.*

DAILY CYCLE OF ACTIVITY: Vesey-Fitz-Gerald (1939) reported that this species was truly nocturnal in that females returned to their nests "at the first light of dawn."

SEASONAL CYCLE: A collection of adults, kindly lent by Dr. Bennett, contained specimens taken during every month of the year except April, November, and December. Presumably, this species is active throughout most if not all of the year.

NEST ASSOCIATES: Although no cuckoo bees were encountered as adults or immatures at Verdant Vale, Bennett recovered larvae of *Odyneropsis apicalis* Ducke from nests in the Nariva Swamp (Rozen, 1966). Adults reared from these larvae had enlarged ocelli, indicating that they too were nocturnal. Other species of *Odyneropsis* in the American Museum collection all have large ocelli so that the genus may consist solely of nocturnal bees. *Triepeolus* associated with *P. arizonensis* had ocelli that were scarcely if at all enlarged. Indeed, the two adults that I captured were flying over the nesting site at a time when there was sufficient ambient light for me to see them. It would be interesting to determine whether these cuckoo bees flew at the very early part of the daily flight period of *P. arizonensis* when there was scarcely any light.





FIGS. 21–23. *Ptiloglossa jonesi.* 21. View of habitat, Portal, Arizona. 22. Egg floating on liquid provisions, top view. Magnification approximately 4×. 23. Cell containing first instar of *Triepeolus* species b. Magnification approximately 5×. Photographed by Dr. Mont A. Cazier and Mr. Martin Mortenson.

Ptiloglossa jonesi Timberlake

LOCALITY: Dr. Mont A. Cazier and Mr. M. Mortenson discovered the nest site of this species on July 24, 1964, on the property of Dr. R. G. Willys in Portal, Arizona. Information concerning this nesting site comes from a number of sources. First, Cazier (personal commun.) has kindly shared considerable data derived from notes and memory about his study. Second, he has generously supplied numerous documentary photographs taken at the nesting site at the time of his investigations. These photographs, some of them reproduced here, reveal facts about nest structure, provisions, larval feeding habits, and various other matters. Third, Cazier during the course of his investigations deposited fragments of cells and cocoons in the collections of the American Museum of Natural History. Together, these resources now permit a substantial understanding of the nesting biology of this species as compared with that of *Ptiloglossa arizonensis.*

DESCRIPTION OF SITE: The nesting aggregation, consisting of approximately 92 active nests, occurred within an elongate area 3 by 5 m., all enclosed in a fenced yard (fig. 21). The ground surface was level to slightly sloping and had few stones, except for a low-lying retaining wall. The soil was a rich loam that had been disturbed. The area was mostly unshaded during the daytime except for a few trees, and the low-lying ground cover consisted of sparse herbaceous plants. Soil contained numerous roots but far fewer stones than the soil at the site of *Ptiloglossa arizonensis.*

DESCRIPTION OF NESTS: Nest entrances occurred in the open, and near, next to, or under such objects on the ground as rocks and small plants. Tumuli apparently surrounded the entrance hole as in *Ptiloglossa arizonensis,* except when washed away by rain. Main burrows, generally vertical, twisted considerably and had the approximate diameter of those of *P. arizonensis.*

Cells were essentially identical in shape with those of *P. arizonensis.* They were 23.0–25.0 mm. in length (three measurements) mea-

sured from the bottom to the floor of the neck; and 11.0–11.5 mm. in maximum diameter (three measurements). The neck on one specimen extended about 8.0 mm. to the closure and had a diameter of 8.0 mm. The diameter of another cell at the level of the neck floor was 9.0 mm. There was no indication of a "plastered" cell wall, and the wall was very smooth as is the case with *P. arizonensis.* The cell lining was conspicuous as in *P. arizonensis* but at least on two specimens it became very thin on the neck surface so that the neck wall was less waterproof than the main cell wall.

PROVISIONING: Cazier (personal commun.) reported that the provisions of this species were a slightly sweet tasting liquid and appeared to be fermenting. Photographs (fig. 22) of cells with eggs and larvae show that at least during early stages provisions are stratified into a lower, more opaque layer and an upper, clear watery one. Foraging of this species was discussed by Linsley (1962) and Linsley and Cazier (1963, 1970).

DEVELOPMENT: In shape, chorion texture, and probably size the eggs of this species appeared identical with those of *P. arizonensis.* They (fig. 22) floated on the surface film in the middle of the cell, with the convex side upward. Cases of more than one egg to a cell were not observed by Cazier (personal commun.). Young and intermediate larvae (fig. 7) floated partly submerged in liquid provisions, just as described for *P. arizonensis.*

Fragments of several cocoons, partly associated with cell walls, showed a circular, nearly flat operculum, 9.0 mm. in diameter (figs. 28, 29). It bore numerous holes, that were not so evenly spaced as in *P. arizonensis.* The fabric between the holes on the upper surface was not fused and plastic-like, as in *P. arizonensis,* but was softer and parchment-like. The filter area on the cocoon consisted of fine silk strands similar to those of *P. arizonensis,* but further description and interpretation of the filter was impossible because of damage.

The cocoon wall did not extend to the bottom of the cell, at least in the two preserved specimens. One cocoon terminated 3.5 mm. from the cell bottom, much as in *P. arizonensis,* and the fecal material was packed into the cell bottom after the cell lining at the bottom had been perforated (or destroyed), presumably by the larva's mandibles. Tiny incisions remained in the cell bottom. Also, just as in *P. arizonensis,* the larva had spun a thick, concave floor over the feces.

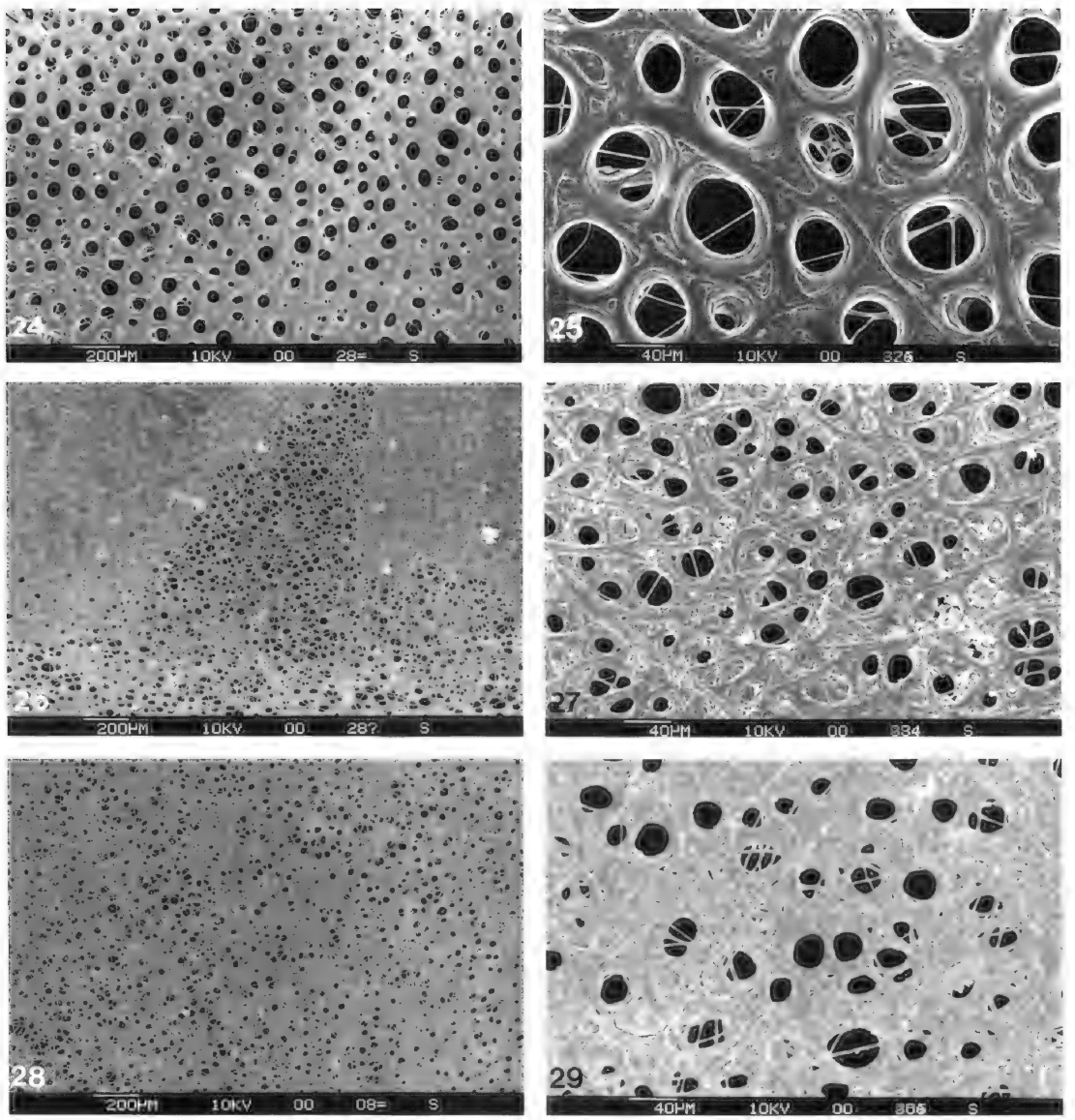
NEST ASSOCIATES: The large *Triepeolus* associated with the nest of *P. jonesi* was the same species that attacks the nest of *P. arizonensis.* Figure 23, kindly supplied by Cazier, shows its first instar floating on the provisions, with its tapering, lateral body tubercules, and large forked pygopod-like structures everted from the last abdominal segment.

Preserved cells that had contained *Triepeolus* revealed the same fecal pattern as the cells of *Ptiloglossa arizonensis* attacked by the *Triepeolus.*

Ptiloglossa guinnae Roberts

Roberts (1971) provided considerable information about the nesting, mating, foraging, and provisioning habits of this species, and kindly supplied a fragment of a single cocoon (fig. 9) with the fecal chamber attached at the bottom. Although the operculum was missing on the specimen, Roberts (1971) fortunately pointed out that the operculum had "colander-like" perforations. This and the following information indicate that the cocoon is not markedly different from those of other *Ptiloglossa.*

The fabric of the cocoon wall above the fecal chamber was composed of a single thin layer of very pale tan silk with multidirectional fibers more fused than in the cocoon of other species so that the material was moderately shiny and transparent. Although the fabric almost certainly adhered to the cell lining, observations concerning this point were not possible because the cell lining was not preserved. The cell wall just above the fecal chamber had silk strands that were somewhat more apparent than those farther above, and these were irregularly pigmented with brown, perhaps indicating a thicker application of silk. Just at the level of the floor, the fibers of the cell wall became parallel to one another and concentric with the bottom point of the cell, all as described for *Ptiloglossa fulvopilosa.* Some of these parallel fi-



FIGS. 24–29. Scanning electron micrographs of diphaglossine cocoon opercula, top view; pictures on left magnified approximately 30×, on right, approximately 150×. 24, 25. *Ptiloglossa arizonensis.* 26, 27. *Ptiloglossa fulvopilosa.* 28, 29. *Ptiloglossa jonesi.*

bers were darker than others, so that the parallel streaking of the cocoon of the fecal chamber was quite apparent, and the tendency of the fabric to tear along parallel lines of weakness was noted. The "rice-paper" effect extended downward for about 3 mm. and gradually changed back to a multidirectional fiber pattern at the cocoon bottom. I could not determine whether the cocoon wall of the fecal chamber was impervious to liquids, nor whether the cell wall had been perforated by the larva. However, the similarity between the fabric of the fecal chambers of *Ptiloglossa guinnae* and *fulvopilosa* was so striking that

I would not be surprised if the chamber and its lining permitted drainage of fecal liquid into the substrate.

The fecal chamber (fig. 9), located at the rounded bottom of the vertical cell as typical for the subfamily, was 4.5 mm. at maximum depth and approximately 8.5 mm. in diameter at the top. The living chamber floor above it was slightly convex, rather than concave, as are those of other known Diphaglossinae. However, this shape may have resulted from the material being preserved in alcohol. Drying of the meconial mass in fecal chambers may cause the concavities observed in other species. The floor was tan, smooth silk and turned up at the edges to meet the cell wall.

Crawfordapis luctuosa (Smith)

Several nesting sites of this species were studied by Otis et al. (1983) in Costa Rica and by Roubik and Michener (in press) in Panama. Both teams of researchers kindly supplied specimens of cocoons for the following description. Roubik and Michener also gave me a copy of their notes, as well as a manuscript which provided information on fecal material that could not have been determined by examining the preserved specimens.

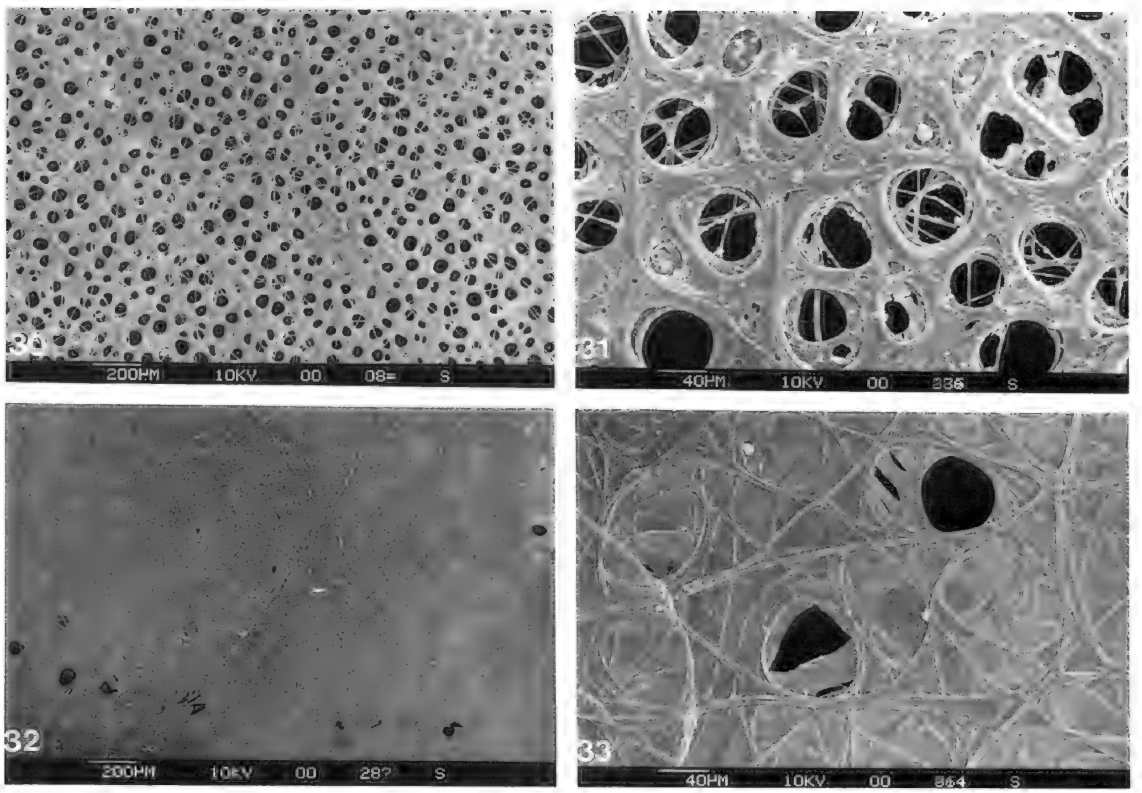
The upper surface of the operculum (figs. 30, 31) of this species was similar to that of *Ptiloglossa arizonensis* except it was medium tan in color, 11.5 to 13.0 mm. (four measurements) in diameter and the sieve holes were somewhat smaller. Whereas the rigid, plastic-like, slightly concave opercular surface and its circular outline closely resembled the same features in *P. arizonensis,* the filter area beneath was thinner as seen from the side than that of *P. arizonensis,* but thicker than that of *P. fulvopilosa.* In cross section (fig. 10) it was composed of oblique sheets of open netting, some of which attached to the undersurface of the operculum. The lower surface of the filter (i.e., the ceiling of the living chamber) was a single dome-shaped sheet of denser, more opaque, parchment-like material that contained small openings that were finer than those of the operculum. The presence of oily material was not detected in any of the cocoons, all of which have been preserved in alcohol. The cocoon was very large, reflecting the large size of the cell. Three measured 32 to 33 mm. long, from the center of the operculum to the bottom of the cocoon, (i.e., bottom of the cell), and two measured 14.0 mm. in greatest diameter. They were similar in shape to those of *Ptiloglossa,* that is, elongate and rounded on the bottom. The operculum angled obliquely in relation to the long axis of the cocoon.

The cocoon wall of semitransparent, tan, parchment-like material resembled in texture that of *Ptiloglossa* and also was closely appressed to the cell lining. Unlike those of *Ptiloglossa* studied here, the walls extended to the bottom of the cells and the nature of the wall fabric was the same throughout. From this and from the large size of the fecal chamber (fig. 10), I conclude that the larva did not perforate or destroy the cell lining, and that the feces, very liquid in nature (Michener, personal commun.; field notes), were deposited without any mechanism to reduce the liquid content of the stored meconial mass. One can only assume that the soil, saturated by the moisture of the elfin forest environment, would not have absorbed such liquid even if there had been no waterproof lining at the bottom of the cell. Michener and Roubik (field notes) commented that the feces were often distinctly stratified into an upper clear liquid layer and a lower equally thick, opaque white or yellow layer (grading to brown above) of firmer material (pollen grains). The floor of the living chamber above consisted of a thick, concave layer of dark brown leathery silk, obviously applied by the larva after defecation. It was similar to the cocoon floors found in *Ptiloglossa.*

Policana albopilosa (Spinola)

Claude-Joseph (1926)[11] treated the biology of this species (as *Policana herbsti* Friese) in considerable detail. Presumably as a result of this study he preserved larvae, pupae, and a small clump of earth containing four cells, all from Correo Nuñoa, Chile. The samples were deposited in the National Museum of Natural History, and the larvae were described by Michener (1953) and McGinley (1981). The

[11] Claude-Joseph, an ecclesiastical name used by H. Janvier for his 1926 publication, was abandoned on his subsequent papers.



FIGS. 30–33. Scanning electron micrographs of diphaglossine cocoon opercula, top view; pictures on left, magnified approximately 30×, on right, approximately 150×. 30, 31. *Crawfordapis luctuosa.* 32, 33. *Policana albopilosa.*

following account of the cells, cocoons, and fecal material was possible because of the preserved cells. The soil was very fine-grained with no pebbles.

DESCRIPTION OF NESTS: The vertical cells were elongate, rounded on the bottom, and, in general, shaped as in figure 11. They ranged in maximum diameter from 7.0 to 8.0 mm. (four measurements) and the cell height in one case was roughly 18 mm., as measured from the bottom of the cell to the approximate level of the closure. The cell wall was very smooth except embossing from the female's pygidial plate was evident here and there over the surface. The cocoon height in two cases was approximately 14.5 and 15.0 mm., measured from cell bottom to the center of the operculum. Cells were lined with a transparent layer of waterproof, shiny material that extended to the cell closure, as characteristic of other known diphaglossines (that is, well above the cocoon operculum). One cell was clearly constructed partly within another that had been previously constructed and lined. A wall of fine soil 0.3 mm. thick separated the bottom of the one from the other. There was no way of determining whether the first cell was from a previous generation or whether the female for some reason abandoned the first cell and started a new one in the same place.

The side tunnel, 4.5 and 5.0 mm. in diameter at the cell entrance (two measurements), curved away from the cell toward the main tunnel (Claude-Joseph, 1926), but its curvature was gentle (illustrated at about a 45-degree angle by Claude-Joseph) as was also revealed on one exit tunnel partly preserved. This curvature was in sharp contrast to the more than 90-degree angle between the long axis of the cell and the lateral of *Ptiloglossa* and perhaps *Crawfordapis.* Claude-Joseph's description of the spiral closure seems to correspond closely with that of *Ptiloglossa ari-*

zonensis, and indeed, the hardened outer spiral of the closure was still clearly evident in two samples.

DEVELOPMENT: There appears to be a major discrepancy between Claude-Joseph's observations on this species and mine on *Ptiloglossa arizonensis* (and for that matter, my interpretations of other species described here). He attributed the construction of the operculum ("la membrane de fermeture"—the closing membrane) to the female's constructing this structure rather than to the spinning of the larva, as is unquestionably the case in *Ptiloglossa arizonensis* and *jonesi.* Indeed, he never discussed the presence of the cocoon at all. Although the similarities of the cocoon of *Policana albopilosa* and that of *Ptiloglossa arizonensis* are too great for me to assume that they are products of different life stages in the two genera, some of his data are so detailed that there may be some basis in fact. The details are as follows: He describes how the female, after oviposition, positions herself at the cell entrance and, with the lobes of the tongue closed together, constructs strands that support a viscous material that forms "the membrane." He further asserts that during construction of the cell closure, bits of soil drop onto the membrane. How he made these rather startling observations is not clear. He also gives details about how the female rubs the metasoma against the cell wall in order to smooth the surface before she uses her tongue to disburse a viscous secretion over the wall. This is another remarkable observation that seems to correspond correctly to how the cell lining is applied in *Colletes,* as reported by Batra (1980). Additional observations need to be made on the cell lining and cocoon construction in *Policana albopilosa* and, at that time, we can determine what role, if any, the female has in the construction of the cocoon operculum.

The preserved cocoon operculum (figs. 32, 33) was a nearly flat surface, slightly beveled downward just at the cell wall. A flange of cocoon material extended up the cell wall from the attachment of the operculum and a number of strands of silk were strung diagonally upward from the operculum surface either to the cell wall or to the flange. The two opercula examined were tan and consisted of dull parchment-like, circular sheets 5.5 and 6.5 mm. in diameter (two measurements). In addition to threads of silk embedded in the matrix, there were small, extremely thin spots that glistened in reflected light, apparently representing ribbon-like strands of silk as shown in figure 33. The mature larva of this species is peculiar for the Diphaglossinae in that the salivary lips are flattened to form a small transverse slit that presumably is capable of forming both threadlike and ribbon-like silk (Michener, 1953; McGinley, 1981). One operculum bore irregularly scattered fenestrations of various sizes over most of the surface; the other had these holes absent in the center, which was about 2.0 by 2.5 mm. Differences in the filters of these two cocoons, as described below, accounted for this dissimilarity. In both cocoons, the number of holes was much fewer than in those of other taxa treated in this paper (see fig. 32). The opercula slanted somewhat in the direction of the cell entrances.

The filter of one cocoon consisted of several well-formed sheets of netting that extended partway across the upper end of the cocoon and were loosely tied with silk strands to the other sheets, to the operculum, or to the dome-shaped lower surface of the filter. The parchment-like lower surface bore numerous randomly scattered small holes that tended to be smaller than the openings of the operculum. The filter on the other cocoon had the lower surface arched upward in the center, so that it adhered to the operculum. Where these two surfaces were contiguous, there were no holes. Elsewhere the holes were similar to those described for the other cocoons. The sheetlike netting was somewhat similar to that of the other cocoon, except, of course, it was not present in the central area.

The cocoon wall, like that of other diphaglossines, was transparent, parchment-like, brown, with numerous strands of silk, all closely adhering to the cell lining. The wall (fig. 11) extended downward, but terminated before reaching the cell bottom. The line of termination seemed to be 2.0 to 3.5 mm. from the bottom (three measurements), but the exact line was hard to determine because the silk floor of the living chamber was dark, opaque, deeply concave, and overlapped the

end of the cocoon wall almost 1 mm. so that it was difficult to determine the lower terminus of the wall. Most of the cell lining of the fecal chamber was clearly perforated by numerous tiny incisions, presumably made by the larva's mandibles, although the lining in this area was not destroyed to the extent of that of *Ptiloglossa arizonensis*. Feces, yellowish in color, were deposited beneath the floor. They contained some fibrous material on their lower surface that may have been hyphae rather than silk strands.

DISCUSSION

The information presented above, when incorporated with the literature accounts of diphaglossine bees, demonstrates that the nesting biology of the subfamily is homogeneous and contrasts to a considerable extent with that of other colletid subfamilies. In spite of similarities, differences in nesting behavior among taxa within the subfamily are also evident. These similarities and differences, together with my interpretation of research areas needing further study, are presented below. Unless otherwise stated, literature citations to species are as follows:

Tribe Caupolicanini:
- *Caupolicana albiventris* Friese—Janvier, 1955
- *Caupolicana funebris* Smith—Claude-Joseph, 1926; Janvier, 1955
- *Caupolicana gayi* (Spinola)—Claude-Joseph, 1926
- *Caupolicana gaullei* Vachal—Janvier, 1955
- *Caupolicana ocellata* Michener—Michener, 1966
- *Caupolicana pubescens* Smith—Janvier, 1933
- *Ptiloglossa arizonensis* Timberlake—present paper
- *Ptiloglossa fulvopilosa* Cameron—present paper; Vesey-FitzGerald, 1939
- *Ptiloglossa guinnae* Roberts—Roberts, 1971; present paper
- *Ptiloglossa jonesi* Timberlake—present paper
- *Crawfordapis luctuosa*[12] (Smith)—Otis et al., 1983; Roubik and Michener, in press

Tribe Diphaglossini:
- *Diphaglossa gayi*[12] Spinola—Janvier, 1933
- *Cadeguala occidentalis*[12] (Haliday)—Claude-Joseph, 1926, as *Policana occidentalis*
- *Policana albopilosa*[12] (Spinola)—Claude-Joseph, 1926, as *Policana herbsti* Friese

ECOLOGY: The Diphaglossinae are restricted to the New World. Their habitats range from desert to near desert conditions on the one hand and to wet tropical rain forest on the other. Some species (*Crawfordapis luctuosa, Ptiloglossa guinnae,* and apparently *P. mexicana* (Cresson) (Roubik and Michener, in press) have been found nesting under cool, wet conditions of montane rain forests in Central America. *Ptiloglossa arizonensis* and *P. jonesi,* though in a desert, are active during the summer rainy period and the claylike soil of the site of *P. arizonensis* was moist. *Ptiloglossa fulvopilosa,* from tropical Trinidad, nests in situations that are sheltered from the rain so that the sites themselves are actually quite dry. Ecological information is not available on the South American taxa discussed by Claude-Joseph (1926) and Janvier (1933, 1955).

All Diphaglossinae are ground nesting. Edaphic requirements for nesting seem varied, with some species apparently preferring sandy situations, others claylike material, and no particular pattern of specificity emerges from limited data on hand. There may, however, be some preference for slope of the nesting surface. *Cadeguala occidentalis, Policana albopilosa,* and some *Caupolicana,* appear to prefer either vertical banks or at least sloping surfaces, whereas *Ptiloglossa* has been found nesting primarily in horizontal surfaces. Ground cover at nesting sites varies from scattered herbs to trees; some sites seem to be exposed throughout the day to the sun, whereas others (e.g., *Ptiloglossa fulvopilosa*) are completely shaded for most of the day.

SOCIAL ORGANIZATION: There is a distinct tendency for individuals to nest gregariously in irregular groups of a few to many nests. Roberts (1971) counted 1348 nests at the site

[12] These genera are monotypic.

of *Ptiloglossa guinnae,* and Otis et al. (1983) found a maximum of 400 nests at one site of *Crawfordapis luctuosa,* although the number fluctuated from season to season and year to year. The *Cadeguala occidentalis* site had 200 burrows, as reported by Claude-Joseph (1926), who also found colonies of *Caupolicana funebris* consisting of "hundreds of galleries." Such groupings in diphaglossine bees often persist for more than one generation, as evidenced by old, vacated cells among the cells containing immature bees, reported here for *Ptiloglossa arizonensis* and *fulvopilosa.* One of the sites of *Ptiloglossa fulvopilosa* may have been the one studied by Vesey-FitzGerald in 1939, in which case it would have been in existence for 26 years. Roubik and Michener's (in press) site of *Crawfordapis* had been present for at least 25 years, and Claude-Joseph (1926) claimed that aggregations of *Cadeguala occidentalis* and *Caupolicana funebris* persisted for a number of years. It would not be surprising if long-term nesting aggregations were characteristic of the entire subfamily.

Evidence reported here and in other literature indicates that the Diphaglossinae are solitary bees (nonsocial), and that there is but a single female to a nest. However, Otis et al. (1983) noted a strong tendency for marked females of *Crawfordapis luctuosa* to visit a number of nests. On the other hand Roubik and Michener (in press), upon excavating nests of the same species during the rain when most bees would be in their nests, never recovered more than a single female in a burrow. Visitations to more than one nest by a single female may in some way be related to double ovipositions found in several cells of *Ptiloglossa arizonensis.* These matters warrant further investigation.

NEST STRUCTURE: A few generalities regarding overall nest structure of the Diphaglossinae can be presented. A copious tumulus, presumably always concentric, is reported for both *Ptiloglossa* and *Crawfordapis* in situations where the species nest in horizontal surfaces, and such tumuli will probably be found characteristic of the subfamily. Tumuli obviously erode rapidly under certain weather conditions such as the high rainfall at the sites of *Crawfordapis luctuosa* and *Ptiloglossa guinnae.*

Nests consist of a single entrance burrow without multiple entrances and without vestibules. Although it is difficult at times to determine the number of cells to a particular nest because of nests grouped close to one another, all species have more than one cell and probably many cells to a nest. The nests in horizontal surfaces are generally deep to very deep. *Diphaglossa gayi,* with cells ranging from 10 to 25 cm. in depth and *Ptiloglossa arizonensis,* from 11 to 36 cm., appear to be among the most shallowly nesting species, whereas *Crawfordapis luctuosa* has burrows descending as far as 120 cm. Nests entering sloping or vertical surfaces may in some cases be shallower than those in horizontal surfaces; the works of Claude-Joseph (1926) and Janvier (1933, 1955) are difficult to interpret in this regard, but may reflect the situation. Otis et al. (1983) diagrammed the main burrows of *Crawfordapis luctuosa* entering vertical surfaces as being short, and those entering horizontal surfaces as being long.

MAIN BURROWS AND LATERALS: Main galleries are large in diameter, no doubt reflecting the large to very large body size of almost all species in the subfamily. These burrows were open in all reported cases, although a plug of soil slightly below the ground surface has been characteristically noticed for *Ptiloglossa arizonensis, guinnae,* and *fulvopilosa,* when the females are in the burrows. Roubik and Michener note that dead females often stoppered the main burrows of *Crawfordapis luctuosa,* but found no other obstructions. Burrows are unlined by female secretion, and only *Ptiloglossa fulvopilosa* apparently constructs a wall of plaster-like soil in the main burrows. Burrows of most taxa descend in an irregular, meandering path that becomes extremely sinuous where there are roots and rocks in the substrate. Burrows appear to be unbranched in all species of *Ptiloglossa.* Although the same may be true for *Crawfordapis luctuosa* as suggested by Roubik and Michener (MS), the diagrams of Otis et al. (1983) suggest that either the burrow branched or the laterals branched, in which case the distinction between main burrow and laterals would have to be defined by whether the tunnel is filled with soil after oviposition.

In all taxa, laterals radiate from the main

burrow in various directions and each ends in a single cell. They are filled with soil after oviposition. Apparent exceptions are *Diphaglossa gayi,* in which the laterals apparently are not filled according to Janvier (1933) and *Cadeguala occidentalis,* for which Claude-Joseph (1926) diagrammed a nest having a grapelike cluster of cells with laterals connected to cell bottoms in some cases. Additional studies of the nests of these two taxa are required to permit an interpretation of their structures in relation to nests of other diphaglossines.

Laterals, in some but not all cases, rise before connecting to cells. This is characteristic of *Ptiloglossa* in general and perhaps of *Caupolicana pubescens* but apparently not of many of the other taxa. Roberts (1971) suggested that such a rise might serve to exclude rain water from entering open laterals and cells. One might expect that such "sink-traps" would be found in nests of *Crawfordapis luctuosa* because of the heavy rains in the high rain forests, but the diagrams of Otis et al. (1983) were ambiguous and observations by Roubik and Michener (in press) were mixed on this matter.

CELLS: Cell orientation for *Ptiloglossa, Crawfordapis, Cadeguala, Diphaglossa,* and *Policana* is vertical, as has also been recorded for all species of *Caupolicana,* with the single known exception of *C. albiventris.* According to Janvier (1955) its cells are not kept perfectly vertical, but lean, the first to the left and the next to the right, so that no two consecutive cells have their axes parallel.

So far as can be determined, the cells of all taxa are large to very large (corresponding to the large size of the bees), elongate, with a diameter somewhat greater than the diameter of the burrow, and circular in cross section. Those of *Ptiloglossa* are unique among bees in that the top part of the cell bends 90 degrees or more, which may also be true of *Crawfordapis luctuosa,* as discussed above. The upper parts of cells of *Caupolicana, Policana albopilosa,* and *Cadeguala occidentalis* are bent, but at a lesser angle than those of *Ptiloglossa.* Curved to strongly curved cell tops, then, seem to be a characteristic of the subfamily.

The literature regarding this matter is confusing, however, because some authors have regarded the curved upper part of the cell as the downturned end of the lateral connecting to the cell. Roberts (1971) studying *Ptiloglossa guinnae* has regarded the cell as only the chamber just below the curved neck, probably, at least in part, because he was unable to define a spiral earthen cell closure. However, such a closure is present in cells of *Ptiloglossa arizonensis* and *fulvopilosa.* The spiral nature of the closure in these two species is so indistinct that it was only on comparison with a series that a consistent spiral shape was revealed. The identity of the cell top of the *Ptiloglossa* that I studied, as well as that of *Policana,* is further confirmed by the presence of the cell lining extending up to, or nearly to, the earthen closure. Roberts diagrammed such a lining for *Ptiloglossa guinnae.*

Another confusion regarding the upper part of diphaglossine cells has been created because Claude-Joseph (1926) and Janvier (1933, 1955) completely misinterpreted the cocoon operculum. He believed that in various genera of South American diphaglossines the upper part of the cell was a silken septum deposited by the mother bee after ovipositioning and before constructing the earthen closure. He regarded the space between the operculum and the closure as an air chamber, large or small depending upon the species. Roberts and Roubik and Michener have correctly recognized the operculum as the upper part of the cocoon. Not only do my observations confirm theirs, but I was able to observe mature larvae of *Ptiloglossa arizonensis* constructing opercula, and it was clear to me that opercula were not present in cells containing eggs, young larvae, or live *Triepeolus* larvae of any age. Claude-Joseph also confused the cocoon wall spun by the larva with the cell lining provided by the female; he never mentioned that any diphaglossine bees spun a cocoon.

The earthen cell walls of *Ptiloglossa, Crawfordapis, Cadeguala,* and *Policana* are very smooth, and this will probably be characteristic of the subfamily in general. Among all taxa, only *Ptiloglossa fulvopilosa* is believed to construct a cell wall using mortar. The meaning of this is unclear, but where it was noted, the substrate consisted of decomposed, schistlike material with a distinct grain,

so that the plaster nature of the wall contrasted with the grain and was not therefore easily misinterpreted.

In all known species, the female bee lines the cell wall with a clear to semitransparent lining that covers the entire cell surface. This lining has sometimes been described as colorless, but Roubik and Michener (in press) referred to it as whitish in *Crawfordapis luctuosa* and Roberts (1971), as tan in *Ptiloglossa guinnae.* This lining, probably in all cases, adheres closely to the cell wall so that there is no air space between it and the wall. Filmlike, it is multilayered, at least in *Ptilogiossa arizonensis,* and is a laminate of a number of identical films, which on fresh cells can be teased apart only with effort. With some species the total lining is sufficiently strong so that it can be removed intact while still holding the liquid provisions. With most species, however, it seems to be weaker, and I found that it could best be examined intact by placing cell and substrate in a dish of water and washing away the soil. The lining is waterproof, an apparent necessity considering the liquid nature of the larval provisions.

This lining seems to be homologous with that of the cells of other colletid subfamilies, and probably is applied with the specialized glossae characteristic of all colletid bees. However, there are no air spaces between the lining and the cell wall nor are its layers separated by air spaces and fibrous strands, as seems to be typical of *Colletes, Scrapter,* Hylaeinae, and Xeromelissinae. The lining of diphaglossine cells therefore lacks the glistening, reflective appearance of these other colletids and much more closely resembles the "varnished" cell surface of other families with a conspicuous, nonwaxlike lining. A few examples of such linings in the American Museum collection include *Protoxaea, Andrena,* and *Nomia.* One wonders then what are the chemical differences and similarities of the cell linings of the Diphaglossinae, of the rest of the Colletidae, and of those other families with nonwaxy linings. And what are the phylogenetic implications?

With the possible exception of the circular wad of cotton-like material attached to the cell lining immediately in front of the earthen closure of *Ptiloglossa arizonensis,* there is no suggestion that female diphaglossines provide a secreted closure to their cells. Certainly, the cell lining is not folded shut at the mouth of the cells as it is in *Colletes.*

Cell closures appear to be more or less poorly consolidated earthen spirals, at least in *Ptiloglossa arizonensis, fulvopilosa, Caupolicana gaullei, albiventris, Cadeguala occidentalis,* and *Policana albopilosa. Ptiloglossa guinnae* and *Crawfordapis luctuosa* are both reported not to have a special spiral earthen closure, although laterals leading to cells are filled with soil. Janvier (1933) stated that *Diphaglossa gayi* has neither an earthen closure nor a filled lateral. According to Claude-Joseph (1926) and Janvier (1933), the closures of *Caupolicana gayi* and *pubescens* consist of a series of flat plates, a description that is difficult to relate to other diphaglossines.

Roberts (1971) broached the subject of gas exchange in the cells of *Ptiloglossa guinnae,* especially with respect to carbon dioxide produced by yeasts in the provisions. He pointed out that the lack of a cell closure may have evolved as a means of facilitating gas exchange. Even in those *Ptiloglossa* where I detected a cell closure, the middle part of the spiral is poorly formed, unconsolidated, and sufficiently fenestrated that its gas exchange capability probably would not be diminished. Gas exchange is also discussed below in relation to the cocoon structure.

Order of cell construction has been discussed by several authors. Janvier (1933) stated that the most recently constructed cells are the lowest in the nests of *Diphaglossa gayi* and *Caupolicana pubescens.* Diagrams of the nests of *Policana albopilosa, Caupolicana gayi,* and *funebris* presented by Claude-Joseph (1926) indicate the same, as do illustrations of the nests of *Caupolicana gaullei* and *albiventris* provided by Janvier (1955). Otis et al. (1983) indicated that the oldest cells of *Crawfordapis luctuosa* are closest to the surface and that still open cells are farthest away. On the other hand, Roberts (1971) stated that "lateral branches are not constructed in any obvious vertical sequence" in *Ptiloglossa guinnae,* and my observations on *Ptiloglossa arizonensis* and *fulvopilosa* suggest that the order is indeterminate, with cells of various ages at different levels in no apparent sequence. Hence, *Ptiloglossa* may differ from

some of the other genera in this regard. Diagrams presented by Claude-Joseph (1926) show a confusing picture of cells in tandem in *Cadeguala occidentalis,* old larvae being both higher and lower than young ones in a single series.

PROVISIONS: A consistent feature of the nesting biology of the Diphaglossinae is the liquid nature of the provisions, which occupy approximately the lower half of the vertical cell. This has been noted with respect to all species of *Ptiloglossa, Crawfordapis luctuosa,* many species of *Caupolicana, Diphaglossa gayi, Cadeguala occidentalis,* and *Policana albopilosa.* At least in *Ptiloglossa,* but possibly also in other taxa, the provisions are stratified initially into a nearly clear liquid (nectar) on top and an opaque mass of wet pollen at the bottom of the cell. There is some indication in *Ptiloglossa arizonensis* that the nectar may be disgorged by the female first, and then the pollen, dropped into the cell later, settles to the bottom. Floating fine droplets of oily material were observed in *Ptiloglossa arizonensis* but have not been reported for other taxa. The provisions change over a period of time, presumably through fermentation in the case of *Ptiloglossa guinnae.* It is not clear whether the "soupy," "ropey," "viscous," or "slimy" nature of the provisions of some *Ptiloglossa* and of *Crawfordapis luctuosa* are the result of aging of the stored food (for example, through fermentation) or whether this consistency is characteristic of newly stored provisions as well. Claude-Joseph (1926) and Janvier (1933, 1955) stated that with *Caupolicana gaullei, pubescens,* and apparently *albiventris,* the pollen forms a pyramidal mass whose base occupies the bottom of the cell while the top emerges like an islet from the liquid. The projecting pollen then becomes the oviposition site. In *Cadeguala occidentalis, Caupolicana funebris,* and apparently *Policana albopilosa* he reported that some pollen sinks to the bottom of the cell and the rest floats on the surface serving as the oviposition site.

OVIPOSITION: Eggs of all species of diphaglossines, so far as we know, are elongate and, at least in the case of *Ptiloglossa arizonensis, fulvopilosa,* and *jonesi* are slightly curved, with the anterior and posterior ends nearly identical in shape. With the reported exception of *Caupolicana funebris,* eggs are found on the surface of the provisions near the center of the cell. Certainly in *Ptiloglossa* and in *Caupolicana gayi* they float on the provisions, but according to Claude-Joseph (1926) and Janvier (1933, 1955) with some other species of *Caupolicana,* as well as with *Cadeguala occidentalis* and *Policana albopilosa,* they rest on pollen that floats on or emerges from the surface of the provisions. Janvier (1933) stated that the egg of *Caupolicana funebris* is suspended from the "membranous wall by a short filament," but the meaning of this is unclear.

DEVELOPMENT: Aside from what little is known of the development of *Ptiloglossa arizonensis* presented here, almost nothing has been reported elsewhere on the development or feeding activities of the larvae of diphaglossine bees except that young larvae of some float on the surface of the provisions. This will probably be found to be characteristic of this subfamily in general, because of the very liquid nature of the early provisions.

COCOON AND FECES: Disregarding the confusion in the works of Claude-Joseph (1926) and Janvier (1933, 1955) with respect to the cell lining and cocoon, all diphaglossines spin cocoons. To the extent that the drawings and diagrams of Claude-Joseph (Janvier) can be interpreted in light of the known cocoons of other diphaglossine taxa, shape and structure of cocoons of the subfamily are unique for any group of bees. No other colletid is known to spin a cocoon, a fact reflected in larval anatomy, in that only the Diphaglossinae have salivary openings produced into projecting lips (McGinley, 1981).

The cocoon has a number of distinctive parts. The cocoon top, here called the operculum, is a nearly flat surface that spans the upper part of the cell. The nature of the fabric of the operculum varies from species to species, as indicated in the first part of this paper. It is of some interest that the opercula of *Ptiloglossa arizonensis, Crawfordapis luctuosa,* and *Caupolicana gayi* (Claude-Joseph, 1926) are very similar, consisting of fused threads of silk forming a rather rigid, plastic-like fabric, whereas the opercula of other *Ptiloglossa* are somewhat softer and more fabric-like. The opercula of all cocoons examined by me, as well as of *Caupolicana gayi,*

are clearly fenestrated with large or small holes. The opercula of *Ptiloglossa, Crawfordapis,* and *Policana* are tilted somewhat toward the cell closure, so that they are not at right angles to the long axis of the main part of the cell. Claude-Joseph (Janvier) implies that the opercula are horizontal in some species that he examined.

The cocoon immediately below the operculum, here termed the filter, is of variable thickness and composition, depending upon the species and perhaps the genus (see above), but often consists of vague sheets of silk extending from the walls of the cocoon toward the middle part of the operculum. Beneath this is a denser layer of soft woven fabric with obvious fenestrations. With some species, including *Ptiloglossa arizonensis,* there is yet another woolly layer beneath that. The bottom of the filter is dome-shaped or at least curved, and forms the ceiling of the chamber containing the developing bee. Claude-Joseph (1926) and Janvier (1933, 1955) described the filter for a number of species of *Caupolicana* as well as for *Diphaglossa gayi.* Although his descriptions are difficult to interpret, they imply that interesting species-specific features may be found among these taxa.

Both Roberts (1971) and Claude-Joseph (1926) broached the matter of gas exchange in the cells of diphaglossine bees. Roberts pointed out that the openings in the operculum may be important for gas exchange in the living chamber of the cocoon. I am inclined to agree with this hypothesis in view of the consistent presence of fenestrations in the opercula and filters of all the species that I have examined. It seems reasonable that the cell wall (sometimes, as in *Ptiloglossa arizonensis,* in claylike soil), the strong cell lining applied by the mother, and the cocoon itself may provide a substantial barrier to gas exchange. Hence, the fenestrated cocoon top, in conjunction with the loose fill of the lateral and the imperfect or absent earthen cell closure may be the only passage for gas. Clearly the physics of this situation needs to be addressed.

Whereas the holes in the operculum and underlying filter can be discussed in terms of their adaptive significance for gas exchange, the presence of the cocoon itself, including the presence of the operculum, the filter beneath it, and the cocoon walls, remains enigmatic. A general presumption has been that cocoon-spinning is a primitive (plesiomorphic) feature because of the ability of numerous wasps, a sister group to bees, also to spin cocoons. Why has group after group of bees lost the ability to spin such overwintering chambers? One can only suppose that the operculum might function as a barrier to the oviposition of mutillids and other parasites, that the filter beneath might also exclude such parasites, and that the thin but leathery cocoon wall has a similar function with respect to predators and parasites in the soil. However, evidence bearing upon this matter is not available, and indeed, the multiple origins of a cocoon-less state in the phylogeny of bees seems to suggest that cocoons are simply not that important.

The cocoon wall of diphaglossines is a thin, pale to dark tan, single-layered structure that adheres so closely to the cell lining that the two are nearly indistinguishable upon excavation. Whereas the nature of the cocoon wall is consistent throughout the known diphaglossines, the lower termination of the wall is another matter that seems to have some phylogenetic and ecological significance, all involved with fecal deposition.

In all diphaglossines so far studied, feces are deposited at the bottom of the cell: all *Ptiloglossa, Crawfordapis luctuosa, Caupolicana funebris* and *pubescens,* and *Policana albopilosa.* In all species whose cocoons I have examined (situation not known for *Ptiloglossa guinnae*), with the single exception of *Crawfordapis luctuosa,* the larva perforates the cell lining in the bottom 3 to 5 mm. of the cell before defecating with the result that the lining near the bottom is no longer waterproof. Some species then construct the cocoon wall only down to that level: *Ptiloglossa arizonensis, Ptiloglossa jonesi,* and *Policana albopilosa.* This seems to be the case for either *Caupolicana funebris* or *pubescens,* or both, from photographs presented by Janvier (1933). Other species spin a parchment-like cocoon down to that level, but below it modify the texture of the cocoon fabric, making it permeable to liquid: *Ptiloglossa fulvopilosa* and *Ptiloglossa guinnae.* Only in *Crawfordapis luctuosa* is the cocoon wall homogeneous

from below the operculum to the very bottom of the cell. In *C. luctuosa* the lower part of the cocoon that houses the fecal material is much larger than that in the other species, and the fecal material is more copious and semiliquid to liquid, in contrast to the dry nature of the fecal material in the other diphaglossines that have been studied in this regard. In these other species, destruction of the lower cell lining almost certainly allows for the drainage of the copious liquid associated with the fecal material and therefore the fecal chamber is small. Hence, in the Diphaglossinae the absence or at least incomplete cocoon bottom seems to be the most widespread character state, and yet the complete cocoon bottom found in *Crawfordapis luctuosa* seems to compare with the complete cocoons found elsewhere among those bees that spin cocoons. Which character state is specialized cannot be determined at this time.

DAILY ACTIVITY: The Diphaglossinae are mostly crepuscular or nocturnal bees, and represent the only subfamily of bees with that predominant tendency. Some authors, particularly Linsley (1962; see his bibliography for other such citations), Linsley and Cazier (1970), Michener (1966), and Roberts (1971) have delineated times of flight, both in the morning and in the evening, for various taxa. Vesey-FitzGerald (1939), Schrottky (1907), Claude-Joseph (1926) and Janvier (1933, 1955) have presented other flight data that, although not as precise as those of the above authors, suggest nocturnal, early morning, and late evening activity for South American forms. Probably the large body size characteristic of the subfamily is functionally correlated with the ability of individuals to fly in cool morning hours.

Caupolicana notabilis (Smith) from the Dominican Republic was collected near midday (Michener, 1966) and therefore may be a diurnal species. *Crawfordapis luctuosa* (Otis et al., 1983, and Roubik and Michener, in press) is clearly active during the main part of the day. The cool, moist condition of its high montane habitat probably approaches the environmental factors associated with other diphaglossine bees that fly very early in the morning or late in the evening.

Although various authors have treated foraging periods of these bees, almost nothing has been recorded concerning times of mating except for Roberts' observations (1971) that *Ptiloglossa guinnae* mates only in the morning.

SEASONAL ACTIVITY: There is little information that presents a meaningful pattern regarding the seasonal activity of diphaglossine bees. So far as is known, *Ptiloglossa arizonensis* has but a single generation a year, and passes the winter as a postdefecating larva that remains slightly active. Other diphaglossine bees in the northern and southern temperate regions may also be univoltine. In the tropics, forms, such as *Ptiloglossa guinnae* and *Crawfordapis luctuosa* seem to fly throughout the year, although the activity of nesting sites may wax and wane seasonally. Although most bees are generally assumed to have at least one generation a year, Janvier (1933) reported that *Caupolicana funebris* takes two years to develop, passing the first winter as a larva and the second as an adult that emerges the following summer. He also stated that *Caupolicana pubescens* usually has a two-year life cycle, with only those individuals that are in the shallower cells emerging in a single year. These in the deeper cells pass the second winter in the adult stage and emerge in the spring. Claude-Joseph (1926) indicated that *Cadeguala occidentalis* overwinters in the adult stage and *Policana albopilosa,* in the larval stage.

CLEPTOPARASITIC BEES: The only parasitic bees associated with the Diphaglossinae are in the nomadine tribe Epeolini. The following are the associations:

Diphaglossine	Parasitic Bee
Ptiloglossa arizonensis	*Triepeolus* species b (present paper)
Ptiloglossa fulvopilosa	*Odyneropsis apicalis* Ducke (present paper)
Ptiloglossa jonesi	*Triepoelus* species b (Rozen, 1966; present paper)
Ptiloglossa guinnae	*Odyneropsis gertschi* Michener (Roberts, 1971)
Caupolicana gayi	*Doeringiella gigas* (Spinola) (Claude-Joseph, 1926, as *Epeolus gigas*)

PROFILE OF THE BIOLOGY OF THE DIPHAGLOSSINAE

The following summary, similar in format to that of fideliine bees (Rozen, 1977), is an attempt to provide a formal synoptic overview of the biological features characterizing this subfamily. It is based primarily on information discussed above, but in addition incorporates data regarding flower preference, mating, and diurnal activity.

NESTING: New World, ground-nesting species inhabiting warm, temperate, semiarid to moist tropical situations. Body size large to very large, corresponding to large to very large size of burrows and cells. Individuals nesting in loose, large, irregular aggregations; nesting sites reoccupied over extended periods of years, at least in some cases. Nesting surface horizontal to vertical, in a variety of substrates. Females excavating own nests rather than using burrows of other insects, etc., but perhaps in some cases reusing main burrows from previous generations; apparently only one female to a nest, but some indication of females visiting more than one nest; nests, at least in horizontal surfaces, deep to very deep. Tumuli dry, loose, concentric on horizontal surfaces; turrets absent. Main burrow circular in cross section, unlined by obvious secreted substances, and usually without special built-in walls (exception, *Ptiloglossa fulvopilosa*); vestibules and multiple entrances absent; burrow path moderately meandering to extremely sinuous; burrows open but in some species with plug of soil near surface; laterals, at least with most species, radiating from main burrow in various directions; their walls like those of main burrows; each terminating in single cell, except perhaps for *Cadeguala occidentalis*; laterals not ending blindly; laterals filled after oviposition except for *Cadeguala occidentalis*. Cells vertical or nearly so, elongate, narrowly rounded below. Upper part of cells more (e.g., *Ptiloglossa, Crawfordapis luctuosa*) or less (*Policana albopilosa*) curved above; cell walls very smooth, usually without masoned surface (except *Ptiloglossa fulvopilosa*); cell lining conspicuous, cellophane-like; this waterproof substance secreted by female bee; lining adhering closely to cell wall and extending evenly from bottom of cell to closure; foreign substances not brought into nest or used in cell construction. Cell closure usually a spiral, often poorly formed in the middle, sometimes apparently a series of overlapping plates, other times apparently without special shape or consistency (*Crawfordapis luctuosa, Ptiloglossa guinnae*).

PROVISIONING: Provisioning habits as yet poorly understood, but many taxa broadly (e.g., *Crawfordapis luctuosa*) or narrowly polylectic; stored provisions liquid, often extremely fluid, in some cases stratified into upper clear layer and lower opaque, pollen-laden layer; provisions apparently changing during ontogeny of larva, in some cases through fermentation, into soupy mixture, but nature and sequence of modification poorly understood. Pollen transported in dry state on female scopa.

DEVELOPMENT: Moderately elongate, somewhat curved egg, one or occasionally two to a cell, floating on surface of provisions or, according to Claude-Joseph (1926) and Janvier (1933, 1955), resting on small islet of pollen emerging from or floating on provisions. Young larvae feeding while on side, with most of body surface submerged; older larvae curled in cells while finishing food. After feeding but before defecation, larvae spinning elaborate cocoon consisting of upper flat plate (operculum) somewhat below the cell closure subtended by more or less thick woolly filter-like area; cocoon wall extending from operculum nearly to or to bottom of cell, depending on taxon. Larvae of most taxa piercing cell lining at very bottom of cell, thereby allowing drainage of fecal liquid into soil (exception, *Crawfordapis luctuosa*); this destruction apparently taking place during cocoon construction. Cocoon extending down approximately to top of pierced lining or, if extending to bottom of cell, nature of cocoon fabric at bottom of cell differing from cocoon wall above pierced lining, presumably allowing for drainage of fecal liquid. Feces deposited in bottom of cell followed by spinning of floor to chamber containing larva so as to separate living chamber from fecal chamber. Postdefecating larvae remaining slightly active during hibernating period, at least in one species (*Ptiloglossa ar-*

izonensis). Cocoon fabric, composed solely of silk (no foreign material), adhering intimately to cell lining along sides of cell.

ADULT ACTIVITY: Bees, predominantly crepuscular (especially matinal) or nocturnal; only *Crawfordapis luctuosa* certainly diurnal. Mating observed over nesting site in some species, in other species on flowers; territorial behavior of males suggested in some species but not in others.

CLEPTOPARASITIC BEES: Only epeoline genera known nest parasites of some Diphaglossinae; some species of *Triepeolus, Doeringiella,* and *Odyneropsis* definitely associate with certain diphaglossine species.

PHYLOGENETIC CONSIDERATIONS

Dr. Radclyffe B. Roberts (*in litt.*) raised several interesting questions after reviewing this manuscript: (1) what evidence presented here suggests that the Diphaglossinae and other colletids are monophyletic and (2) what biological information points to a relationship between the Diphaglossinae and major groups of short-tongued bees other than the Colletidae.

Relationship of the Diphaglossinae to Other Colletids: The inclusion of the diphaglossines in the family must continue to be based on adult and larval anatomy. If true colletids, then the diphaglossines would appear to be a sister group of the rest of the family because of their cocoon-spinning habits, unique for the Colletidae but a primitive feature in higher Hymenoptera. The distinctive and conspicuous cell lining of all colletids, including that of the Diphaglossinae, is the main biological synapomorphy uniting the group. This lining is associated with the characteristic glossae of the adults. However, there are obvious differences with respect to the number of layers in the lining, the structure of these layers, and the cell closure involving the lining, from one subfamily to another. We do not know yet the nature and therefore the similarities and differences of the chemical composition of the linings of most of the colletid taxa.

Relationship of the Diphaglossinae to Other Bee Taxa: The higher classification of the Apoidea has long been vexing. Most recently, two families have been recognized that might be considered relatives of the Diphaglossinae—the Oxaeidae (Rozen, 1965b) from the New World, and the Stenotritidae (McGinley, 1980) from Australia. Both families are composed of relatively few large-sized bees that resemble, at least superficially, the Diphaglossinae. Biological information does not seem to align either family with the diphaglossines. Roberts (1973) provided the first major study of the nesting biology of a member of the Oxaeidae, *Oxaea flavescens* (Klug). From his studies and also from my own unpublished data on *Protoxaea gloriosa* Fox, the only possible synapomorphies between this family and the diphaglossines are the vertical cell and the fact that both groups are parasitized by epeolines. There are substantial differences between the two with respect to food consistency, cell shape, probably cell lining, and larval behavior not to mention anatomical differences in both adults and larvae. Houston (1975) presented an interesting account of the biology of *Stenotritus pubescens* (Smith), as he did more recently for the related *Ctenocolletes* (Houston, MS), and Houston and Thorp (MS), for *Stenotritus greavesi.* Numerous aspects of the nest architecture, provisions, and larval behavior reveal no synapomorphies with the Diphaglossinae. However, *Stenotritus pubescens* flies one-half hour before sun-up for about three hours; hence, it is a matinal bee not unlike most diphaglossines. At least most species of *Ctenocolletes* are truly diurnal, as is *Stenotritus greavesi.* In sum, the relationships of the Stenotritidae and Oxaeidae to other bees including the Diphaglossinae remain obscure.

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