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## Early Nesting Biology of the Bee *Caupolicana yarrowi* (Cresson) (Colletidae: Diphaglossinae) and Its Cleptoparasite *Triepeolus grandis* (Friese) (Apidae: Nomadinae)

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### ABSTRACT

The first part of this publication, written by a group of participants in Bee Course 2018, results from the discovery of three nests of *Caupolicana yarrowi* (Cresson, 1875) at the base of the Chiricahua Mountains in southeastern Arizona. The nests are deep with branching laterals that usually connect to large vertical brood cells by an upward turn before curving downward and attaching to the top of the chambers. This loop of the lateral thus seems to serve as a “sink trap,” excluding rainwater from reaching open cells during provisioning. Although mature larvae had not yet developed, an egg of *C. yarrowi* was discovered floating on the provisions allowing an SEM examination of its chorion, the first such study for any egg of the Diphaglos-

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sinae. Larval food for this species at this site came from *Solanum elaeagnifolium* Cav. (Solana-ceae). Nests were parasitized by *Triepeolus grandis* (Friese, 1917) (Epeolini), which previously was known to attack only *Ptiloglossa* (Diphaglossinae: Caupolicanini).

The subterranean nest cells of the desert bee *Caupolicana yarrowi* (Colletidae), which are enveloped by a casing of hardened soil that easily separates from the surrounding matrix, are discussed in a separate appendix. Chemical analysis revealed the casing to be rich in reducing sugars, indicating that the mother bee had regurgitated floral nectar onto the rough interior walls of the cell cavity before smoothing and waterproofing them. This novel use of nectar in nest construction is compared with that of other bee species that bring water to a nest site to soften soil for excavation.

## INTRODUCTION

Here we present data and observations on the nesting biology including phenology, immature stages, and nest associates of *Caupolicana yarrowi* (Cresson, 1875) (Colletidae: Diphaglossinae: Caupolicanini). Three active *C. yarrowi* nests were discovered in the ground by students and instructors participating in the 20th annual presentation of the Bee Course, a 10 day intensive course on bee identification, biology, and conservation, based at the Southwestern Research Station near Portal, Cochise County, Arizona. Our findings come from the discovery and subsequent excavation of these nests (fig. 1) by a subset of class participants on August 28 and 29, 2018, near Paradise, Cochise County, Arizona (31.9325°N, -109.2083°W).

Members of the Caupolicanini are large, solitary, ground-nesting bees composed of three genera found only in tropical and subtropical America. Herein, the higher classification of the Colletidae follows that of Michener (2007), although the reader should be aware that a very different classification has been proposed by Moure et al. (2007).

Although the three nests were actively being used by female *C. yarrowi* and therefore incomplete, the bees were far enough along in the nesting season to reveal much about their nest architecture, cell construction and orientation, and provisioning. One nest yielded an egg of *C. yarrowi*, which is described below with SEM images of its chorionic microsculpture, the first such pictures for any Diphaglossinae. They also demonstrated for the first time that nests of *C. yarrowi* are attacked by the cleptoparasite *Triepeolus grandis* (Friese, 1917) (Apidae: Nomadinae: Epeolini) through the recovery of its first instar from one of the cells. Thus, this cleptoparasite is now known to attack two genera within the Caupolicanini since M.A. Cazier and M. Mortenson had discovered it (as *Triepeolus* species b) as a cleptoparasite of *Ptiloglossa jonesi* Timberlake, 1946 (Rozen, 1984).

Diphaglossinae is the basal subfamily of Colletidae and is mostly, though not exclusively, New World in distribution. The species can be found along a gradient of climates from hot, arid deserts to montane forests of the wet tropics (Rozen, 1984). One tribe found within the subfamily is the Caupolicanini, well known for the prevalence of dim-light foraging as reported by numerous authors (e.g., Linsley and Cazier, 1970; Roberts, 1971; Rozen, 1984; Sarzetti et al., 2013). Our present knowledge of Diphaglossinae biology centers on their nest architecture and immature stages throughout their range from New Mexico south to Argen-



FIGURE 1. Satellite image of nesting area with individual nest locations identified, demonstrating wide distributions of nests. Inset: location of nest sites in southeast Arizona.

tina (Roberts, 1971; Otis et al., 1982; Rozen, 1984; Rozen and Rozen, 1986). Some species nest singly, while others form loose aggregations. *Crawfordapis luctuosa* (Smith, 1861) has been observed to form large, perennial aggregations that can remain active for over two decades (Roubik and Michener, 1984). Nesting similarities among species can be found in a sinuous tunnel architecture as well as in cell construction and immature stages: all known members of Diphaglossinae build vertical cells with curved necks; store liquid or semiliquid provisions, some of which have been observed to ferment (Roberts, 1971), inside a cell lined with a cellophanelike material; and, unlike those of other colletid subfamilies, mature larvae spin cocoons (Rozen, 1984; Otis et al., 1982; Sarzetti et al., 2014). Knowledge about nest associates is scant, although Linsley and Cazier (1970) and Rozen (1984) reported on the epeoline cleptoparasites of *Ptiloglossa* and *Caupolicana*.

#### NESTING BIOLOGY

The *C. yarrowi* nesting area was located at an elevation of 1670 m in an oak-juniper savanna that, in part, consisted of an active cemetery and a steep, gravelly wash along an east-west axis (fig. 1). The site has a mean annual temperature of 13.7° C and receives 379 mm of rain each year (Western Regional Climate Center, <http://wrcc.dri.edu/cgi-bin/rawMAIN.pl?caaswr>). Nonwoody vegetation at the site is dominated by grasses (height 20–50 cm) with scattered patches of *Solanum elaeagnifolium* Cav., some small *Opuntia* sp. and other cacti, *Agave* sp., along with roadside herbaceous plants such as *Argemone* sp., and *Helianthus* sp. Of note, an extensive population of *S. elaeagnifolium* extending over an area of 300 m<sup>2</sup> was flowering in the cemetery during our study.

TABLE 1. Composition and texture of soil samples taken from two nests of *Caupolicana yarrowi* from nesting sites near Paradise, Cochise Co., Arizona, in 2018.

	Texture	% sand	% silt	% clay
Nest 2a	sandy clay loam	48.3	27.9	23.8
Nest 2b	loam	45.7	30.0	24.3
Nest 3	sandy clay loam	54.5	24.7	20.8

All three nests discovered were scattered over gently sloping areas with low vegetation. No nests were found on the wash banks, one nest (Nest 1) (fig. 1) was found in the border of the cemetery far removed from any grave sites, and the final two were discovered in the savanna (Nest 2, Nest 3) (fig. 1). In the cemetery the soil was heavy, rich in organic matter, and free of rocks. The nests located in the savanna (figs. 12, 13) were built in soil of sand, silt, and clay with large (5–20 cm) rocks near the surface and becoming evenly moist beneath the rocky layer (>20 cm). Subsequent to the fieldwork, B.N.D. had the Cornell Soil Health Lab analyze two soil samples gathered from Nest 2 and one from Nest 3. Table 1 reports the texture of the samples and the percentage of particle composition.

Morning observations of female *C. yarrowi* were made both at nest entrances and on *S. elaeagnifolium* between 05:30–07:00 A.M. Individuals observed at nests marked for excavation were collected, identified by J.G.R., and deposited either in the individual personal collections of S.K.K. and N.N.D., in the Cornell University Insect Collection (CUIC), or in the bee collection of the American Museum of Natural History (AMNH). No males of the species were observed. Additionally, no visits were made to the site in the late afternoon or evening. We observed *C. yarrowi* exclusively collecting pollen by sonicating flowers of *S. elaeagnifolium*, although Linsley and Cazier (1970) indicated that this species is polylectic. On our first visit to the site, we observed *Ptiloglossa arizonensis* Timberlake, 1946, foraging alongside *C. yarrowi*. However, on August 29, we found only *P. arizonensis* foraging in the cemetery. Linsley and Cazier (1970) report a similar finding and interpreted it as *P. arizonensis* excluding *C. yarrowi* from large resource patches. Since we never observed *C. yarrowi* foraging elsewhere, we cannot say for certain the activity we observed was the result of the competition between the two species. By 7:30 A.M. on August 29, 2018, the second day of the study, the majority of diaphaglossine foraging had ceased. Interestingly, a single *C. yarrowi* female was observed foraging at 9:30 A.M. in the cemetery when no other bees were detected on the resource patch. As both mornings progressed, *C. yarrowi* and *P. arizonensis* were accompanied on *S. elaeagnifolium* by *Bombus sonor* Say, 1837, and *Xylocopa californica arizonensis* Cresson, 1879.

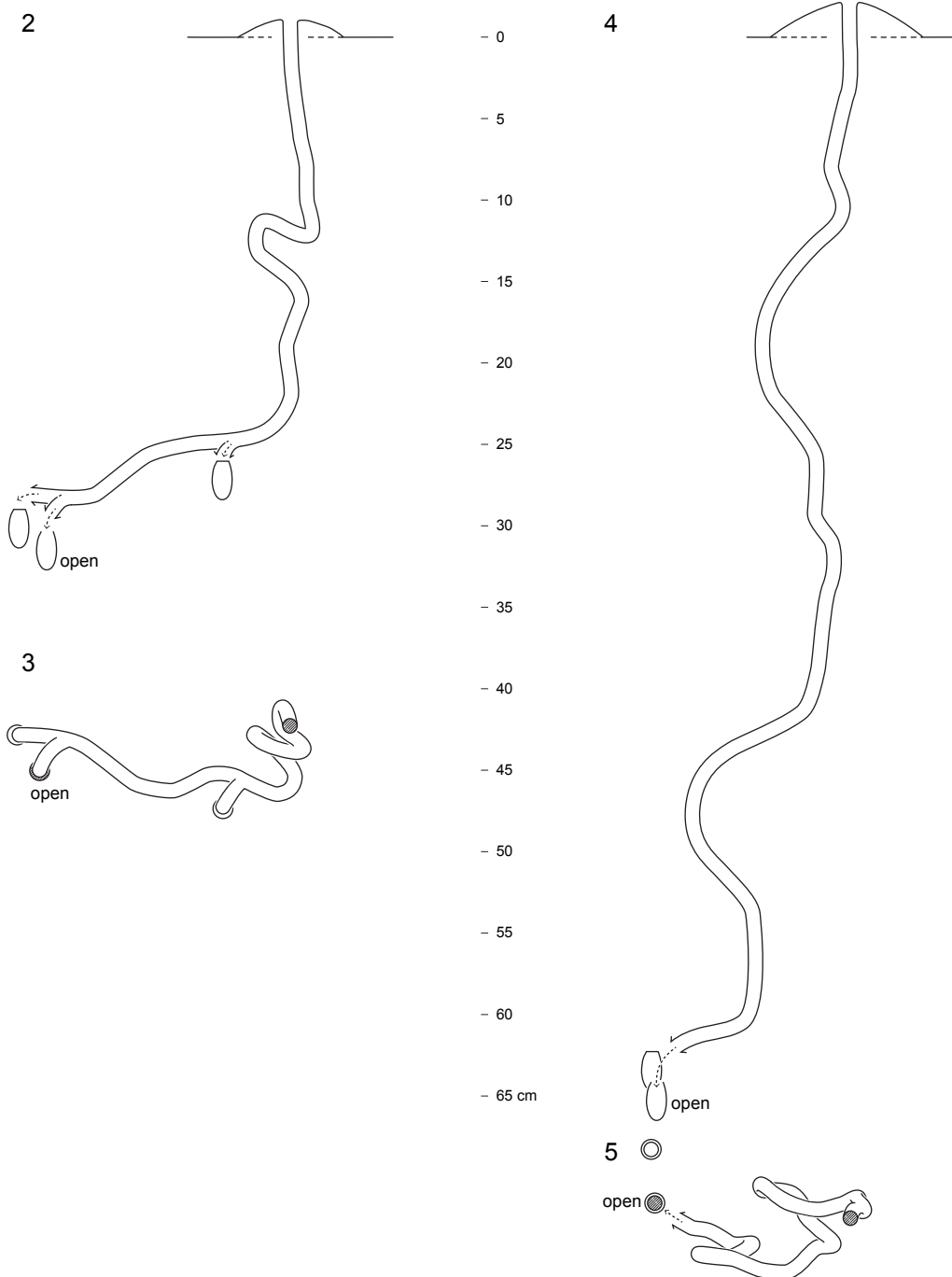
To document our observations of *C. yarrowi* early nesting phenology in Arizona, we compiled all available museum records of *C. yarrowi* from Symbiota Collections of Arthropods Network (SCAN; <http://scan-bugs.org/portal/collections/>) and the National Museum of Natural History. Only unique collection events (day and location) were recorded, resulting in a dataset of 123 records that shed light on the phenology and geographic distribution of *C. yarrowi*. Importantly, these records indicate only that *C. yarrowi* was flying on a certain day, not necessarily nesting. Still, we are able compare our observations to the larger window of activity during which nesting occurs. We analyzed males and females together.

*Caupolicana yarrowi* has been collected as far south as 21° N in Jalisco, Mexico and as far north as 35° N in New Mexico (figs. 6, 7). Thus, *C. yarrowi* nests in the desert and dry tropics, as is the case with Caupolicanini relatives *P. arizonensis*, *P. jonesi*, and *Caupolicana (Zikanapis) tucumana* (Moure, 1945) (Linsley and Cazier, 1970; Sarzetti et al., 2013). This contrasts with the two known species of *Crawfordapis* that nest exclusively in the wet tropics (Otis et al., 1982; Roubik and Michener, 1984). *C. yarrowi* nests throughout the summer, with 75% of collection events occurring between June 15 and September 15. These data (fig. 8) suggest that *C. yarrowi* is univoltine, as is the case with other Diphaglossinae except for *Crawfordapis*. Further, variation in flight phenology cannot be attributed to latitude; *C. yarrowi* adults collected in the southern part of its range were not collected at a significantly different day of the year than those collected in the north (Generalized Linear Model, slope = -0.64 day/degree latitude; Likelihood Ratio Test  $\chi^2 = 0.30$ , df = 1,  $p = 0.58$ ).

To gain an understanding of their architecture, Nests 1 and 2 were excavated using the methods outlined by Rozen (2018) by exposing one side of each nest to reveal the descending tunnel and subsequently locating each of its branches and their paths as excavation continued. To better see the tunneling, white powder (talcum powder or dry plaster of Paris) was blown onto the tunnel walls with a plastic squeeze bottle as they were exposed. A series of diagrams were made during the excavation. Using these preliminary sketches, K.R.U. created a master diagram of each nest (figs. 2, 4) in lateral view. Each is here accompanied by another diagram showing the distributions of the terminal cells in dorsal view (figs. 3, 5). Cells, all vertical and with curved entrance tunnels, and their contents were diagrammed and examined with hand lenses before being set aside for future inspection. Three additional cells were roughly measured in the field and found to be about 2–2.5 cm long. Three cells in moderately good condition and some extra fragments were also set aside to be inspected later. Architectural configuration of a nest can be understood by examining the side view and top view diagrams simultaneously.

Nest 3 was excavated with the goal of harvesting *C. yarrowi* cells by following the tunnel straight down. Thus, the method differed from that of Rozen (2018). At the surface it started with a compacted tumulus forming an open turret. A few drops of water placed on the interior wall of the entrance were readily absorbed. The tunnel descended vertically for 33.5 cm in an irregular path and then angled down 45° to a depth of 56.5 cm where it connected to an open cell entrance. The cell's contents were liquid at the bottom with a ring of dry pollen suspended above. The cell lacked any discernable fermentation odor; therefore, we cannot confirm the report of Roberts (1971). No immature stages were recovered.

We hypothesize that the depths of the cells in Nests 1 and 2, their far separation from one another, and the overall nest architectures, provide the immatures and provisions considerable protection from discovery by subterranean predators and parasites. Chances of cell flooding from heavy rain downpours during the nesting seasons are likely avoided by the long, sinuous unlined tunnels leading to them. As indicated in the Discussion, below, there is also evidence of possible protection provided by waterproof, curved “sink traps” at cell entrances (as identified and well diagrammed by Roberts, 1971: fig. 2, for *Ptiloglossa guinnae*



FIGURES 2-5. Diagrams of Nests 1 and 2 showing branching pattern, lengths and depths of tunnels, and positions of cells to one another. Note that the connections of cells to laterals are indicated only stylistically, because we were not fully aware of sink traps at the time of excavation. 2, 3, Nest 1, side and top views. 4, 5, Nest 2, side and top views.

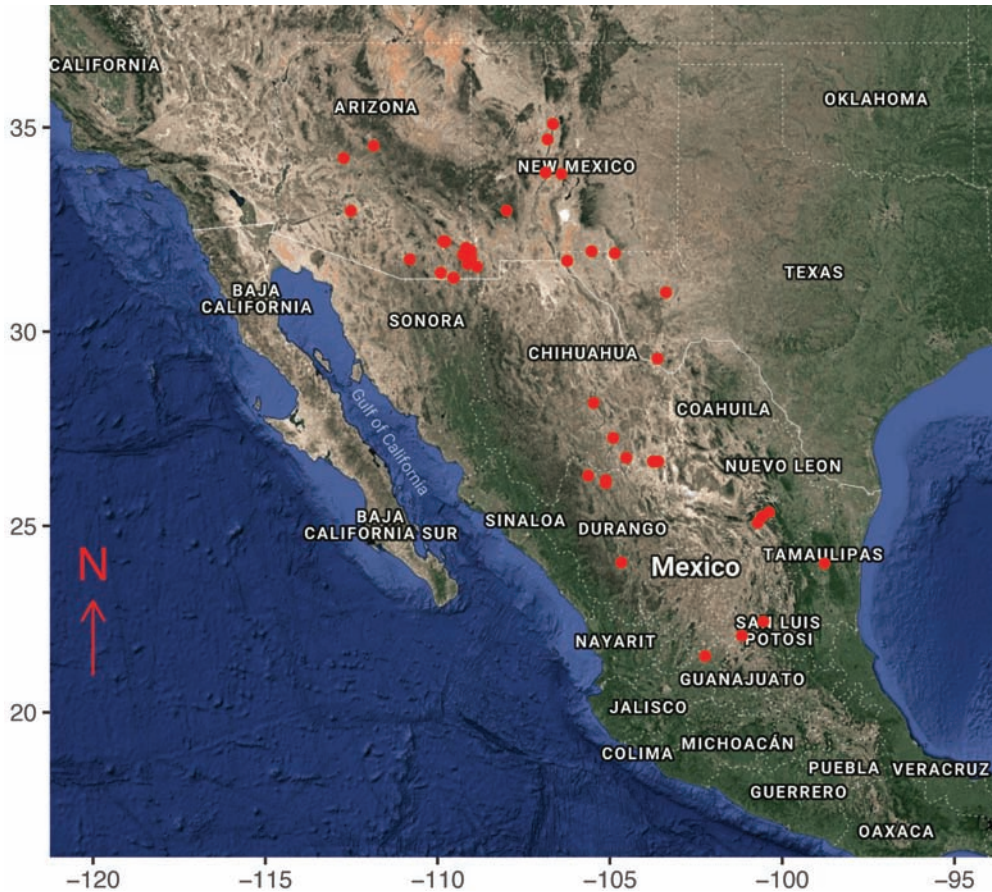


FIGURE 6. Distributional map of collection localities of *Caupolicana yarrowi*.

Roberts, 1971). Interestingly, in his paper, Roberts reported that this species never provides a cell closure after oviposition.

The three cells that were put aside for detailed study were somewhat fractured as a result of our excavation. The three included much of the cell lumen intact while one also included much of the curved connection of the side tunnel, permitting a more in-depth understanding of the cell architecture (fig. 14). All revealed a good deal of the hard cell wall, as well as the cell surface and cellophanelike inner coating of the cell. In all, the cell walls as well as the entrance tunnels were composed of soil that was consistently more consolidated and harder than the surrounding substrate. Almost certainly this was a result of the female's application of a transparent, nonreflective, hardening agent either during excavation or soon after. The soil particles adhered to one another without any evidence of mechanical manipulation such as tamping. Thickness of walls ranged from 2 mm to more than 5 mm. When a hard piece of tunnel or cell wall (but not cell surface, described below) was dropped into water, the piece immediately disassembled into soil particles, indicating that the hardening material is water-soluble. Soil particles consisted of fine brownish flakes and an assortment of very small to minute rocklike pebbles.



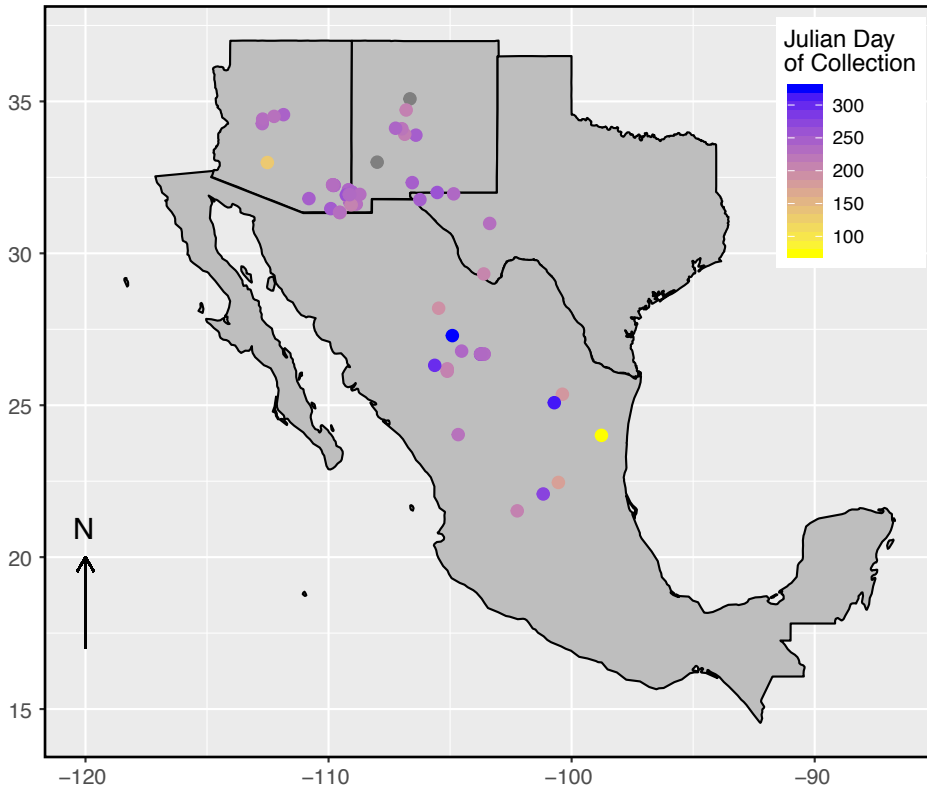


FIGURE 7. Distributional map of *Caupolicana yarrowi* coded by color to reveal relative times of collections of adult specimens.

A paper on Colletidae nesting biology by Eduardo Almeida (2008) pointed out that Torchio et al. (1988) may have been correct that the responsible hardening agent of colletid cell walls actually consists of two secretions. One secretion from the salivary gland and one from the Dufour's gland are applied sequentially and alternatively. The first secretion is hypothesized to account for the extensive hardening of the wall and the second results in the polymerization of the other secretion at the cell's inner surface. Although Torchio et al. (1988) assumed that the Dufour's gland was responsible for the resulting polymerization, a comparison of the relative sizes of the two glands might be necessary for confirmation, in consideration of the large amount of soil that is hardened. When this matter was expressed to J.H. Cane, he responded (e-mail message to J.G.R.: 1/07/2019): "Since it [the secretion] hardens the soil but is water-soluble, I would also consider that it might be regurgitated nectar whose sugars are binding the soil particles. That would allow for the volume of liquid that would be needed to permeate that amount of soil without invoking the existence of some voluminous gland other than the Dufour's, plus something watery would wick into the soil in a way that an oily secretion would not." Intrigued by his response, we sent to him a large piece of cell wall, which he then tested (see appendix. His conclusion: "The presence of substantial amounts of sugar in the hardened soil casing sur-

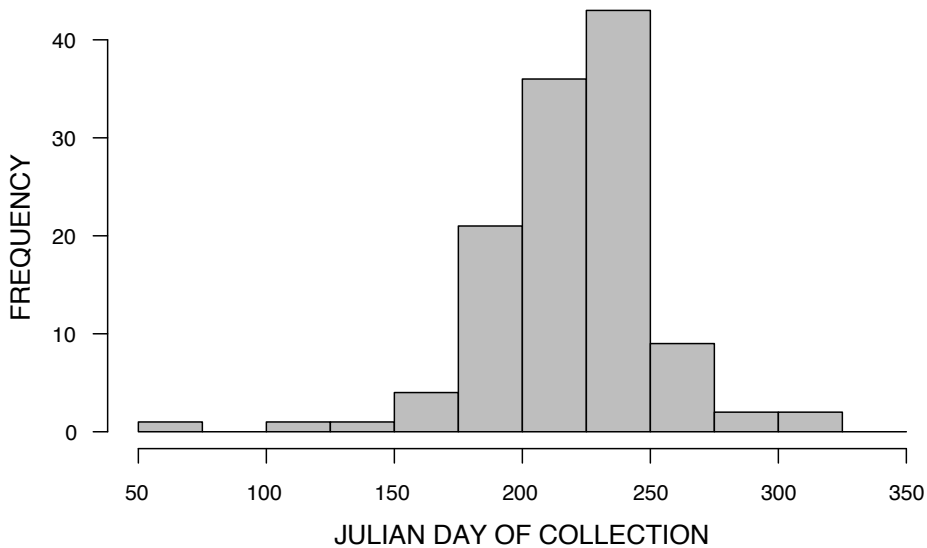


FIGURE 8. Histogram based on dates of collection of adult *Caupolicana yarrowi* throughout its range.

rounding the empty *C. yarrowi* nest cell implies that the applied liquid was regurgitated floral nectar.”

Oval in shape, the three cells appeared to be identical (fig. 14) in that the cell lumen was about 25 mm long measured from the posterior end to the center of the entrance, and 12 mm in maximum diameter, which was at midlength. The entrance tunnel, about 6 mm in diameter, was strongly curved. The inner surface of the cell was covered with a shiny, waterproof, nearly transparent lining, so the soil particles were clearly visible. The lining, often referred to as cellophanelike, adhered to the soil surface but could easily be peeled with forceps. The brown surface thus exposed (figs. 15, 16) was remarkably smooth and so thin that it might be termed a veneer. It did not show any of the irregularities of the broken surfaces of the hard cell wall immediately beneath (fig. 16). When tested with a water droplet, the droplet was slowly absorbed over a period of seven minutes (fig. 15). The complex structure of the cell surface seems unusual and requires further study.

#### IMMATURE STAGES

The lack of mature larvae and pupae and the paucity of cells per nest clearly indicated that the 2018 nesting season for this species had only recently started. Except for a single small immature feeding larva, the only immature *C. yarrowi* found was an egg, described below. Previously, nests of only *Caupolicana gayi* Spinola, 1871 (Claude-Joseph, 1926) and *Caupolicana ocellata* Michener, 1966 (Rozen and Rozen, 1986) had been discovered, and only that of *C. gayi* contained a single mature larva. The specimen was collected and first described by Claude-Joseph (1926) and then redescribed by Michener (1953) after it had been loaned to him from the Smithsonian Institution. The specimen, subsequently borrowed by J.G.R., was badly preserved, so that only the mandible



FIGURES 9–13. Nests 1 and 2. **9.** Students and instructors examining Nest 1 at entrance to cemetery as Danforth (black hat) prepares to excavate. **10.** Close-up of excavation of Nest 2 looking toward Nest 1 identified by arrow on grassy horizon. **11.** Open entrance of Nest 1 surrounded by tumulus. **12.** Excavation of Nest 1 underway with arrow pointing to Paradise Road. **13.** Excavation of Nest 2, lateral view, showing use of white powder to clearly follow tunnel descent and tracking measurements.

could be identified; its distinctive, projecting mandibular cusp had been well documented as figures 42 and 43 by Michener (1953). It clearly distinguishes the mandible of *C. gayi* from those of other known caupolicanine larvae.

In addition to the mature larva of *C. gayi*, the mature larvae of other members of the tribe Caupolicanini have been well described and illustrated by McGinley (1981): *Ptiloglossa fulvopilosa* (Cameron, 1903) (McGinley, 1988: figs. 5–7), *P. guinnae* (McGinley, 1988: figs. 12, 13), *P. arizonensis* (as *P. species B*) (McGinley, 1988: fig. 14) and by Otis et al. (1982: figs. 11–13) for *Crawfordapis luctuosa*.

#### EGG OF *CAUPOLICANA YARROWI*

##### Figures 17–22

Although the following is the first formal description of an egg of any Diphaglossinae that provides SEM images of external chorionic microstructure, there are sufficient references and illustrations to suggest that there is probably little variation in the general shape and form of eggs among those of various included taxa. See Rozen (1984) for a detailed account of the earlier studies on eggs of the subfamily.

**DESCRIPTION:** Egg ( $n = 1$ ) length 4.15 mm; maximum width (midbody) 0.875 mm. Shape (fig. 17) elongate, slender, slightly curved, with anterior end (identified by outline of embryonic head as well as by position of micropyle) slightly narrower than posterior end; both ends rounded except middle of anterior end bearing low micropylar mound. Chorion generally smooth, slightly reflective under low stereoscopic magnification; under high magnification, chorion uniformly reticulate, without areas of smooth chorion. Under SEM magnification (fig. 17, uneven appearance due to SEM preparation), reticulate pattern resulting from elevated ridges of chorion arising from smooth lower chorionic surface; these ridges forming interlocking pattern of mostly hexagons<sup>8</sup> (figs. 18–22); each angle of hexagon rising farther from base than sides of hexagons, each appearing like an elevated dried raisin; sides of hexagons also with weak concavities. The micropyle under SEM magnification (figs. 22, 23) consisted of a small cluster of pores surrounded by converging elongate hexagons.

<sup>8</sup> Eggs of many bee taxa have an exposed fine surface pattern on their egg chorions in the form of a distinct, elevated, geometric pattern of hexagons. Toward the anterior end of the egg these so-called “hexagons” become increasingly elongate the closer they approach the egg tip. In doing so, their six-sided shape gradually elongates and changes so that the forms are no longer six-sided though still referred to as “hexagons” because their six-sided patterning persists over most of the chorion.

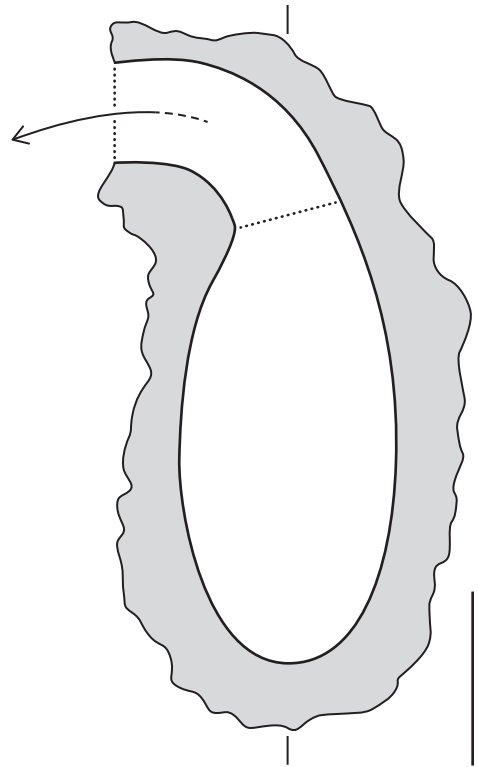


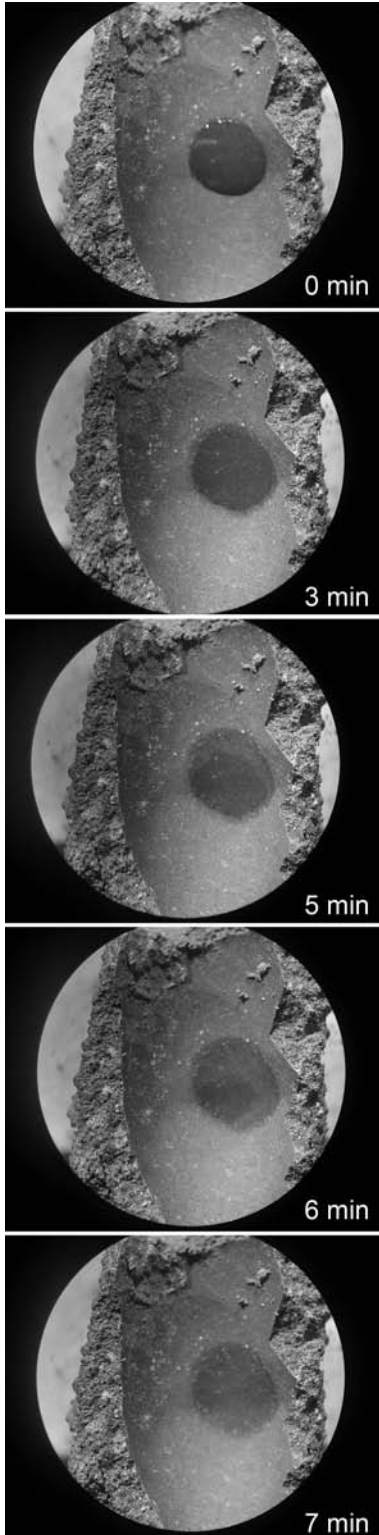
FIGURE 14. Diagram of brood cell and extensive cell wall of *Caupolicana yarrowi*, lateral view.

MATERIAL STUDIED: 1 egg, found floating on surface of provisions in Nest 1.

#### NEST ASSOCIATES

We observed *T. grandis* adults active in the early morning hours when female *C. yarrowi* forages. In one case, we saw an adult *T. grandis* sitting 10 cm from a *C. yarrowi* nest entrance, although we did not observe any *T. grandis* entering or exiting a *C. yarrowi* burrow. Two adult *T. grandis* were collected and are maintained in CUIC and in the personal collection of N.N.D. Upon excavation of Nest 1, a first larval instar of *T. grandis* was observed on the wall of an opened cell and made quick movements when probed with forceps. Upon further inspection of the cell, we found no evidence of the host egg or larva. A first instar of this species had previously been photographed floating on the surface of the provisions in the nest of *P. jonesi* (fig. 23).

Furthermore, Rozen (1989a) recognized that *T. grandis*, originally known only from the type, was actually a common, often collected species from Arizona and New Mexico as well as from northern Mexico. Later the same year, Rozen (1989b) described its first instar along with those of other *Triepeolus* and *Epeolus* first instars that were available. Details presented in that paper revealed that the shape of the first instar larval head in lateral view, the presence and structure of the incurved mandibular edge, and the presence and sizes of lateral body tubercles as well as those of the last abdominal segments demonstrate a wide range of features enabling various species to be distinguished from one another. The first instar specimen (fig. 23) found in Nest 2 of *C. yarrowi* agreed in many respects with these same features of first instars of *T. grandis* described by Rozen (1989a, see fig. 1–9 therein), that, up to then, had been a cleptoparasite associated only with *Ptiloglossa* (Caupolicanini). However, it should be noted that the sides of the head capsule



FIGURES 15. Series of time-lapse photographs of cell of *Caupolicana yarrowi* from which the cellophanelike lining has been removed, exposing the smooth, veneerlike surface on which a drop of water was placed and allowed to be absorbed for a period of 7 min, leaving only a damp spot on the wall. This suggests that even without waterproof cell lining, the veneerlike surface layer may help control cell wall sorption rate.

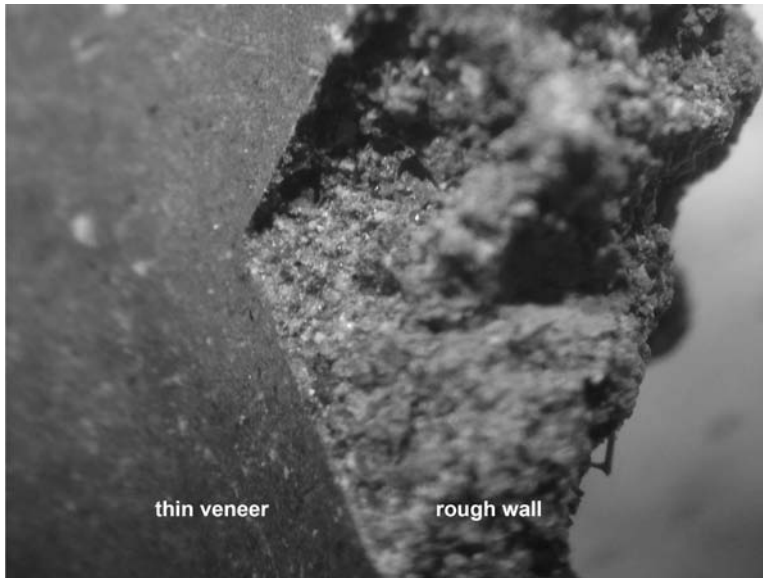


FIGURE 16. Close-up right-angle chip in cell of *Caupolicana yarrowi* in figure 15 demonstrating thinness of the veneerlike subsurface on left, contrasting with rough wall material on right.

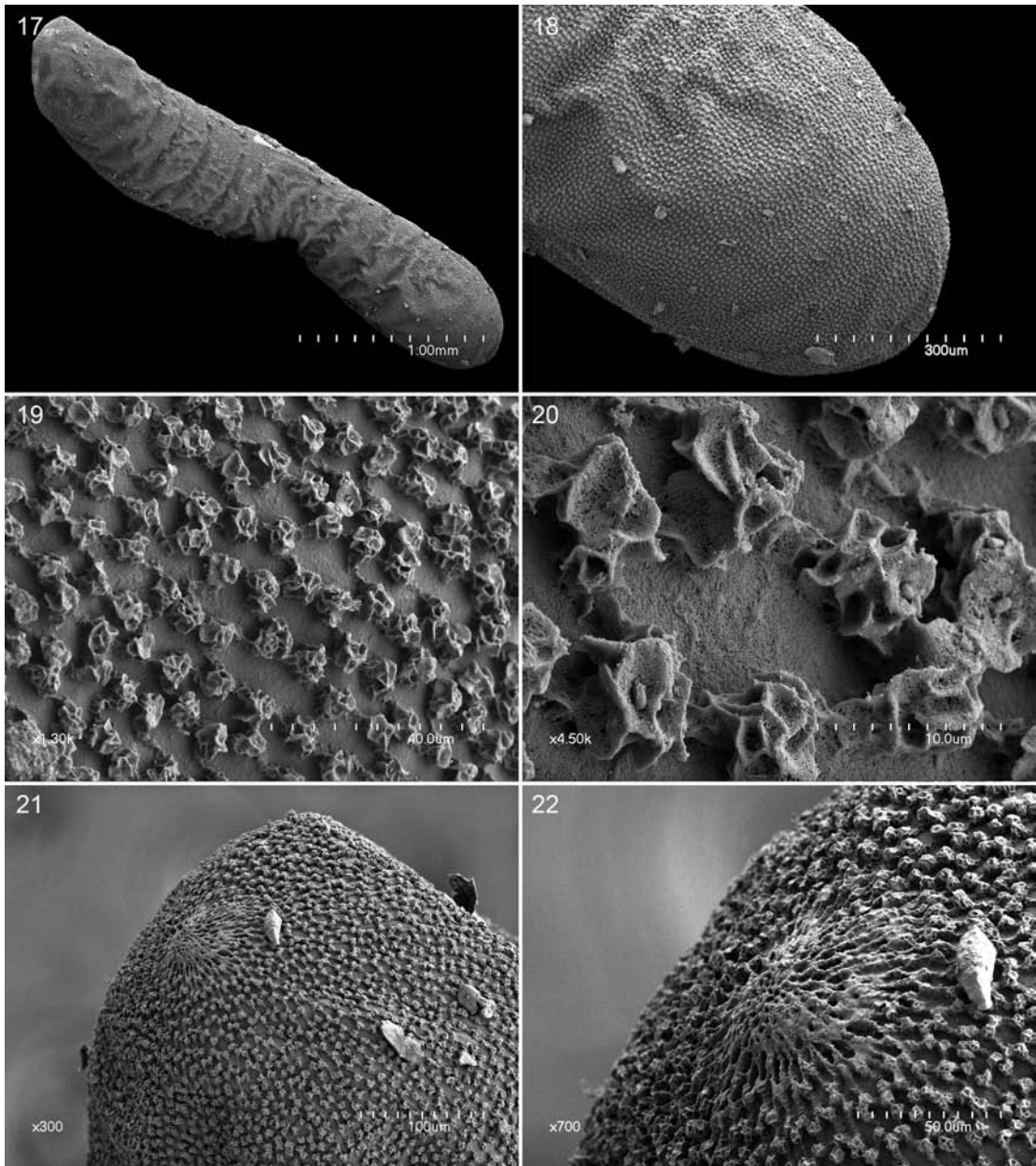
in figures 24 and 25 converge slightly apically compared with those of Rozen (1989b: figs. 1, 2). Furthermore, the head capsule (figs. 24, 25, 27) is slightly shorter than that in Rozen (1989b: figs. 1, 2, 6). Also, in lateral view, the dorsal surface of the head capsule (fig. 27) is more strongly curved than that in Rozen (1989b: fig. 6). On the other hand, the incurving opposing mandibular edges (fig. 26) closely parallel those shown in Rozen (1989b: fig. 2). Because the differences noted could be illustration errors, a comparison with specimens from the early study was made and indicated that the differences are real but likely intraspecific variation. A careful inspection of adult *Triepeolus* from the site revealed that all were *T. grandis*.

Other cleptoparasites that attack Caupolicanini belong to the genera *Doeringiella* (host: *Caupolicana*) (Michener, 2007) and *Odyneropsis* (host: *Ptiloglossa*) (Rozen, 1966a, 1994b). Both of these parasitic genera, like *Triepeolus*, belong to the Epeolini.

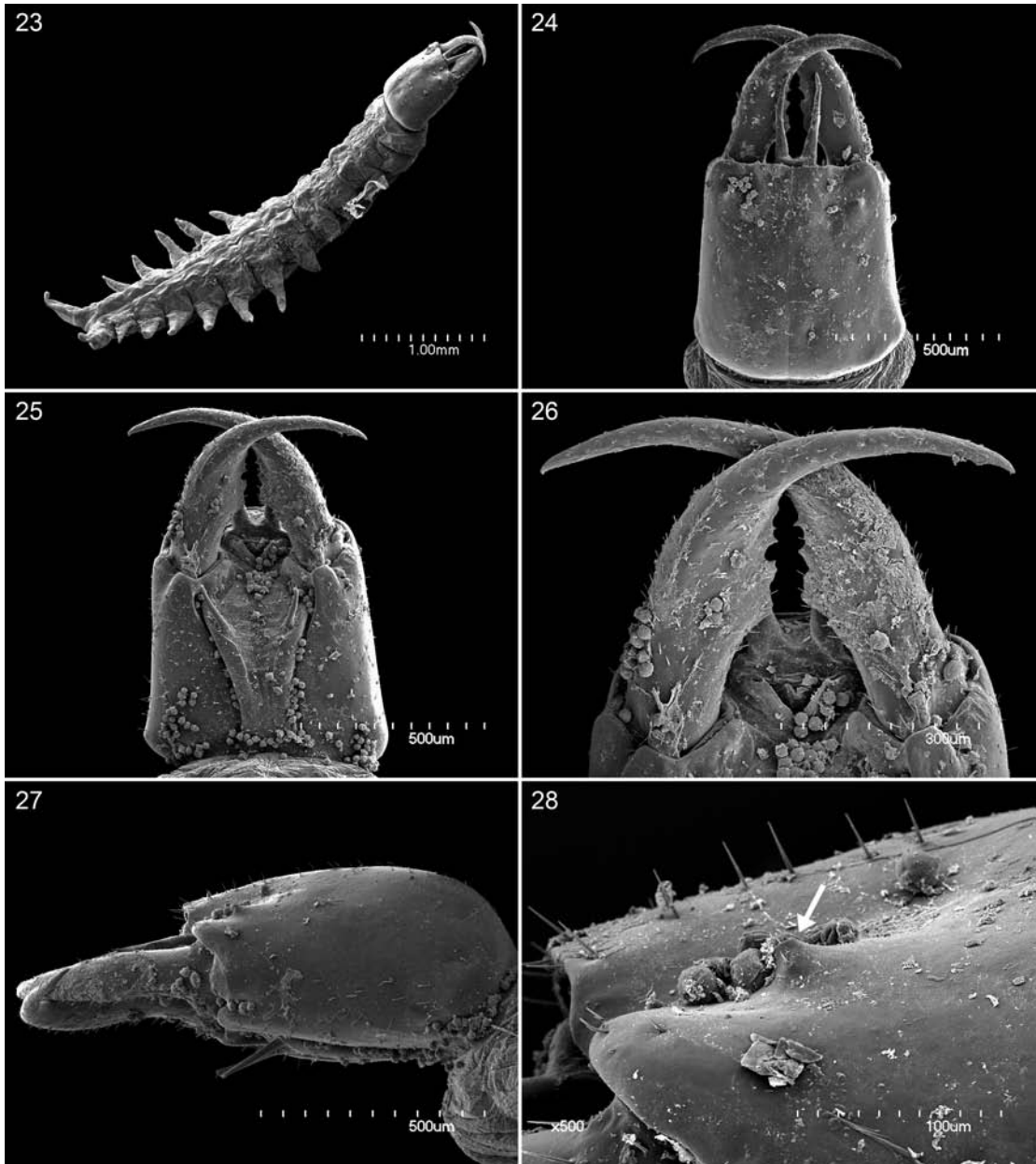
## DISCUSSION

Future investigations of *C. yarrowi* should include descriptions of other immature stages, most importantly that of the mature larva, although an understanding of the changes in larval anatomy from one instar to the next would be interesting. Information on nest architecture presented here is substantial. Nest cell orientation and depth are clearly presented. Cell walls (figs. 14–16) are obviously thick and provide strong protection against predators or parasites, and we are now obtaining some information (see appendix) as to how females form cell walls, which are more consolidated than the surrounding soil.

Cells are elongate, vertical, and presumably nearly symmetrical around their long axis, and lateral tunnels leading to them bend downward to attach to cell tops. Sarzetti et al. (2013) revealed



FIGURES 17–22. SEM micrographs of egg of *Caupolicana yarrowi*. 17. Entire egg, anterior end to upper left. 18. Posterior end showing external reticulate pattern of chorion and absence of this pattern at extreme end. 19. Close-up of reticulations. 20. Even closer, showing hexagonal microsculpturing as described in text. 21. Anterior end of egg showing slightly protruding micropyle. 22. Close-up of micropyle.



FIGURES 23–28. SEM micrographs of the first larval instar of *Triaepolus grandis*. **23.** Entire larva after critical-point drying, anterior end right. **24, 25.** Head dorsal and ventral views, showing slight narrowing of sides toward anterior end. **26.** Close-up of mandibles, ventral view, showing opposing jagged incurved edges characteristic of this species. **27.** Head lateral view, showing dorsal outline curving toward posterior end. **28.** Close-up of anterior edge of head capsule, with antenna identified (arrow).



through an excellent comparative study of five diphaglossine taxa (*C. (Z.) tucumana*, *Ptiloglossa tarsata* (Friese, 1900), *Ptiloglossa matutina* (Schrottky, 1904), *Cadeguala albopilosa* (Spinola, 1851), and *Diphaglossa gayi* Spinola, 1851<sup>9</sup>) that nests of most of these taxa had laterals that rose sharply just before dropping downward and connecting to the tops of the cells. Thus, there is a hooked-shape connection of the lateral to the cell. Laterals of only *Cadeguala albopilosa* lacked this configuration in that they did not suddenly turn upward before bending to the cell top. Earlier Roberts (1971: fig. 2) had recognized the upward loop at the distal end of laterals of *P. guinnae* and had referred to this connection as a “sink trap.” This is a configuration of the tunnel at the cell entrance that protects the cells from flooding during heavy rains. In testing the hypothesis that cells were protected from flooding by these traps, Sarzetti et al. (2013), using values of mean annual precipitation (MAP) in geographic regions where the species were known, found inconsistent support for Robert’s (1971) hypothesis. Some species in regions of low MAP values exhibit sink traps, while at least one species in an area of high MAP values appears to completely lack the structure.

However, here we suggest that MAP is not a valid estimate of the potential harmful effect of precipitation on a species that actively nests for only a month or so during a year. Presumably a sink trap is important in avoiding rainwater invading cells that are being provisioned and thus before the lateral is backfilled by the nesting female. Although *C. yarrowi* is found in a dry region of the world, its nesting period occurs in the brief but often extremely drenching monsoon thunderstorms of the wet time of the year, which, of course, account for the late summer flowering of the host plant(s) that provide food.

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<sup>9</sup> Not to be confused with *Caupolicana gayi* Spinola, 1851.

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## APPENDIX

### USE OF NECTAR BY THE DESERT BEE *CAUPOLICANA YARROWI* (COLLETIDAE) IN CELL CONSTRUCTION

James H. Cane<sup>10</sup> and Jerome G. Rozen, Jr.

**INTRODUCTION.** Some ground-nesting bees characteristically nest in soils that are so dry and hard packed that they resist biting and digging efforts. It has long been known that, to facilitate excavation, some of these bee species regurgitate liquid from their crops onto the hard soil surface, thereby softening the soil for digging (e.g., *Anthophora*, Malyshev, 1935). Investigators who found the liquid's source discovered the bees imbibing surface water, either at a nearby puddle or a seep <10 m distant. Taxa include species of *Anthophora* (Rau, 1929; Norden, 1984) and three genera of Emphorini, being species of *Ptilothrix* (Rust, 1980; Martins et al., 1996), *Melitoma* (Linsley et al., 1980), and *Diadasia* (Neff et al., 1982). These species also construct surface turrets or chimneys with the wetted soil. The turrets of one species, *D. rinconis* Cockerell (1897), were found to contain sugar, suggesting the use of regurgitated floral nectar rather than mere water to soften the soil from which the turret was made (Neff and Simpson, 1992).

Nests of the large summer-flying desert bee *Caupolicana yarrowi* (Cresson, 1875) were excavated, revealing nest cells with glossy interiors and hard external earthen casings that readily separated from the surrounding soil, as reported above (in the main body of this article). Several considerations plus the report of *D. rinconis* led to a suspicion that the soil comprising the casing had been impregnated with regurgitated nectar rather than water. The fact that the main tunnel walls were not hardened strongly suggests that the surrounding soil was soft enough for conven-

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tional excavation without the mother bee adding water or nectar. Impregnation of the cell walls instead seems to serve in molding and shaping of the walls prior to application of the water-repellent lining. Once hardened, it could also exclude some parasites and predators.

**METHOD.** To detect reducing sugars (e.g., glucose, fructose) in the hardened soil casings surrounding nest cells, a standard colorimetric sugar assay was chosen that uses dinitrosalicylic acid (DNS) (Miller, 1959). The DNS assay was used to quantify the reducing sugars that constitute most nectars (glucose and fructose) in a broad survey of Finnish wildflower communities (Käpylä, 1978). A 3 g soil fragment representing about 2/3 of the casing of an unprovisioned nest cell of *C. yarrowi* was prepared for assay by first brushing away a few stray pollen grains, leaving a clean shiny interior surface. The fragment was placed in 5 ml of distilled water, where it quickly disintegrated. After the soil suspension settled overnight, the water was decanted through filter paper. Sodium sulfite was added to remove dissolved oxygen, and, after heating with DNS, the resulting color was stabilized using Rochelle salts. Color absorbance was read by spectrophotometer at 575 nm. To account for slight lingering turbidity of the soil extract, the absorbance of the unreacted extract was subtracted from the reaction product. Reacted glucose standards of incrementing dilution were used to estimate sugar concentration in the soil extract.

**RESULTS.** Pieces of the thin, shiny lining of the cell could be peeled away from the smooth soil interior. It seems that the Dufour's gland secretion, which forms the polymerized cell lining (Cane, 1983), had not penetrated the surrounding soil. Ready disintegration of the casing in water further indicated the absence of any waxy, oily, or hydrophobic binding agent such as that of the Dufour's gland secretion. The reaction of DNS with the soil extract yielded a dark reddish-brown solution indicative of reducing sugars (e.g., nectar sugars glucose and fructose). Interpolating extract absorbance values from that of the glucose standards, we estimate that the extract from the soil casing fragment contained 28 mg of sugar, or a calculated 38 mg for the original intact soil casing of the entire nest cell.

**DISCUSSION.** Species of four genera of ground-nesting bees have been observed regurgitating liquid at their nest entrances, presumably to soften hard soil surfaces for excavation. Until this study, water has been the observed or inferred liquid, apart from one instance of sugar detected in the turret of a species of *Diadasia* (Neff and Simpson, 1992). For nests in the wild, this soil-wetting activity can be observed only at the soil surface and not underground. The liquid added to the roughened cell walls by a *C. yarrowi* female apparently enabled her to sculpt the remarkably smooth interior cell surface over which she could brush the thin shiny waterproof film that is polymerized from the macrocyclic lactones in her Dufour's gland secretion (Cane, 1981).

The presence of substantial amounts of sugar in the hardened soil casing surrounding each empty *C. yarrowi* nest cell implies that the applied liquid was regurgitated floral nectar, a seemingly profligate use of a precious resource. How much time and travel would a female expend acquiring enough nectar to wet the interior surfaces of the roughened cell walls? Nectar foragers of a similarly large bee, the bumble bee *Bombus vosnesenskii* Radoszkowski, returned to their nest carrying on average 25 mg of sugar as nectar (Allen et al., 1978). We expect that in just two foraging trips, a like-sized female of *C. yarrowi* could return with nectar enough in her crop to have accumulated the 38 mg of sugar that we found in the hard soil casing surrounding a nest cell. We

do not know when in the day female *C. yarrowi* excavate their nest cells and smooth their walls, but presumably such work would be preceded by several foraging trips for nectar to then use for softening the hard desert soil substrate in which they were hollowing out nest cells.

To be efficient, it would seem that *C. yarrowi* would need a generous nectar source for use in soil preparation, for which there is some precedent among other big desert bees. Another crepuscular desert bee, *Ptiloglossa jonesi* Timberlake, 1947 (Diphaglossinae), lives near this *Caulopolicana* nesting site. Its females sonicate nectarless flowers of *Solanum* for pollen, but their scopal loads also contained stray grains of the large and distinctive pollen of *Agave* (J. Cane and S. Buchmann, personal obs.). This clue suggests that the plentiful nectar available from locally flowering, bat-pollinated *A. palmeri* was the source of nectar in their provision masses. Female *C. yarrowi* also buzz *Solanum* for pollen (Linsley and Cazier, 1970), and so are accustomed to seeking additional floral hosts for nectar when using this pollen resource. Other flowering species that *C. yarrow* commonly uses, such as *Larrea tridentata*, provide both pollen and nectar (Linsley and Cazier, 1970). Both bat and hawkmoth-pollinated flowers offer generous nectar flowers, at least in early morning. Both bee species also reportedly forage for nectar in late afternoon (Linsley and Cazier, 1970) when they may be preparing the next morning's nest cell.

The use of regurgitated nectar to manipulate nesting soils is novel for bees, who otherwise metabolize nectar as a carbohydrate or energy source. We suspect that the behavior is not unique to *C. yarrowi*, however, but may manifest in some other desert bees. The need for smoothed nest cells is near universal among the many bees that thinly coat their nest cells with the hydrophobic secretion of their Dufour's gland (Cane, 1981). Many bees find damp soil layers in which to prepare their nest cells, but for those restricted to dry nesting soils, exogenous water sources may be needed. In xeric habitats, sources of surface water are often widely scattered and unreliable. In substituting floral nectars for nest cell construction, bees such as *C. yarrowi* can have greater flexibility in their choice of nesting site. The use of regurgitated water by bees for working soils underground will be challenging to detect, but a sensitive sugar assay like DNS can reveal the use of nectar for this purpose. Some bee collections (e.g., AMNH) include associated nest cell materials, which could be surveyed for nectar sugars using the DNS assay. Besides softening of surface soils to enable excavation, regurgitated nectar applied to subterranean nest cells could both aid the bee in shaping, molding, and smoothing the interior soil surfaces of nest cells, as well as in long-term fortification of cell walls against penetration from exterior threats.

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