

Preliminary Study of the Bumble Bee
Bombus griseocollis,
Its Eggs, Their Eclosion,
and Its Larval Instars and Pupae
(Apoidea: Apidae: Bombini)

JEROME G. ROZEN, JR.,¹ COREY SHEPARD SMITH,¹
AND DENNIS E. JOHNSON

ABSTRACT

This paper describes and illustrates the egg, fifth, first, and fourth larval instars, as well as the female pupa of *Bombus* (*Cullumanobombus*) *griseocollis* (DeGeer), all collected from a single nest in June 2017 in Wisconsin. In so doing, attempts are made to understand the biological significance of the anatomical and behavioral features of these various life stages.

INTRODUCTION

A recent study by Rozen et al. (2017) identified spicules on first instars of certain solitary bees as well as of *Apis mellifera* L. thought to enable their larvae to extricate themselves from the confines of their chorions during eclosion. It also reported that these spicules, termed hatching spines, did not occur on some groups of cleptoparasitic bees. Because the study did not explore the presence of these spines on social bees other than *A. mellifera*, we record here an investigation into the egg and its eclosion in a bumble bee and to add to it information regarding its other immature stages.

¹ Division of Invertebrate Zoology, American Museum of Natural History.

On June 29, 2017, D.E.J. collected and preserved all immature stages of *Bombus* (*Cullumanobombus*) *griseocollis* (DeGeer) that he found in the wall insulation of a local garage that was being demolished in Chippewa Falls, Chippewa County, Wisconsin. The nest contained an approximate total of 101 individuals represented by larvae, pupae, and eggs (proportion of live and dead eggs uncertain). Despite this large total, representation of larvae whose stadia could be judged was low, as indicated in table 2. In accordance with the study of this species by Stephen and Koontz (1973) that there are five larval stadia, only a single specimen could be identified as presumably a second instar and another instar, probably the third instar (but possibly the fourth) was entirely missing. To further cloud the issue, body spicules were discovered on all larval instars. Since those found on the smallest larva are slender, tapering, and curved just as they are on later larval instars (and do not match the short, triangular, flattened, sharp-pointed, white-tipped hatching spines found only on the first instars of *Centris bicornuta* Mocsáry (Rozen, 2017b: fig. 5) or of *Apis mellifera* L. (Rozen, et al., 2017: figs. 14, 15, 29), it is uncertain that they assist in hatching. Thus the study is not capable of shedding light on the use of larval hatching spines in this species of *Bombus*, its original focus. However, the study has a broader purpose as indicated above, namely, to understand how all immature stages of this species are adapted to accomplish all that needs to be done to survive. With the wealth of specimens that D.E.J. has collected, we present here some unsuspected insights in the developmental biology of this species including how the first instar without hatching spines emerges from its egg.

METHODS

Eggs and larvae were killed and then preserved and stored in Kahle's solution. All SEM micrographs were captured with a Hitachi S5700 in the Microscopy and Imaging Facility of the American Museum of Natural History. Microphotographs were taken with a Canon Power Shot A2300 camera hand held to one of the eyepieces of a Leitz Wetzlar stereomicroscope. All specimens of *Bombus griseocollis* treated herein resulted from a single collecting event (table 1). Unless stated otherwise, specimens examined are listed therein. Maximum and ranges of head widths of larval instars are present in table 2.

EGG

Figures 1–22

DESCRIPTION: Average length of eggs ($n = 10$) preserved in Kahle's solution 2.91 mm, range 2.8–3.1 mm; maximum diameter (at midlength) 0.92 mm, range 0.8–1.0 mm; average length of eggs ($n = 2$) after critical point drying 2.45 mm, range 2.4–2.5 mm. Color white (fig. 1). Form (figs. 1, 2) moderately robust, slightly curved with anterior end (identified by position of micropyle) somewhat more rounded than more pointed posterior end. Chorion of large middle part of egg thick, ridged, and strongly reticulate as seen on dried specimen (fig. 2), only moderately reflective, while chorion at extreme anterior and posterior ends thin, transparent, smooth, flexible, and highly reflective; borders of these two surfaces sharply identified because of contrasting different chorionic thicknesses and textures. Micropyle difficult to identify with

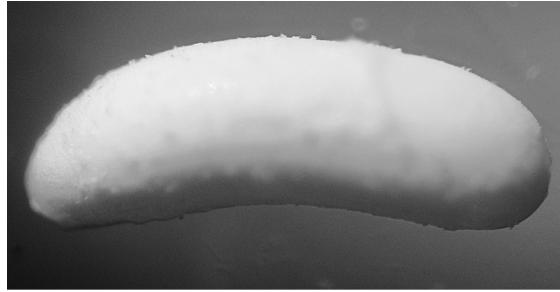


FIGURE 1. Microphotograph of egg of *Bombus griseocollis*, anterior end at right.

stereoscope but visible with SEM on anterior end of egg (figs. 4, 12–17), often as a small, indistinctly pitted oval area with only a few apparent holes surrounded by radiating wrinkles, but on one specimen (fig. 7) with a tight cluster of distinct holes, discussed below.

MATERIAL STUDIED: Approximately 17 specimens examined with SEM, all collected June 29, 2017.

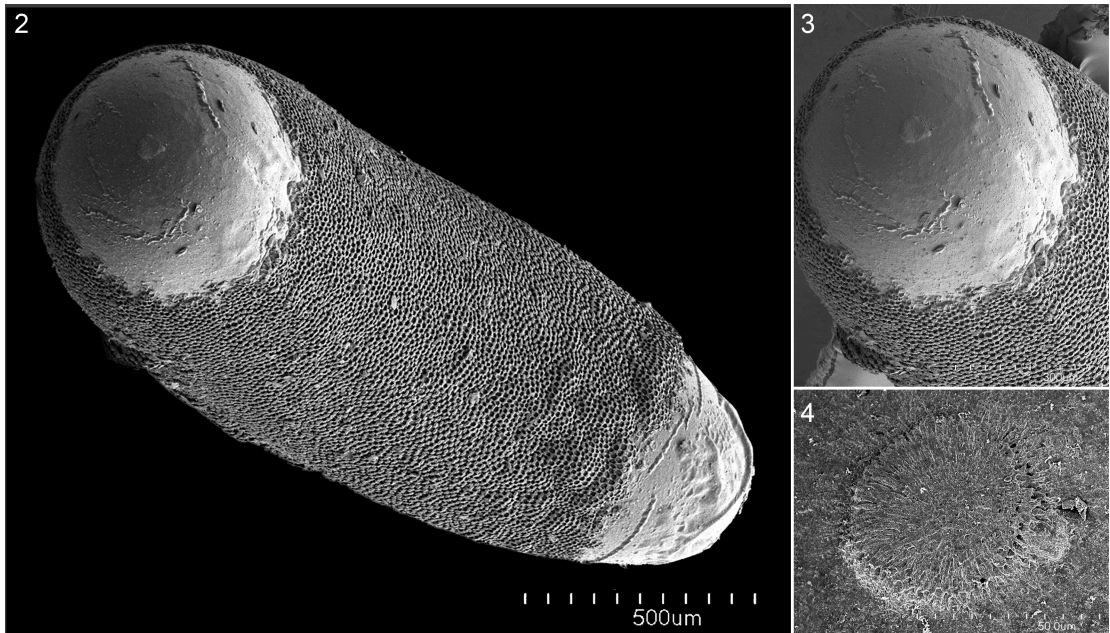
DISCUSSION OF EGGS

Stephen and Koontz (1973: fig. 1) provided a good illustration depicting egg shape and recorded the egg length of this species as being a bit longer (length = 3.1 mm) than reported here. However, their description of the egg was incorrect in two ways. First, assuming that the part of the visible embryo was the head end, they mistook the orientation of the egg in stating that its small end was the anterior end. Now with the use of an SEM (figs. 12–17) the rather obscure configuration of the micropyle is clearly visible on the surface of the chorion, identifying the broad end as the front end.²

Second, they (1973: 14) described the chorion as being “shiny, translucent, unreticulated.” Clearly this is inconsistent with the present interpretation based upon SEM imaging (figs. 2, 5). Likely their observations had been made while the eggs were immersed in liquid (water or alcohol) in which case the distinctiveness of the finely reticulated, rigid, thick surface of the extensive midpart of the egg, and a small, shiny, flexible, thin surface at the two ends of the egg appear much less contrasting (contrast, for example, fig. 1, a microphotograph, with figs. 2 and 5, which are SEM micrographs). Of note, the study by Stephen and Koontz (1973) was carried out 35 years ago, before SEM imaging was widely available. In other respects their study was, and still is, outstanding in detail, interpretation, and presentation.

This patterning of egg textures as revealed by SEM technology has not been noted earlier for any group of bees. Its distinctiveness raises the question of adaptive function: does it have a selective advantage? Does an egg with this double pattern of two chorionic textures function

² The identification of the front end of a bee egg can also be determined by the orientation of the oocyte in the ovary. When eggs are deposited, they are always released from the ovary posterior end first. Thus, the posterior end of the mature oocyte passes from the ovarian follicle into the lateral oviduct first, followed by the rest of the oocyte. Similarly it then enters the median oviduct to be deposited in the brood chamber, again posterior end first.



FIGURES 2–4. SEM micrographs of egg of *Bombus griseocollis*. 2. Entire egg (anterior end up left) demonstrating smooth transparent chorion at short anterior and posterior ends and long stretch of reticulate, rough chorion in the middle. 3. Close-up of anterior end showing the small elevation of the micropyle located centrally. 4. Close-up of the micropyle elevation, with some micropylar opening visible along upper periphery.

differently from an egg with either a uniform thin, smooth surface or one with a uniform thicker, reticulated surface?

After pondering these questions, a possible explanation was identified, assuming that the emerging first instar egresses through the front end of the chorion. When the emerging first instar moves forward through the tube of rigid reticulate chorion, the thin, flexible chorion of the rear end can collapse, thereby avoiding resistance formed from creating a vacuum in the rear end of the egg as the larva moves forward. It is hoped that this explanation can be tested by watching live larvae egressing. As pointed out by Rozen et al. (2017), with many but not all bees, a hatching egg splits along the sides of the chorion, but there is no evidence of such ruptures along the sides of the eggs and egg chorions found in the current sample. This is not surprising since hatching spines were not detected. Thus, the lack of hatching spines and the presence of an extremely thick, ridged chorion appear to be functionally related phenomena.

Currently, the mechanisms permitting first instars that emerge through the front end of this egg or the eggs of any of the parasitic apids (Rozen et al., 2017) have yet to be discovered.

Because of the confusion regarding the polarization of the egg of this species, we examined a good many eggs to be certain of the position of their micropyles. In most cases the micropyles were so indistinct that they could be identified only with the use of an SEM. In almost all specimens so examined they appeared as indistinctly pitted areas, in some cases only questionably with openings into the lumen of the egg. On one specimen (fig. 7), however, a distinct, sharply delineated cluster of openings was apparent. On seeing this, we decided to take a random sample of 10 more eggs

TABLE 1. Estimates of numbers of immature specimens of *Bombus griseocollis* recovered from single nest, collected in Chippewa Falls, Chippewa County, Wisconsin, June 29, 2017, D.E.J., collector.

Eggs (unhatched, either dead or alive)	42
Larvae (all ages, presumably alive)	37
Pupae (presumably alive)	22
Total	101

TABLE 2. Mean larval head widths and ranges (in mm) of *Bombus griseocollis*, assuming five larval instars. Number of data in parentheses. Figures in the bottom row are those supplied by Stephen and Koontz (1973; here, "S. & K.") and are referenced in Discussion of Larval Instars.

Instar	1	2	3	4	5
Mean	0.63 (6)	0.73 (1)	–	1.14 (6)	1.95 (15)
Range	0.61–0.68	0	–	1.05–1.2	1.88–2.0
S. & K.	0.58–0.63–0.75	0.76–0.9	0.9–1.25	1.6–2.1	

from the available supply, resulting in the following: All eggs revealed the micropylar area in the middle of the broad anterior end with radiating surface channels surrounding them. It is hypothesized that these channels may function as sperm guides directing stored spermatozoa, when released by the female, toward the micropyle aperture(s) during oviposition. The center of micropylar area on the additional specimens showed considerable variation in the number of clearly opened entrances, suggesting that figure 7 was merely an extreme case in the range of variation.

A single egg (figs. 10, 11) that had lost its chorion while in laboratory preparation was completely smooth except it revealed a single central small, pitlike indentation, which was considered likely to be the ingress channel through the vitelline membrane for spermatozoa during fertilization. The SEM images of the structure are presented here with the hope that the hypothesized functioning of this structure can be tested at a later date.

LARVAL INSTARS OF *BOMBUS GRISEOCOLLIS*

Because last larval instars are typically used to describe the larvae of bees, the detailed description of the fifth instar is presented first, followed by comparisons with other available larval instars. As pointed out by Michener (1953), the last stage larva of this species was described by Ritcher (1933) under the name *Bremus separatus* (Cresson).

FIFTH INSTAR

Figures 23–36

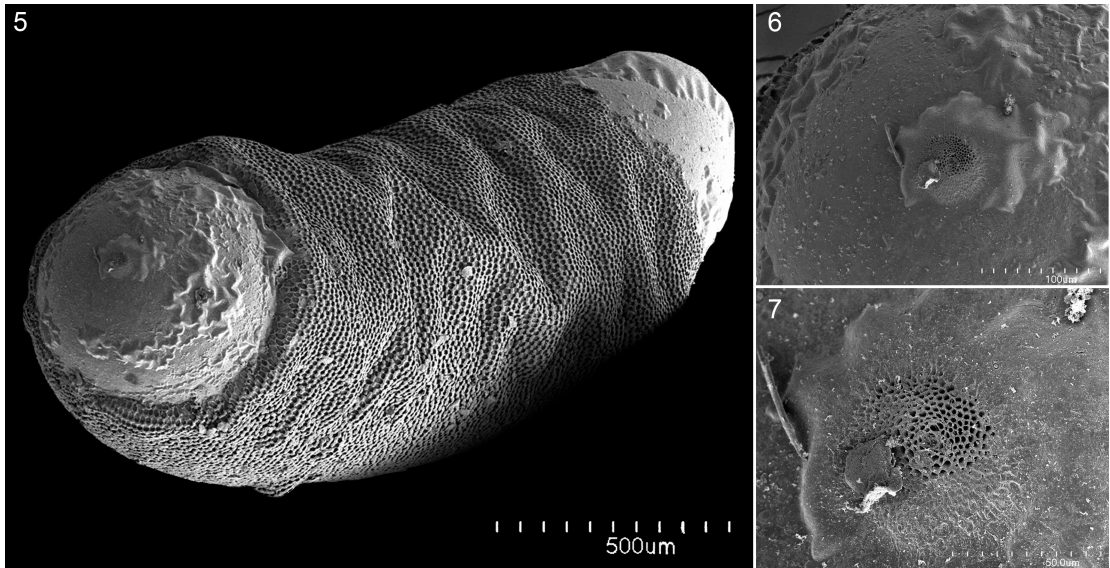
DESCRIPTION: Head (figs. 26, 27, 31–36): Integument of head capsule faintly pigmented except articulating points of mandibles with hypostomal ridge distinctly pigmented; areas just above epistomal ridge with vague blotches of slightly darkish pigmentation; maculations of clypeus variable, in that sometimes clypeus with single large, dark to nearly black inverted

Δ -shaped maculation medially of upper part (fig. 31), occasionally accompanied by another such median maculation on junction of epistomal ridge with median coronal ridge of head capsule (fig. 32), other times scarcely pigmented below epistomal ridge (fig. 33), a feature that may or may not depend on age of the fifth instar. Labrum faintly pigmented on dorsal surface and sides at base (figs. 31–33); darkening of apical edge resulting from narrow apical band of dark pigmentation showing through from adoral surface. Prelabium bearing strongly pigmented, subapical, sclerotic ring sublaterally above including narrow dorsal bridge flecked with irregular dark marks; pigmentation of ring gradually diminishing toward venter; extreme labial apex unpigmented except for palpi and salivary lips; apex of labrum bearing small, distinct dark mark medially, located internally on salivary duct behind salivary lips. Integument of head capsule and mouthparts with scattered, moderately short, setiform sensilla.

Head size extremely small relative to body size of large individuals (figs. 23–25); front of head in lateral profile (fig. 26) relatively flat below narrow vertex, so that frons, clypeus, and labrum closely aligned and angling sharply from jutting labiomaxillary region; head capsule moderately broad with summit of vertex faintly bilobed in frontal view (fig. 27). Tentorium incomplete, presumably because of impending pupation; posterior tentorial pits normal in position; posterior thickening of head capsule moderately well developed, only slightly bending forward medially as seen in dorsal view; coronal ridge pronounced but unpigmented on cleared head capsule, extending to intersection of epistomal ridge (fig. 27), diminishing somewhat partway down; hypostomal ridge well developed, pigmented, without dorsal ramous. Parietal bands long, deep, but not well defined. Antennal prominences scarcely developed (fig. 26); antennal papilla small, but projecting, and longer than basal diameter, bearing perhaps three sensilla. Vertex narrowly rounded in lateral view; frontoclypeal area not projecting beyond labrum (fig. 26); labral sclerite not evident; forward-directed labral tubercles absent but labrum slightly swollen on each side.

Mandible (figs. 34–36) apically darkly pigmented, nearly black; at intermediate length gradually becoming lighter toward base except for two darkened areas, i.e., one at each articulation with head capsule (figs. 31–33); mandibular shape (figs. 34–36) stout, rather short, with long axis slightly curving upward in inner (fig. 34) or outer (fig. 36) views; apex with pronounced scoop-shaped apical concavity (fig. 35) on inner surface occupying approximately two-fifths of mandibular length; mandibular apex rounded bearing single subapical tooth partway to base of dorsal edge of concavity; this tooth slightly splayed outward apically; edge of concavity moderately smooth except for short series of several fine teeth along ventral edge (arrow); outer surface of mandible somewhat uneven midlength and basal area of outer surface bearing several widely spaced, fine setae on small tubercles (fig. 36: arrows).

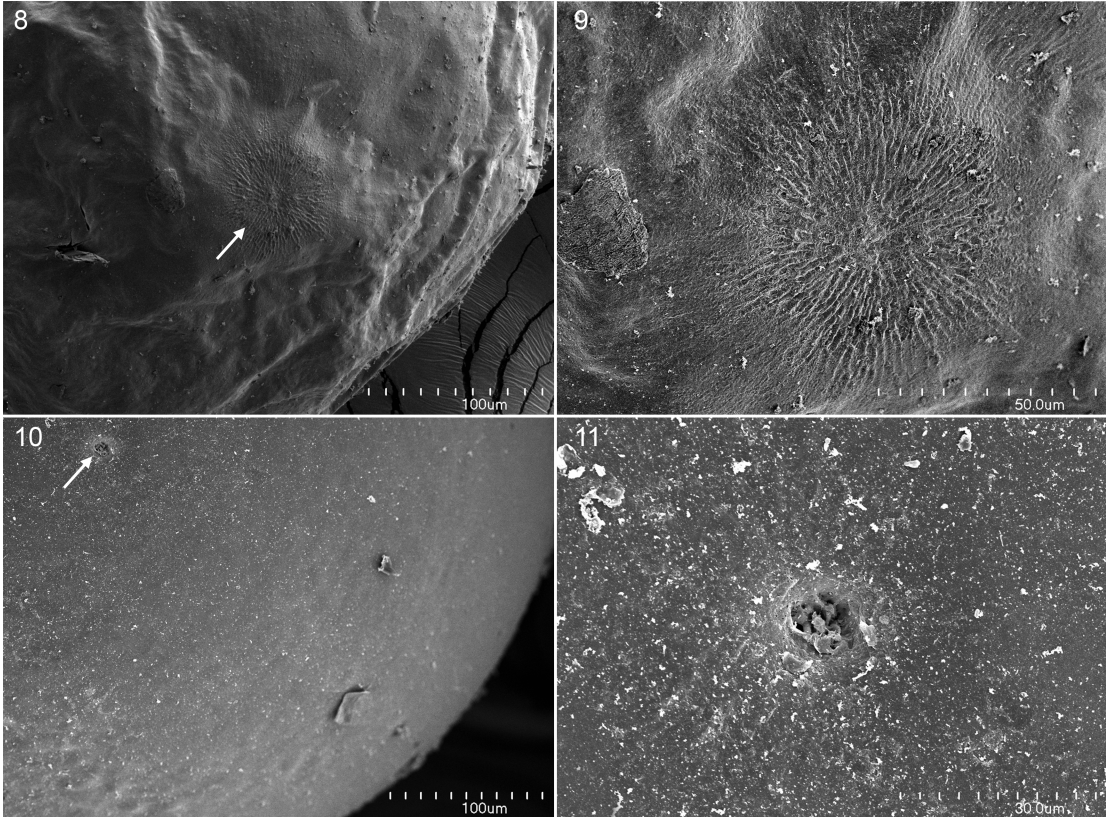
Labiomaxillary region produced and not greatly fused; pigmentation described below and visible in figures 31–33; labium projecting somewhat beyond maxilla in lateral view (fig. 26). Maxillary apex not bent mesad; palpus apical in position, elongate, more than twice as long as basal diameter, pigmented; galea represented by stout but short seta arising from small tubercle on maxillary apex short distance mesad from palpus; stipes apically partly ringed by darkish sclerotization except extreme apex unpigmented; articulating arm of stipes pronounced, darkly



FIGURES 5–7. SEM micrographs of another egg of *Bombus griseocollis*. 5. Entire egg (anterior end left), again with micropyle centered in middle of front end. 6. Close-up of elevation. 7. Further close-up, demonstrating large number of possible openings.

pigmented; basal articulation of stipes to cardo faintly pigmented; cardo more or less pigmented. Strongly projecting labium divided into prementum and postmentum, and bearing apically projecting broad lips of slitlike salivary opening extending full length separating labial palpi; labial palpus with length more than twice basal diameter. Hypopharynx pronounced, weakly bilobed, strongly spiculate.

Body: Integument generally densely covered with extremely fine, minute, sharp-pointed spicules except for inter- and intrasegmental creases; on midbody segments (approximately abdominal segments 3 and 4) spicules on outer surface of lateral abdominal swellings gradually becoming denser, longer than elsewhere on segment, so that ventral surface of swelling most densely covered; thoracic segments each with pair of small, apically pigmented tubercles dorsally (figs. 23–25, 28–30) as characteristic of corbiculate taxa; spiracles exhibiting faintly pigmented atrial rims and spines (see below), elsewhere integument unpigmented. Postdefecating larva at first robust presumably with body tapering at both ends as seen laterally and dorsoventrally (fig. 24), but, with approaching pupation, abdomen becoming physogastric with internal development of pupal anatomy; hence at first most body segments with pronounced separation of swollen caudal annulets contrasting with recessed cephalic annulets (as in predefecating larva, fig. 23); with approaching pupation, cephalic and caudal annulets of mesosoma become more elongate and uniform in diameter with internal development of pupal mesosoma while metasoma swells by shrinking in length although distinction of cephalic and caudal annulets still partly maintained (figs. 24, 25); as measured from above, dorsal breadth of abdominal segment 5 approximately twice that of thoracic segment 3 shortly before pupation (see Remarks below regarding this change in body form). Spiracle of postdefecating larva with



FIGURES 8, 9. Distant (arrow) and close-up views of the micropyle of yet another egg, this one revealing no certain opening. FIGURES 10. Front of egg of *Bombus griseocollis* that has lost its chorion, revealing small opening into yolk (arrow) in the vitelline membrane through which spermatozoa are presumably funneled to fertilize the egg. 11. Close-up of entrance.

shallow atrium, about half as deep as wide when viewed externally; atrial wall gradually darkening as it approaches tracheal opening thereby imparting slightly tannish hue to sclerotized spiracle under low magnification; center of innermost end of atrium with short sclerotized ring to which is attached flexible, collapsible tube of thin transparent chitin several times as long as atrial depth; inner end of tube attaching to trachea (hence, flexible nature of tube and indeed entire spiracular arrangement closely matching figure in Rozen, 2017a: fig. 4).

MATERIAL STUDIED: See table 1.

REMARKS: The change in body form of the fifth instar described above and illustrated by figures 24 and 25 obviously occurs after the meconial mass has been discharged. Accordingly, Stephen and Koontz (1973) referred to it as a “prepupa.” While this terminology is correct and logical, a problem arises in that many bees have a long period of diapause after defecation during which they overwinter in a near dormant state until the following year. They too are often referred to as “prepupae.” One should be certain to indicate which phenomenon is under consideration: one is a brief larval stage undergoing rapid metabolic changing; the other is a larva undergoing almost no metabolic activity.

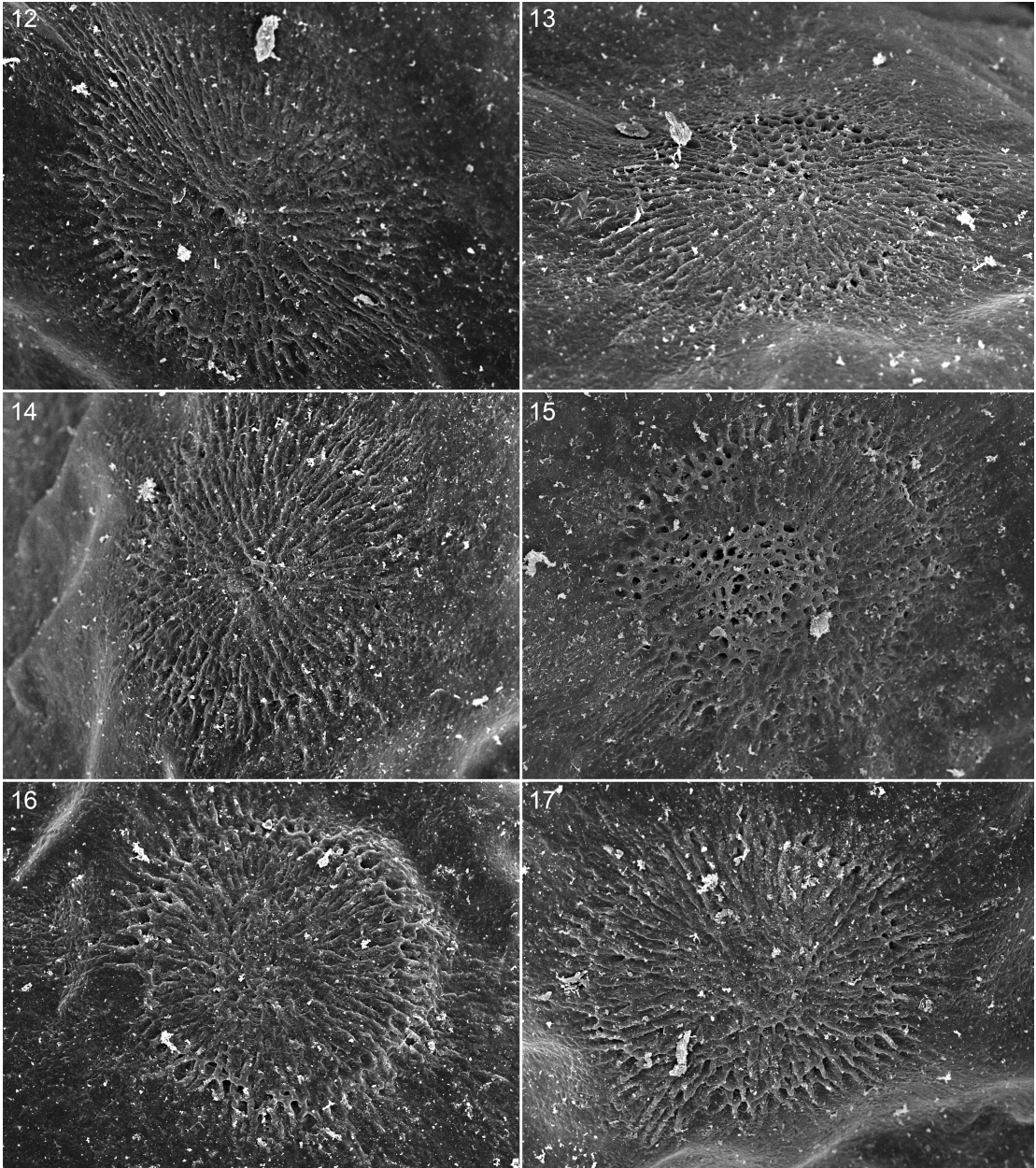
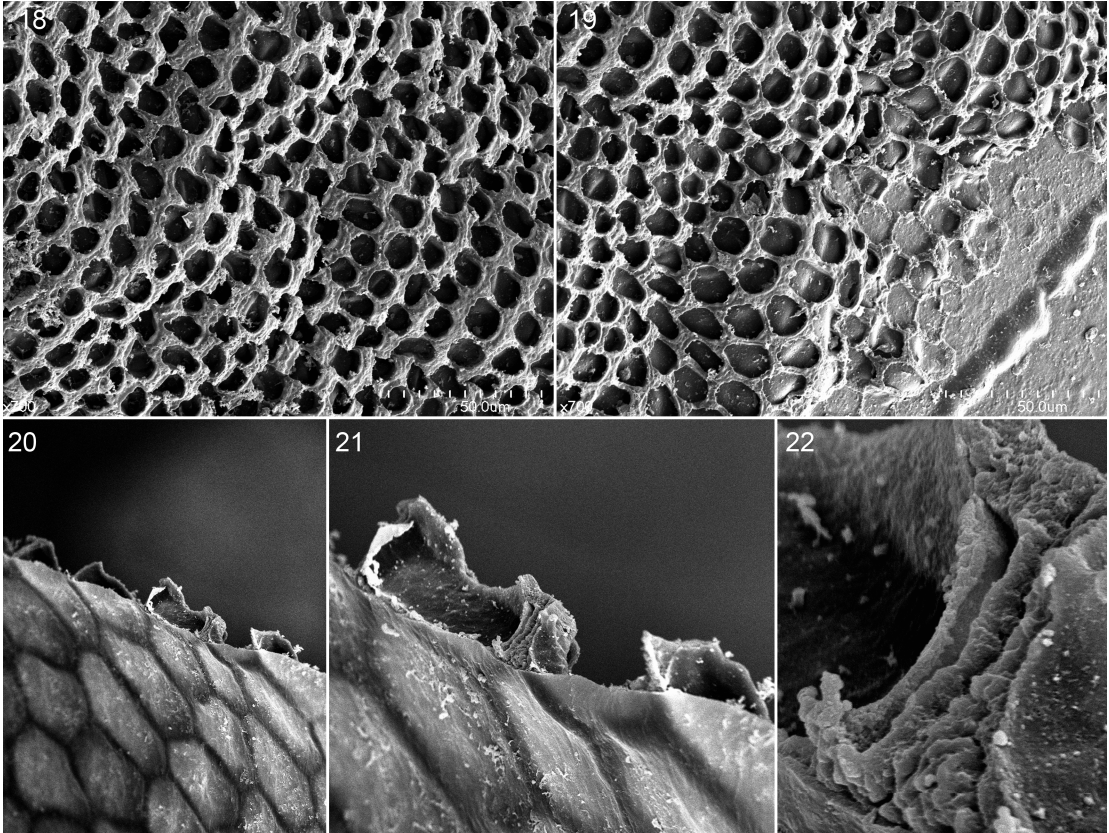


FIGURE 12–17. SEM micrographs of micropylar areas on other eggs of *Bombus griseocollis* showing variation in radiating channels and in apparent number of openings.

A somewhat different interpretation of the term “prepupa” appears to be employed by Salmah et al. (1996) and others who worked on stingless bees (Meliponini). They identified distinctive anatomical stages of the immature bee without reference to when immatures molt. Their so-called larval stage is divided into three substages based on size increments seeming to correspond to the first three larval instars of other holometabolous insects (assuming, as



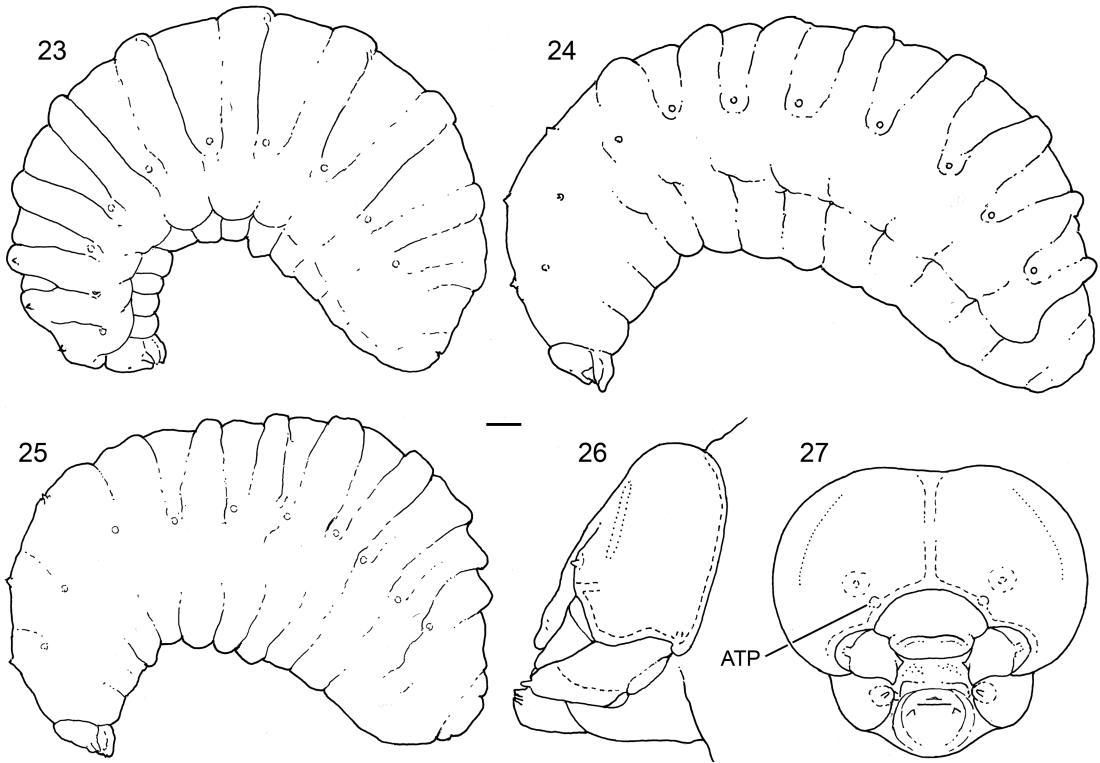
FIGURES 18–22. SEM micrographs of reticulate chorion. **18.** Close-up of exterior surface of reticulate chorion. **19.** Same, except where reticulate chorion meets smooth chorions. **20.** Edge of split of reticulate chorion with thin inner surface below and edge of outer structure along upper side. Note reticulate pattern of surface structure revealed through thin inner surface, **21.** Close-up of central area in figure 20. **22.** Close-up of parallel perpendicular lamellae from central area of figure 20 demonstrating complexity of outer ornamentation.

they did, that there are a total of three larval instars). The next stage they termed the pupal stage, divided into six substages, the first of which they termed the “prepupa,” with head, thorax (mesosoma), and abdomen (metasoma) distinguishable but without legs or other appendages. Hence, their definition would seem to correspond to the late stage mature larva of *B. griseocollis* (fig. 25), here recognized as the final pharate stage of the last larval instar. And, finally, they recognized five other substages of the pupal stage, all with antennae, legs, wings, based mostly on successive degrees of pigmentation and wing development.

FIRST INSTAR

Figure 37

The following description follows a similar format as that of the fifth instar but emphasizes differences between the two forms, thereby avoiding repetition as well as some obvious differences that are to be expected. However, unexpected similarities are also presented.



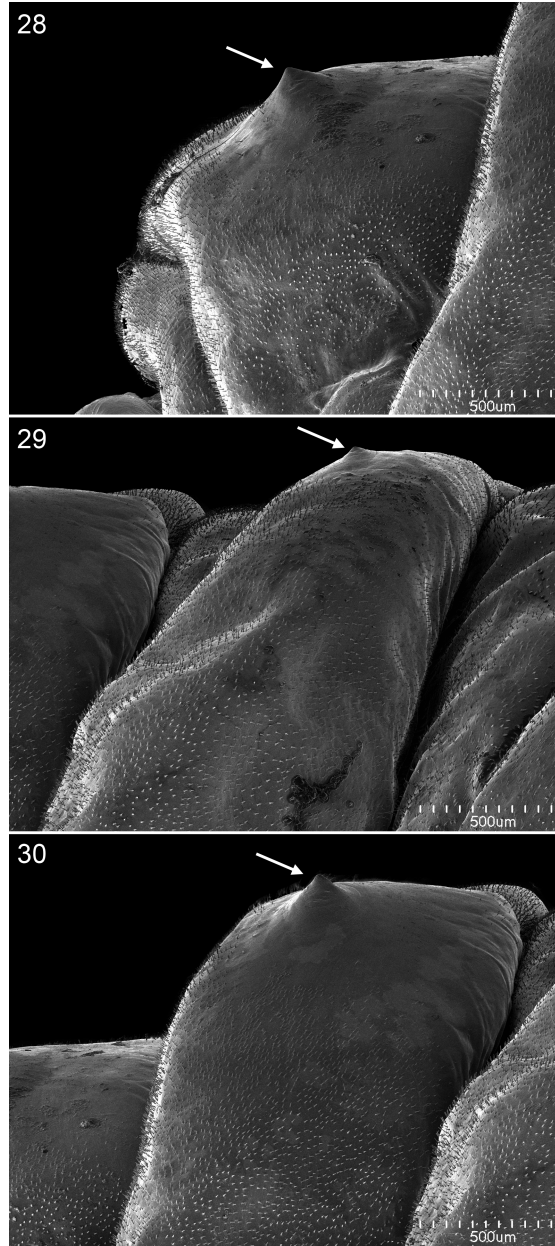
FIGURES 23–25. Diagrams of fifth larval instars of *Bombus griseocollis*, lateral views, all to same scale. Scale = 1.0 mm, referring to figs. 23–25. Variation in body size reflecting natural variability in this stage. **23.** Pre-defecating larva. **24.** Early postdefecating larva with thoracic segmentation starting to elongate. **25.** Late post-defecating larva with pupal metasoma starting to swell abdomen. Scale = 1.0 mm. FIGURES 26, 27. Close-up of head of fifth larval instar of *B. griseocollis*, lateral and front views respectively.

DESCRIPTION: Body posture usually strongly curved to almost circular, similar to that of the first instar of *A. mellifera*.

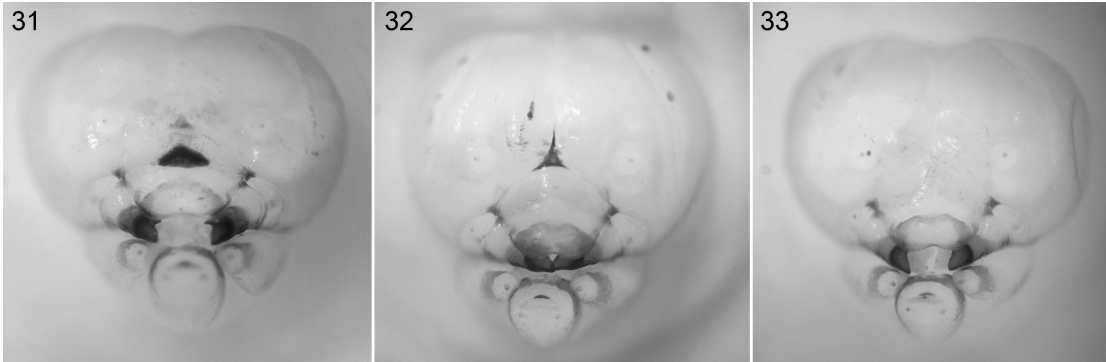
Head: Integument unpigmented except mandibles faintly pigmented apically, their articulation points minutely pigmented, and hypostomal ridge a dark line; after clearing and before staining, all sclerotized areas (head and spiracles) visually better defined, suggesting faint pigmentation.

Head size large relative to body size (fig. 37), globose; front of head in lateral profile strongly, evenly curved, so that vertex curving into gently curved frons and clypeus that bend into relatively recessed labial maxillary region. Tentorium well developed, complete; internal head ridges well developed. Parietal bands long, evident. Antennal prominences not evident. Vertex well rounded in lateral view. Labrum slightly swollen on each side.

Mandible faintly pigmented, with dorsal subapical tooth questionably expressed; apical concavity evident. Maxillary apex bending strongly mesad far beyond palpus; galea questionably identified at extreme tip of maxilla; maxillary and labial palpi short, about as long as basal diameter. Pre- and postlabium distinctly expressed. Salivary opening scarcely visible, without projecting lips.



FIGURES 28–30. SEM micrographs of tops of pro-, meso- and metathorax, respectively of fifth larval instar of *Bombus griseocollis* showing left tubercles (arrows) of paired dorsal tubercles on caudal annulets, lateral view.



FIGURES 31–33. Microphotographs of front of head of *Bombus griseocollis* showing variation in clypeal pigmentation.

Body: Much of integument also extensively covered with vestiture of fine spicules; Body form linear, far less swollen than that of fifth instar. Spiracles with atrial rim circular; atrial inner surface with pattern of equally spaced large spicules.

SECOND INSTAR

This instar was represented by a single specimen and was examined with an SEM after critical-point drying and sputter coating. It seemed to agree in most ways with the first instar, but an important difference is its somewhat greater head width (table 2).

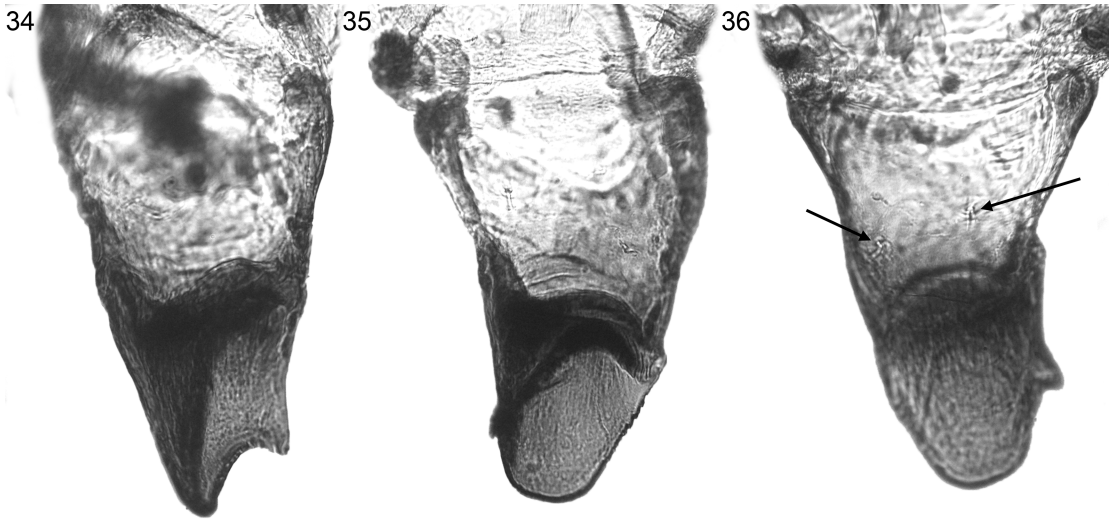
FOURTH INSTAR

Figure 38, 40

It was uncertain at first whether the specimens described here belonged to the third or fourth larval instar. Then, the discovery of extremely small, paired dorsolateral tubercles on each of the thoracic segments mirrored those observed on the fourth instars in a large series of specimens of *Bombus (Fervidobombus) pennsylvanicus* (DeGeer) collected by C.D. Michener in 1950, currently on long-term loan from the University of Kansas. Such tubercles identically positioned, though larger and more or less pigmented, are found on the last larval instar of all corbiculate bees. Their appearance as minute, nearly colorless tubercles on fourth instars of *B. griseocollis* was first recorded by Stephen and Koontz (1973: 15).

DESCRIPTION: Head: Integument of head capsule colorless except faintly pigmented along hypostomal ridge, distal part of maxilla, and labium; mandibular apex moderately pigmented. Integument of head capsule and mouthparts with unremarkable, short, setiform sensilla.

Head size relative to body size unremarkable (fig. 37). Tentorium complete. Parietal band long, recessed, but difficult to see because of lack of sharply defined edges. Antennal prominences scarcely developed; antenna and antennal papilla small but papilla elongate, projecting farther than its basal diameter. Vertex in lateral view more broadly rounded than that of last



FIGURES 34–36. Microphotographs of right mandible of fifth larval instar of *Bombus griseocollis* dorsal, inner, and ventral, and outer views, respectively (arrows pointing to setae in outer view).

instar; labrum viewed in lateral profile projecting beyond frontoclypeal area, but labral apex, maxillary apex, and labium projecting equally.

Mandible very much as described for fifth instar except smaller, pigmentation much paler, subapical tooth somewhat smaller and not displayed.

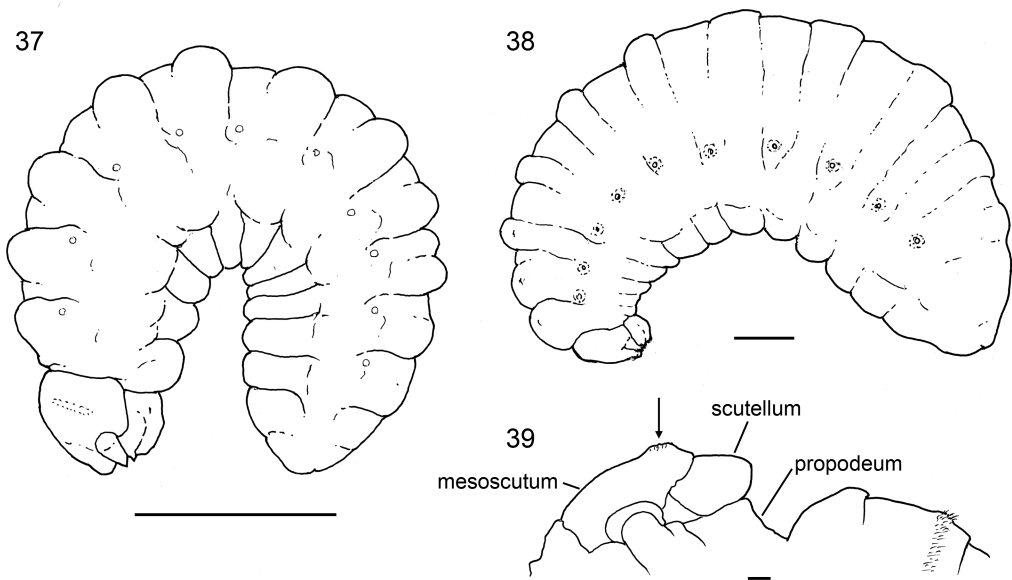
As in fifth instar and unlike that of first instar, maxillary apex not bent mesad, palpus apical in position; galea evident, slightly projecting obliquely mesad of palpus, bearing several sensilla.

Body: Integument mostly covered with small to minute spicules (except for deep integumental folds which lack spicules) but broad irregular band of elongate spicules (fig. 40) occurring along ventral surfaces of lateral body swellings mostly on abdominal segments, well below spiracular line; these spicules tapering evenly and at least four times longer than basal width but far less dense than spicules elsewhere.

MATERIAL STUDIED: See table 1.

DISCUSSION OF LARVAL INSTARS

Data from the current study in table 2 were based on the assumption that the species had five larval instars. Importantly, they were recorded before they were compared with and analyzed in light of the study by Stephen and Koontz (1973). On the basis of maximum head width (referred to as “head diameter” by Stephen and Koontz), the specimens appeared to group into four categories presumably representing four instars. After the discovery of the presence of small, paired dorsal thoracic spines on both the third as well as the fourth group (i.e., fifth instar), it was concluded that these two stages were consecutive, making the third group the fourth instar (assuming that there are five larval stages). Therefore the third instar was not represented. Hence, specimens of instars one and two and also four and five were available for study. However, when compared with the



FIGURES 37–39. Diagrams of 37. first and 38. fourth larval instars, and of 39. dorsal surface of pupal mesosoma and metasoma of *Bombus griseocollis*, respectively, lateral views drawn to different scales. Arrow pointing to paramedian mesoscutal tubercles on pupa. Scales = 1.0 mm.

information published by Stephen and Koontz (1973) (repeated here as the last row of table 2) the interpretation of first and second instars is inconsistent between the two studies. They claimed the head width of the first instar was 0.58 mm whereas the current study reported 0.61–0.68 mm as the range of the first instar, which falls within the range of the second instar in the former study. However, the single reading (0.73 mm) of the head of the second instar in the current study also falls in their second instar grouping. The difference between their head width reading (0.58 mm) and the smallest head width of the current study (0.61 mm) is only 0.03 mm, scarcely a large number.

However, there is other evidence to consider. The midintestine of the cleared first instars described above contained a substantial amount of pollen, certainly indicating the specimen had fed before being preserved. On examination of some solitary and cleptoparasitic bees in which hatching spines are involved with eclosion, Rozen et al. (2017) postulated that the second instar is the first stage to ingest pollen as well as nectar. Thus, this observation indicates either the first instar of *B. griseocollis* feeds substantially on pollen, or the specimen was actually a second instar. If the latter is correct, where is the first instar? Rozen et al. (2017) indicated that in a number of solitary bees the first instar is, in fact, pharate within the chorion, and actually the second instar emerges leaving behind both the chorion and the exuviae of the first instar. Evidence of this was examined among the vacated and broken remains of eggs from the current study without success. Additional studies of nest contents of this or other *Bombus* species are required to resolve this matter.

The elongate maxillary apex of the presumed first instar and its bending toward the midline of the head contrasts with the unbending maxillary apices of both the fourth and fifth instars. That of the second instar appears, however, to represent an intermediate condition. What is the explanation of this uncommon sequence?

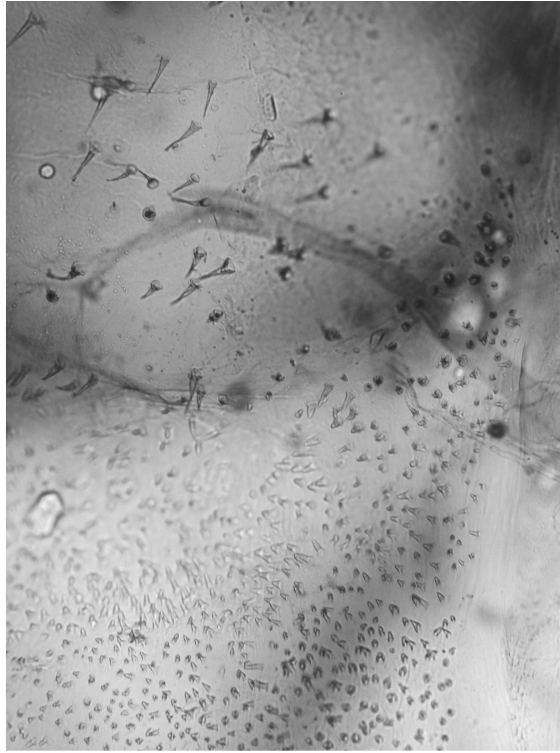


FIGURE 40. Microphotograph of cleared abdominal segment of fourth larval instar of *Bombus griseocollis*, anterior end right, with long, tapering, posteroventrad directed spicules of lower surface of lateral body swelling above and much smaller, denser ventral body spicules below.

Display of elongate spicules on the lower side of lateral body swellings (e.g., fig. 40) is a noteworthy feature among bee larvae for which there is currently no certain explanation. Since their distribution might increase ovoid patterning of the body on the surface of a semifluid substrate, it might be used to maintain body orientation relative to the surface on which it feeds or to move forward by twisting its body from side to side? However, the curved long axis of the body on preserved specimens would seem to argue against such explanations. Also, why do these giant spicules appear on the second instar rather than the first? Observations on live larvae in their cells should be revealing.

With so many questions unanswered concerning this species there is an obvious need for further investigations of its developmental biology.

PUPA OF *BOMBUS GRISEOCOLLIS*

Figure 39

All 22 pupae recovered were females, easily identified by 12 antennal segments, scopal configuration, and genitalia. The following treatment was based on the characters employed in Michener (1954).

DESCRIPTION: Integument without setae. Head without tubercles on vertex, frons, or scape. Lateral angle of pronotum rounded, not produced as spine; posterior lobe of pronotum slightly produced sublaterally; mesoscutum with pair of low, paramedian tubercles close to scutellum (fig. 39); mesoscutellum strongly produced, lobelike overhanging metanotum (fig. 39). Tegula and wings without tubercles. All legs with each coxa and trochanters bearing apical tubercle. Metasomal terga 2–6 with posterior subapical row of conspicuous spicules³ characterized by elongate hollow basal area and threadlike pointed apex; this apex possibly more than twice as long as basal part.

MATERIAL STUDIED: See table 1.

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³ Note that, contrary to Michener (1954: Table I), female pupae of *B. griseocollis* do have spicules on metasomal tergum 6.

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