

Larval Anatomies, Eggs, and Developmental Biologies of *Centris bicornuta* and *Epicharis albofasciata* (Apoidea: Apidae)

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ABSTRACT

This paper presents detailed comparative descriptions of the mature larvae and eggs of *Centris* (*Heterocentris*) *bicornuta* Mocsáry and *Epicharis* (*Epicharoides*) *albofasciata* Smith as representatives of two genera that are closely related. It strongly suggests that both species, while developing, pass through five larval instars; because the first instar remains mostly pharate within the chorion, it is only as a second instar that it begins to consume provisions and increase in size. There follows an account of how each species changes in functional anatomy from one instar to the next and how each instar of one species compares with the same instar of the other.

In response to a recently published paper (Martins and Melo, 2016), which suggested that the tribe Centridini may be polyphyletic because some taxa within *Centris* share features with corbiculate genera, it is pointed out that all corbiculate genera uniquely share an apomorphy: they bear small paired, elevated, finely setose, sclerotized, and usually pigmented apical tubercles on the thoracic segments of mature larvae. Such thoracic tubercles are unknown in the Centridini or elsewhere among bees.

INTRODUCTION

This study compares the larval stages of *Centris* (*Heterocentris*) *bicornuta* Mocsáry and those of *Epicharis* (*Epicharoides*) *albofasciata* Smith, as representatives of the two related genera comprising the apid subfamily. Understandably, most studies of bee larvae have centered on

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the mature last larval instar. That instar is the largest and therefore most easily examined and distinguished from earlier stages since all immature bees defecate only as last larval instars. Hence, feces in a cell or even in the larval proctodaeum identify the larva as the last larval instar. Also, this stage has the greatest longevity of any instar (in part because it is the usual stage during which diapause takes place), so it is most likely that collected larvae will be in this stage. By comparison, there have been comparatively few studies examining earlier larval stages, except perhaps for cleptoparasitic bees in which the first or even later instars are indicative of hospicidal activities.

This investigation was initiated not only to present formal descriptions of the mature larvae of these two species as exemplars of their genera, but importantly also to explore the differences and similarities of all larval stages by examining the variation between the instars within each of the species as well as by comparing the same instar of each species. It was well known beforehand that last larval instars of *Centris* spin cocoons whereas those of *Epicharis* do not (Rozen, 1965), and their last larval instars have distinctive differences in their labiomaxillary regions indicative of whether they are, or are not, cocoon producers (Rozen, 1965; Camargo et al., 1975). Mature *Centris* larvae exhibit features that enable them to produce and apply silk to the brood chamber wall: labiomaxillary regions with elongate palpi and strongly produced, projecting labium and wide salivary lips, and conspicuous articulating arms of stipes enabling pronounced prementum to be thrust forward while silk is being extruded. The elongate palpi possibly help in the placement of silk. In contrast, mature larvae of *Epicharis* larvae have labiomaxillary region with much shorter palpi, labium less strongly produced, a narrower salivary opening lacking projecting lips, and scarcely discernable articulating arms of stipes.

Following the descriptions of the last larval instar of *C. bicornuta* and *E. albofasciata*, the anatomical features of the earlier instars are explored. This is made possible by the extensive collections of the immature stages of these two species currently held at the American Museum of Natural History. Because these collections also included eggs, egg anatomies are also recorded.

Nests and nesting biology of *Centris bicornuta* have been described in some detail along with nests of other cavity-nesting *Centris* by Vinson et al. (2010). However, the nesting biology of *Epicharis albofasciata* had gone unreported until the start of this investigation. Examining the specimens brought to mind notes prepared by me and my late wife, Barbara L. Rozen, after we examined a substantial nesting aggregation near Hollis Reservoir on Trinidad in 1968. These notes have now been expanded, and reinterpreted considering recent studies of related groups (Rozen, 2016).

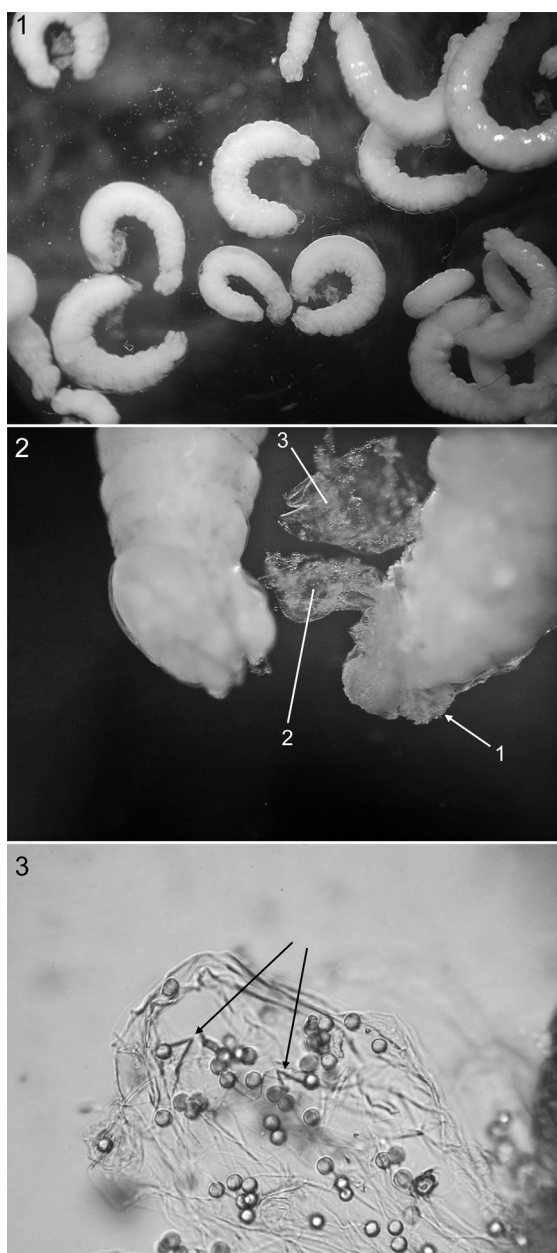
When this manuscript was initially drafted, the genera *Centris* and *Epicharis* were considered to comprise the tribe Centridini in accordance with Michener (2007). When that version of the manuscript was submitted for publication, one of the anonymous reviewers kindly pointed out that Martins and Melo (2016) through a detailed molecular study had recently announced that this version of the tribe Centridini was paraphyletic in relation to the corbiculate bees. Thus, there currently exist two interpretations of the phylogeny of this part of the Apidae. In the Discussion section below some observations are presented that bear upon this matter.

METHODS AND ANALYSIS

This investigation is based upon a recent mass collection of specimens of all stages of *C. bicornuta* from Costa Rica and the above-mentioned collection of *E. albofasciata* from Trinidad. Specimens of *C. bicornuta* were the first to be examined. In attempting to understand the number of larval instars, both eggs and larvae still partly encased in chorion were set aside. The remaining larvae were roughly sorted by head width and formed four discrete groups on the basis of size. Those with the largest heads varied greatly in body size and obviously represented the last larval instar. This suggested that the three remaining larval groups represented the first three larval instars, thus indicating that this species had a total of four larval instars. This conflicted with the author's recent studies (e.g., Rozen et al., 2006; Rozen et al., 2010; Rozen, 2011, Rozen and Hall, 2011, 2012) reporting that the Apoidea have a total of five larval stages.

This problem started to resolve under close examination of a specimen of *C. bicornuta* (fig. 2) from the group with a larger head size compared with individuals from the other two groups, making it to be the presumed third instar. Adhering to the outside of the posterior venter of this specimen were the cast exuviae of two earlier instars, each with paired, pigmented, sclerotized mandibles (presumed to be the remains of the second and first instars). In addition, an amorphous, nonsclerotized, and unpigmented mass of exuviae also clung. After careful removal and separation of the three exuviae, the sclerotized conditions of the mandibles and head carinae on two of them became clear, and on the third mass two acute though nonsclerotized points (fig. 3) were discernible: might these be mandibular remnants of the true first instar, which would identify the two other cast exuviae as the second and third instars? The author then remembered an earlier study (Rozen, et al., 2011: 9–10) on the ground-nesting, Costa Rican *Centris* (*Centris*) *flavofasciata* Friese in which it had been clearly documented that during hatching from the egg, the chorion splits along the spiracular line on each side of the first instar and then the first instar sheds its exoskeleton. Thus, its exuviae and remnants of the chorion were simultaneously slipped off to expose the second instar. The lateral splitting of the chorion along the sides of the body is presumably caused by the swelling of the body due to ingested amniotic fluid as well as fluid from the surface of the food mass through a small opening created at the front of the egg chorion. It is hypothesized that this swelling forces a row of fine, sharp spicules that runs just above the spiracles along the two sides of the first instar to rupture the chorion. These spicules do not occur on the second instar, suggesting they are a signature of the first instar. Thus, the duration of the first instar is extremely brief, mostly pharate within the chorion, and the larva's growth negligible since no pollen has been ingested. Consequently, the first instar is easily overlooked. As Rozen et al. (2011) pointed out, similar observations were made for a species in the Eucerini (Rozen, 1964) and another in the Tapinotaspidini (Rozen et al., 2006) (both Apidae).

Unsuccessful attempts were made to find the row of spicules on the specimen of *C. bicornuta* first examined. However, on another specimen from which the chorion had slipped away from the first instar's cast exoskeleton, the row of sharply pointed spicules was visible (figs. 4, 5), supporting the hypothesis that, within at least some non-cleptoparasitic Apidae, **first instars exist only briefly, do not feed on pollen, and therefore do not grow in size, but serve as a staging vehicle for the emergence of the active, feeding, and consequently growing second instar.**



FIGURES 1–3. Fourth instars of *Centris bicornuta*. 1. Numerous individuals demonstrating strongly curved shape in lateral view. 2. Close-up of fourth instar with cast exuviae of three earlier instars (arrows 1, 2, 3). 3. Cast exuviae of first instar removed from fourth instar showing (arrows) pointed apices of mandibles.

After the examination of the extensive material of *C. bicornuta*, the collection of *E. albofasciata* was similarly studied with the sorting of material based on head width as described above. Again four categories were identified. This then led to a study of field notes and sketches on the nesting biology of this species drafted at the time of collection in 1968 and now prepared as a manuscript (Rozen, 2016) that provisionally concludes that *E. albofasciata* also has five larval instars, the first of which is pharate within the chorion and does not increase in size. This conclusion has recently been supported by Gaglianone et al. (2015: 406), who reported that for several other species of *Epicharis*, the cast exuviae of the first instar “(evident due to spiracles and spicules)” was identified in the chorion while the hatched larva was the second instar.

Table 1 represents the head widths in mm of the larval instars of *C. bicornuta* and *E. albofasciata*. Averages refer to all specimens examined. Those specimens came from a sampling of specimens that were estimated to represent the maximum size range within each instar. Head widths of first instars of *C. bicornuta* could not be measured since none was exposed. However, the widths of the broadest part of the anterior end (where the embryonic head is at its maximum width) of seven eggs were measured giving an average of 0.5 mm and a range of 0.45–0.55 mm, probably sufficiently close to the average and range of the second instar of the species to further support the conclusion the first instar is indeed pharate, nonfeeding, and therefore nongrowing (see table 1). Similarly, in the case of *E. albofasciata* the anterior end of four eggs measured 0.71, with a range of 0.7–0.75, which overlapped the head width of the second instar (see table 1).

TABLE 1. Comparison of maximum head widths (mm) of larval instars of *Centris bicornuta* and *Epicharis albofasciata*.

Instar	<i>Centris bicornuta</i>			<i>Epicharis albofasciata</i>		
	Average	Range	Total Data	Average	Range	Total Data
5th	1.625	1.45–1.7	(9)	1.748	1.625–1.825	(6)
4th	1.1625	1.075–1.25	(6)	1.3393	1.275–1.4	(7)
3rd	0.8188	0.8–0.85	(4)	1.025	1.0–1.075	(6)
2nd	0.5464	0.5–0.6	(7)	0.7510	0.725–0.775	(8)
1st ^a	–	–	–	–	–	–

^a Pharate in chorion; see discussion of table in Methods and Analysis.

Below, the mature larvae *C. bicornuta* and *E. albofasciata* are formally described. Afterwards, a detailed comparison of the other larval stages of these two taxa is presented, followed by the formal descriptions of the eggs of these two taxa. At the end there is a brief Discussion.

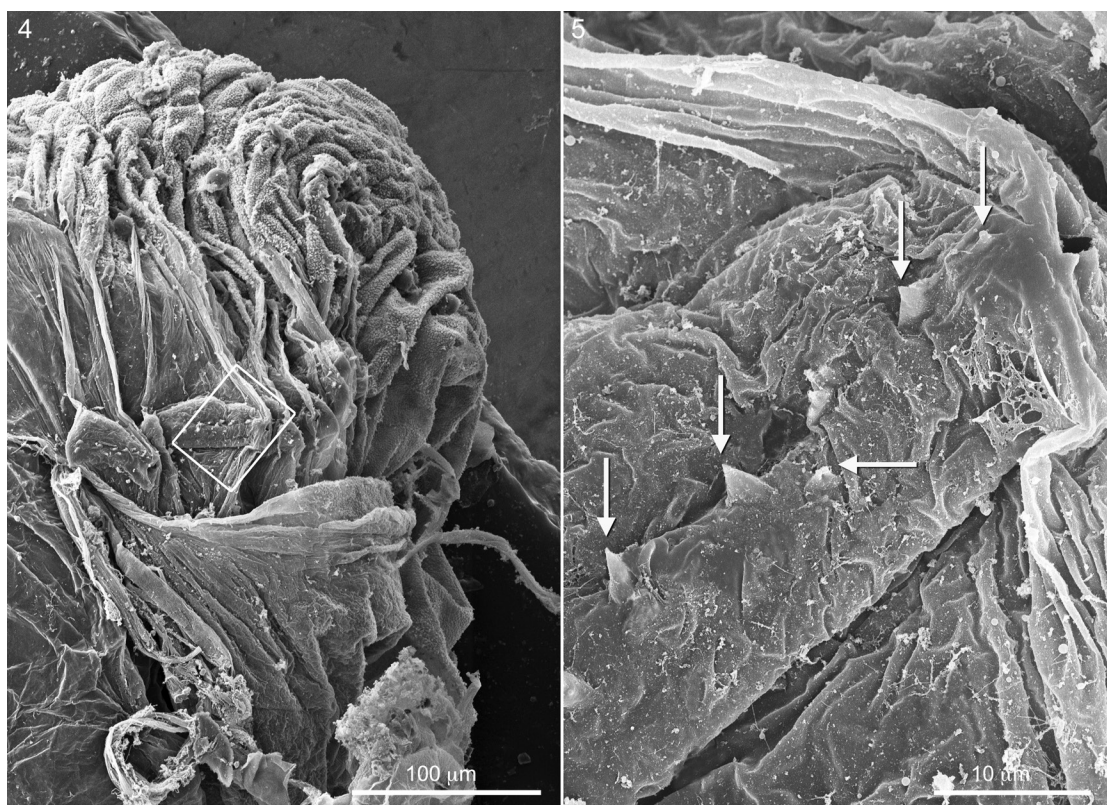
FIFTH LARVAL INSTAR OF *CENTRIS (HETEROCENTRIS) BICORNUTA* MOCSÁRY

Figures 6–13, 20, 21

DIAGNOSIS: Features relating to cocoon production presented in the Introduction will separate all mature *Centris* larvae from those of *Epicharis*. Contrasting the mature larva of *C. bicornuta* with that of *E. albofasciata* one can see that the labrum of *C. bicornuta* (fig. 11) is produced both apically and ventrally compared with *E. albofasciata* (fig. 19), and on the same figures, the antennal papilla of *C. bicornuta* is elongate, unlike that of *E. albofasciata*. While the postdefecating larvae of the two species tend to be elongate (figs. 6, 16), only the larva of *E. albofasciata*, exhibit the dorsolateral linear row of spines on many body segments, a feature also seen on the predefecating form (fig. 17). Whereas spiracular atria are extensively armed with spines and the primary tracheal opening guarded by collar spines in *E. albofasciata* (figs. 22, 23), no such heavy ornamentation is visible in spiracles of *C. bicornuta* (figs. 20, 21).

The distinction of larval *C. bicornuta* relative to previously described mature larvae of *Centris* may be relevant here. The earliest larval descriptions of the genus *Centris* involved four species (Rozen 1965) with the first having the name of *C. (Hemisiella) lanipes* (Fabricius) based on specimens given to C.D. Michener by J.S. Moure and on which Michener and Lange (1958) described its nesting biology. Moure+ (in litt., June 28, 1966) informed J.G.R. that the correct name for those specimens was *C. tarsata* Smith, but the species is now known as *Centris (Hemisiella) trigonoides* Lepeletier (Ascher and Pickering, 2016).² The described larvae of that species were predefecating, but their postdefecating form might well have been similar to the more linear form like that of *C. bicornuta* (fig. 6). Certainly, the large, well-rounded, uniform teeth along the dorsal apical edge of the scoop-shaped apical mandibular concavity (Rozen, 1965: figs. 40, 41) of *C. tarsata* sets this larva apart from all others treated here. Larvae of *C. (C.) aenea* Lepeletier, *C. (Ptilotopus) derasa* Lepeletier, and *C. (Melacentris) rufosuffusa* Cockrell are robust forms, and therefore distinct from the more slender and linear larva of *C. bicornuta*.

² This species is referred to subgenus *Heterocentris* in Michener (2007).



FIGURES 4, 5. SEM micrographs of cast exuviae of the abdomen of the first instar of *Centris bicornuta* on which were found fine, sharply pointed spicules extending as a row across body segments just above spiracular line, and rectangular area of figure 4, enlarged to show spicules (arrows) and spiracle, respectively.

In addition to the larval treatment of those four species, descriptions of mature larvae of the ground-nesting *Centris* (*Paracentris*) *caesalpiniae* Cockerell and *C. (Paracentris) pallida* Fox were published more recently (Rozen and Buchmann, 1990). Despite numerous similarities between *C. bicornuta* and these other two species mentioned in the following description, the smaller size (see scale bar, fig. 6) as well as the slenderer body form of *C. bicornuta* will distinguish it from both *C. caesalpiniae* and *C. pallida* (Rozen and Buchmann, 1990: figs. 43, 49).

The following account is based on both postdefecating and predefecating larvae of *C. bicornuta*.

HEAD (figs. 10, 11, 14, 15): Integument with scattered short setiform sensilla especially abundant on labral apex; dorsal surface of maxilla, lateral epipharyngeal surface, and hypopharynx densely spiculate with sharp-pointed spicules. Mandibles and mandibular articulations moderately pigmented except mandibular apices deeply pigmented; articulation arms of stipital sclerites elongate, distinctly pigmented; remaining parts of head scarcely pigmented.

Head size moderate relative to body size; head capsule broad with summit of vertex flattened or even slightly depressed as seen in frontal view (fig. 10). Tentorium incomplete because of impending ecdysis; posterior tentorial pits normal in position; posterior thicken-

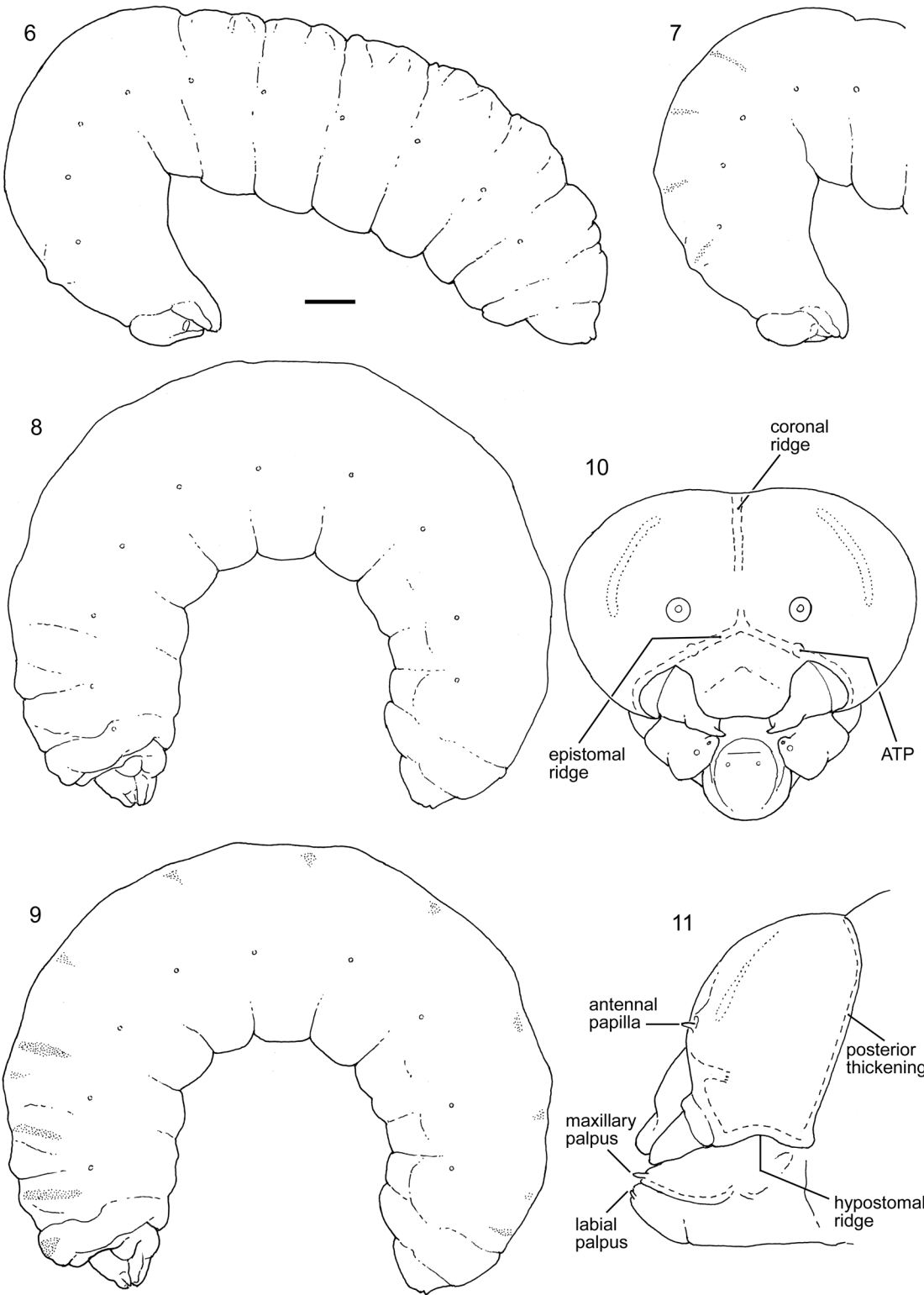
TABLE 2. Distribution of linear rows of spines on last larval instars of four species of *Epicharis*. Meta = metathorax; numbers refer to abdominal segment numbers; + indicates presence of sublateral row of at least two or more spines on each side of caudal annulet.

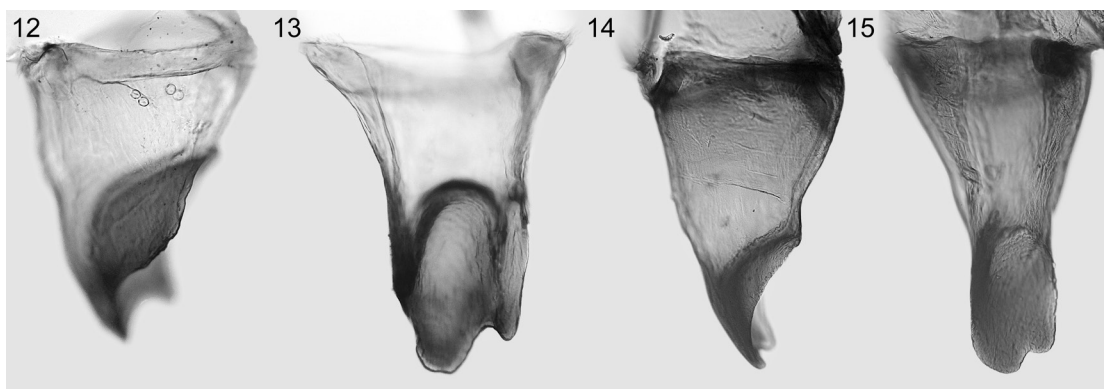
Species	Meta	1	2	3	4	5	6	7	8	9	10
<i>E. albofasciata</i>	+	+	+	+	+	+	+	+	+	+	0
<i>E. fasciata</i>	0	0	+	+	+	+	+	+	0	0	0
<i>E. picta</i>	+	+	+	+	+	+	+	+	+	+	0
<i>E. rustica</i>	0	+	+	+	+	+	+	+	+	+	+

ing of head capsule moderately well developed, only slightly bending forward medially as seen in dorsal view; coronal ridge pronounced on cleared head capsule though unpigmented, interrupted below but evident again just before intersection with median section of median epistomal ridge (fig. 10); hypostomal ridge well developed, without dorsal ramus. Parietal bands faint depressions, not illustrated. Antennal prominences weakly developed; antennal papilla small but projecting, somewhat longer than basal diameter, bearing three sensilla. Vertex evenly rounded in lateral view; frontoclypeal area not projecting beyond labrum; labral sclerite not evident; forward-directed labral tubercles absent; labral margin faintly bilobed in frontal view.

Mandible massive, apically short as seen in outer or inner views (fig. 13) and thus contrasting with that of *E. albofasciata* (fig. 15), with pronounced scoop-shaped apical concavity, apically rounded and bearing single subapical tooth as described for *C. caesalpiniae* (Rozen and Buchmann, 1990) except mandibular setae not on tubercles, dorsal edge of apical concavity roughened but lacking toothlike projections and apical concavity as seen in inner view tapering somewhat apically. Labiomaxillary region produced and not greatly fused; labium projecting somewhat beyond maxilla in lateral view. Maxillary apex not produced and bent mesad; cardo and stipes evident though not deeply pigmented; stipital rod deeply staining as does articulating arm of stipes; palpus apical, elongate, more than twice as long as apical diameter; galea elongate though distinctly shorter than maxillary palpus, at least as long as basal diameter bearing several elongate setae. Strongly projecting labium divided into prementum and postmentum, and bearing apically projecting lips of slitlike salivary opening; labial palpus with length more than twice basal diameter. Hypopharynx normal in size, bilobed, exceeded by labiomaxillary region; hypopharyngeal groove deeply impressed.

BODY: Because integumental patterning of setae, spicules, and integumental composition is complicated, larval form is here treated first. Postdefecating larva (fig. 6) elongate with thorax and first abdominal segment strongly bent in lateral view and intersegmental lines obscure; in contrast rest of abdomen strongly linear with intersegmental constrictions obvious and with cephalic and caudal annulets identifiable from abdominal segments 3–8; body contour of both post- and predefecating larvae without elevated pigmented dorsal tubercle. Predefecating larva (fig. 8) also elongate, usually continuously curved, but with dorsal intersegmental constrictions reduced, as are intrasegmental boundaries separating caudal and cephalic annulets giving dorsal surface of larva smoother appearance compared with postdefecating form. After clearing and staining with Chlorazol Black E, thoracic segments and abdominal segment 1 of both post- and predefecating forms exhibit distinctive staining patterns (figs. 7, 9); pair of transversely linear, darkly stained streaks





FIGURES 12–15. Microphotographs of right mandibles: **12, 13.** *Centris bicornuta*; **14, 15.** *Epicharis albofasciata*, in maximum dorsal view and inner view, respectively.

positioned toward posterior margin of each thoracic segment and abdominal segment; these streaks clearly on low elevations and easily visible on predefecating larva and certainly positioned on caudal annulets of mesothorax and abdominal segment 1; similar paired streak on abdominal segment 8 toward the rear of the segment. Darker staining indicating that the integument has increased thickness but is not naturally pigmented nor different in composition from integument elsewhere. In addition to this dark-staining pattern, abdominal segments 1–8 with faint but distinct pattern of paired patches occupying low, paired swelling on dorsal surface of cephalic annulets; each patch roughly as long as wide and this surface finely, closely spiculate. Spiracle of postdefecating larva with wide atrium with slightly projecting rim, strongly tapering to primary tracheal opening, with walls distinctly concentrically ringed (figs. 20, 21) (but making peritreme difficult to detect); subatrium short (figs. 20, 21); in postdefecating larva flexure mostly collapsed (fig. 20); in predefecating larva, flexure strongly annulated with some rings swollen (fig. 21).

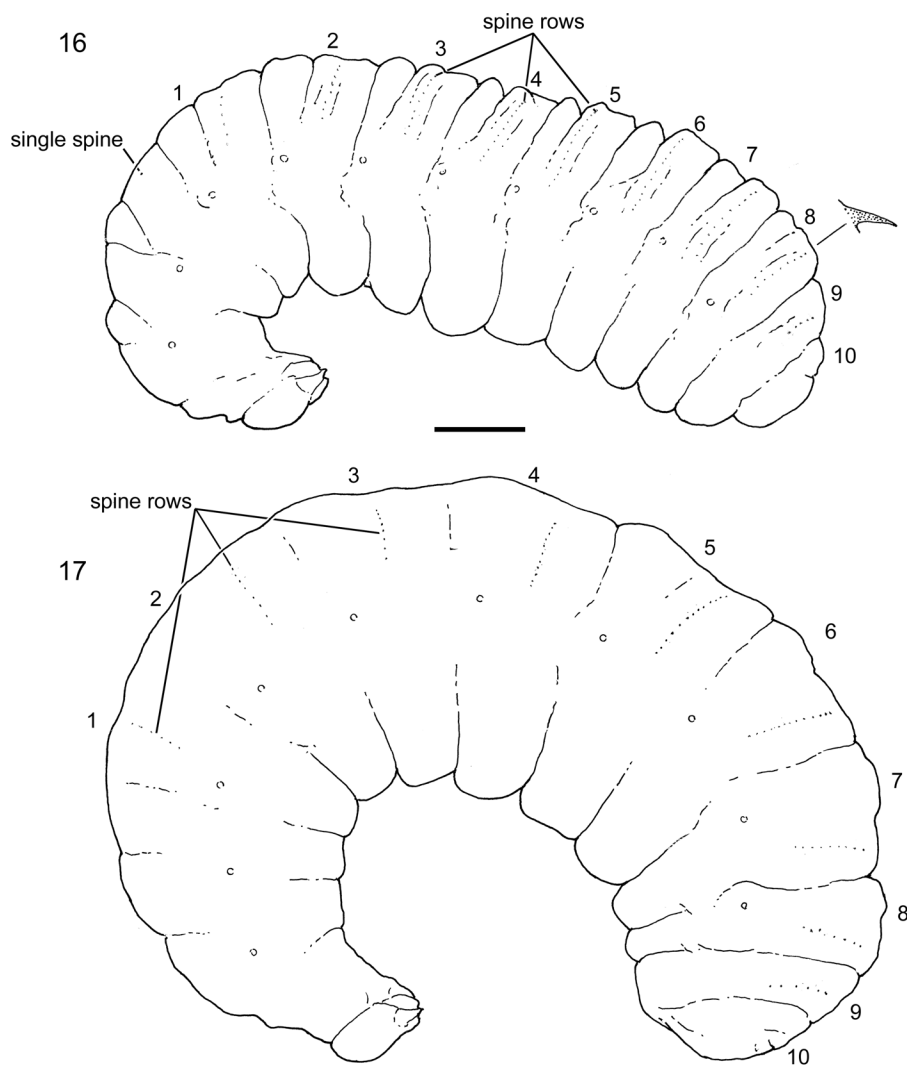
MATERIAL STUDIED: Four postdefecating larvae: **Costa Rica:** Guanacaste Prov., ONR, II-5-2009 (Frankie Group). Seven postdefecating larvae: **Costa Rica:** Guanacaste Prov.: 9 km NW Bagaces: Hacienda Monte Verde, I-30-2009 (Frankie Group). Four predefecating larvae: **Costa Rica:** Guanacaste Prov.: 9 km NW Bagaces, II-17-2009 (Frankie Group). Seven fourth larval instars: **Costa Rica:** Guanacaste Prov.: 9 km NW Bagaces, II-17-2009 (Frankie Group).

FIFTH LARVAL INSTAR OF *EPICHARIS* (*EPICHAROIDES*) *ALBOFASCIATA* SMITH

Figures 16–19, 22, 23

DIAGNOSIS: For generic distinction and contrasting larval features of *E. albofasciata* and *C. bicornuta*, see Diagnosis of *Centris bicornuta*.

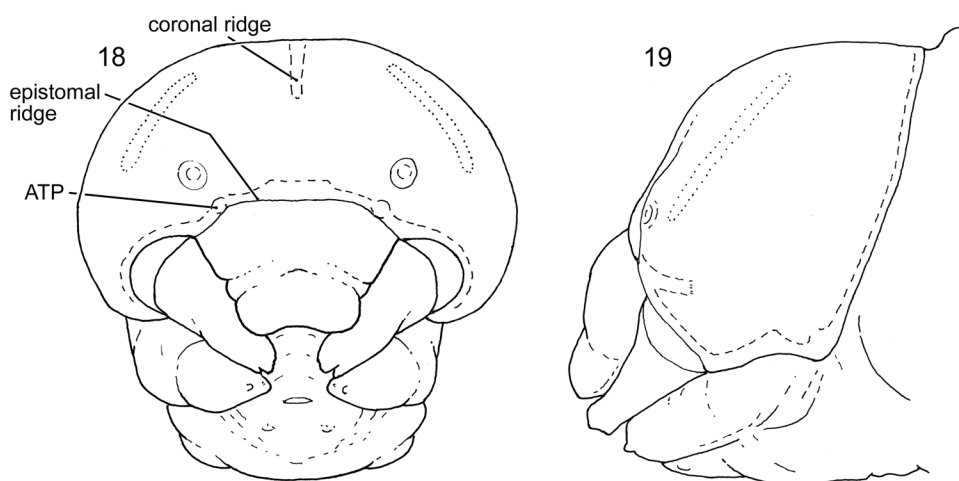
FIGURES 6–11. Mature larva of *Centris bicornuta*. **6.** Entire postdefecating larva, lateral view. **7.** Same except showing only anterior part of body, with stippled areas indicating staining pattern there when treated with Chlorazol Black E; abdominal segment 8 (not shown) with similar stain streak. **8.** Entire predefecating larva, lateral view. **9.** Same, with stippled areas indicating staining pattern when treated with Chlorazol Black E. **10, 11.** Head, frontal and lateral views, respectively. Scale line (figs. 6–9) = 1.0 mm.



FIGURES 16, 17. Mature larvae of *Epicharis albofasciata*, lateral view, to same scale; scale line = 1.0 mm. **16.** Postdefecating larva **17.** Predefecating larva.

Few species of larval *Epicharis* have been collected and only those of *Epicharis* (*Hoplepicharis*) *fasciata* Lepeletier and Serville, *Epicharis* (*Epicharana*) *rustica* (Olivier) have been described (Rozen, 1965). In addition, predefecating specimens of *Epicharis* (*Epicharoides*) *picta* (Smith) (Brazil: Minas Gerais: Viçosa, Feb. 2012) were available, thanks to W.A. Werneck, who collected them. Although there appears to be little specific variability in the genus, most species can be differentiated based on the distributions and patterning of the sublateral linear rows of pigmented spines on the caudal annulets of certain body segments as shown in table 2.

In addition to the presence or absence of spines indicated in table 2, some species have larger and darker spines on certain segments. Furthermore, intensity of pigmentation varies as to whether a larva is a pre- or postdefecating form; the older the larva, the darker the spines.



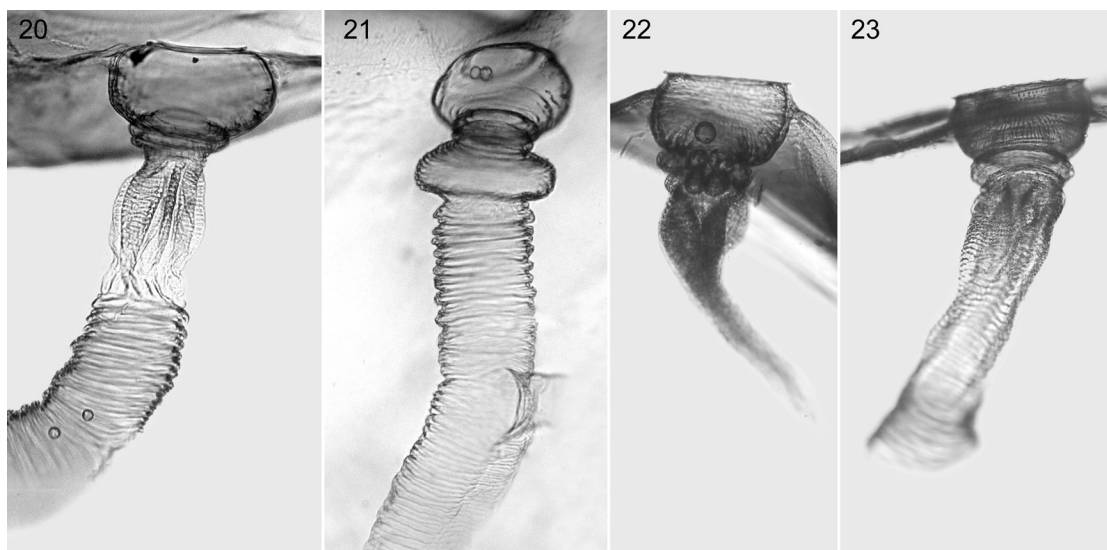
FIGURES 18, 19. Head of mature larva of *Epicharis albofasciata*, frontal and lateral views.

All species in the genus known so far possess these spines, which presumably assists them in activities within the brood cell. Since no earlier instar of any of them has these spines, their functions obviously relate only to the last instar, perhaps in some way to accommodate their large body size.

DESCRIPTION: Head (figs. 18, 19): Vestiture about as described for *C. bicornuta* except speculation, particularly of postmentum, more pronounced. Pigmentation pattern as described for *C. bicornuta*, but all parts of integument faintly pigmented.

Head size moderately small relative to body size, especially apparent on predefecating form; head capsule broad with summit of vertex forming even curve as seen in frontal view (fig. 18). Tentorium incomplete on postdefecating form because of impending ecdysis, but complete and well developed on predefecating form; posterior tentorial pits normal in position; posterior thickening of head capsule well developed, not bending forward medially as seen in dorsal view; coronal ridge pronounced on cleared head capsule though unpigmented, fading out about halfway between vertex and midsection of epistomal ridge (fig. 18); hypostomal ridge well developed, without well-developed dorsal ramus. Parietal bands faint depressions. Antennal prominences not developed; antennal papilla not projecting, appearing nearly as flat disc, bearing three sensilla. Vertex evenly rounded in lateral view; frontoclypeal area not projecting beyond labrum; labral sclerite not evident; forward-directed labral tubercles absent; labral margin faintly bilobed in frontal view.

Mandible massive, apically elongate as seen in outer or inner views (fig. 15) thus contrasting with *C. bicornuta* (fig. 13), with pronounced scoop-shaped apical concavity, apically rounded and bearing single subapical tooth; mandibular setae numbering about six on outer surface, not on tubercles; dorsal edge of apical concavity roughened but lacking toothlike projections and apical concavity as seen in inner view tapering somewhat apically. Labiomaxillary region produced and not greatly fused; labial apex exceeding maxillary apex in lateral view. Maxillary apex neither produced nor bent mesad; cardo and stipes evident though not deeply



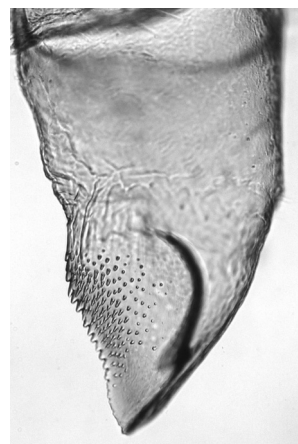
FIGURES 20–23. Microphotographs of spiracles of postdefecating and predefecating larvae: **20, 21.** *Centris bicornuta*; **22, 23.** *Epicharis albofasciata*, respectively.

pigmented; stipital rod deeply staining as does articulating arm of stipes; palpal length less than basal diameter; projecting galea not evident although inner maxillary angle truncate, densely spiculate. Labium divided into prementum and postmentum, bearing slitlike salivary opening overhung by projecting dorsal surface of labium; labial palpus only faintly projecting. Hypopharynx normal in size, bilobed, barely exceeded by labiomaxillary region; hypopharyngeal groove deeply impressed.

BODY: Body surface extensively covered with uniformly fine spicules; setal and spine distribution treated below. Postdefecating larva (fig. 16) elongate with thorax and first abdominal segment strongly bent in lateral view as described for *Centris bicornuta*, but unlike that taxon, intersegmental lines as well as lines separating annulets incised, quite distinct (fig. 16); abdomen linear, rather robust, with intersegmental constrictions as well as separation of cephalic and caudal annulets strongly expressed (fig. 16). On all abdominal segments except for 10, caudal annulets bearing paired, elevated, elongate, transverse ridges, each bearing row of darkly pigmented, posteriorly curved spines; these ridges and their spines positioned sublaterally on each segment from level of spiracles dorsally and fade out well before dorsal midline; spines on abdominal segments 9 and 8, most pronounced and often posteriorly curved as indicated (fig. 16, enlarged spine); spines on preceding segments becoming increasingly smaller; thoracic segment 3 with single pair of spines. Predefecating larva (fig. 17) also elongate, usually continuously curved in lateral view, and with abdominal segments swollen, so that intersegmental boundaries appearing weakly expressed dorsally and annulation boundaries and transverse ridges mostly nonapparent, giving dorsal surface of larva smoother appearance compared with postdefecating form; sublateral transverse rows of spines present, but tending not to be as darkly pigmented as spines on postdefecating form. After clearing and staining with Chlorazol Black E, thoracic segments and abdominal segment 1 of both post- and predefecating forms

exhibiting little distinctive staining pattern although on predefecating larva apices of sublateral tubercles identified by very narrow, slightly darkened band. Body setae of mature larvae consisting of sparse, fine setae restricted to summits of sublateral ridges on caudal annulets of most abdominal segments; these setae transparent contrasting with darkly pigmented and thicker spines with which they are intermixed. Spiracle of postdefecating larva with wide, shallowly urn-shaped atrium wider than deep, slightly projecting beyond body wall, and with distinct rim; atrial wall concentrically ringed with spiculate ridges (fig. 22); peritreme also pigmented, narrow, so that spiracular opening wide, distinctly wider than primary tracheal opening; subatrium short, about same length as depth of atrium; flexure (as defined by Rozen, in press) strongly collapsed; spiracle of predefecating larval (fig. 23) showing long uncollapsed flexure connected to trachea.

MATERIAL STUDIED: More than 10 pre- and postdefecating larvae: **Trinidad:** Hollis Reservoir, III-10-1968 (F.D. Bennett). More than 50 postdefecating and 50 predefecating larvae: **West Indies:** Trinidad: Hollis Reservoir near Valencia, various dates 1968 (J.G. and B.L. Rozen).



FIGURES 24. Microphotograph of mandible of fourth instar of *Epicharis albofasciata*, ventral view, showing denticles on inner surface of apical concavity.

EARLY INSTARS OF *CENTRIS BICORNUTA* AND *EPICHARIS ALBOFASCIATA*

Early instars, though not the first, of *C. bicornuta* tend to be strongly curved in lateral view (fig. 19), so that in some cases they are circular in outline, with the head occasionally overlapping abdominal segment 10. In *E. albofasciata* the body is only moderately curved during the first three stadia while the fourth instar tends to be semicircular. Since these are the postures of larvae killed and preserved in Kahle's solution, they are not mechanically constrained by cell shape, although their shapes may be adaptive to cell shape and feeding along the circumference of the sloping surface of the provision mound.

With both species cuticular pigmentation is reduced in all but the last larval instar. However, both the pigmentation and sclerotization of the mandible is evident on all but the first instar. Although overall cranial pigmentation is primarily seen in the last instar of *E. albofasciata*, it is slightly discernible in the fourth instar but not at all in any instar of *C. bicornuta*. However, it is of considerable interest to note that the presence of internal head ridges, while generally not strongly pigmented remain evident on all instars (except unknown for the first instar) when specimens are cleared and stained. These ridges (as identified on head capsules of the last larval instars, figs. 5, 6, 13, 14) include the entire epistomal ridge between and laterad of the anterior tentorial pits, hypostomal ridge, posterior thickening of the head capsule, and the coronal ridge from the posterior thickening to halfway to the median epistomal ridge. Considering the variation among bee larvae in the length and presence of the coronal ridge

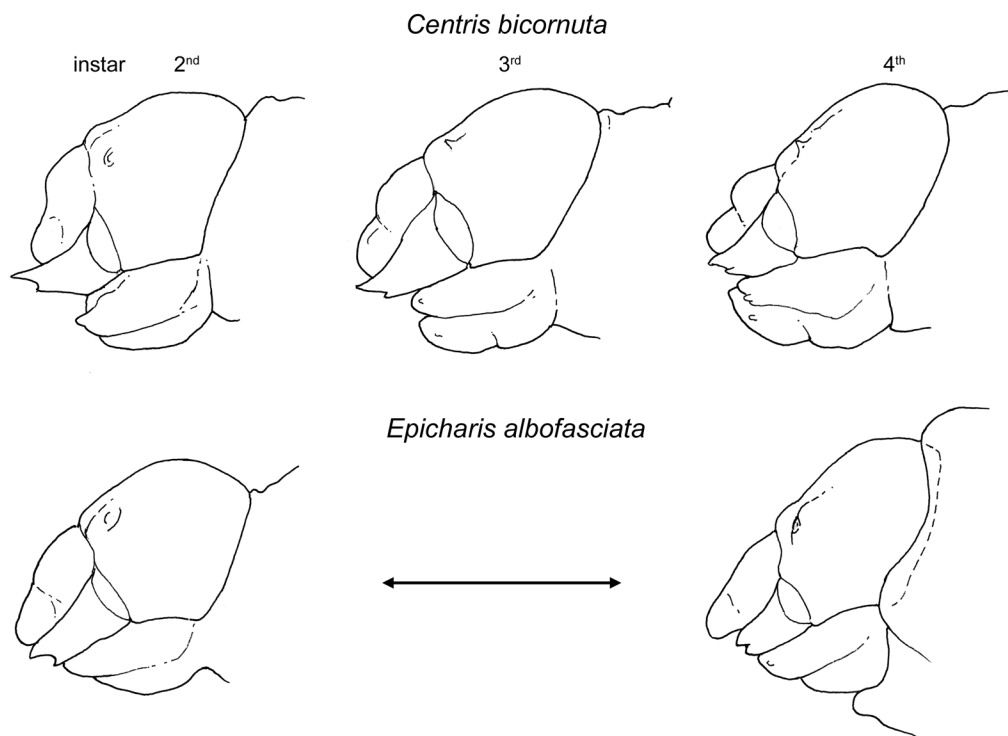


FIGURE 25. Diagrammatic lateral views of heads of instars 2–4 of *Centris bicornuta* and instars 2 and 4 of *Epicharis albofasciata* drawn to approximate same size demonstrating changes in shape through developmental stages.

and in the presence of the median portion of the epistomal ridge, uniform presence of these features in the centridine larvae seems to suggest a close relationship of the two included genera.

The pronounced antennal papilla of the second and third larval instars of *C. bicornuta* are not especially elongate, but that of the fourth instar projects about as far as its basal diameter, thus approaching the condition of the last larval instar. With *E. albofasciata*, the second through fourth instars exhibit weakly defined antennae in general with papillae that are nonelevated and therefore similar to that of the last larval instar.

The mandible of *C. bicornuta* changes little beyond size from the second to fourth instar. The mandible is characterized by having two sharply pointed apical teeth and a long apical concavity that becomes progressively more pronounced with each molt. In addition, there is a faint patterning of fine denticles along the dorsal apical edge of the dorsal apical tooth. The postdefecating larva is dissimilar in that the apical teeth become more rounded, probably due in large part to wear in that those of the predefecating form are distinctly more sharply pointed than those of the postdefecating form. However, during the final larval stadium, the denticles along the dorsal apical edge are absent. The difference in the two forms also involves an increase in apical mandibular pigmentation. Similar phenomena occur in the mandible of *E. albofasciata*. However, there is also a more unusual change in *E. albofasciata*: the second and third

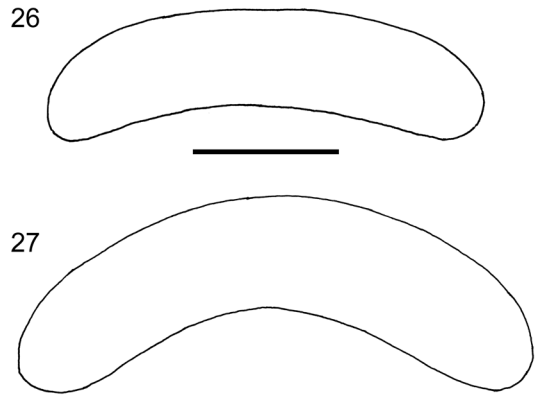
instars are characterized by having sharp denticles that extend along the dorsal apical edge of the concavity and then into the inner surface of the concavity until they fade out at the middle of the concavity (fig. 24). How these denticles function is unknown, although their position clearly suggests that they are involved with food ingestion.

Not surprisingly the labiomaxillary regions of *C. bicornuta* and *E. albofasciata* undergo a major change in appearance through development. In early instars they tend to be similar, but the changes that take place that accommodate cocoon production in the last instar of *C. bicornuta* create a greater change in that species compared with *E. albofasciata*, as demonstrated in figure 25 and illustrations of the last larval instar of each species (fig. 11 and fig. 19). This is evident in lateral profile when comparing the head of second instar *C. bicornuta* with that of *E. albofasciata* (fig. 25). Furthermore, the apices of the labium are positioned nearly identically during the early instar stages, but when comparing the same structures of last larval instars, the labium of *C. bicornuta* is seen to project far forward (fig. 11) in contrast with that of *E. albofasciata* (fig. 19). The change in *C. bicornuta* enables the larva of the species to thrust the salivary opening forward to apply silk to the cell wall with its apically positioned salivary gland opening.

The maxillary and labial palpi of the fourth instar of *C. bicornuta* are slightly greater in length than their respective fifth instar lengths, and they taper apically their entire length. The galea of this instar is a clearly evident, small, tapering projection, obviously smaller than the maxillary palpus. The salivary opening is extremely broad (i.e., distinctly greater in width than half the width of labial apex on cleared specimen), with lips questionably faintly expressed. In the second and third instars the width of the salivary opening is clearly broader than half the width of the prementum. When viewed laterally (fig. 25) the labiomaxillary regions of instars 2–4 of *C. bicornuta* increase in size relative to the head capsule size of the instar whereas there seems less change in relative size of the labiomaxillary regions of the instars of *E. albofasciata*.

Considering the reduced maxillary and labial palpi of the fifth instar of *E. albofasciata*, their near absence as projecting structures in earlier stages is not surprising. Although the salivary opening is transverse, it is about one-third of the maximum width of the prementum, comparatively shorter than that of *C. bicornuta* in all instars. Interestingly, the second instar of *E. albofasciata* exhibits a somewhat elongate maxillary apex that appears to bend toward the median line of the head capsule.

Although the spiracular atrium of second instar spiracles of *C. bicornuta* is only questionably ornamented, those of the third and fourth instars are certainly somewhat concentrically



FIGURES 26, 27. Diagrams of eggs of *Centris bicornuta* (above) and *Epicharis albofasciata* (below), lateral view, front end to left, to same scale. Scale line = 1.0 mm.

ringed, but all entirely lack spiculation. On the other hand, second to fifth larval instars of *E. albofasciata* are clearly spiculate, with those of the final two instars most strongly so.

In conclusion, the larval instars of these two representative species of *Centris* and *Epicharis* are quite different from one another as documented in the descriptions of the last larval instars. However, the development of each through its individual stages appears as a gradual process.

MATERIAL EXAMINED: *Centris bicornuta*: Five second instars; ca. 25 third instars; ca. 25 fourth instars: **Costa Rica**: Guanacaste Prov.: 9 km NW Bagaces, II-17-2009 (Frankie Group).

Epicharis albofasciata: Thirty-eight second instars, 21 third instars, and 15 fourth instars: **West Indies**: Trinidad: Hollis Reservoir near Valencia, various dates 1968 (J.G.R. and B.L. Rozen).

EGG OF *CENTRIS* (*HETEROCENTRIS*) *BICORNUTA* MOCSÁRY

Figures 26, 28, 29

DIAGNOSIS: Eggs of *C. bicornuta* and *E. albofasciata* are similar in shape, color, gross structure, and overlap in size, but are easily distinguishable by differences in chorionic microstructure. That of *C. bicornuta* consists of a pebbled surface (fig. 29), and that of *E. albofasciata*, an elevated latticework (fig. 30). Furthermore, the micropyle of *C. bicornuta* (figs. 28, 29) is surrounded by a radiating network of low elevated ridges that are absent in *E. albofasciata* (fig. 30).

DESCRIPTION: Most specimens cream color, but several pale bluish (presumably a postmortem change). Chorion smooth, uniform but nonreflective as seen by stereomicroscope, but strongly, uniformly pebbled in SEM examination (fig. 29). Shape (fig. 26) gently curved, elongate, tending to be parallel sided, but some slightly larger at posterior end; rounded at both ends; polarity not evident without SEM examination or embryo orientation. Length 2.9–3.4 mm; maximum diameter 0.6–0.7 mm. Micropyle evident only on SEM examination, found at anterior end associated with an elevated network of ridges leading to dense cluster of micropylar pores (figs. 28, 29).

MATERIAL STUDIED: Nineteen eggs: **Costa Rica**: Guanacaste Prov.: 9 km NW Bagaces, II-17-2009 (Frankie Group).

EGG OF *EPICHARIS* (*EPICHAROIDES*) *ALBOFASCIATA* SMITH

Figures 27, 30

DIAGNOSIS: See Diagnosis of egg of *C. bicornuta*, above.

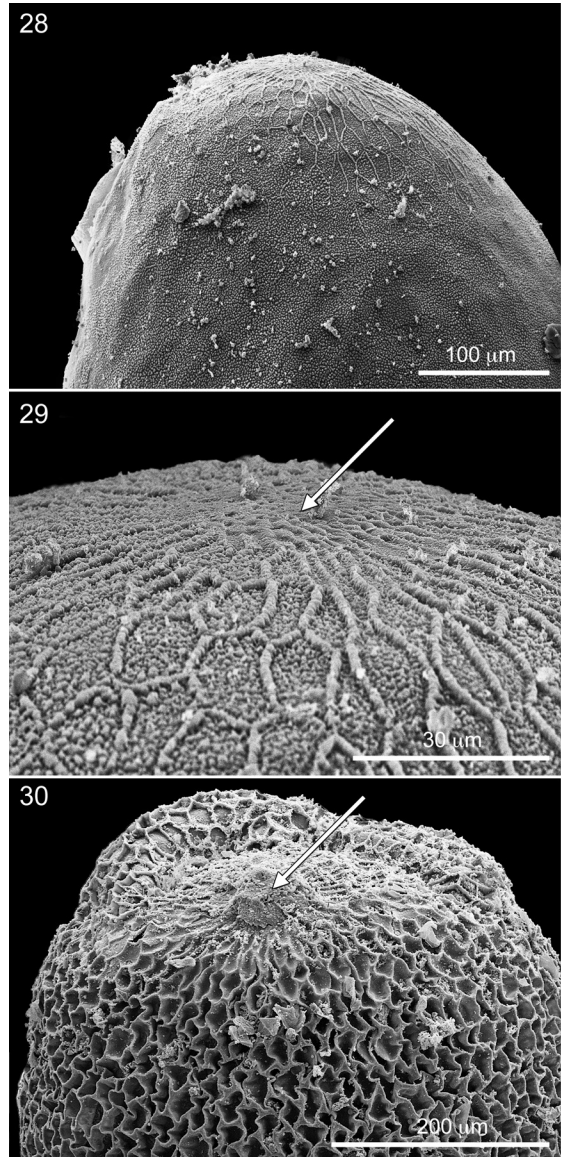
DESCRIPTION: Specimens uniformly pale cream color. Chorion minutely reticulate at least at ends; under SEM, chorion strongly reticulate with pronounced elevated boundaries (fig. 30). Overall shape of egg elongate, gently curved, tending to be parallel sided (fig. 27), but some slightly swollen at one end; rounded at both ends. Length 2.8–3.5 mm; maximum diameter 0.7–0.9 mm (sample of 10 of 31). Micropyle not evident without SEM examination, but with SEM micropylar area identified with small mound surrounded by converging reticular boundaries (fig. 30); micropyle openings obscure; egg polarity not evident without SEM examination or embryo orientation.

MATERIAL STUDIED: 31 eggs: **Trinidad**: Hollis Reservoir near Valencia, III-2-1968 (J.G.R. and B.L. Rozen).

DISCUSSION

The number of larval instars reported for bees has been variable, usually four or five ((Michener, 2007). Here it is reported that within at least the Apidae, certain species of the Centridini, Tapinotaspidini, and probably Eucerini have five larval instars, although the first instar is mostly pharate within its chorion; this stage is not one of feeding and growing, but serves as a vehicle for the emergence of the second instar, which is the first stage during which the larva actively feeds on provisions, leading to growth in size. Investigations by others (e.g., Stephen and Koontz, 1973) have convincingly confirmed that other apid tribes also display five larval instars. Special attention will need to be directed to the cleptoparasitic taxa since first instars of many have been repeatedly identified as active, host-killing, nonpharate creatures that are fully emerged from their chorions and that move about in their cells until they encounter and kill eggs or larvae of their host bees. So far, only in the Megachilidae do we know of sedentary cleptoparasites that are still covered by their chorion when they attack their hosts: several species in the subgenus *Allocoelioxys* of the genus *Coelioxys* (Ferton, 1896; Rozen and Kamel, 2008) and one species in the genus *Radoszkowskiana* (Rozen and Kamel, 2007). However, first instars of at least two species of *Mesoplia* are known to attack hosts while the nearly empty, vacated chorion is still attached to their terminal segments (Vinson et al., 1987; Rozen, 1991; Rozen et al., 2011).

Many of the differences between the two larval representatives treated here clearly involve whether they are, or are not, cocoon spinners. However, some of the differences do not reflect production of a cocoon. For example, the sublateral linear rows of pigmented spines on the caudal annulets of certain body segments of all known larvae of *Epicharis* probably aide their huge mature larvae to move in the cell to



FIGURES 28, 29. SEM micrographs of egg of *Centris bicornuta*. 28. Anterior end showing micrograph and elevated ridges radiating from it before they disappear. 29. Close-up of micropyle, showing pebbled texture of chorion and micropyle (arrow). FIGURE 30. SEM micrographs of egg of *Epicharis albofasciata*, showing micropyle (arrow) and texture of elevated latticework.

orient for defecation and/or for adult emergence. One wonders whether traction against the smooth, waxlike surface to the cell wall of *E. albofasciata* (Rozen, 2016: fig. 14) will eventually be the explanation. More difficult to evaluate are differences in certain structures related to perception. Antennal papillae project in the case of *C. bicornuta*³ as do all of its palpi, in contrast to these structures of *E. albofasciata* and its congeners whose larvae have been described (Rozen, 1965). The antennal papilla projects on none of its instars, and the palpi scarcely project at all. Perhaps observation of feeding larval representatives of the two genera will be revealing.

As indicated in the Introduction, a recent paper by Martins and Melo (2016) concluded that the tribe Centridini as used by Michener (2007) was paraphyletic as evaluated through a detailed molecular study. One of the external reviewers of the current manuscript requested an evaluation that might support one or the other interpretation of the monopoly of the tribe. While it should be understood the purpose of this study was to explore the developmental larval anatomy of these two genera, the request of the reviewer seems appropriate:

(1) Is there evidence supporting the monophyly of the tribe Centridini sensu Michener (2007)?

(2) Are there characters that support the combined genera *Centris* and *Epicharis* as a distinct lineage, i.e., distinct at the level of tribe?

The answer to the second question is brief: ability and anatomical modifications to spin cocoons could be a good tribal characteristic, but obviously is not necessarily so (e.g., in the Rophitinae (Halictidae) species of *Dufourea* spine cocoons, those of *Conanthalictus* do not); in *Anthophorula* (Apidae) some species of winter generations spin cocoon while those of the summer generation of the same species do not spin cocoons (Michener, 2007).

The response to the first question is longer, in part because it introduces an anatomical character of corbiculate larvae that has gone unreported. Representatives of all corbiculate tribes (Euglossini, e.g., Rozen, 2016: figs. 1, 6, 17, 27; Bombini, e.g., Michener, 1953: fig. 248; Meliponini, e.g., Michener, 1953: fig. 266; and Apini, e.g., Michener, 1953: fig. 275) as fifth larval instars exhibit mostly small, paired, usually pigmented, elevated dorsal tubercles bearing fine setae on the three thoracic segments (as well as of the first abdominal segment of *Euglossa*). Such tubercles are lacking in both *Centris* and *Epicharis*, thereby separating the corbiculate taxa from the monophyletic tribe Centridini. These tubercles tend to become more pigmented the longer the specimen has been in the last instar, so that those of newly eclosed fifth instars tend to be only slightly pigmented. All tubercles are found on the posterior dorsolateral part in the same relative position on all thoracic segments on all corbiculate specimens, attesting to their homology.

Apis mellifera (listed above as Michener, 1953: fig. 275) is a notable exception in some ways. Its tubercles, though bearing scattered fine setae, are never naturally pigmented, although on specimens cleared and stained with Chlorazol Black E, tubercles are clearly identified. Those

³ Although the antennal papilla of the mature larva of *Centris bicornuta* is conical and projecting (though small), the projecting shape is not a consistent characteristic for the genus as observed in various species whose larvae have been treated (Rozen, 1965; Rozen and Buchmann, 1990). However, projecting palpi and galea are persistent features of all known mature *Centris* larvae.

of the pronotum are large and strongly transverse while those of the following thoracic segment are somewhat smaller than those of the pronotum (Michener, 1953: fig. 275).

Because these tubercles have been identified in all recognized tribes of corbiculate bees for more than 50 years, they are a well-established, if little known, autapomorphy of mature corbiculate bee larvae. This character has not been detected in any other bee larva including those of *Centris* and *Epicharis* and thereby supports the recognition of the tribal status of these two genera, assuming that mature larvae of all species of these two genera will eventually be found to lack elevated thoracic tubercles.

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