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Proventricular structure in the bee tribe Augochlorini (Hymenoptera: Halictidae)

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Abstract

Characters of the digestive tract have received little attention in modern phylogenetic analyses regarding relationships among bees, in part because studies on the internal morphology of bees generally concentrate on physiological and behavioural aspects. This paper presents a comparative study of the proventricular structure, analysed with scanning electronic microscopy, in bees of the tribe Augochlorini. Eleven species of Augochlorini were analysed as the ingroup, versus one each of Halictini and Caenohalictini, and two of Crabronidae as outgroups. The presence of a long columnar proventricular fold is an apomorphy for Augochlorini, whereas a proventricular fold with a keel-shaped structure at the moving lip level is an apomorphy for Augochlorina. Together these data corroborate the monophyly of Augochlorini and Augochlorina, respectively.

Keywords: Insects; Morphology; Gut; Foregut; Proventriculus

Introduction

In Hymenoptera, the foregut consists of the pharynx, oesophagus, crop or honey sac, and proventriculus (Chapman 1998). The proventriculus is the most specialised part of the foregut, and lies between the honey sac and midgut (Snodgrass 1956). It is subdivided into three parts: an anterior end that protrudes into the honey sac lumen forming the proventricular bulb and which consists of four lips forming a x-shaped opening; a midpiece or neck in the crop/midgut boundary; and a posterior cardiac valve situated in the midgut lumen (Cruz-Landim and Rodrigues 1967; Serrão and Cruz-Landim 1995).

As the proventriculus is ectodermic in origin, it is lined by a sclerotized cuticular intima. At the proventriculus bulb level, this cuticle is highly sclerotized and generally many hairs arise from the upper parts of the lips to form a comb. This comb filters solid particles from the crop contents down to the midgut as a result of muscular action. Bailey (1952) and Peng and Marston (1986) have described this filtering mechanism.

Bailey (1952), Gibbs (1967), Lebrun (1985), Lebrun and Lequet (1985), and Caetano (1988) have reported anatomical variation in the insect proventriculus, which they related to feeding habits of the respective insects. However, phylogenetic relationships may be important in determining variation in insect guts (for a review see Terra and Ferreira 1994).

The living bees with species found in Brazil have been classified in five families: Colletidae, Halictidae, Andrenidae, Megachilidae, and Apidae (Roig-Alsina and Michener 1993; Alexander and Michener 1995; Engel 2000, 2001a, 2005).

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Halictidae is one of the more basal lineages of bees, being the sister group to all other bees except Colletidae (Alexander and Michener 1995; Engel 2001a). The family is relatively large, consisting of approximately 3500 described species in three subfamilies (sensu Engel 2005), but in Brazil only Halictinae and Rophitinae are represented (Alexander and Michener 1995; Engel 2000; Silveira et al. 2002). Within Halictinae, the tribe Augochlorini shows great diversity in behaviour patterns such as levels of sociality and nest architecture (Sakagami and Michener 1962; Eickwort and Sakagami 1979). Thus, Halictidae is an important group of bees that should be well known for the understanding of bee phylogeny and behaviour.

Data sets for reconstructing phylogenetic trees of bees are, for the most part, based on external morphology, behavioural characters, DNA sequences or fossils (e.g. Cameron 1991; Sheppard and McPherson 1991; Roig-Alsina and Michener 1993; Alexander and Michener 1995; Costa et al. 2003; Drumond et al. 2000; Engel 2000, 2001a, 2001b, 2004; Serrão 2005). Anatomical, histological or cytological data are rarely incorporated, and there have been only a few studies, even though these characters show strong congruence with phylogenetic hypotheses based on external character systems (e.g. Bilinski et al. 1998; Bitsch and Bitsch 1998; Klass 1998; Kubrakiewicz et al. 1998; Strausfeld 1998; Buschbeck 2000; Ehmer and Hoy 2000; Serrão 2000; Peixoto and Serrão 2001; Martins and Serrão 2002, 2004; Yeates et al. 2002).

There are no papers concerning the structure of the digestive tract in Augochlorini. As part of an ongoing study, therefore, the present paper deals with proventricular structure in augochlorine bees, observed with the scanning electron microscope. The aim of the study is to show that proventricular characters provide insight into relationships among bees.

Material and methods

Original observations reported in this paper were based upon bees dissected as freshly killed specimens and subsequently transferred to 4% paraformaldehyde at phosphate buffer 0.1 M, pH 7.4. The proventriculi were isolated from dissected guts, the pieces dehydrated in ethanol, transferred to hexamethyldisilazane (HMDS) for 5 min, and air-dried at room temperature prior to gold-covering (Nation 1983).

The 11 species of Augochlorini (ingroup), one of Halictini, one of Caenohalictini, and two of Crabronidae (outgroups) used in this analysis are listed in Table 1.

Two analyses were performed: (1) external structure of the proventricular bulb, for which intact pieces were used; and (2) internal structure, for which a longitudinal section of the bulb was made prior to scanning electron microscopy procedures.

Table 1. List of taxa included in the analysis (classification after Engel 2000, 2005)

Species
Family Halictidae Thomson
Tribe Augochlorini Beebe
Subtribe Corynurina Engel
Rhinocorynura briseis (Smith, 1853)
Subtribe Augochlorina Beebe
Infratribe Megaloptita Moure
Augochloropsis aurifluens (Vachal, 1903)
Augochloropsis cockerelli Schrottky, 1909
Augochloropsis electra (Smith, 1853)
Augochloropsis pattens (Vachal, 1903)
Augochloropsis (*Paraugochloropsis*) sp. (Schrottky 1906)
Infratribe Augochlorita Beebe
Augochlora (*Augochlora*) sp. (Smith 1853)
Augochlora (*Augochlora*) *amphitrite* (Schrottky, 1910)
Augochlora (*Oxystoglossella*) *thalia* Smith, 1879
Infratribe Megaloptidiita Engel
Megommation insigne (Smith, 1853)
Pseudaugochlora graminea (Fabricius, 1804)
Tribe Caenohalictini Michener
Caenohalictus serripes Ducke, 1908
Tribe Halictini Thomson
Dialictus sp. (Robertson 1902)
Family Crabronidae Latreille
Psenulus aztecus R. Bohart and Grissell, 1969
Crabro sp. (Linnaeus 1758)

Comparing proventriculus morphology among solitary bees, ten characters (see Appendix A) were evaluated for phylogenetic considerations. Character polarity was determined using outgroup comparison (Watrous and Wheeler 1981; Maddison et al. 1984; Nixon and Carpenter 1993). Character states represented in the outgroup were considered as plesiomorphic, and coded (0). Cladistic analyses were performed using the computer programme PAUP, version 4.0b10 (Swofford 1998) employing the star-decomposition search option. Results were illustrated using TreeView version 16.6.

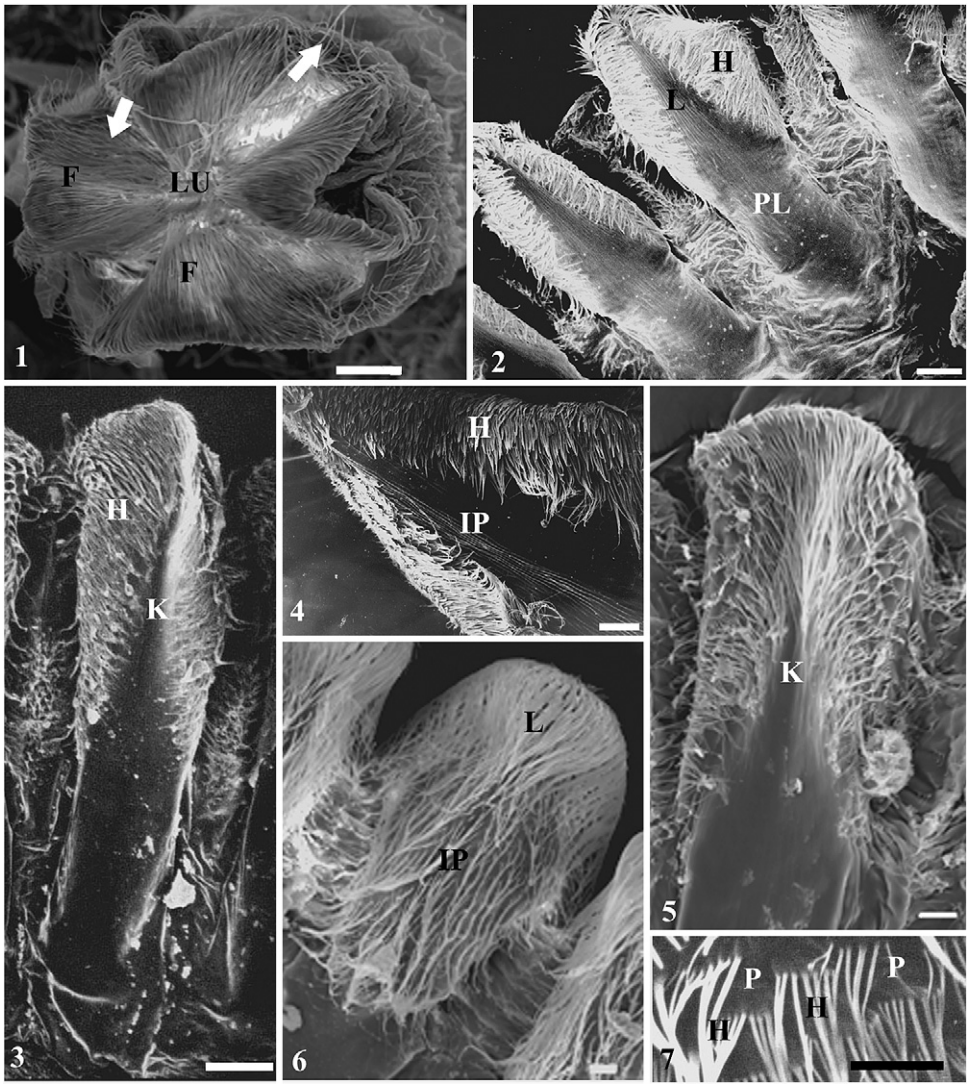
Results

In all studied species, the proventricular bulb shows the same basic morphological patterns. It is formed by four thick folds of the proventricular wall, with the cuticle coating external to the apex, where a great number of hair-like projections are present (Fig. 1).

Each proventricular fold can be divided in two regions: the apical region constituting the moving lips with hairs, and the basal region constituting the basal plate, without hairs but covered by a chitinous cuticle (Fig. 2).

In Augochlorini each fold is formed by a chitinous piece, which is columnar (Fig. 2). In the subtribe Augochlorina, the hairs at the level of the moving-lips section are placed laterally, never covering the innermost part (Figs. 3 and 4). The innermost part is elevated, forming a keel-shaped structure (Figs. 3 and 5). In the subtribe Corynurina, this moving-lips section is flattened and completely covered by hairs of two sizes (Figs. 6 and 9).

Plates with 7–10 cuticular projections form the hair-like projections of the cuticle in the proventriculus of Augochlorini (Fig. 7). These projections are of two sizes: those present in the external tip of the proventriculus bulb are short and spine-like, whereas those in the



Figs. 1–7. Scanning electron micrographs of proventriculus structures. **1.** Apical view of proventriculus bulb of *Augochloropsis cockerelli* showing the four proventricular folds (F) with hair-like projections (arrows) of the cuticle; LU = lumen; scale bar = 150 μm. **2.** Inner view of proventricular fold of *Pseudaugochlora graminea* showing moving lips (L) with hair-like projections (H), and basal plate (PL) without hairs; scale bar = 50 μm. **3.** Inner view of proventricular fold of *Augochloropsis pattens*, Megaloptita, showing hairs (H) placed laterally, and innermost part forming keel-shaped structure (K); scale bar = 50 μm. **4.** Inner view of proventricular fold of *Megommation insigne,* Megaloptidiita, showing hair (H) placed laterally, never covering innermost part (IP); scale bar = 50 μm. **5.** Inner view of proventricular fold of *Augochlora (Augochlora) amphitrite*, Augochlorita, showing keel-shaped structure (K) in innermost part of proventricular fold; H = hairs; scale bar = 50 μm. **6.** Inner view of proventricular fold of *Rhinocorynura briseis,* Corynurina, showing hairs covering entire surface of innermost part (IP) and moving lip (L); scale bar = 50 μm. **7.** Hair-like projections of cuticle in proventriculus of *Augochloropsis aurifluens*, showing hairs (H) formed from plates (P); scale bar = 10 μm.

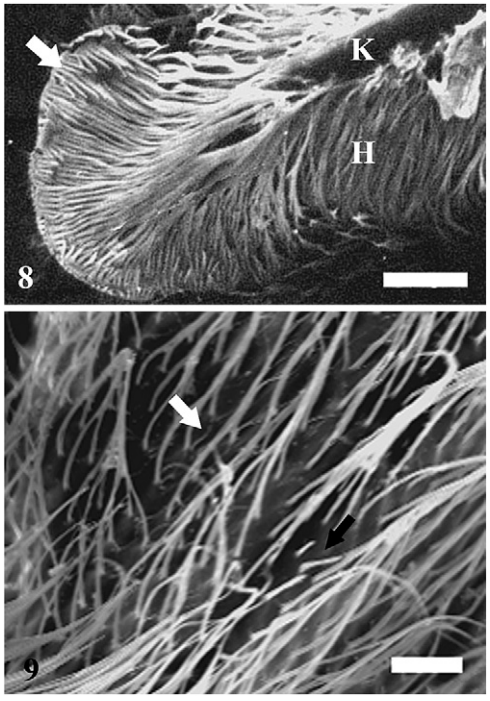


Fig. 8–9. Scanning electron micrographs of hair-like projections. **8.** *Pseudaugochlora graminea* with short, spine-like cuticular projections (arrow) in apex, and long, hair-like projections (H) in lateral portion of proventricular fold; K = keel-shaped structure; scale bar = 20 μm. **9.** *Rhinocorynura briseis* with short, hair-like projections (arrows) scattered among long ones; scale bar = 20 μm.

lateral portion are longer and hair-like (Fig. 8). However, Corynurina have small hairs scattered among the long hairs (Fig. 9).

The proventriculus bulb characters described in the Appendix A are expressed in the studied taxa as shown in Table 2. Parsimony analysis of these proventricular characteristics exclusively results in trees with length 7, CI 1.0, and RI 1.0 (strict consensus shown in Fig. 10). The Halictidae clade (node A) is supported by elongate hairs on the cuticular plate (character 8). Node B (= Augochlorini) is supported by character state 5-1, whereas monophyly of the subtribe Augochlorina (node C) is supported by a distinct character state (keel-shaped proventricular fold). *Rhinocorynura*, a representative of Corynurina, is supported by the autapomorphy of character state 10-1.

Discussion

The aspects of the proventricular fold in Halictidae studied herein support the hypothesis of Serrão (2000) that the proventricular fold with moving lips containing hairs and a basal plate where hairs are lacking is a symplesiomorphy for bees, because similar proventricular

Table 2. Matrix of proventriculus character states; for explanation of characters see the Appendix A

Taxon	Character									
	1	2	3	4	5	6	7	8	9	10
Augochloropsis aurifluens	0	0	0	1	1	1	0	1	0	0
Augochloropsis cockerelli	0	0	0	1	1	1	0	1	0	0
Augochloropsis electra	0	0	0	1	1	1	0	1	0	0
Augochloropsis pattens	0	0	0	1	1	1	0	1	0	0
Augochloropsis (*Paraugochloropsis*) sp.	0	0	0	1	1	1	0	1	0	0
Augochlora (*Augochlora*) sp.	0	0	0	1	1	1	0	1	0	0
Augochlora (*Augochlora*) *amphitrite*	0	0	0	1	1	1	0	1	0	0
Augochlora (*Oxystoglossella*) *thalia*	0	0	0	1	1	1	0	1	0	0
Megommation insigne	0	0	0	1	1	1	0	1	0	0
Pseudaugochlora gaminea	0	0	0	1	1	1	0	1	0	0
Rhinocorynura briseis	0	0	0	0	1	0	0	1	0	1
Caenohalictus serripes	0	0	0	0	0	0	0	1	0	0
Dialictus sp.	0	0	0	0	0	0	0	1	0	0
Psenulus aztecus	0	0	0	0	0	0	0	0	0	0
Crabro sp.	0	0	0	0	0	0	0	0	0	0

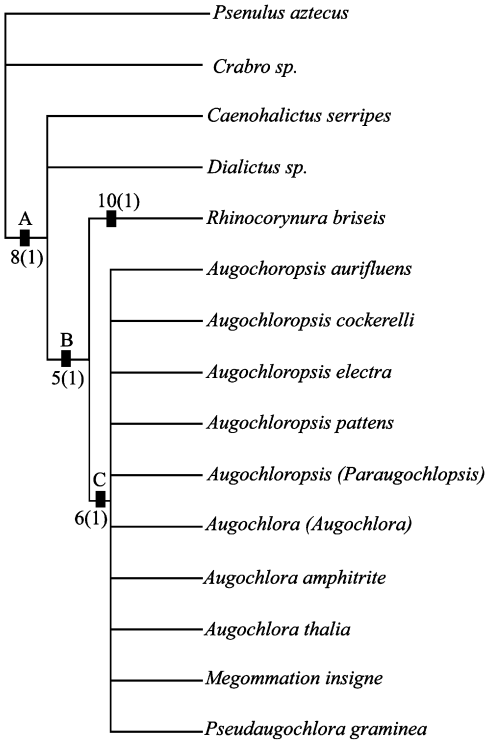


Fig. 10. Phylogenetic relationships based on proventricular characteristics (TL = 7, CI = 1.00, RI = 1.00).

bulbs are present in other bee families as well as in other Hymenoptera such as ants, wasps, and spheciform wasps (Caetano 1984; Von Zuben and Caetano 1994; Serrão and Cruz-Landim 1995; Serrão 2000).

Occurrence of hairs never covering the innermost part of the moving lip is found also in *Apis mellifera*, but in the honey bee the innermost part of the lip is flat and the proventricular fold triangular (Peng and Marston 1986; Serrão 2000).

Hairs arising from the cuticular plate can be considered as plesiomorphic for Halictidae, because similar cuticular projections of the proventricular fold are present in other bees, such as some Apidae, in spheciform wasps (Serrão 2000, 2005) and Vespidae (Von Zuben and Caetano 1994).

Cruz-Landim and Rodrigues (1967) suggested that proventricular variation in bees can be related to their different sociality levels. However, the present work as well as data from Serrão (2000, 2005) suggests that there is no relation between sociality and proventricular structure, because it is similar in bees with different levels of sociality.

Although the phylogenetic reconstructed for Augochlorini presented here is certainly not robust owing to the low number of characters and species, the long columnar proventricular fold (character state 5-1) supports monophyly of Augochlorini. In the tribes Caenohalictini, Halictini, and other outgroups the folds are short columnar. Thus, the above-mentioned apomorphy may be added to the five synapomorphies proposed by Engel (2000) as supporting monophyly of the tribe. The keel-shaped aspect of the proventricular basal plate (state 6-1) supports monophyly of the subtribe Augochlorina. In the primitive subtribe Corynurina, this proventricular feature is flattened (6-0), supporting Corynurina as the sister-group of Augochlorina as hypothesised by Engel (2000). Unfortunately, the small sampling of other Halictinae does not permit determination of the sister-group to Augochlorini. Nevertheless, the present study demonstrates the phylogenetic utility of proventricular studies in bees.

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Appendix A

Annotated list of proventriculus characters

1. Number of folds forming the proventriculus wall: (0) four; (1) six.
2. Covering of the proventricular bulb tip: (0) covered by cuticle; (1) without cuticle.
3. Aspect of cuticle in the moving lips: (0) with hairs; (1) without hairs.
4. Hairs on inner surface of moving lips: (0) covering entire surface; (1) placed laterally; never covering the innermost part.
5. Shape of proventricular fold: (0) short columnar; (1) long columnar.
6. Aspect of innermost fold surface: (0) flattened; (1) keel-shaped.
7. Shape of hairs: (0) hairs arising from a cuticular plate; (1) thread-shaped hairs arising individually.
8. Shape of hairs in the cuticular plate: (0) spine-like projections; (1) elongate.
9. Shape of hairs in the external apex and the moving lips: (0) all hairs similar; (1) with various shapes.
10. Shape of hairs in the moving lips: (0) all hairs similar; (1) with various shapes.

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