

HISTOLOGY OF THE ILEUM IN BEES (HYMENOPTERA, APOIDEA)

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ABSTRACT

In most hymenopterans, the ileum extends as a long tube from the midgut to the rectum, and shows no marked anatomical specialization. The function of the ileum is not fully understood, although the presence of an epithelial layer of cuboidal or columnar cells with apical and basal plasma membrane infoldings suggests that this organ may be involved in water and nutrient absorption. In this work, we investigated the ileal morphology of 47 species of bees in the Andrenidae, Apidae (Apini, Meliponini, Xylocopinae, Centridini, Bombini, Eucerini, Euglossini, Tapinotaspidini, Exomalopsini), Halictidae and Megachilidae, as part of a study to understand the physiological and phylogenetic importance of this organ. In all cases, the ileum consisted of an epithelium containing cuboidal or columnar cells that usually had basal nuclei and apical plasma membrane infoldings, although there were variations in cell shape, position of the nucleus, degree of chromatin condensation and cuticle thickness. The epithelial cells were covered with a cuticle and transverse sections revealed the presence of 4-6 folds projecting into the lumen. The cell cytoplasm below the apical plasma membrane infoldings contained numerous vacuoles of different sizes. A single layer of circular muscle was located beneath the epithelium. The histological organization of the ileum suggested a role in the absorption of luminal solutes as a mechanism for regulating the hemolymph and the general osmotic balance of these insects. However, there was no relationship between the structural organization of the ileum and the degree of social development and/or phylogeny of the bees.

Key words: Digestive tract, morphology, osmotic control, physiology

INTRODUCTION

The digestive tract of insects is divided into foregut, midgut and hindgut. The foregut and the hindgut are of ectodermal origin whereas the midgut is endodermic [6]. In bees, the foregut consists of the pharynx, esophagus, crop and proventriculus, the midgut corresponds to the ventriculus and the hindgut is divided into ileum and rectum [3,4,10,22,34,39]. Various morphological and biochemical aspects of the digestive tract of bees have been studied [7,13,15-17,23,25,29,32,41]. However, the ileum had received less attention because of the general belief that the midgut is responsible for food digestion and nutrient absorption whereas the rectum is responsible for osmotic control [32,35-37].

The ileum of bees is a long tube interposed between the midgut and the rectum but shows no visible (macroscopic) anatomical specialization throughout

its length. Although its precise function remains to be established, the ileum may be involved in water and nutrient absorption [8]. In the stingless bee *Melipona quadrifasciata anthidiodes*, the epithelial cells of the ileum have four types of microvilli, indicating that there may be some form of compartmentalization in the absorption of organic solutes; this finding, together with other ultrastructural features, is also suggestive of a digestive function [9,10].

Morphological variations in the digestive tract of insects have been related to differences in their feeding habits and degree of sociality [1,4,11,12,18]. However, phylogenetic relationships may also influence insect gut morphology [29,32,33,42]. In this context, studies designed to compare the histological structure of the ileum in different species of bees are required to test the hypothesis that variations in this organ are related to phylogeny and/or the degree of sociality in these insects. The purpose of this study was to compare the histology of the ileum in bees belonging to several tribes of four families in order to assess the foregoing relationships.

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MATERIAL AND METHODS

Forty-seven species of bees from the Andrenidae, Apidae (Apini, Meliponini, Xylocopinae, Centridini, Bombini, Eucerini, Euglossini, Tapinotaspidini, Exomalopsini), Halictidae and Megachilidae were studied (Table 1). The bees were collected while foraging on flowers in the towns of Viçosa (Minas Gerais state) and Boituva (São Paulo state) and transported to the laboratory where they were killed by freezing. The bees were subsequently dissected using a stereomicroscope and the digestive tract was removed, immersed in insect saline solution (0.1 M NaCl, 0.1 M Na₂HPO₄, 0.1 M KH₂PO₄) and then transferred to Zamboni solution [40] for 4 h. After fixation, the hindgut was isolated and the length of the ileum was measured using a micrometer eyepiece (Olympus).

For histological analysis, the tissues were dehydrated in an ethanol series, embedded in glycol metacrylate (JB4 EMS historesin, Warrington, PA, USA), sectioned into 4-µm-thick sections and stained with hematoxylin and eosin (HE). The Feulgen reaction was done to identify nuclear DNA [24]. The thickness of the cuticle lining the lumen of the ileum was measured with a micrometer eyepiece. After fixation and dehydration, another set of ilea was transferred to hexamethyldisilazane for 5 min, air dried, sputtered with gold, and examined with a LEO VP1430 scanning electron microscope operated at 15 kV.

The size of the bees was determined by measuring the intertegular distance (distance between the forewing tegulae) [5] with a micrometer eyepiece. The morphometric data for 30 species (Table 2) were analyzed by analysis of covariance (ANCOVA) to determine the correlation between body size, ileal length and degree of sociality.

RESULTS

The ileum corresponded to the proximal portion of the hindgut, with the transition from the distal midgut to the ileum being marked by a valve located just above the opening of the Malpighian tubules (Fig. 1A,B). The ileal lumen was lined by a cuticle that extended throughout its entire length and included the rectum (Fig. 1C,D). The posterior distal region of the ileum protruded into the rectum where it formed a valve-like structure (Fig. 1C). Transverse sections showed that the epithelium consisted of 4-6 folds that bulged into the lumen (Fig. 2A). Externally, there was a single layer of circular muscle formed by several muscle fibers (Fig. 2C,D), and interposed between this layer and the ileal epithelium was a distinct subepithelial space that was larger at the base of the epithelial folds where the muscle layer was interrupted to allow entrance of the tracheae (Fig.

2B). The single layered epithelium consisted of only one cell type that varied from cuboidal to columnar. All of the specimens examined had flattened cells at the ileal-rectal interface (Fig. 1C), with the ileal epithelial cells usually having apical plasma membrane infoldings and basal nuclei (Fig. 3A).

Although the structural organization of the ileal wall was similar in all of the species examined here, there were some variations in epithelial cell shape, position of the nucleus, degree of chromatin condensation and cuticular thickness. Thus, for example, *Apis mellifera*, *Cephalotrigona capitata*, *Exomalopsis auropilosa*, *Melipona rufiventris*, *M. compressipes*, *M. eburnea*, *Nannotrigona testaceicornis*, *Partamona helleri*, *Paratrigona lineata*, *Plebeia remota*, and *Tetrapedia nigratarsis* had cuboidal epithelial cells (Fig. 3A,B) while the other species had columnar cells (Figs. 3C,D and 4A,C).

The cuticle that lined the epithelium throughout its entire length varied in thickness from 0.7 µm to 4.6 µm in *M. scutellaris* and *Xylocopa frontalis*, respectively (Table 2). In the epithelial cells of *Augochloropsis* sp., *Melipona capixaba* and *M. scutellaris*, the nucleus was located centrally (Fig. 3C), while in the other species it was located in the basal portion of the cells (Fig. 4A). In *Augochloropsis* sp., *Bombus morio*, *Centris violacea*, *Epicharis flava*, *Euglossa mandibularis*, *Exomalopsis auropilosa*, *Friesomelitta varia*, *Geotrigona* sp., *Megachile* spp., *Megalopta* sp., *Paratrigona lineata* and *Xylocopa frontalis* the chromatin was decondensed (Figs. 3D and 4A,B), although in many other species some regions of condensed chromatin were seen (Figs. 3A-C and 4C). The Feulgen reaction showed that the nucleus had many regions of condensed chromatin, with the cytoplasm containing numerous vacuoles of different sizes, the presence of which deformed the nucleus (Figs. 3A,B and 4B,C). These vacuoles occurred throughout the entire length of the ileum, except in *A. mellifera*, *E. mandibularis*, *F. varia*, *Melipona quadrifasciata anthidioides*, *M. bicolor* and *P. helleri*, in which they accumulated in the posterior half of the organ. Apical plasma membrane infoldings that reached the medium portion of the cell were also observed (Fig. 3A).

The size of the ileum generally increased with body size (Table 2) (ANCOVA: $F_{1,27} = 64.39$; $p < 0.001$), but there was no relationship with the degree of sociality (ANCOVA: $F_{1,27} = 0.86$; $p < 0.361$) (Fig. 5).

Table 1. List of the taxa studied.

Family	Tribe	Species
Apidae	Apini	<i>Apis mellifera</i> Linnaeus, 1758
		Meliponini
		<i>Cephalotrigona capitata</i> (Smith, 1854)
		<i>Frieseomelitta flavicornis</i> (Fabricius, 1798)
		<i>Frieseomelitta varia</i> (Lepeletier, 1836)
		<i>Geotrigona</i> sp.
		<i>Lestrimelitta limao</i> (Smith, 1836)
		<i>Melipona asilvai</i> (Moure, 1971)
		<i>Melipona bicolor</i> (Lepeletier, 1836)
		<i>Melipona capixaba</i> (Moure & Camargo, 1995)
		<i>Melipona compressipes</i> (Fabricius, 1804)
		<i>Melipona eburnea</i> (Friese, 1900)
		<i>Melipona quadrifasciata anthidiodes</i> (Lepeletier, 1836)
		<i>Melipona quinquefasciata</i> (Lepeletier, 1836)
		<i>Melipona rufiventris</i> (Lepeletier 1836)
		<i>Melipona scutellaris</i> (Latreille, 1811)
		<i>Nannotrigona testaceicornis</i> (Lepeletier, 1836)
		<i>Paratrigona lineata</i> (Lepeletier, 1836)
		<i>Paratrigona subnuda</i> (Moure, 1947)
		<i>Partamona helleri</i> (Friese, 1900)
		<i>Plebeia remota</i> (Holmberg, 1903)
		<i>Ptilotrigona</i> sp.
		<i>Scaptotrigona postica</i> (Latreille, 1807)
	<i>Trigona spinipes</i> (Fabricius, 1793)	
	Bombini	
	<i>Bombus atratus</i> (Franklin, 1913)	
	<i>Bombus morio</i> (Swederus, 1787)	
	Euglossini	
	<i>Eufriesea violacea</i> (Blanchard, 1840)	
	<i>Euglossa (Glossura) imperialis</i> (Cockerell, 1922)	
	<i>Euglossa mandibularis</i>	
	<i>Eulaema nigrata</i> (Lepeletier, 1841)	
	<i>Exaerete smaragdina</i> (Guérin-Méneville, 1845)	
	Xylocopini	
	<i>Xylocopa frontalis</i> (Oliver, 1789)	
	<i>Xylocopa (Schoenherria) subcyanea</i> (Perez, 1901)	
	Centridini	
	<i>Centris violacea</i> (Lepeletier, 1841)	
	<i>Epicharis flava</i> (Friese, 1900)	
Apidae	Eucerini	<i>Thygater analis</i> (Lepeletier, 1841)
		<i>Thygater palliventris</i> (Friese, 1908)
	Exomalopsini	<i>Exomalopsis auropilosa</i> (Spinola, 1853)
	Tapinotaspidini	<i>Tetrapedia nigratarsis</i> (Moure & Mucci, 1998)
Halictidae	Augochlorini	<i>Augochlora</i> sp.
		<i>Augochloropsis</i> sp.
		<i>Megalopta</i> sp.
		<i>Pseudaugochlora graminea</i> (Fabricius, 1804)
		<i>Rhinocorynura briseis</i> (Smith, 1853)
Andrenidae	Oxaeini	<i>Oxaea flavescens</i> (Klug, 1807)
Megachilidae	Megachilini	<i>Megachile aureiventris</i> (Schrottky, 1902)
		<i>Megachile (Leptorachis) paulistana</i> (Schrottky, 1902)
		<i>Megachile (Cressoniella) rava</i> (Vachal, 1808)

Table 2. Length, cuticular thickness and intertegular distance of the ileum in bees.

Species	Ileum (mm)	Intertegular distance (mm)	Cuticular thickness (μm)
Apidae			
<i>Apis mellifera</i>	3.66	3.00	1.40
<i>Cephalotrigona capitata</i>	1.85	2.11	1.56
<i>Frieseomelitta varia</i>	0.77	1.24	2.46
<i>Geotrigona</i> sp.	1.27	1.61	2.20
<i>Lestrimelitta limao</i>	1.13	1.62	2.88
<i>Melipona eburnea</i>	4.25	3.08	2.52
<i>Melipona quadrifasciata anthidiodes</i>	5.03	3.45	2.08
<i>Melipona rufiventris</i>	3.61	2.55	1.20
<i>Melipona scutellaris</i>	6.57	2.73	0.70
<i>Paratrigona lineata</i>	0.66	1.18	-
<i>Paratrigona subnuda</i>	0.64	1.00	-
<i>Partamona helleri</i>	1.75	1.93	1.96
<i>Ptilotrigona</i> sp.	1.94	1.48	3.92
<i>Scaptotrigona postica</i>	2.13	1.91	2.92
<i>Trigona spinipes</i>	1.74	1.71	3.48
<i>Bombus atratus</i>	11.92	4.24	4.52
<i>Bombus morio</i>	13.58	4.55	-
<i>Eufriesea violacea</i>	6.83	4.45	2.17
<i>Euglossa (Glossura) imperialis</i>	6.46	3.52	1.10
<i>Eulaema nigrita</i>	11.17	5.59	1.96
<i>Exaerete smaragdina</i>	8.23	4.43	1.96
<i>Xylocopa frontalis</i>	15.64	7.82	4.60
<i>Xylocopa (Schoenherria) subcyanea</i>	3.26	3.14	4.30
<i>Centris violacea</i>	6.26	5.20	3.16
<i>Epicharis flava</i>	12.85	4.90	2.48
Halictidae			
<i>Augochlora</i> sp.	1.24	1.63	2.36
Andrenidae			
<i>Oxaea flavescens</i>	7.06	6.17	2.28
Megachilidae			
<i>Megachile aureiventris</i>	3.54	3.50	1.68
<i>Megachile (Leptorachis) paulistana</i>	1.87	2.60	2.08
<i>Megachile (Cressoniella) rava</i>	3.12	2.63	2.50

DISCUSSION

The presence of a single layer of circular muscle in the species of Apoidea investigated here differed from that in some species of Lepidoptera, which have two muscle layers (external circular and inner longitudinal muscles) [20] and Coleoptera, in which the inner muscle layer is circular and the external layer is longitudinal [38]. The presence of a single layer of circular muscle has also been reported in ants [43]. These findings indicate that the ileum has a uniform structure within the Hymenoptera, although additional studies in wasps are necessary to confirm this.

A comparison of the variations in ileal structure with the phylogeny of bees, as previously done by

Roig-Alsina and Michener [31], Peixoto and Serrão [29] and Serrão [33] for other characteristics, revealed no relationship between these two parameters, which suggested that the ileum is not a useful character for phylogenetic analyses in bees.

The main function of the insect hindgut is to modify the composition of the primary urine produced by the Malpighian tubules by releasing K^+ , Na^+ and Cl^- into the lumen, thereby promoting the influx of water and of substances to be excreted [6]. This modification occurs mainly in the rectum but apparently begins in the ileum, which is efficient in absorbing water and ions, and in regulating acid-base balance and nitrogen excretion [14,19,30]. The

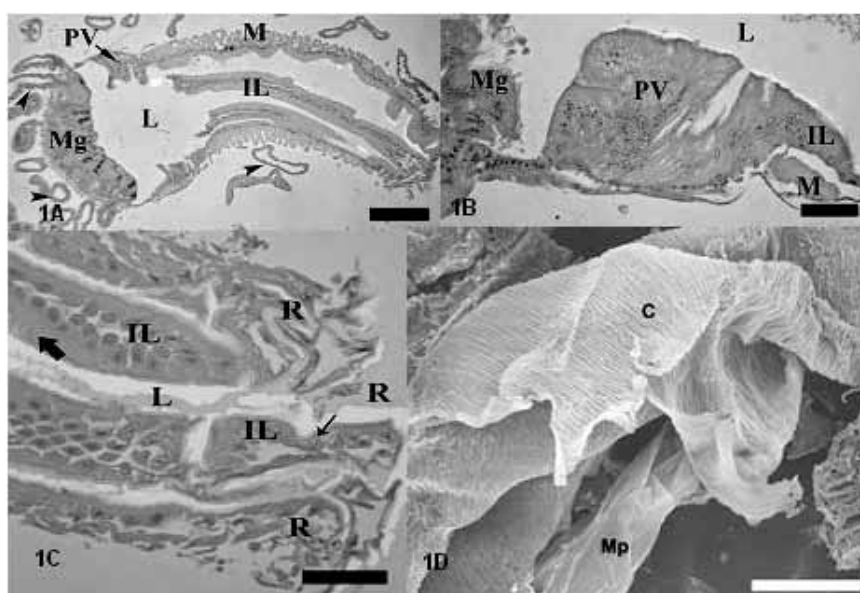


Figure 1. A. Section of the ileum of *Melipona eburnea* showing the transition from midgut (Mg) to ileum (IL) and the pyloric valve (PV). Arrowheads - Malpighian tubules, M - muscle. Bar = 50 μ m. B. Detail of the pyloric valve (PV) in the ileum (IL) of *Eufriesea violacea*. L - lumen, M - muscle, Mg - midgut. Bar = 30 μ m. C. Transition area from the ileum (IL) to the rectum (R) in *Bombus morio* showing the columnar cells (large arrow) becoming flattened (small arrow). L - lumen. Bar = 40 μ m. D. Scanning electron micrograph of the ileal lumen of *Melipona quadrifasciata anthidioides* showing the cuticle (C) that lines the organ. Mp - peritrophic membrane. Bar = 100 μ m.

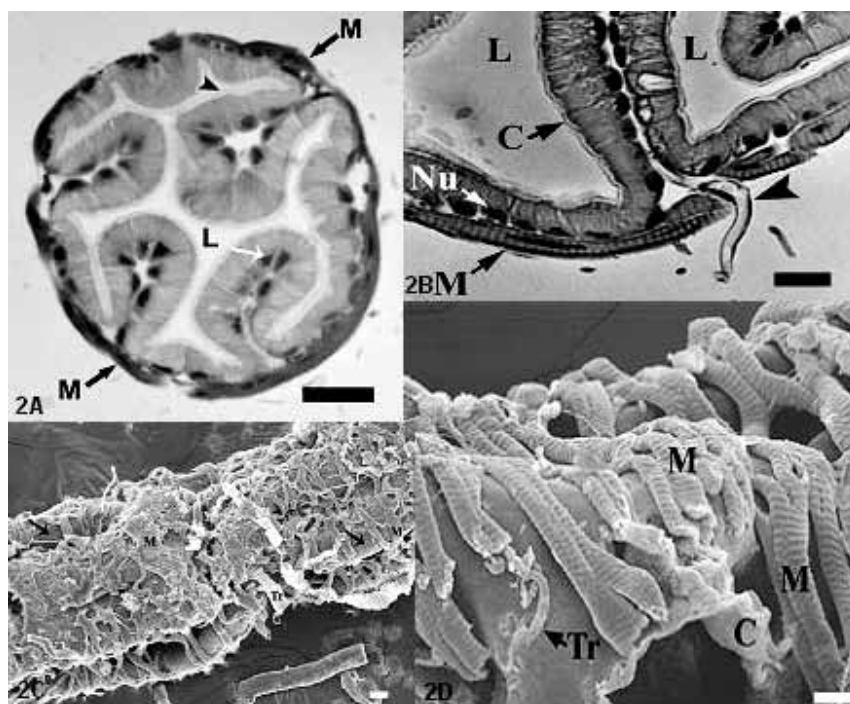


Figure 2. A. Transverse section of the ileum of *Thygater palliventris* showing the epithelial folds and the layer of circular muscle (M). Arrow - nucleus of epithelial cell, arrowhead - cuticle, L - lumen. Bar = 50 μ m. B. Detail of the ileum of *Eufriesea violacea* showing the muscular layer (M) interrupted by the entrance of tracheae (arrowhead). C - cuticle, L - lumen, Nu - nucleus. Bar = 40 μ m. C. Scanning electron micrograph of the ileum of *Melipona quadrifasciata anthidioides* showing the layer of circular muscle fibers (M) that surrounds the organ. Arrow - epithelium of the ileum, Tr - trachea. Bar = 20 μ m. D. Scanning electron micrograph of the ileum of *Apis mellifera* showing a detail of the circular muscles (M). Part of the cuticle (C) that covers the epithelium of the organ internally can be seen. Tr - trachea. Bar = 20 μ m.

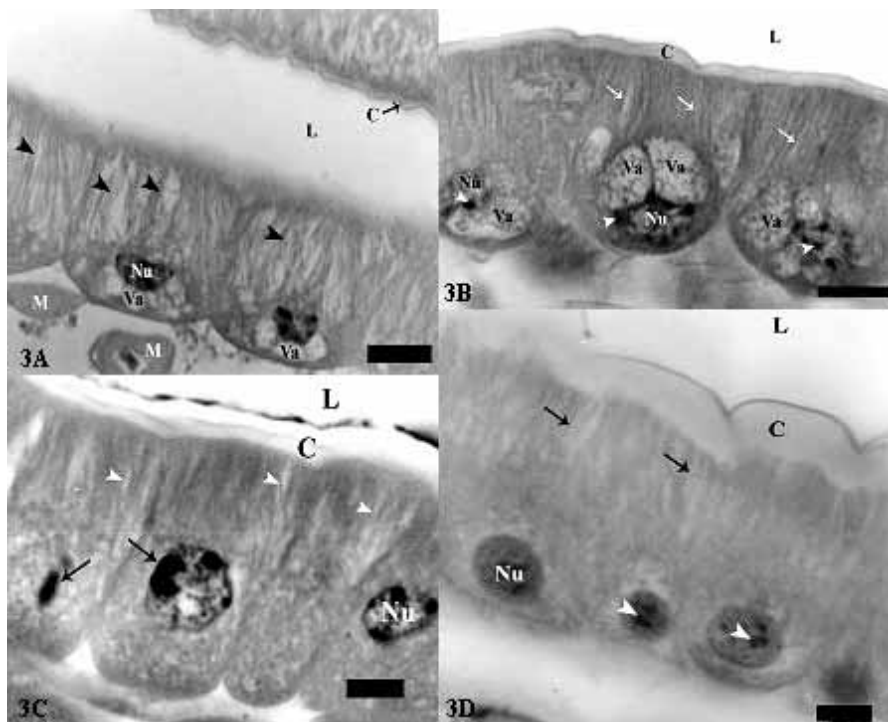


Figure 3. A. The ileum of *Melipona eburnea* showing the epithelium with cuboidal cells and the apical plasma membrane infoldings (**arrowheads**) extending to the middle portion of the cell. **C** - cuticle, **L** - lumen, **M** - muscles, **Nu** - nucleus, **Va** - vacuoles. Bar = 10 μ m. **B.** The ileum of *Cephalotrigona capitata* showing the epithelium with cuboidal cells, basal nucleus (**Nu**) and condensed chromatin (**arrowheads**). Note the apical plasma membrane infoldings (**arrows**) and large vacuoles (**Va**). **C** - cuticle, **L** - lumen. Bar = 10 μ m. **C.** The ileum of *Melipona capixaba* showing the centrally located nucleus (**Nu**) of the epithelial cells and the condensed chromatin (**arrows**). The arrowheads indicate the apical plasma membrane infoldings. **C** - cuticle, **L** - lumen. Bar = 10 μ m. **D.** The ileum of *Bombus morio* showing the epithelium with columnar cells and thick cuticle (**C**). The arrows show the apical plasma membrane infoldings. **Arrowheads** - chromatin condensed, **L** - lumen, **Nu** - nucleus. Bar = 10 μ m.

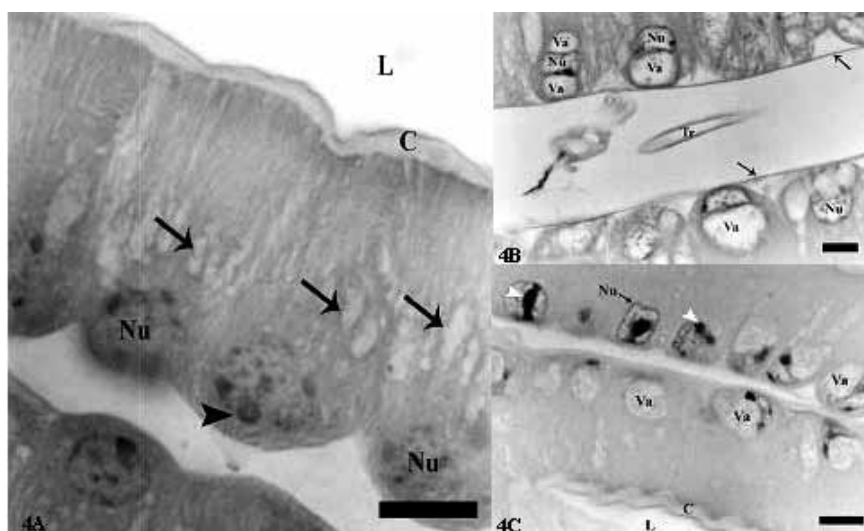


Figure 4. A. The ileum of *Centris violacea* showing the epithelium with columnar cells, basal nucleus (**Nu**) and condensed chromatin (**arrowhead**). Note the presence of vacuoles (**arrows**) in the cytoplasm. **C** - cuticle, **L** - lumen. Bar = 10 μ m. **B.** The ileum of *Megachile (Cressoniella) rava* showing the epithelium and nuclei containing condensed chromatin (**Nu**). Note that the nuclear shape is distorted by the accumulation of vacuoles (**Va**). **Arrow** - basal lamina, **Tr** - trachea. Bar = 10 μ m. **C.** The ileum of *Eulaema nigrita* showing deformations of the nucleus (**Nu**) caused by large vacuoles (**Va**) in the cytoplasm. **Arrowhead** - condensed chromatin, **C** - cuticle, **L** - lumen. Bar = 10 μ m.

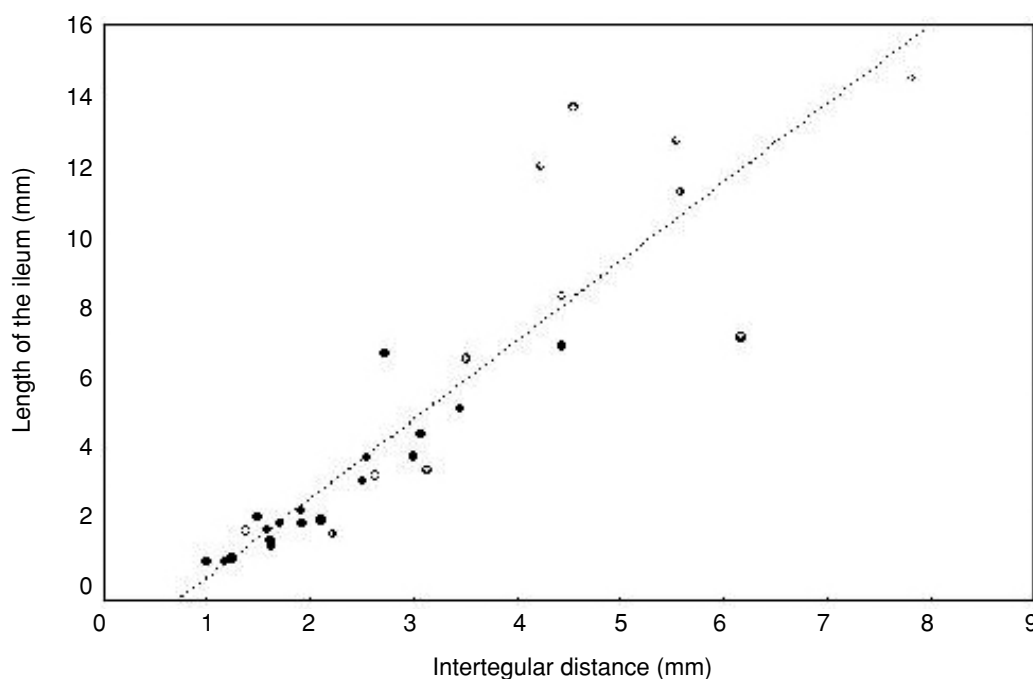


Figure 5. Analysis of covariance (ANCOVA) showing the relationship between ileal length, bee size (intertegular distance) and the degree of sociality in the species studied. O - solitary bees, ● - social bees.

presence of apical plasma membrane infoldings and the accumulation of vacuoles within the cells seen in the bees studied here suggest that the ileum plays a role in nutrient absorption. This conclusion agrees with the classic function of the ileum in modifying the primary urine produced by the Malpighian tubules [43].

The primary urine that arrives in the ileum is isosmotic compared to hemolymph, but its volume and concentration decrease and increase, respectively, during transit through the ileum, via a process that is dependent on active ion pumping into the epithelial cells [2]. The presence of basal and apical folds in the ileal epithelium is compatible with this view since this specialization is typical of epithelia involved in active transport. However, in insects that feed on nectar, the primary urine is hypo-osmotic compared to hemolymph [26,27]. *Xylocopa capitata* bees have a moderately concentrated hemolymph and dilute urine, and because of the low level of ions they ingest these bees need to conserve ions rather than water in order not to suffer water stress [27]. In the butterfly *Pieris brassicae*, the filtrate that arrives in the ileum from the Malpighian tubules is hypo-osmotic compared to hemolymph and there is a decrease in the K^+ concentration of the primary urine as it passes through the ileum, thereby ensuring that enough K^+ remains in the hemolymph to support fluid secretion

by the Malpighian tubules [26]. These findings indicate that, in the absence of an osmotic gradient, there may be ion absorption without water flow.

Since bees feed on nectar, it is reasonable to suppose that the primary urine of these insects is hypo-osmotic compared to hemolymph. Hence, to maintain their osmotic balance, bees need to absorb ions from the primary urine without a corresponding water flow. Such minimal coupling between solute and water transport has been observed in the salivary glands of *Calliphora erythrocephala* (Diptera) and in the Malpighian tubules of *Rhodnius praliocus* (Hemiptera), both of which produce hypo-osmotic fluids by ion resorption from an initially isosmotic secretion [21,28]. In *Pieris brassicae*, K^+ is reabsorbed passively by the ileum and the low water permeability of this epithelium maintains the osmotic gradient [26].

The morphological aspects of the ileum in described here, such as the infolding of the apical plasma membrane and the presence of vacuoles in the basal region of these cells are indicative of solute absorption without water flow. In a hypothetical model of this physiological mechanism, solutes would be absorbed from the ileal lumen and stored temporarily inside vacuoles, thereby avoiding the influx of water that would dilute the hemolymph and alter the osmotic balance in these insects.

In conclusion, the results of this study indicate that although the ileum of bees present some variations among species, this organ has not relationships with the sociality degree and phylogeny of bees being its morphological features related with the osmotic control in all species of bees studied.

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