HISTOLOGICAL AND ULTRASTRUCTURAL CHARACTERISATION OF THE STOMACH AND INTESTINE OF THE OPISTHOBRANCH BULLA STRIATA (HETEROBRANCHIA: CEPHALASPIDEA)

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ABSTRACT

In order to obtain more data for a comparative analysis of the digestive system in opisthobranchs, the stomach and intestine of Bulla striata were studied with light and electron microscopy. A 3D-model of the stomach and its connections with the posterior oesophagus, digestive gland ducts and intestine was created from a series of histological sections. The U-shaped stomach is just a segment of the digestive tube without any external distinction from the intestine. Internally, the stomach is characterized by the presence of a typhlosole and many mucus-secreting cells that are strongly stained by PAS reaction and alcian blue. Significant amounts of proteins were not detected in the mucus-secreting cells of the stomach, but protein-rich secretory material was found in the apical region of another type of secretory cells present in both stomach and intestine. The end of the typhlosole can be considered the transition point between the stomach and intestine. Mucus-secreting cells are also abundant in the intestine and all of them stain with alcian blue. However, most mucus-secreting cells of the intestine are not significantly stained by PAS reaction, but contain more proteins than the mucus-secreting cells of the stomach. The granular cells with a large number of small electron-dense secretory vesicles containing proteins and neutral polysaccharides were found only in the intestine. The available data show that despite some anatomical and histological differences several cell types are identical in the digestive systems of Aplysia depilans and B. striata.
INTRODUCTION

In recent years, molecular approaches have been used to investigate opisthobranch phylogeny (Wägele et al., 2003; Grande et al., 2004; Vonnemann et al., 2005; Malaquias et al. 2009a; Dinapoli & Klussmann-Kolb, 2010; Jörger et al., 2010). Due to these efforts, a new vision of the relationships among opisthobranch clades, and between these and other heterobranch gastropods is now emerging. The molecular data do not support the monophyly of the traditional opisthobranchs, but a clade (Euopisthobranchia) comprising the Umbraculoidea, Cephalaspidea, Runcinacea, Anaspidea (or Aplysiomorpha) and Pteropoda has received a good support (Klussmann-Kolb et al., 2008; Dinapoli & Klussmann-Kolb, 2010; Jörger et al., 2010). In this clade diets are very diversified: umbraculoideans feed on sponges, anaspideans and runcinaceans are herbivores, pteropods can be carnivores (Gymnosomata) or omnivores (Thecosomata), and cephalaspideans include both herbivorous and carnivorous species (Kohn, 1983; Wägele & Klussmann-Kolb, 2005; Malaquias et al., 2009b).
Dietary specialization and the related modifications of the digestive system were considered fundamental aspects in opisthobranch evolution (Thompson, 1976; Mikkelsen, 2002; Malaquias et al., 2009b), but in spite of that the digestive systems of these animals is not fully investigated yet. Some light microscopy studies dedicated to the digestive system of cephalaspideans were published many years ago (Fretter, 1939; Rudman, 1971, 1972b, 1972c). The radular teeth and gizzard plates of some species were studied using SEM (Gosliner, 1994; Malaquias & Reid, 2008), whereas histochemistry and TEM were used to investigate the digestive system only in a very limited number of species. The digestive system of Aplysia is fairly well studied, with several articles reporting histochemical and ultrastructural aspects of its organs (Taïeb & Vicente, 1999; Lobo-da-Cunha, 1999, 2000; Taïeb, 2001; Lobo-da-Cunha, 2001, 2002; Lobo-da-Cunha & Batista-Pinto, 2003, 2005, 2007). The salivary glands and the oesophagus of the herbivorous cephalaspidean Bulla striata have already been studied with histochemical and ultrastructural methods (Lobo-da-Cunha & Calado, 2008; Lobo-da-Cunha et al., 2010a, 2010b), but the digestive system of carnivorous cephalaspideans has just started to be investigated with the same level of detail (Lobo-da-Cunha et al., 2009). To our best knowledge, the digestive systems of umbraculoideans and pteropods were never studied by TEM, and in runcinaceans only the digestive gland was studied at the ultrastructural level (Kress et al., 1994).

In order to obtain more data for a comparative analysis of the digestive system in opisthobranchs, the stomach and intestine of B. striata were studied with light and electron microscopy methods.

**MATERIALS AND METHODS**

Specimens of Bulla striata Bruguière, 1792 about 2-3 cm in length were collected in Ria de Alvor and Ria de Faro, two estuaries in the South coast of Portugal. Stomach and intestine samples collected from 5 animals were processed for light and electron microscopy as reported below.

**Morphology**

For light microscopy, tissue pieces containing the stomach, initial portion of the intestine and part of the digestive gland were fixed for 24 h in Bouin solution, dehydrated in increasing concentrations of ethanol and embedded in paraffin. Middle and terminal segments of the intestine were processed in the same way. Tissue sections were stained with haematoxylin and eosin. For transmission electron microscopy (TEM), samples of stomach and intestine were fixed for about 2 h at 4°C in 2.5% glutaraldehyde and 4% formaldehyde (obtained from hydrolysis of para-formaldehyde), diluted with 0.4 M cacodylate buffer pH 7.4 (final buffer concentration 0.28 M). After washing in buffer, samples were postfixed with 2% OsO₄ buffered with cacodylate, dehydrated in increasing concentrations of ethanol and embedded in Epon. Semithin sections (2 µm) for light microscopy were stained with methylene blue and azure II. Ultrathin sections were stained with uranyl acetate and lead citrate, before being observed in a JEOL 100CII transmission electron microscope operated at 60 kV.

**Histochemistry**

The tetrazonium coupling reaction for protein detection was applied to 2 µm sections of Epon embedded stomach and intestine fragments. These semithin sections were treated with a 0.6% solution of H₂O₂ for 10 min., to remove the osmium tetroxide fixative from the tissue. After washing in water, sections were treated for 10 min with a freshly-prepared 0.2% solution of fast blue salt B in veronal-acetate buffer pH 9.2, washed in water and treated for 15 min with a saturated solution of β-naphthol in veronal-acetate buffer pH 9.2 (Ganter & Jollès, 1970). The PAS reaction for polysaccharide detection was applied to sections of paraffin embedded pieces and to semithin sections of Epon embedded fragments. After oxidation with 1% periodic acid for 10 min, tissue sections were washed with water and stained with Schiff reagent for about 15 min (Ganter & Jollès, 1970). Alcian blue staining was applied to sections of paraffin embedded material.
detection of carboxylated polysaccharides, sections were stained for 20 min. with 0.5% alcian blue in 3% acetic acid (pH 2.5). To demonstrate the presence of sulphated polysaccharides, sections were stained for 20 min. with 0.5% alcian blue diluted in a HCl solution with pH 1.0 (Ganter & Jollès, 1970). Sections of paraffin embedded tissues were subsequently stained with haematoxylin, washed, dehydrated and mounted with DPX. Epon sections were washed, air dried and mounted with DPX.

Figure 2:
Histology and histochemistry of the stomach of *B. striata*. A. Transverse section through the middle of the U-shaped stomach (st) showing the typhlosole (arrows). Numerous PAS positive secretory cells (arrowheads) can be seen in the stomach epithelium, but not in the long digestive gland duct (ld). Digestive gland tissue (dg) surround the stomach. B. A few PAS positive secretory cells (arrowheads) can be seen in the epithelium of the long digestive gland duct (ld) at the connection point with the stomach (st). C. Detail of the stomach epithelium showing lining epithelial cells (asterisk) and PAS positive bottle-shape secretory cells (arrows). The unstained zone (arrowhead) in the basal region of these mucus-secreting cells corresponds to the nucleus. D. Alcian blue stains stomach mucus-secreting cells (arrows) that are also present in the typhlosole (ts). E. Stomach mucus-secreting cells (arrowheads) are not significantly stained by the tetrazonium coupling reaction, but this technique revealed protein-rich secretory material at the apex of another type of secretory cells (arrow). nu - nuclei. F. Apical secretory material is not stained by PAS reaction (arrow), but glycogen deposits in neighbour cells are strongly PAS positive (arrowhead).

**Cytochemistry**

For polysaccharide detection by TEM, ultrathin sections collected on copper grids were treated with a 5% solution of tannic acid for 10 min, briefly washed in water, stained with 2% uranyl acetate for 10 min and finally washed in water (Sannes *et al*., 1978).

For localization of acidic polysaccharides by TEM, the colloidal iron method described by Knight
and Lewis (1992) was used. A stock solution of colloidal iron was prepared adding 1 ml of a 25% FeCl₃ solution drop-by-drop to 50 ml of boiling water. The dark red stock solution was filtered and dialysed. Just before use, 1.5 ml of stock solution were diluted with 7.5 ml of an acetic acid solution with a pH about 1.8. Ultrathin sections were collected on gold grids and stained for 5 min with the diluted colloidal iron solution. The grids were washed in 10% acetic acid and finally in distilled water, before being observed with the transmission electron microscope.

**RESULTS**

**Anatomy, histology and histochemistry**

The stomach of *B. striata* is embedded in a narrow tip of the digestive gland (Fig. 1A). On the surface of the digestive gland it is possible to see one long straight duct that opens into the stomach near the connection point between the stomach and the posterior oesophagus (Fig. 1A). In order to disclose what lies underneath the surface of the digestive
A gland, a 3D model was created from a series of 200 histological sections stained with haematoxylin and eosin. To obtain an unobstructed view of the stomach and its connections with the posterior oesophagus, digestive gland ducts and intestine, the digestive gland tissue was excluded from the model (Fig. 1B-D). In the model it is possible to see a short digestive gland duct that opens into the top of the stomach, behind the terminal portion of the posterior oesophagus (Fig. 1C-D). In the animal, this short digestive gland duct is completely covered by digestive gland tissue being invisible on the surface. The U-shaped stomach is just a segment of the digestive tube without any external distinction from the anterior portion of the intestine that circles around the tip of the digestive gland, being visible at its margin (Fig 1A-D). In the largest animals used in this study, the intestine was about 2 cm long.

Two sections of the U-shaped stomach can be seen when this organ is transversely cut (Fig. 2A). The stomach wall forms a typhlosole along the inner curve of this U-shaped organ, and a very large number of secretory cells are present in the stomach epithelium (Fig. 2A). On the other hand, secretory cells are rare in the epithelium of digestive gland ducts (Fig. 2A-B). The lining epithelium of the stomach is formed by thin ciliated and non-ciliated columnar cells interspersed with two types of secretory cells. Bottle-shaped mucus-secreting cells are very abundant, with
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a thin apical neck and a larger basal region containing the nucleus (Fig. 2C-E). Much less frequent are the cells with an apical mass of secretion (Fig. 2E-F). The histochemical properties of both cell types can be seen in Table 1. A thin muscular layer surrounds the stomach epithelium.

In the intestine the typhlosole is absent (Fig. 3A). This structure ends not far after the middle of the digestive tube segment that goes from the curve of the U-shaped stomach to the first curve of the intestine around the tip of the digestive gland (Fig. 1C). Most intestine epithelial cells possess cilia, but some are non-ciliated (Fig. 3B). The most abundant secretory cells present in the intestine are the granular cells and mucus-secreting cells. The granular cells are thinner and contain a large number of small secretory granules most of them in the cytoplasm above the nucleus, which is positioned approximately in the centre of the cell (Fig. 3B-D). The histochemical properties of this cell type are included in Table 1. Mucus-secreting cells of the intestine are bottle-shaped (Fig. 3C-E), but the histochemical techniques show that two kinds of mucus secreting cells are present in the intestine (Table 1). In sections stained by both PAS reaction and alcian blue, it can be seen that the larger mucus-secreting cells (mucous cells II, in Table 1) are stained by alcian blue showing a light blue colour. The thinner ones (mucous cells I, in Table 1) are stained by both procedures appearing with a dark blue coloration in result of the superposition of light blue and the magenta colour of PAS positive cells, but none are stained by PAS reaction alone (Fig. 3F). However, the change in the staining pattern of mucus-secreting cells occurs gradually at the transition zone between the stomach and the intestine. After the end of the typhlosole the number of PAS positive cells gradually diminishes in the digestive tube, and after the first curve of the intestine only about one third of the mucus-secreting cells are PAS positive (Fig. 3E). In addition to these cell types, the cells with an apical mass of protein-rich secretory material found in the stomach epithelium are also present along the intestine in small amounts (Table 1). A thin layer of muscular cells surrounds the epithelium of the intestine (Fig. 3B).

Ultrastructure and cytochemistry

The lining epithelium is identical in both the stomach and the intestine. It is formed by thin elongated cells with a border of microvilli between 2 and 3 µm long, and most of them also have cilia (Fig. 4A). Vesicles, multivesicular bodies, some lysosome-like bodies and several mitochondria can be seen in the supranuclear region (Fig. 4A-C). A few lipid droplets can be found in these epithelial cells, but glycogen is the main reserve substance filling a substantial part of the cytoplasm in some cells (Fig. 4D).

The mucus-secreting cells of the stomach are filled with a large number of secretory vesicles of variable dimensions with low or median electron-density. They can be oval or irregular in shape (Fig. 5A) and some of them may be fused forming larger compartments with secretory material. The vesicles contain very fine filaments forming a reticulate pattern that can be denser in some vesicles than in others (Fig. 5B). The secretory vesicles are moderately stained by tannic acid-uranyl acetate method for polysaccharide detection (Fig. 4D), and with the colloidal iron method for acid polysaccharide detection iron particles are distributed over the secretory material contained in the vesicles (Fig. 5C). These cells possess several Golgi stacks formed by a large number of flat cisternae, mainly located around the nucleus (Fig. 5A-B). A few mitochondria and rough endoplasmic reticulum cisternae are also present. Very frequently, an intraepithelial nerve terminal was seen in direct contact with the basal region of a mucus-secreting cell. In ultrathin sections of these zones the basal lamina has a reticulated appearance, and some perforations are clearly visible in the basal lamina (Fig. 5D).

In the intestine, the mucous cells I are similar to the mucous cells of the stomach. However, the majority of intestinal mucous cells (type II) possess
large electron-lucent vesicles in which the secretory material forms a web of fine filaments linking small cores with low electron-density (Fig. 6A). These vesicles also fuse with each other, but the substances they enclose are not significantly stained by the tannic acid-uranyl acetate method. On the other hand, the secretory material is strongly marked by colloidal iron particles. Very fine strings of iron particles cover the thin web of filaments and a high concentration of iron particles is seen around the cores of secretory material (Fig. 6B). The concentration of iron particles on these cores creates a pattern with coarse spots, which is
very different from the more uniform distribution of iron particles in the secretory vesicles of the stomach type of mucous cells. The secretory vesicles fill the cytoplasm almost completely and usually just some Golgi stacks can be seen in peripheral zones of the cytoplasm. These Golgi stacks formed by many flattened cisternae resemble the ones observed in the stomach mucus-secreting cells.

Cells with an apical mass of secretory material were observed in ultrathin sections of both stomach and intestine epithelia. These cells possess some

Figure 6:

Ultrastructure of intestinal secretory cells. A. Intestinal mucous cell containing secretory vesicles with a web of very fine filaments (arrowheads) connecting small cores of secretory material (arrows). nu - nucleus. B. In the secretory vesicles, colloidal iron particles attach to the thin web of filaments (arrowheads) and around the cores of secretory material (arrows). C. Cell containing an apical mass of secretory material (asterisks) showing some microvilli at the apex (arrowhead). The secretory material of the granular cells has a higher electron density (arrows). D. Apical region of a granular cell showing microvilli (asterisk), multivesicular bodies (arrows), and many electron-dense secretory vesicles (arrowheads).
microvilli and their apical region is typically filled by a membrane bound mass of secretory material with a very irregular shape and median electron-density, that seems to result from the fusion of smaller vesicles (Fig. 6C). Material with identical electron-density and texture is also present in round vesicles and Golgi stack cisternae. Several small Golgi stacks and many rough endoplasmic reticulum cisternae are present in these cells that also contain a few lysosome-like bodies.

The intestinal granular cells are characterised by a large number of spherical secretory vesicles containing electron-dense material. These vesicles are very abundant in the cytoplasm above the nucleus, reach a diameter about 0.8 µm and never fuse with each other (Fig. 6D). The cell apex is covered by microvilli about 2 µm long. Some multivesicular bodies and small electron-lucent vesicles are usually present in the apical region, but endoplasmic reticulum cisternae are not abundant (Fig. 6D). The nucleus is located in the central part of the cell. Below the nucleus only a small number of secretory vesicles are present, because the basal region of these cells is filled with very deep cell membrane invaginations associated with a large number of mitochondria.

As in the stomach, intraepithelial nerve terminals attached to the basal region of secretory cells and a reticulated basal lamina with perforations were also observed in the intestine of *B. striata*.

**DISCUSSION**

Cephalaspideans and Anaspideans are two opisthobranch clades with a close phylogenetic relationship (Dinapoli & Klussmann-Kolb, 2010; Jörger *et al.*, 2010), including the herbivorous species *B. striata* and *Aplysia depilans*, respectively. With the existing anatomical, histochemical and ultrastructural data, it is now possible to make a more detailed comparative analysis of the digestive system of these two species. Both possess long ribbon-shaped salivary glands, starting from the posterior part of the buccal mass and ending near the gizzard. Moreover, histochemical and ultrastructural studies revealed that the salivary glands of these species have in common two types of mucus-secreting cells, named granular mucocytes and vacuolated mucocytes. Nevertheless, the ciliated cells present in the salivary glands of these species are different, being secretory
in *A. depilans* and non-secretory in *B. striata* (Lobo-da-Cunha, 2001, 2002; Lobo-da-Cunha & Calado, 2008). Few mucous cells were found in the oesophagus and crop of *A. depilans* while in *B. striata* mucous cells are very abundant in the anterior and posterior oesophagus (Lobo-da-Cunha & Batista-Pinto, 2005; Lobo-da-Cunha et al., 2010a, 2010b). Both species have a gizzard, although with a different number of hard plates. In *A. depilans*, a filter chamber is positioned between the gizzard and the stomach, containing many acicular teeth to prevent the entrance of larger algal fragments into the stomach (Howells, 1942; Fretter & Ko, 1979). In *B. striata*, the gizzard is followed by the posterior oesophagus, but some acicular teeth are present in the most anterior region of the posterior oesophagus (Lobo-da-Cunha et al., 2010b).

In both species the stomach is embedded in the digestive gland and linked to it by ducts, and the intestine is attached to the digestive gland for most of its length. Nevertheless, substantial anatomical differences can be found in the stomach, which in Aplysiidae ends in a caecum that does not exist in *B. striata* (Howells, 1942; Fretter & Ko, 1979). A caecum is also present in the stomach of the Thecosomata, which are closely related with the Anaspideans (Klussmann-Kolb & Dinapoli, 2006).

Mucus-secreting cells are very abundant in the stomach epithelium of these opisthobranchs. However, the secretory material of these cells is rich in proteins in *A. depilans* (Lobo-da-Cunha & Batista-Pinto, 2003) and that is not true for the stomach of *B. striata* (Table 1). Despite that, in each of these species stomach mucous cells form secretory vesicles with low or medium electron-density containing thin filaments of secretory material (Lobo-da-Cunha & Batista-Pinto, 2003). In addition to these mucus-secreting cells, the stomach epithelium of *B. striata* contains cells specialised in protein secretion, whereas in the stomach of *Aplysia* just the mucous cells have been reported so far (Howells, 1942; Lobo-da-Cunha & Batista-Pinto, 2003). In the stomach of the carnivorous cephalaspideans *Philine aperta* and *Scaphander lignarius* just one type of mucus-secreting cells has been reported (Fretter, 1939). However, the carnivorous cephalaspidean *Melanochlamys cylindrica* and the herbivore *Bulla quoyi* seem to be devoid of secretory cells in the stomach (Rudman, 1971, 1972c). On the other hand, the stomach epithelium of *Haminoea* is more complex, containing additional types of secretory cells (Fretter, 1939; Rudman, 1971). The stomach epithelium of the herbivore *Haminoea hydatis* includes a cell type characterized by an apical accumulation of secretory material (Fretter, 1939), that could be related to the cells found in the stomach and intestine of *B. striata* also containing a mass of secretory material in the apical region. Among the Acteonidae, secretory cells of the mucous type were reported in the stomach of some species, but not in others (Fretter, 1939; Rudman, 1972a).

Mucus-secreting cells are allegedly responsible for the lubrication of the luminal surface of the digestive tube and were also found in the stomach of many other gastropods (Bolognani Fantin et al., 1982; Roldan & Garcia-Corrales, 1988; Soto et al., 1990; Leal-Zanchet, 1998). However, only a few papers reported ultrastructure aspects of the stomach cells of gastropods (Pipe, 1986; Triebskorn, 1989; Boer & Kits, 1990; Leal-Zanchet, 2002; Lobo-da-Cunha & Batista Pinto, 2003; Martin et al., 2010).

In the intestine, *A. depilans* and *B. striata* contain granular cells and mucus-secreting cells (Table 1). The former are characterized by the presence of electron-dense secretory vesicles containing proteins and neutral polysaccharides, and could be classified as serous cells (Lobo-da-Cunha & Batista-Pinto, 2007). In the intestine of *B. striata*, the extensive system of cell membrane invaginations associated to a large number of mitochondria in the basal region of the granular cells suggests an intense active transport in these cells. In the intestine of *A. depilans* just one kind of mucous cells was detected (Lobo-da-Cunha & Batista-Pinto, 2007), while in *B. striata* some are PAS
positive and others PAS negative (Table 1). In addition to this difference in PAS reactivity, the mucous cells of B. striata intestine can be distinguished by the content of the secretory vesicles and by the pattern of colloidal iron staining. They may represent two sub-types of mucous cells or different stages of cell maturation. The secretory vesicles containing a web of thin filaments linking small cores of secretory material in the intestinal type of B. striata mucous cell resemble the vesicles described in mucus-secreting cells of the anterior oesophagus of this species (Lobo-da-Cunha et al., 2010a).

The cells with an apical mass of secretion observed in the stomach and intestine of B. striata were not reported in Aplysia. These cells belong to a different type, and can easily be distinguished from the granular cells by the difference in electron-density of the secretory material, absence of PAS staining and fusion of secretory vesicles creating the large mass of secretory material in the cell apex, an aspect never observed in the granular cells of both species (Lobo-da-Cunha & Batista-Pinto, 2007).

Two types of secretory cells were also reported in the intestine of the opisthobranchs A. punctata (Howells, 1942), B. quoyi (Rudman, 1971), S. lignarius (Fretter, 1939) and in some species of Philine (Fretter, 1939; Rudman, 1972b), but although their characterization was based only on light microscopic observations, those cells seem to correspond to the intestinal mucous and granular cells of B. striata and A. depilans (Lobo-da-Cunha & Batista-Pinto, 2007). However, in Haminoea zelandiae just one type of secretory cell was reported in the intestine (Rudman, 1971). Ultrastructural observations of the intestinal epithelium of the marine snail Nerita picea revealed secretory cells very similar to the granular cells of B. striata and A. depilans (Pfeiffer, 1992). In the limpet Patella vulgata, the intestinal epithelium contains club-shaped protein secreting cells rich in rough endoplasmic reticulum cisternae and its secretion coats the fecal rods with a protein layer, preventing them from disintegrating (Bush, 1988). These cells are also similar to the granular cells of B. striata and A. depilans, which may have similar functions, but the secretory granules in P. vulgata club-shaped cells are not stained by PAS reaction. However, considering the data that support an absorptive function for the intestine, secretion of digestive enzymes cannot be ruled out for intestinal cells containing proteins in their secretory vesicles. Two or more secretory cell types were encountered in the intestine of pulmonate gastropods, including mucous cells and other secretory cells (Triebskorn, 1989; Boer & Kits, 1990; Franchini & Ottaviani, 1992; Leal-Zanchet, 1998, 2002).

The presence of many intraepithelial nerve terminals in close association with the base of secretory cells in both stomach and intestine of B. striata put in evidence the importance of nervous control over the activity of these parts of the digestive tract, as previously reported in other species of gastropods (Bush, 1988; Boer & Kits, 1990; Lobo-da-Cunha & Batista-Pinto, 2003).

The columnar cells of the stomach and intestine lining epithelium are also identical in B. striata and A. depilans. Lysosomes with arilsulphatase activity are very conspicuous in the supra-nuclear region of stomach and intestine columnar epithelial cells of A. depilans and probably are responsible for the intracellular digestion of substances collected from the lumen by endocytosis (Lobo-da-Cunha & Batista-Pinto, 2003, 2007). The digestive gland is recognized as the main sites of digestion and nutrient absorption in gastropods, but it was demonstrated that absorption of nutrients occurs along the digestive tract (Walker, 1972; Orive et al., 1979). According to some authors, the function of the gastropod intestine is the consolidation of fecal pellets (Mikkelsen, 1996). However, the presence of a microvillous border, lysosomes and the accumulation of lipids and glycogen in epithelial cells of the digestive tract of gastropods were considered as signs of an absorptive function (Roldan & Garcia-Corrales, 1988; Boer &
Kits, 1990). In addition, the observation of vesicles and multivesicular bodies that are usually related with endosomes in the apical region of epithelial cells strongly supports the existence of an endocytic activity in the stomach and intestine of B. striata. The thin muscular layer in the intestine wall of B. striata suggests that ciliary action is an important factor for the movement of fecal matter. A partial typhlosole extending a short distance into the intestine was considered the plesiomorphic state in cephalaspideans, but its absence in the intestine of Bulla and other euopisthobranch genera was previously reported (Mikkelsen, 1996).

To conclude, the available data show that despite some differences the digestive systems of A. depilans and B. striata present several similar anatomical aspects and many of their cell types are identical. It will be interesting to see if some characters of the digestive system such as cell types are phylogenetically relevant or related with dietary specialization. However, for a cladistic analysis it will be necessary to have detailed ultrastructural and histochemical information on digestive system cells of more species. Additionally, the diversity of cell types, especially in the intestine of B. striata, suggest the secretion of different specific substances that would be interesting to study further in order to improve our knowledge about the physiology of the digestive process in opisthobranchs. The application of histochemical methods in semithin sections and the ultrastructural study have proved to be very valuable for the detection and characterization of those cell types, allowing a better understanding of the digestive system in opisthobranchs.

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