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Light and electron microscopy study of the digestive system in the Euopisthobranchia (Mollusca, Gastropoda)

Lobo-da-Cunha, A.[*/**], Oliveira, E.[*], Alves, A.[*] and Calado, G.[***]

[*] Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal
[**] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal
[***] Lusophone University of Humanities and Technologies, Lisbon, Portugal

Email: alcunha@icbas.up.pt

The Euopisthobranchia is a clade of marine gastropods including herbivorous and carnivorous species. Since herbivory seems to be their ancestral condition [1], the species that changed feeding habits must have gone through some transformations during the adaptation to a carnivorous diet. To understand the evolution of their digestive system we are trying to find out how the histological and ultrastructural features are correlated with diet and phylogeny. The salivary glands, esophagus and crop were studied by light and electron microscopy in three herbivores (*Aplysia depilans*, *Bulla striata* and *Haminoea navicula*) and three carnivores (*Philine quadripartita*, *Aglaja tricolorata* and *Philinopsis depicta*). Secretory cells were characterized with histochemical techniques applied to semithin sections (PAS reaction for polysaccharides; alcian blue at pH 2.5 for carboxylic acid polysaccharides; alcian blue at pH 1 for sulphated acid polysaccharides; tetrazonium coupling reaction for proteins). Colloidal iron was used to detect acid polysaccharides by TEM (Fig. 1). Salivary glands comprise secretory and ciliated cell. Cell types and the main features of their secretory vesicles are given in Table 1. In these herbivores, salivary glands are long and ribbon-shaped, being largely formed by mucocytes with protein secreting granular cells as a minor component. Both granular mucocytes (Fig. 1) and vacuolated mucocytes have very similar histochemical and ultrastructural features in *A. depilans* and *B. striata*. Considering the phylogenetic position, diet [2] and our results, it seems that their salivary glands might be more closely related to the ancestral type of salivary glands in the Euopisthobranchia, in which acid polysaccharides are the major secretory products. In *H. navicula* (Figs. 2-3) and *P. quadripartita* acid polysaccharides are also the main secretory products, but granular cells secreting proteins are more developed in the carnivore *P. quadripartita* (Fig. 4). In carnivores of the family Aglajidae (*A. tricolorata* and *P. depicta*) the short salivary glands are formed by different cell types (Fig. 5) and seem to be the most derived type of salivary glands in the Euopisthobranchia, in which secretory cells secrete large amounts of proteins mixed with acid polysaccharides. However, more species must be investigated to verify these hypotheses. Cells with a very large vacuole surrounded by a very thin layer of cytoplasm were observed in the connective tissue of the crop or esophagus of the three carnivores. Cell membrane invaginations are common in these cells. The detection of calcium in the vacuole using the pyroantimonate method for TEM points to a relationship with the calcium cells of the connective tissue of pulmonate gastropods. These cells could provide ions for buffering the pH of body fluids [3]. Since the vacuolar cells were not found in the digestive tract of the herbivorous euopisthobranchs, they might be a trait of the esophagus and crop in carnivorous euopisthobranchs.

[1] Göbbeler, K. and Klussmann-Kolb, A. *Thalassas*, **27**, 121–154, 2011.
[2] Malaquias, M.A.E. *et al. J. Zool.*, **227**, 88–97, 2009.
[3] Sminia, T. *et al. Neth. J. Zool.*, **27**, 195–208, 1977.

Table 1. Salivary glands of herbivorous (H) and carnivorous (C) euopisthobranchs.

Species / Diet	Secretory cells	Features of secretory vesicles
Aplysia depilans **H**	Granular cells	Proteins and neutral polysaccharides; electron-dense
	Granular mucocytes	Acid polysaccharides and proteins; metachromatic; electron-dense and electron-lucent components
	Vacuolated mucocytes	Acid polysaccharides; electron-lucent
Bulla striata **H**	Granular mucocytes	Acid polysaccharides and proteins; electron-dense and electron-lucent components
	Vacuolated mucocytes	Acid polysaccharides; electron lucent
Haminoea navicula **H**	Granular cells	Proteins and neutral polysaccharides; electron-dense
	Granular mucocytes	Acid polysaccharides; metachromatic; with filaments
	Vacuolated mucocytes	Acid polysaccharides and proteins; electron-dense and electron-lucent components
Philine quadripartita **C**	Granular cells	Proteins and neutral polysaccharides; electron-dense
	Granular mucocytes	Acid polysaccharides; metachromatic; electron-lucent
	Vacuolated mucocytes	Acid polysaccharides; electron-lucent
Aglaja tricolorata **C**	Granular cells	Proteins and neutral polysaccharides; electron-dense
	Alveolar cells	Proteins and acid polysaccharides; electron-dense
	Cells with dense vacuoles	Proteins and acid polysaccharides; electron-dense
Philinopsis depicta **C**	Granular cells	Proteins and acid polysaccharides; electron-dense
	Cells with apical vacuoles	Proteins and acid polysaccharides; electron-dense

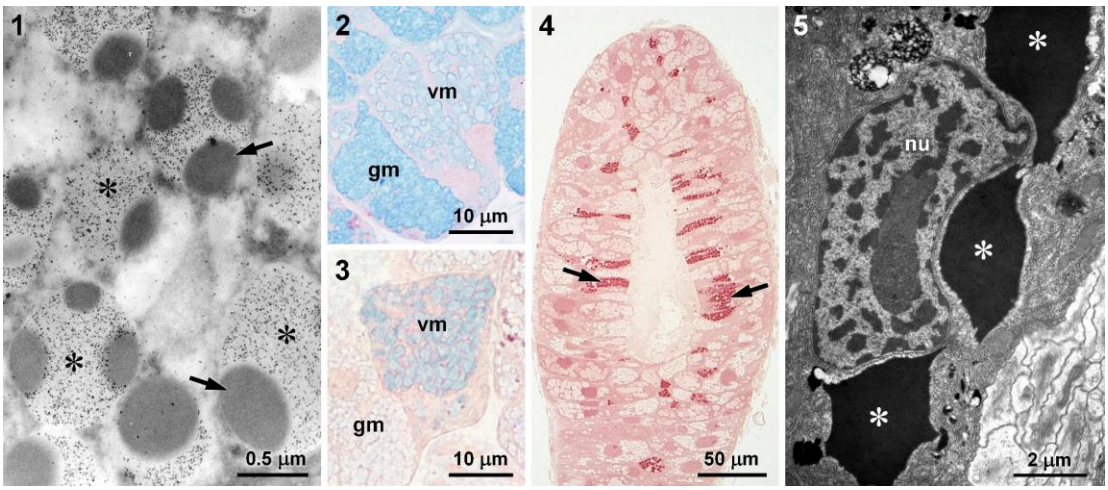

Fig. 1 *B. striata*; colloidal iron particles are attached to acid polysaccharides in the electron-lucent region (asterisks) of the secretory vesicles of granular mucocytes, the electron-dense component of the vesicles (arrows) is made of proteins. **Fig. 2** *H. navicula*; granular mucocytes (gm) and vacuolated mucocytes (vm) stained by alcian blue at pH 2.5. **Fig. 3** *H. navicula*; the vacuolar mucocytes (vm) are stained by alcian blue at pH 1, but granular mucocytes (gm) are not. **Fig. 4** *P. quadripartita*; granular cells (arrows) stained by the tetrazonium reaction for protein detection. **Fig. 5** *A. tricolorata*; secretory cell with vacuoles containing highly electron-dense substances (asterisks); nu - nucleus.

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