

# Vacuolar cells seem to be a special trait of the esophagus and crop of carnivorous cephalaspideans (Euopisthobranchia)

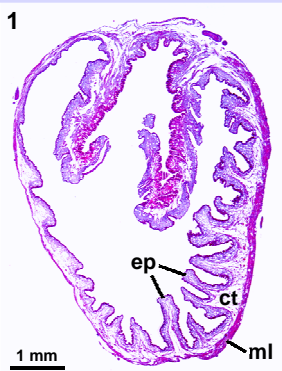
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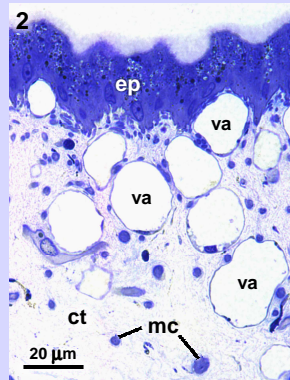


Vacuolar cells were first reported in the crop connective tissue of the carnivorous cephalaspidean *Philinopsis depicta* (Lobo-da-Cunha *et al.* 2011). These cells also exist in the crop of *Aglaja tricolorata* and in the esophagus of *Philine quadripartita*. But, so far, similar cells were not found in the digestive tract of herbivorous cephalaspideans. Calcium detection in the vacuole of these cells supports a relationship with the calcium cells of the connective tissue of pulmonate gastropods. According to Sminia *et al.* (1977), calcium cells provide ions for buffering the pH of body fluids.

## Crop of *Philinopsis depicta*



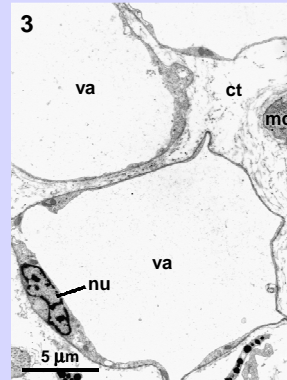
1 - Transverse section of crop stained with hematoxylin and eosin.



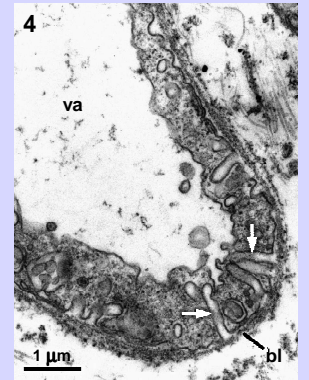
2 - Semithin section stained with methylene blue and azure II.

A very thin layer of cytoplasm surrounds the huge vacuole of these cells. They are more abundant close to the epithelium, but were never seen in direct contact with the lumen of the crop.

**Abbreviations:**  
 bl - basal lamina  
 ct - connective tissue  
 ep - epithelium  
 mc - muscle cells  
 ml - muscular layer  
 nu - nucleus  
 va - vacuolar cells  
 ve - vesicles

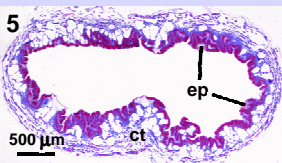


3 - Vacuolar cells observed by transmission electron microscopy.

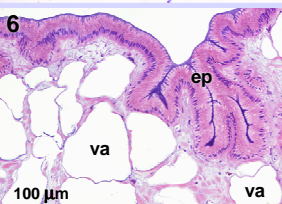


4 - Cell membrane invaginations (arrows) are common in vacuolar cells.

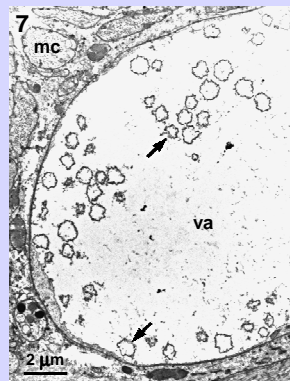
## Crop of *Aglaja tricolorata*



5 - Crop section stained with Masson's trichrome.



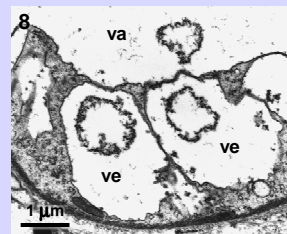
6 - Crop section stained with hematoxylin and eosin.



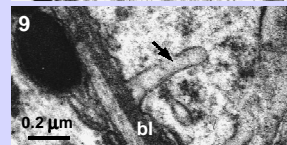
7 - The vacuolar cells of *A. tricolorata* contain electron-dense loops (arrows) in the vacuole and in vesicles.

With the pyroantimonate method for TEM, calcium was detected within the vacuole and vesicles of vacuolar cells. With this technique, electron-dense deposits are formed in structures that contain calcium, even if present in very low amounts (Fig. 10). Control sections were treated with an EGTA solution, which removes the calcium pyroantimonate deposits (Fig. 11).

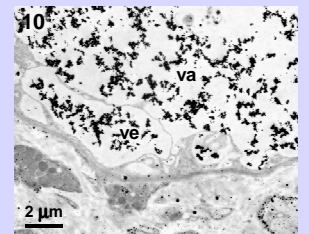
Control sections were treated with an EGTA solution, which removes the calcium pyroantimonate deposits (Fig. 11).



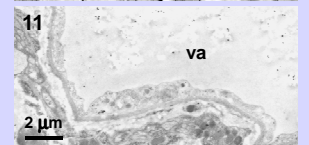
8 - Vesicles with loops. 9 - Cell membrane invagination.



9 - Cell membrane invagination.



10 - Calcium detection. 11 - Control of calcium detection.

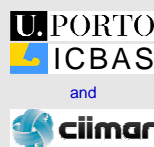


11 - Control of calcium detection.

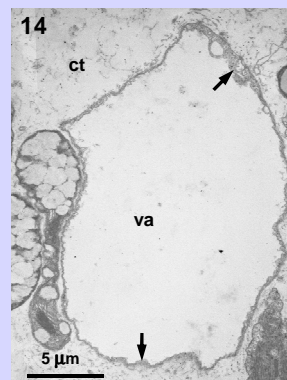
## References:

Lobo-da-Cunha, *et al.* 2011. Microscopical study of the crop and oesophagus of the carnivorous opisthobranch *Philinopsis depicta* (Cephalaspidea, Aglajidae). *Journal of Molluscan Studies*, 77: 322–331.  
 Sminia, *et al.* 1977. Structure and function of the calcium cells of the freshwater pulmonate snail *Lymnaea stagnalis*. *Netherlands Journal of Zoology*, 27: 195–208.

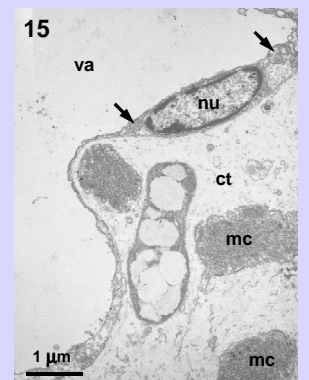
This work was supported by:



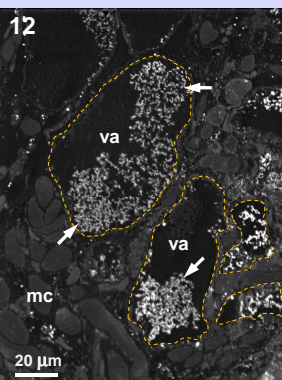
## Esophagus of *Philine quadripartita*



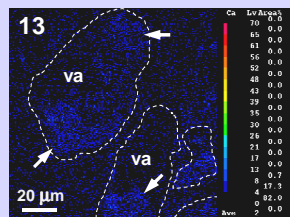
14 - A very thin layer of cytoplasm (arrows) surrounds the large vacuole of these cells.



15 - Detail of a vacuolar cell, showing the nucleus and a few mitochondria (arrows).



12 - Backscattered electrons image showing calcium pyroantimonate deposits (arrows).



13 - Intensity X-ray distribution map of calcium, obtained with an electron microprobe equipped with WDS detectors. Although in low concentration, calcium is present in the vacuolar cells (arrows), and its distribution corresponds to the deposits observed in backscattered electrons images.