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Ultrastructure of the earthworm calciferous gland. A preliminary study

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The earthworm species belonging to the Lumbricidae family (Annelida, Oligochaeta) posses a complex oesophageal organ known as “calciferous gland” which secretes a concentrated suspension of calcium carbonate. Previous studies have demonstrated the non-crystalline structure of this calcareous fluid representing an interesting example of biomineralisation [1].
Among the family members, the species of the genus *Lumbricus* show the most well developed glands, consisting of two glandular portions in segments XI and XII and two oesophageal pouches in segment X where the crystallization process takes place in the form of calcite crystals which are then released into the gut lumen and eventually to the soil (Fig. 1).
Since they were first described in 1829 earthworm calciferous glands have intrigued anatomists and physiologists (including Darwin) and a number of studies on the structure and function of this gland have been publised with special focus on *Lumbricus terrestris*, a well represented species in the British Isles [2]. Consequently, very little information is available on other members of the Lumbricidade family. Here we show the ultrastructure of the calciferous gland of a widespread earthworm species in the Iberian Peninsula, *L. friendi*.
Live specimens were anesthetised, dissected and small blocks of the calciferous gland were immersion-fixed in 2% paraformaldehyde - 2.5% glutaraldehyde in 0.24 M phosphate buffer (pH 7.2), postfixed in 1% OsO_4, dehydrated in a graded acetone series and embedded in Spurr. Methylene blue-stained semithin sections were studied and appropriate areas re-trimmed, ultrathin sectioned and stained in a 2% aqueous uranyl acetate solution and Reynolds lead citrate. Sections were examined on a JEM1010 TEM (JEOL) and photographed using a Gatan Orius CCD camera.
Our preliminary results show that, in cross section, the glandular portions (in segments XI and XII) consist of lamellae disposed radially to the oesophagus. Each lamella consisted of two layers of a secretory epithelium with a blood sinus in between. In addition, is not unusual to find extracellular formations of variable size in between lamella as well as calcite crystals (Fig. 2).
The TEM analyses revealed that the cells of the lamella were irregular in shape with their apical surface showing a number of indentations and/or club-shaped extensions. Furthermore, their nucleus appears to be irregular and euchromatic (Fig. 3a). It is also noticeable that the cell basal area is extremely folded and contains abundant mitochondria and membranous infoldings. This morphology could well represent an adaptation for absorption. If this is the case it would be possible for the calcium in the blood to enter through the basal infoldings and then to be transported and discharged at the peripherical or apical cell surface.
The cytoplasm shows a great number of oval to elongated mitochondria together with extensive Golgi complexes containing stacks of lamellae (Fig. 3b). Vesicles, scattered ribosomes, multivesicular bodies, rough and smooth endoplasmic reticulum and curved structures membranous are often observed. The presence of the rough endoplasmic reticulum and the Golgi complex is usually related to the synthesis and modification of proteins. Our results suggest that the organic

matrix surrounding the spherulites is possible secreted by them. Interestingly, several osmiophilic cytoplasm inclusions of varying electron density, size and shape (often round) and enclosed by membranes are usually scattered at the periphery of the cytoplasm (Fig. 4). Additionally, electron-translucent areas can also be found.
The secretion in the form of 'spherulites', at different mineralisation stages, appear to be concentrated in the extracellular environment between lamellae. Interestingly, they are usually wrapped by an organic matrix which possibly plays a role in their further transformation to solid (Fig. 3a).

References

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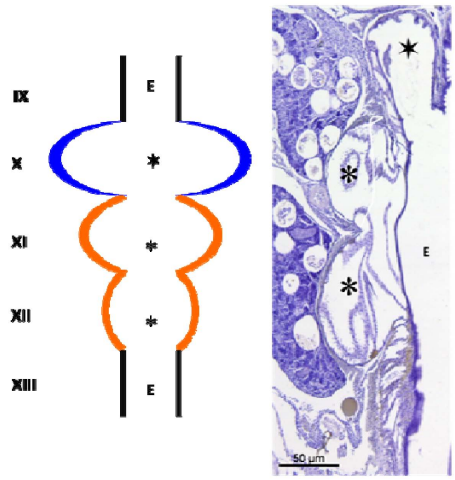

Fig. 1. Schematic diagram and longitudinal section of the calciferous gland of *L. friendi* showing the two pairs of glandular enlargements in segments XI and XII and the anterior oesophageal pouches in segment X which open to the oesophagus (E). Hematoxylin.

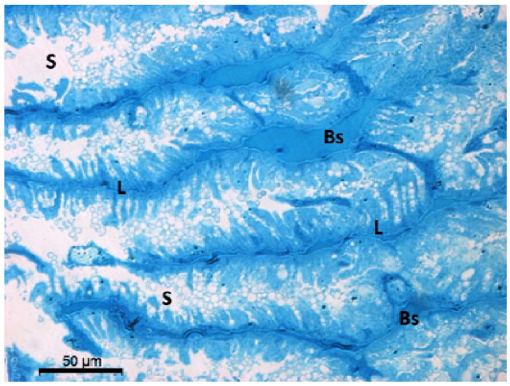

Fig. 2. Semithin section (methylene blue stain) showing the glandular portions of the gland. The secretory lame (L) are disposed radially to the oesophagus. Bs: blood sinus. S: spherulites.

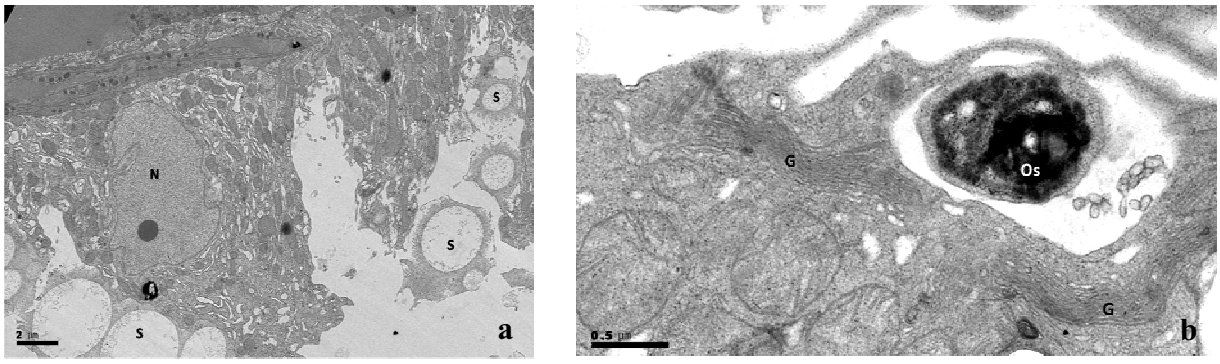

Fig. 3. TEM micrograph showing the general structural organisation of the epithelium. Note (a) the presence of the spherulites (S) in the interlamellar space wrapped by the organic matrix and (b) the high abundance of mitochondria and the extensive Golgi complexes (G). Os: osmiophilic cytoplasm inclusions.

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