Description of calcareous structures of the apoda holothurian *Synaptula hydriformis* (Lesueur 1824)

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Echinoderms usually have a rigid body wall with an elaborate magnesium-rich calcite endoskeleton (Raup 1966). In the Holothuroidea class, the integumental skeleton consists of microscopic ossicles formed within multinucleated syncytial sclerocytes present in the dermal layer of the body wall (Stricker 1986). These ossicles may show a great variety of forms from the simplest, such as small rods, to the most elaborate ones such as forms that vary from perforate and ornamented plates. The calcareous ring, a structure formed by calcified plates that surround the pharynx, is also part of the endoskeleton of these animals (Conand 1990). These calcareous structures have great importance for the systematic character of species identification of holothurians (Pawson and Fell 1965). In this work, the ossicles and other calcareous structures of the apoda holothurian, *Synaptula hydriformis* (Lesueur, 1824), were studied in confocal and scanning electron microscopy.

Adult specimens of *S. hydriformis*, collected in the Canal de São Sebastião in Sao Paulo, Brazil, were reared in a laboratory at the Centro de Biologia Marinha (Universidade de São Paulo) in the same city. Specimens were relaxed in 7.5% $MgCl_2$ solution in seawater (1:1) and preserved in 70% alcohol. For scanning by electron microscopy (SEM), the ossicles were isolated by digesting the body wall fragments using a solution of NaOH, and transferred to double-face tape in stubs and coated with gold. The material was observed in a TSM 940 Zeiss microscope. To study the development of the ossicles using confocal microscopy, living adults were immersed in a container filled with a solution of seawater and tetracycline-HCl (fluorescence marker), with food, for five days (modified from Stricker 1985). The sea cucumbers were returned to the rearing containers for the same period of time. Then, all of the animals were relaxed and preserved for analysis in a LSM Zeiss microscope. Specimens of *Synaptula hydriformis* were deposited in Museu de Zoologia (Universidade de São Paulo) - MZUSP 153 (Echinodermata).

The main ossicles of this species are composed of two parts: a plate and an anchor, distributed all over the skin (Hendler et al. 1995). The various stages in the formation of anchors and plates observed in *Synaptula hydriformis* essentially coincide with the developmental patterns reported for others species of synaptid holothurians (e.g. Clark 1907; Woodland 1907). As in *Leptosynapta clarki* (Stricker 1985), *S. hydriformis* has several isolated anchors, but fully developed plates without anchors are rarely found. In this species, each anchor is usually attached to a plate and measures about 120 µm long, reaching up to 170 µm in some specimens. First, the anchor is formed as a small baton. After the baton has grown longer, the flukes and the stock of the anchor are formed (Fig. 1). The plates are first formed after the anchors are well developed but, in a few cases, plate

Figure 1.
Scanning electron microscopy: Sequence of anchor formation in *Synaptula hydriformis*.

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formation begins before the flukes and stocks appear. The plate is first formed as a small bar that lies near and somewhat perpendicular to, the middle of the anchor's longest axis. Further bifurcation of this structure combined with the fusion of calcium carbonate curved deposits form a whole plate, which measures 95–130 µm (Figs. 2 and 5a,b,c). Others calcareous structures are the batons of the tentacles (the miliary granules) and the oral ring plates. The batons are observed in every margin of the tentacles, and measure 63 µm on average (Fig. 3). The miliary granules, which are usually grouped, measure from 4.82–15.35 µm, and can be found in the corporal wall (Figs. 4a and 5d). Oral ring plates can be of two types: radial and interradial. The first plates are about 339.4 µm wide, and the interradial ones, 208.9 µm (Figs. 4b and 4c). It was observed that, in this species, the ossicle formation and the development of the calcareous structures occur throughout the whole life (including adult stage).

Holothurian species are classified by analyses of tentacles together with the format of the calcareous ring and, mainly, by the form and size of the ossicles. Therefore, this study is an important taxonomic tool, as knowing the sequence of ossicle development makes it possible to identify holothurians that present ossicles in different degrees of formation for this species.

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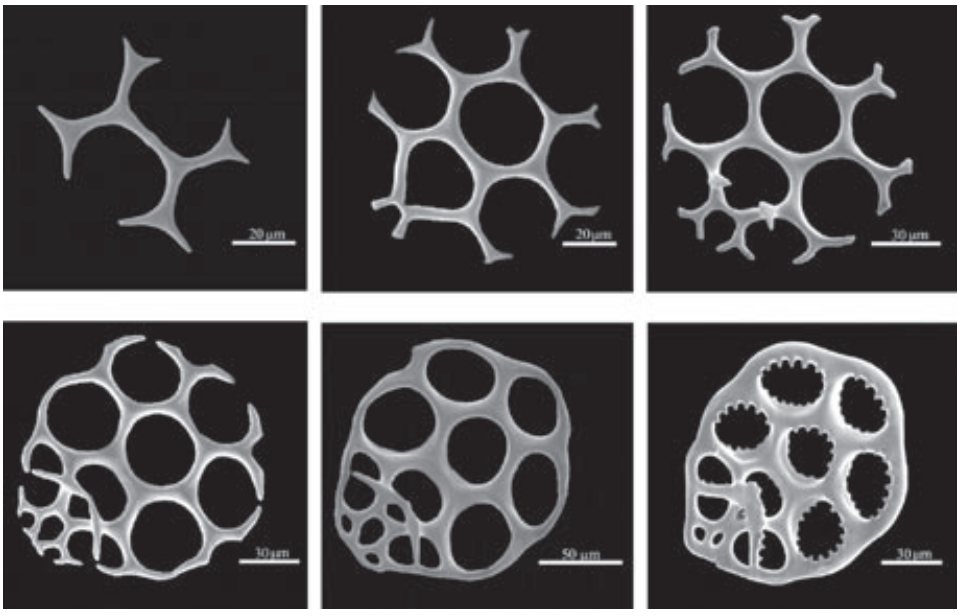

Figure 2. Scanning electron microscopy:
Sequence of plate formation in *Synaptula hydriformis.*

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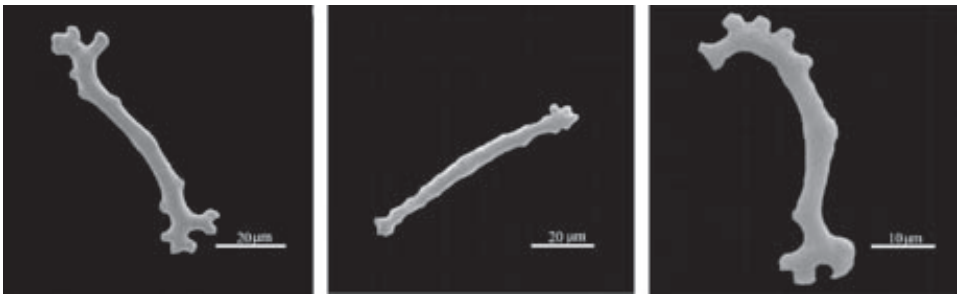

Figure 3. Scanning electron microscopy: Baton of the tentacles.

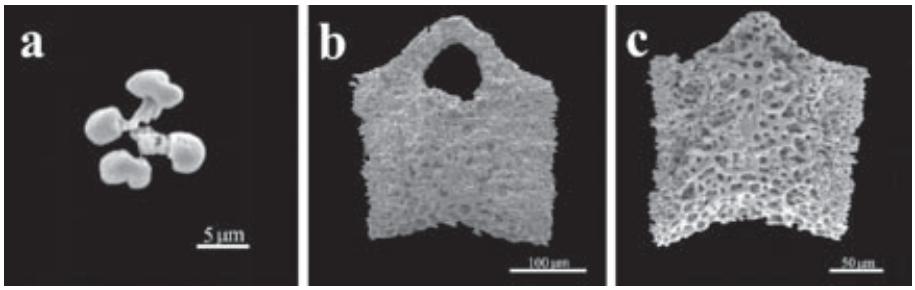

Figure 4. Scanning electron microscopy: a. miliary granule; b. piece radial of oral ring; c. piece interradial of oral ring.

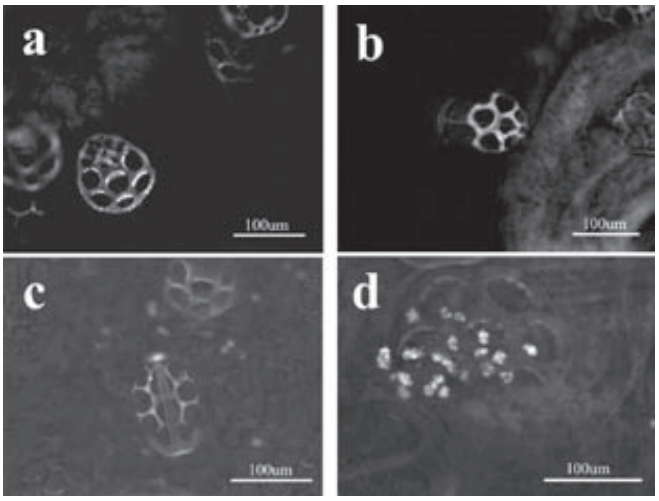

Figure 5. Confocal microscopy: a., b. and c. plate formation; d. miliary granule.