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Tube ultrastructure of *Pomatoceros americanus* (Polychaeta, Serpulidae): implications for the tube formation of serpulids

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Abstract. The inner tube layer of *Pomatoceros americanus* has a complex oriented ultrastructure, which cannot be explained by the standard granular secretion model, predicting a largely unoriented structure of the tube. In the lamello-fibrillar structure of the inner tube layer, the crystallization axis of crystals has a uniform orientation, which is not continuous through successive growth increments. The complex biomineral structures of *P. americanus* suggest a matrix-controlled crystallization model rather than solidification of a slurry with calcareous granules deposited on the tube aperture.

Key words: Serpulidae, biomineralization, tube ultrastructure, tube formation.

INTRODUCTION

Among polychaetes, calcareous tubes occur in serpulids, sabellids, and cirratulids (Fischer et al. 2000; Vinn et al. 2008a). Serpulids construct their tubes from a mixture of calcium carbonate and an organic matrix (Hedley 1956a; Neff 1971a, 1971b). Swan (1950) was the first to identify the tissues responsible for the secretion of calcium carbonate in serpulids. He described a pair of exocrine glands embedded in the subepithelial connective tissue of the ventro-lateral peristomium under the fold of the collar in *Ficopomatus enigmaticus* (Fauvel, 1923) (as *Mercierella*). Later, detailed histological and histochemical studies of these glands in *Pomatoceros triqueter* (Linnaeus, 1758) were made by Hedley (1956a) and Vovelle (1956), who named them calcium-secreting glands (respectively *glandes à calcaire*). The other glandular area is the epithelium of the ventral shield surrounding the opening of the calcium-secreting glands (Hedley 1956b; Neff 1971a, 1971b; Simkiss & Wilbur 1989). The calcium-secreting glands and their functioning have been described in detail for various serpulid species (Hedley 1956b; Neff 1971a; Nott & Parkes 1975; Vovelle et al. 1991).

The study by Neff (1971a, 1971b) has been used as a 'standard' model of serpulid tube formation; he described the secretion of calcium carbonate in *Pomatoceros americanus* Day, 1973 (as *P. caeruleus* (Schmarda 1861)) using a transmission electron microscope (TEM). According to Neff (1971a), the secretory products of the calcium-secreting glands in *P. americanus* have the form of cubic or rhombohedral granules with average

dimensions of 0.15–0.2 µm on a side. The granules are composed of a fibrous organic matrix in which needle-like low magnesium calcite crystals are deposited (Neff 1971a). According to this model, the calcareous granules contribute importantly to the formation of the tube in which the animal lives. The granules reach the exterior of the animal as a slurry that solidifies sufficiently slowly to allow the undersurface of the collar, which is folded back over the aperture of the tube, to mould the calcite-saturated mucus, shaping the end of the tube. This appears to invoke two new phenomena that are more generally associated with the building industry, namely, the solidification of previously prepared granules and the controlled setting of this material. The resulting mineral tube is largely lacking orientation of its fine structure (Simkiss & Wilbur 1989).

Tube formation in sabellids and cirratulids takes place by a mineralization system, in which an organic matrix and calcium ions are secreted by an epithelium. The serpulid opercular plate also is secreted by an organic matrix-mediated system (Bubel 1983; Vinn et al. 2008c). This plate consists of an outer cuticle and two calcified layers, all formed by a single layer of epithelial cells; the organic components of the opercular plate play a major role in the organization of the inorganic components. Oriented structures of the opercular plate can be explained by control of an organic matrix (Bubel 1983).

Indeed, the majority (54%) of the 44 serpulid species studied (out of a total number of about 350 species) have an unoriented tube ultrastructure (Vinn et al. 2008b), which could be considered as in concordance with the

standard granular secretion model. However, serpulids possess not exclusively unoriented, but very diverse oriented tube ultrastructures as well (Vinn et al. 2008b). These oriented tube structures are present in many other serpulid species and cannot be explained by the standard carbonate slurry model (cf. Weedon 1994). Vinn et al. (2008a) have hypothesized that oriented structures in serpulid tubes have been secreted in the same ways as in mollusc shells, based on their ultrastructural similarity. In simple oriented prismatic structures the crystallization axis has a uniform orientation and is continuous through successive growth increments (Vinn et al. 2008b, figs 5A, B, 9I). In complex oriented structures the crystallization axis of crystals has a uniform orientation, which is not continuous through successive growth increments (Vinn et al. 2008b, figs 4E, F, 5C, D, 9J). Trends in the evolution of tube ultrastructure in serpulids implicate that complex oriented structures have evolved from unoriented structures (Vinn et al. 2008b).

As mentioned above, the species *Pomatoceros americanus* played a crucial role in the research history of the tube formation in serpulids, leading to the ‘standard model’. Therefore, the focus of the present paper is detailed re-investigation of the situation displayed in this species. The aim of this paper is (1) to identify secretory granules in the tube ultrastructure of *P. americanus* and (2) to find whether the tube ultrastructure of *P. americanus* is unoriented as would be predicted from the standard granular secretion model supposed by Neff (1971a) for this species. The ultrastructure of *P. americanus* was compared with that of the congeneric *P. triqueter*, which is known to have a lamello-fibrillar tube ultrastructure (Weedon 1994).

MATERIAL AND METHODS

Material was collected intertidally or by diving, trawling, or dredging; it was fixed in (buffered) formalin 4% and

later transferred to ethanol 70% for museum deposition (Table 1). *Pomatoceros americanus* and *P. triqueter* tubes were cut using a razor blade. Pieces of tubes were then oriented and mounted in Canada balsam for machine grinding. Sections of tubes were polished and etched in a 1% solution of acetic acid for two minutes. All preparations were gold sputtered (with a few nanometers thick layer of gold) prior to SEM investigation. The operculum of *Spirobranchus giganteus* (Pallas, 1766) was bleached with NaHCl before SEM. Studies were performed on a Hitachi S-4300 SEM, equipped with an Inca EDX system, at the Swedish Museum of Natural History, Stockholm, and on Zeiss 940D SEM, equipped with SAMx SDD EDX, at the Department of Geology, University of Tartu. The beam was operated at 5–10 kV and 1 nA.

RESULTS

We did not find secretory granules (0.15–0.2 µm on a side), such as described by Neff (1971a), in the tube wall of *P. americanus* (Fig. 1A–D). Instead, the tube of *P. americanus* has two layers, the outer irregularly oriented prismatic and the inner lamello-fibrillar. The border between these two differently oriented layers is transitional. The irregularly oriented prismatic structure (Fig. 1C) is formed by elongate crystals of prismatic shape, which are irregularly oriented within each growth increment. They are 0.5–0.8 µm thick and 2.0–3.0 µm long. The lamello-fibrillar structure (Fig. 1B, D) is formed by elongate crystals of prismatic shape, which have a uniform orientation within each growth increment, but a different orientation in adjacent growth increments. They are 0.3–0.4 µm thick and 2.0–3.5 µm long. Thin (>0.5 µm) organic sheets, parallel to secretion surface, are present in the tube ultrastructure of *P. americanus* (Fig. 1A–D). The sheets are located at an interval of 5–30 µm from each other. As opposed to that of *P. americanus*, the

Table 1. Locality information of the studied serpulid specimens

Species	Collection number	Locality information
<i>Pomatoceros americanus</i>	ZMA V.Pol. 5009	On <i>Argopecten gibbus</i> -shells taken from the trawler <i>Ensign</i> , trawled 10 miles east of Bony, R'4' (Knuckle Bony) off Cape Lookout Shoals, 10–20 m, 15.03.1971, Duke Marine Laboratory, Beaufort, North Carolina, U.S.A., legit W. Kirby-Smith, det. G. van Ee, 1978
<i>P. triqueter</i>	TUG 1232-2	Sweden, Tjämnö Marine Biological Laboratory, legit, det. T. Dahlgren, 2004
<i>Spirobranchus giganteus</i>	ZMA	Reef, little sand; 11 m. From limestone and coral, Netherlands Antilles, Curaçao, Boca Hulu, SE, 14.09.1970 legit, det. H. A. ten Hove, Sta. 2041A

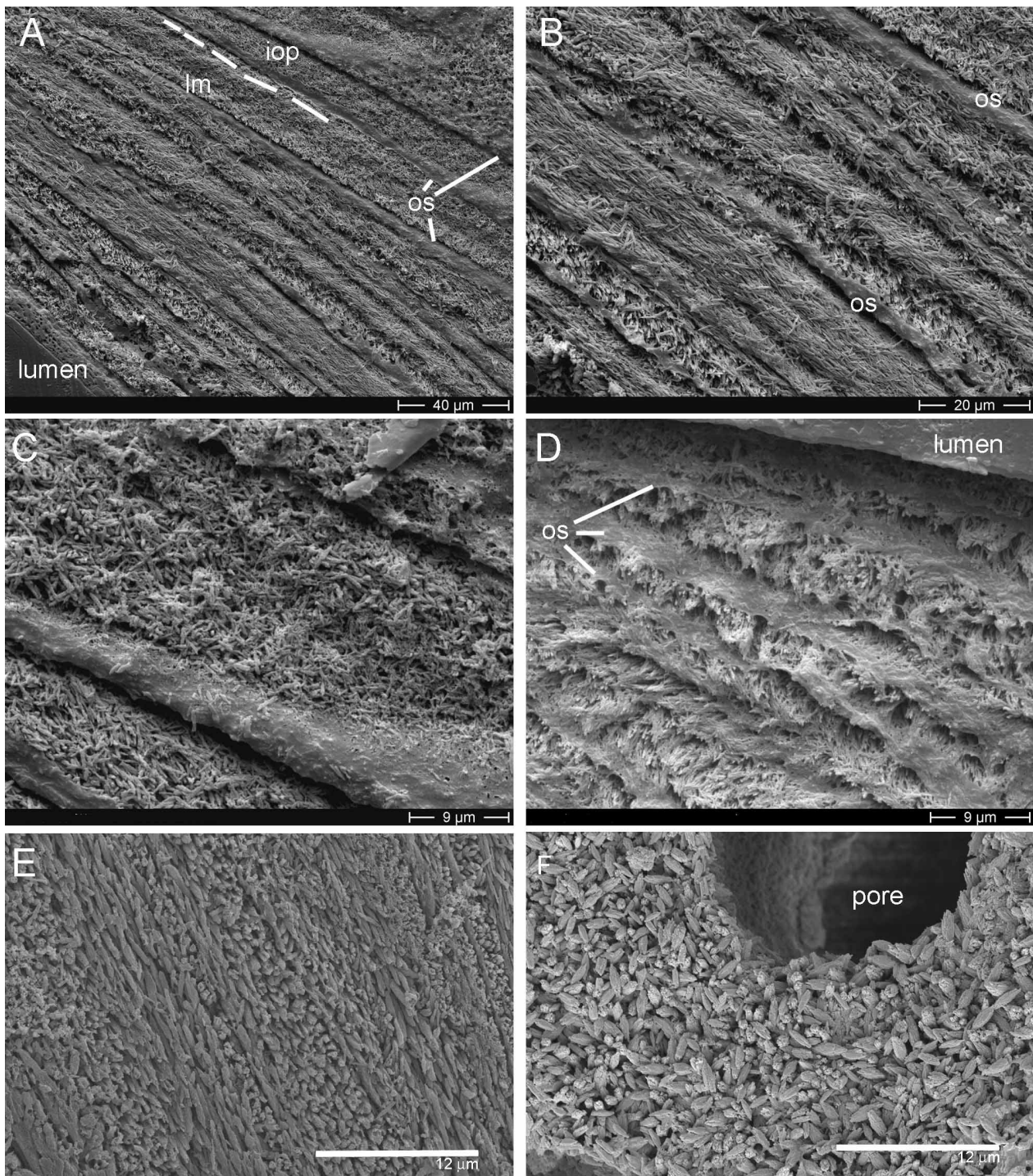


Fig. 1. A–D, *Pomatoceros americanus*. A–C, transverse section, polished and treated with 1% acetic acid for 2 min.: A, two-layered tube, showing a transitional boundary between outer and inner layer; B, inner tube layer with lamello-fibrillar structure, showing different orientation of crystals in different growth increments and organic sheets; C, outer tube layer with irregularly oriented prismatic structure. D, inner tube layer with lamello-fibrillar structure, showing organic sheets parallel to growth increments; longitudinal section, polished and treated with 1% acetic acid for 2 min. E, *Pomatoceros triquetrum*, lamello-fibrillar structure, showing different orientation of crystals in different growth increments; transverse section, polished and treated with 1% acetic acid for 2 min. F, *Spirobranchus giganteus*, outer layer of the calcified opercular plate, irregularly oriented prismatic structure; treated with NaHCl for 20 min. Abbreviations: iop, irregularly oriented prismatic structure; lm, lamello-fibrillar structure; os, organic sheets.

tube of *P. triqueter* is single-layered and has a lamello-fibrillar structure only (Fig. 1E). The calcareous operculum of *Spirobranchus giganteus* is composed of an inner oriented prismatic and an outer irregularly oriented prismatic layer (Fig. 1F).

DISCUSSION

We have not found a granular structure (e.g. agglomerates of individual needle-shaped crystals) in the tube of *P. americanus*. The entities of the tube of *P. americanus* ultrastructure are crystals, which are an order of magnitude larger than that of the secretory granules described by Neff (1971a) from the lumen of the calcium-secreting glands. If the granular slurry model is correct, either the secretory granules have to be recrystallized into larger entities during transport from the gland to deposition or, alternatively, *P. americanus* possesses a different secretion model than proposed by Neff (1971a, 1971b). This question is even more complicated because the dimensions of secretory granules reported in the original study by Neff (1971a, figs 14–21) using untraditional dimension (150–200 μm – milli-micrometre) have afterwards been reprinted in textbooks on biomineralogy as 150–200 μm (e.g. Simkiss & Wilbur 1989). The size of the granules on photographs in Neff (1971a, figs 14–21) is 15–40 μm and, assuming the respective magnification shown in the same figures (72 000–107 500 times), are, in fact, on average 0.15–0.40 μm in size, which is at least an order of magnitude smaller than the size of crystals observed in the wall of *P. americanus*.

According to the standard slurry deposition model (Simkiss & Wilbur 1989), the tube ultrastructure can only be irregularly oriented (unoriented). However, the lamello-fibrillar ultrastructure of the inner tube layer of *P. americanus* is a complex oriented structure. The lamello-fibrillar structure cannot be explained simply by deposition of secretory granules over the tube aperture to be moulded in shape by a collar. Weedon (1994) suggested that the lamello-fibrillar structure in *P. triqueter* is perhaps the result of the moulding of calcite-saturated mucus in forward and backward applications. This explanation seems unlikely considering the microscopic scale of the granules and the precise orientation of crystals in the tube wall of *P. triqueter* (Fig. 1E). Different tube layers could not be treated differently in back and forward movements of the calcite-saturated mucus. Thus, the occurrence of two layers in *P. americanus* and even three in *Hydroides dianthus* (Vinn et al. 2008b) cannot be explained by the way suggested by Weedon (1994). However, hypothetically one could suggest that it could be done by compaction of the fibrous organic matrix around the elongate crystals during the solidification.

The latter way is unknown in animal biomineralization and we consider it unlikely. We hypothesize here that the lamello-fibrillar tube structure in serpulids is formed in a similar way as the lamello-fibrillar structure of molluscs (Carter et al. 1990), where the organic matrix and calcium ions are secreted from a secretory epithelium and crystallization is mediated by the organic matrix too. The orientation of crystals in the lamello-fibrillar structure in *P. americanus* and *P. triqueter* is supposed to be controlled by molecular mechanisms in the organic matrix. We suggest that the outer layer, with its irregularly oriented prismatic structure, is in general formed in the same way as the lamello-fibrillar layer (e.g. as in molluscs), because there is a gradual transition between these two layers. The calcareous endplate of the operculum of *Spirobranchus giganteus*, also directly secreted by an epithelium (that of the opercular ampulla), shows an irregularly oriented prismatic structure too (Fig. 1F). It may be relevant that tabulae, as produced by the abdomen of *P. triqueter* (see Vinn et al. 2008b), show a fine homogeneous (irregularly oriented) structure as well. Hedley (1958) supposed that the ventral abdominal epithelium is the main source of the calcium involved, maybe supplemented by calcium originating from mucous cells in the abdomen.

There are alternative ways to explain the calcified secretory granules described (Neff 1971a) in the lumen of the calcium-secreting glands in *P. americanus*:

- (1) The worm actually produces calcium saturated mucus in the glands. The mucus is then deposited on the tube aperture, where crystallization of structure is controlled by an organic matrix as in molluscs. The calcified granules may only be an artifact of fixation and formed after the death of the worm.
- (2) If calcified secretory granules are not an artifact of fixation, then they must be dissolved and recrystallized before deposition of the material on the tube aperture. Consecutively, an oriented structure is formed from the mucus, regulated by the organic matrix.

CONCLUSIONS

Our study shows that a complex oriented ultrastructure of the inner tube layer of *P. americanus* cannot be explained by the standard granular secretion model, which should have resulted in a largely unoriented structure of the tube. Instead, in the lamello-fibrillar structure of the inner tube of *P. americanus* the crystallization axis of crystals has a uniform orientation, which is not continuous through successive growth increments. We suggest that the complex biomineral structures of *P. americanus* imply a matrix-controlled crystallization model rather than solidification of a slurry with calcareous granules deposited on the tube aperture.

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***Pomatoceros americanus*'e (Polychaeta, Serpulidae) koja peenstruktuur ja selle tähendus serpuliidide koja moodustumise mõistmisel**

Olev Vinn, Kalle Kirsimäe ja Harry A. ten Hove

Pomatoceros americanus'e koja sisemine kiht on keeruka orienteeritud struktuuriga, mida ei saa seletada standardse granulaarse sekretsiooni mudeli abil, mis eeldab orienteerimata koja struktuuri. *P. americanus*'e keerukad biomineraalsed struktuurid on tõenäoliselt tekkinud orgaanilise maatriksi poolt kontrollitud kristallisatsiooni käigus, mitte aga lubigraanulite toru suudmele tsementeerimise teel.