



Ultrastructure and tube formation in Ceriantharia (Cnidaria, Anthozoa)

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ABSTRACT

Cnidomes are an important feature that diagnoses groups of Cnidaria. Our paper describes how these structures are used in the formation of the tubes of tube *Anemones*, Ceriantharia. The structure of the tube was observed using SEM and the organization of the tubules are described for members of the three families of Ceriantharia. Additionally, the mode of production of the tube from animals that had their tube removed is described.

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1. Introduction

Ceriantharians (tube dwelling *Anemones*) are tube-forming cnidarians that live in marine reef- and benthic communities. Although some individuals can be found on hard substrates, barely fixed by the proper tube, the tubes are usually described as being dead-end sacks that are vertically inserted into unconsolidated sediments (Tiffon, 1987; Stampar et al., 2014a). These tubes reach a thickness of approximately, 1–3 cm in adults or older juvenile individuals, whereas, they are usually thinner in small species or juveniles (Tiffon, 1987; Stampar et al., 2010). Some considerable differences in tube length between species have also been noted. *Cerianthus vogti* Danielssen (1890) and *Isarachnanthus nocturnus* (den Hartog, 1977), for example, have a very long tube or a system of branching tubes (den Hartog, 1977; Jensen, 1992), while *Ceriantheomorpha brasiliensis* Carlgren (1931) and *Pachycerianthus schlenzae* Stampar et al. (2014b) have tubes of almost the same

size as the polyp (Stampar et al., 2010, 2014b). Species such as *Ceriantheopsis americanus* (Agassiz in Verrill, 1864) generally have conical and distally pointed tubes with a closed and narrow end region (Frey, 1970).

The production of the tube is initiated shortly after larval settlement (metamorphosis), as noted by Sanzo (1939) while studying a specimen of *Cerianthus* sp. In another study, Nyholm (1943) describes that ectodermal glandular cells of the larvae begin to secrete mucus. The part of oral disk covered with labial tentacles also secretes mucus, but this quickly migrates to the top of the column of the animal. Previously, mucus production was thought to be main responsible for tube construction, while cnidae were considered to be much less important. However, some authors suggested that a type of cnida identified as “atrich” (or atrichous isorhiza) commonly found in the column ectoderm could be largely involved in tube formation (Carlgren, 1940; Schmidt, 1972, 1974). While studying *C. americanus* (Cerianthidae), Mariscal et al. (1977) proposed that this abundant cnida was different from the cnidae previously known, and named it the ‘ptychocyst’. The tubule of this type of cnida is in fact totally devoid of spines, thus, representing a true atrichous. The authors also noted the existence of granular material adhering to the cnidocyst’s sticky everted tubules. However, the main feature that distinguishes the ptychocyst is that its tubule is folded inside the capsule, while the tubules of spirocysts and nematocysts are helicoidally organized. Despite their thorough

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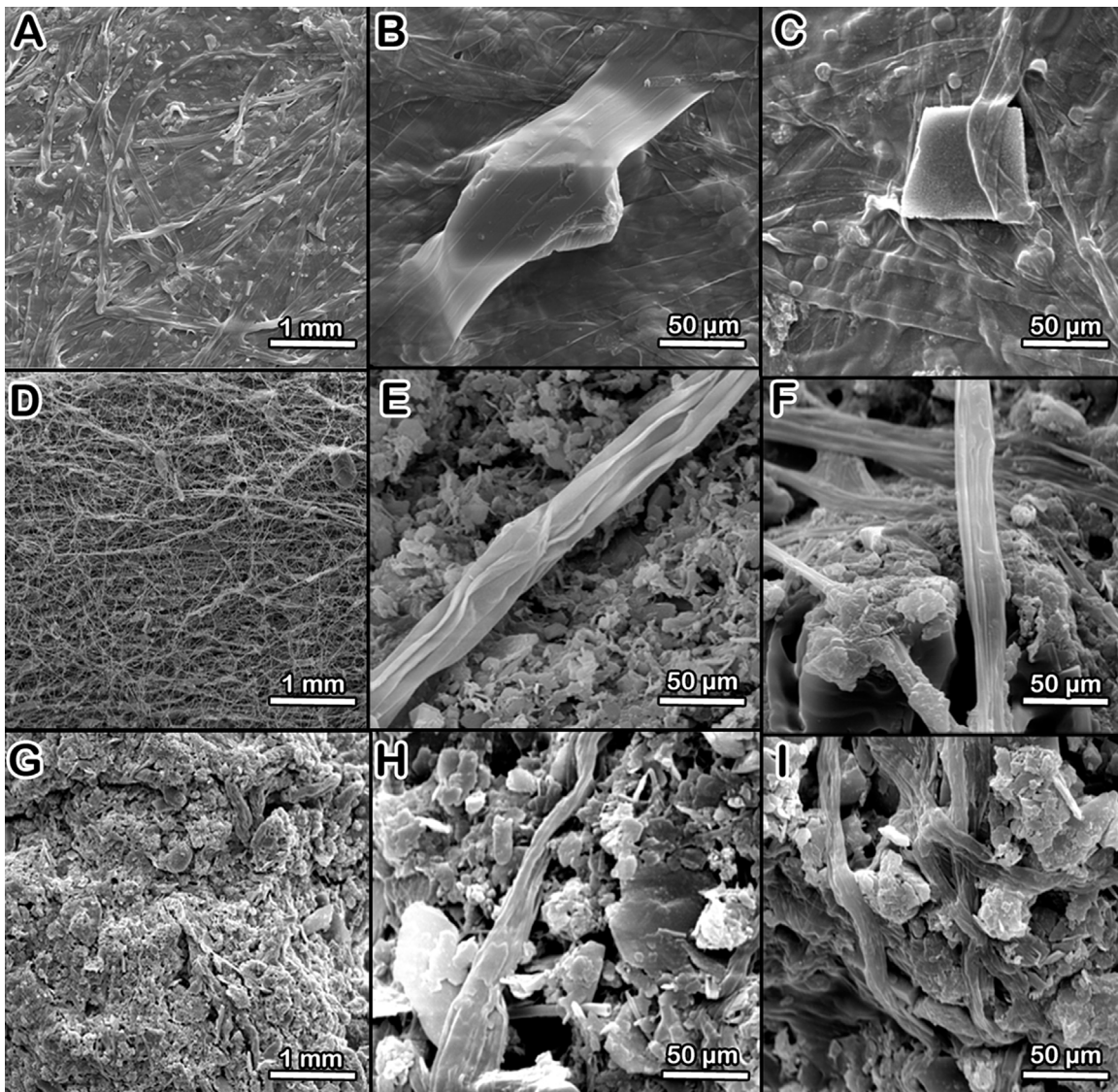


Fig. 1. Ceriantharian tubes observed by scanning electron microscopy (SEM). (A and B) – Cerianthidae (*C. brasiliensis*); (C) – Cerianthidae (*P. schlenzae*); (D–F) – Arachnactidae (*I. nocturnus*); and (G–I) – Botrucnidiferidae (*B. novergicus*).

study of the newly proposed cnida's morphology, Mariscal et al. (1977) did not explore its role in tube formation and did not mention whether it was organized in a similar way in other groups of Ceriantharia.

Mariscal et al. (1977) briefly discussed the construction of the tube in an adult cerianthid. They described the layered construction of the tube of *C. americanus*, suggesting that ptychocysts are periodically added to the interior of the tube during the specimen's life. They also reported the occurrence of an opaque spot in the middle of the column, basically composed of filaments of cnidae; and suggested that it creates a collagenous network which is used as basis for the mucus (glycoproteins) or for the filaments themselves to build the tube (Emig et al., 1972; Mariscal et al., 1977). Tiffon (1987), however, based on his own data and also on data of Boisseau (1952), raised again the argument that the filaments alone cannot form the ceriantharian tube, but may only be responsible for a smaller structural part of a tube built mainly of mucus secretion. Therefore, in order to resolve this controversy, a protocol was established to verify the ultrastructure of the tubes in species of the three currently recognized families of Ceriantharia (Arachnactidae, Botrucnidiferidae, and Cerianthidae). Also, the reconstruction

of the tube in a species of the family Cerianthidae was observed under laboratory conditions.

2. Materials and methods

2.1. Examined species

Two specimens of each of the following species were selected for study: Botrucnidiferidae, *Botrucnidifer novergicus* Carlgren, 1912 from Trondheimsfjorden, Norway (Norwegian University of Science and Technology, Museum of Natural History and Archaeology – NTNU 40502 and 40507); Cerianthidae, *C. brasiliensis* (São Sebastião Channel, Brazil – GenBank AB859831) and *P. schlenzae* (Guarapari, Brazil – Museu de Zoologia da Universidade de São Paulo – MZUSP – 1949 and 1950); Arachnactidae, *I. nocturnus* (São Sebastião Channel, Brazil – GenBank AB859832). The specimens of *B. novergicus* were obtained from museum collections (see above) and these specimens were preserved in formalin and then transferred to ethanol 90%. The other specimens were obtained through field collection by scuba diving (localities and references above and in Stampar et al., 2014a). These specimens were preserved in

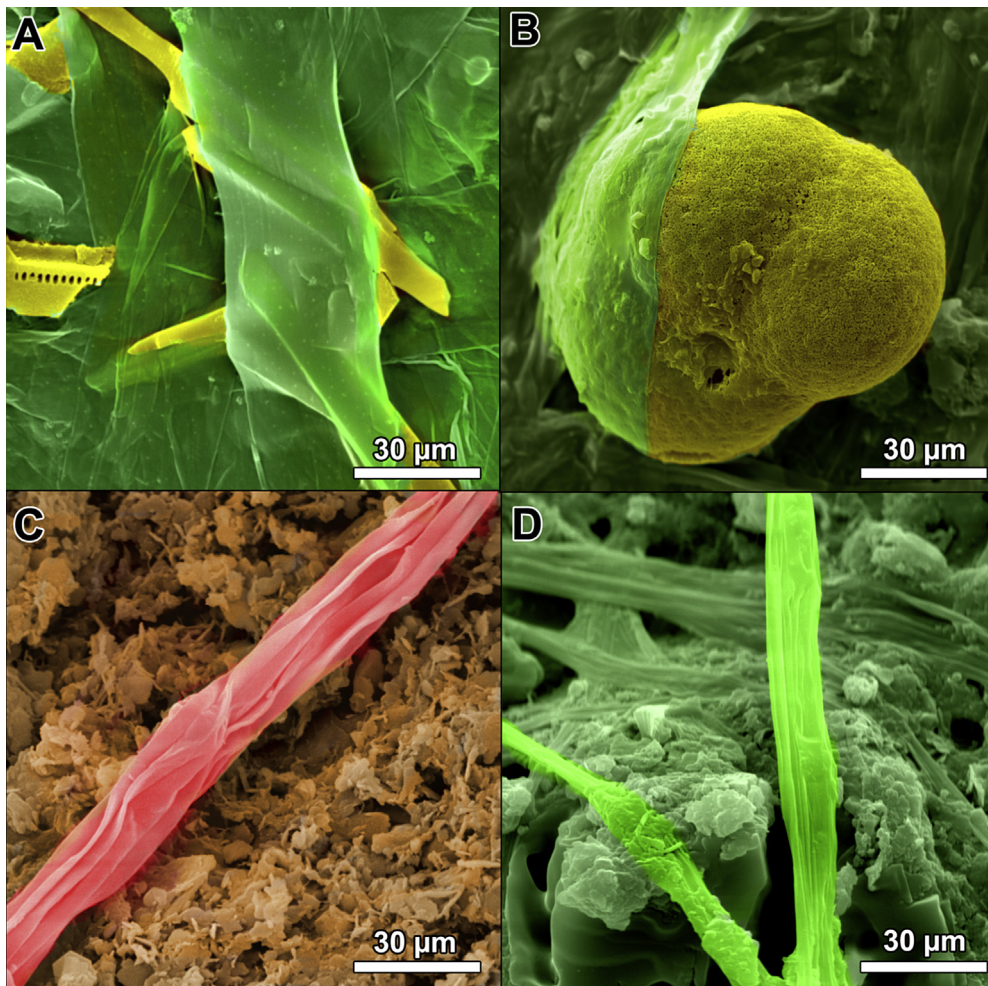


Fig. 2. Detail of ptychocyst filaments (green) observed under scanning electron microscopy (SEM), and artificially colored. (A and B) – Cerianthidae (*C. brasiliensis*); (C and D) – Arachnactidae (*I. nocturnus*). (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article.)

formalin (4%) and then transferred to ethanol 90%. Identification of the material followed the references available for the genera (Carlgren 1912; Spier et al., 2012; Stampar et al., 2010, 2012, 2014b).

2.2. Ultrastructure of the ceriantharian tube

Square pieces (5 mm) were cut from the tubes of each specimen. These pieces were washed in filtered tap water for 24 h and then transferred to ethanol solutions in an ascending series (10–100% by steps of 10) for 1 h at each grade. They were subsequently prepared for scanning electron microscopy (SEM) following Danilatos (1988). The tube pieces were critical-point dried, mounted on stubs, sputter coated with a 10 nm thick gold layer in a Balzers S-SCD 050 sputter coater, and examined under a Zeiss DSM 940 SEM.

2.3. Tube formation in Ceriantharia

Two specimens of *C. brasiliensis* (8 and 9 cm long) were kept alive in natural seawater in glass containers. The tubes of both specimens were completely removed and the polyps' construction of a new tube was observed every 5 min for 14 h and then every 6 h for 15 days (videos and photos). Water circulation was turned off to avoid interference in the movement of specimens and/or of the material produced. The new tubes were observed under a stereomicroscope Nikon SMZ 1000 with Infinity 1 digital camera (videos and photos).

3. Results

3.1. Ultrastructure of the ceriantharian tube

From the SEM observations it was possible to distinguish two different patterns in the arrangement of the ptychocysts tubules on the tube of the three families (Figs. 1 and 2). The first pattern was observed in the specimens of the family Cerianthidae (Fig. 1A–C, Fig. 2A–B), where the ptychocyst tubules form flat adhesive tapes arranged in overlapping layers, creating an arrangement similar to manufactured fabric. It is noteworthy that only a small amount of sediment was used in the construction of this fabric-like tube. The ptychocysts' tubules act like an adhesive tape that only rarely trap sand grains (Fig. 1B) and/or foraminifera tests (Fig. 1C, Fig. 2A–B).

A completely different pattern was observed in tubes from specimens of the two other families, Arachnactidae (Fig. 1D–F, Fig. 2C–D) and Botrucnidiferidae (Fig. 1, E–H). Here the ptychocyst tubules work as a driftnet, trapping much sediment between the tangled filaments, instead of forming a fabric-like tube.

3.2. Tube formation in Ceriantharia (Cerianthidae) (Fig. 3A–F)

Two specimens of *C. brasiliensis* were removed from their tubes and put immediately into glass containers to verify further tube production. We observed regionalized release of ptychocyst tubules just below the marginal tentacle crown until a slight

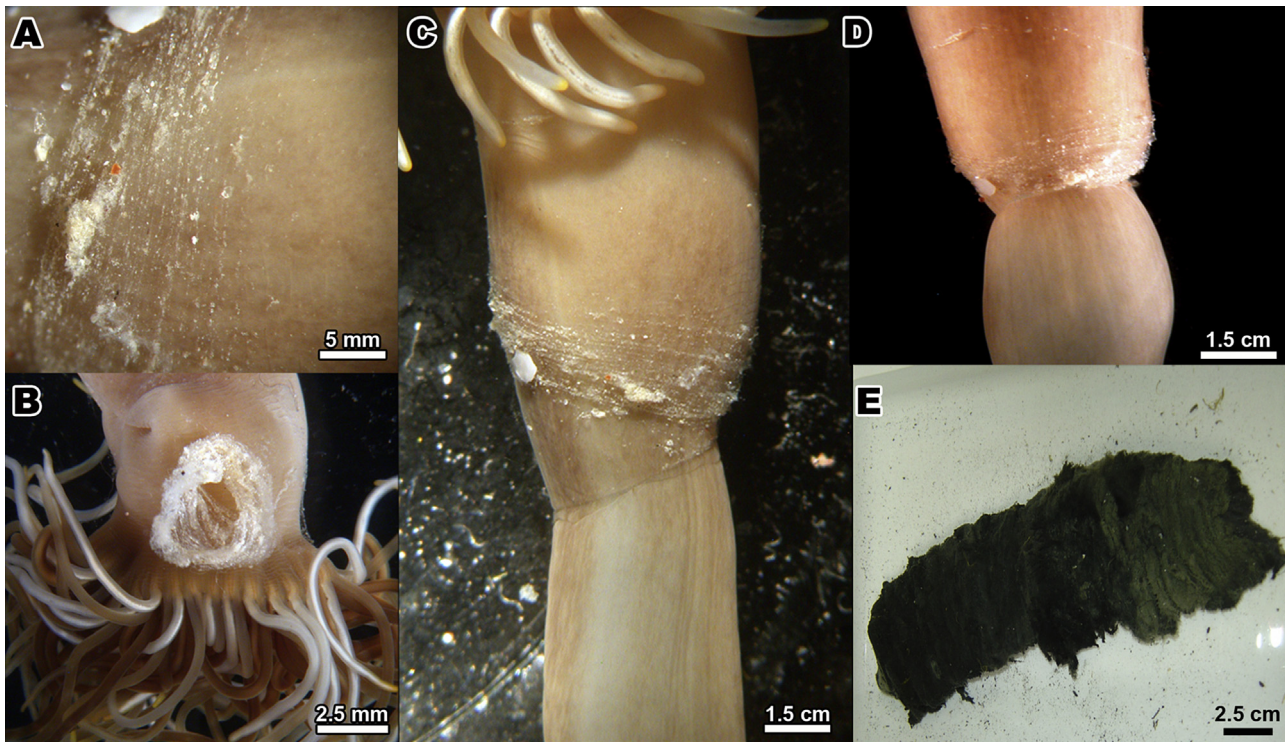


Fig. 3. Detail of *C. brasiliensis* tube formation. (A) – ptychocyst filaments release from the region below the tentacles; (B) – detail of ptychocyst filaments creating a preliminary tube after 20 min; (C and D) – two moments of entrainment of ptychocyst filaments by the constriction of the body; and (E) – Original tube from which the specimen was removed.

whitish area in the first third of the polyps' column (Fig. 3B). From the point where the previous tube was removed, the release of tubules started and followed a constant rate. At each observation (every 5 min) the formation of a ring of tubules was observed (Fig. 3C–E). After four observation events (20 min) it was possible to verify the existence of an already thicker ring, of approximately 1 cm diameter (Fig. 3B). This released material (in rings) was carried to the aboral region of the polyp by ciliary and peristaltic movements of the polyp column (Fig. 3C–E). In less than 12 h the specimens were able to construct a tube that covered almost their entire column, although it was very delicate and would probably not provide great protection against predators. After 7–10 days the tube had reached a thickness of 0.4 mm. At this stage it is possible that the tube could provide protection as it already stayed erect without the expansion of polyp.

Based on the observed tubes in the collected individuals and also on aquarium cultivated ones, it was possible to confirm that there was no closed end in *C. brasiliensis* specimens. The tube was entangled from the middle to the terminal part, which was left open (Fig. 3F). This conclusion is fairly obvious considering the method of production of the tube, in a single area next to the tentacles and always in the shape of rings, and therefore, making it impossible to produce a closed tube.

4. Discussion

The cnidomes are one of the most used set of characters in identification and definition of many groups of Cnidaria, although the character has been met with criticism as well (see Schmidt, 1972; Fautin 2009; Acuña et al., 2003, 2004). Within Ceriantharia, the use of cnidomes as a diagnostic character was applied to Atlantic species of *Isarachnanthus* only, and it turned out that the usefulness of the character was questionable (Stampar et al., 2012). The conclusion is that using cnidomes as the only single character is not a precise way to identify *Isarachnanthus* species.

Contrarily, at higher taxonomic levels the cnidome can provide taxonomically significant information. The two ceriantharian clades suggested by den Hartog (1977), Penicularia and Spirularia, are currently valid and are primarily defined by the difference in types of nematocysts. However, one major issue in Ceriantharia was never addressed: the definition of the family Cerianthidae. Until now, this family had no morphological character that distinguished it from Botrucnidiferidae, except for the lack of the structure botrucnidiae (Daly et al., 2007). This study describes the unique arrangement of the ptychocyst tape-like tubules in Cerianthidae for the first time.

All species of the family Cerianthidae have complex and membranaceous tubes that reach a thickness up to 3 cm. In contrast, members of Arachnactidae and Botrucnidiferidae present simpler tubes that almost dissolve or disaggregate when handled. These tube differences have an important implication in the species' habits. The species of Cerianthidae build resistant tubes that are generally vertically orientated and unbranched. These features are not observed for Arachnactidae species, which have ramified tubes, usually with a horizontal orientation in the sediment (van Beneden, 1924; den Hartog et al., 1977; Stampar – personal observation). Available information on Botrucnidiferidae tubes is scarce.

While it was observed that the tube of *C. brasiliensis* has an open end, Frey (1970) reported that tubes of some species, as *C. americanus*, are closed in their basal region. This structural difference may indicate different tube formation strategies, which shows that generalizations for subclass Ceriantharia may be premature and that this type of information should be examined in different genera. The open-ended tube may provide a way to escape from predators, as there is no limit to how much the polyp can retract itself into the tube. Predation can be avoided by *C. brasiliensis*, which can escape through the lower opening of the tube by releasing water through its anal pore. This type of escape would be useful in species of the North Atlantic, where predators (sea slugs) enter the tube in search for cerianthid prey (Shepard et al., 1986).

Some morphological aspects of Ceriantharia tubes have been studied, but many other still need to be explored and unraveled. The scarce information on tube formation makes it an interesting subject, even if it is considered to be a unique structure among anthozoans. Also, it is remarkable that even adult ceriantharian specimens are able to completely reconstruct a tube if needed. The unique mode observed and described here can be interpreted as a behavior, and, therefore can be used as a character in evolutionary studies (Japyassú et al., 2006). Another poorly studied and important aspect is the associated fauna occurring in the ceriantharian tubes (e.g. Emig et al., 1972; Stampar et al., 2010; Vieira and Stampar, 2014), and in particular the anchoring method that these symbionts use to attach to the tubules of the tubes.

5. Conclusions

This study presents data on very relevant and poorly known aspects of Ceriantharia. The tube produced by tubules of pythocysts is a type of “fabric” that has not previously been studied. This type of bioproduct can be extremely useful for various unknown applications (see more in Silva et al., 2012). Furthermore, the tube construction may provide differential taxonomic characters that are specific for groups within Ceriantharia.

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