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The biocomposite tube of a chaetopterid marine worm constructed with highly-controlled orientation of nanofilaments

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ABSTRACT

The ultrastructure of the self-constructed tube housing of the bioluminescent marine worm, *Chaetopterus* sp. reveals that the bio-nanocomposite tube comprises of multiple non-woven plies of multi-axially oriented organic nanofilaments (ø 50–1100 nm) cemented together by an unstructured organic matrix binder. The thin-walled, impermeable tubes are bio-inspirational for conventional pipe technology. Orientation distribution analyses revealed that the dominant orientation angles of nanofilaments in the tube were 0°, ±45° and ±65°, which correlate well with optimal winding angles for 'man-made' fibre reinforced composite pipes subjected to specific loading conditions. Such a use of high aspect ratio nanofilaments in multi-axial laminates would impart toughness and flexibility to the tube structure, and facilitate rapid tube growth. While the tube production mechanism is not entirely known at this stage, our time-lapse studies show that, contrary to generic assumptions in literature, the worm actively, rapidly and sporadically produces and expands the tube.

1. Introduction

Unlike errant polychaetes, the majority of sedentary marine polychaetes spend their entire post-larval existence within self-constructed tubes that are partially burrowed beneath the seabed (as in chaetopterids), if not cemented to hard substrates (as in serpulids). The external tubular structure not only facilitates protection and hiding as antipredator adaptations [1], but also provides a microenvironment that the annelids totally depend upon [2–6].

1.1. The tubiculous polychaete, Chaetopterus variopedatus

Chaetopterus variopedatus animals typically live in un-branched U-shaped tubes, where both inhalant and exhalant openings protrude above the seafloor sediment (Fig. 1a). In its tube habitat, *C. variopedatus* functions as an industrious 'transfer pump' [7]. By actively pumping a directional current of water through their tube, they obtain both oxygen and food, and remove excrements and contaminants (Fig. 1a) [2–4,6]. In fact, *C. variopedatus* lack appropriate respiratory and feeding structures, such as respiratory pigments and protrusible tentacular apparatus, to support out-of-tube living [2–6], and it has been shown that their filter-feeding mechanism using a mucous bag (Fig. 1a) is entirely dependent upon their water-pumping activities [4]. Consequently, the chaetopterid *C. variopedatus*, like other sedentary polychaetes, expends over half of its total production energy in tube production, rationing the rest between somatic growth and gamete production [8,9].

Of course, the design and construction of the tube have evolved to support the worm. While chaetopterid tubes have attracted some interest since the study of Enders [10], research has principally focussed on the tube building and cleaning behaviour of the worms [2,3,10] and the function of the tube itself [4] with only limited notes and anecdotal observations on the tube ultrastructure [11–13]. Here, we examine the complex oriented ultrastructure of *Chaetopterus* tubes from both a biological, evolutionary perspective and from a materials science perspective. The discussion is complementary to our recent analysis of the thermo-mechanical properties of the sturdy tube wall biomaterial, which exhibits remarkable stability over a broad temperature range, both in the dry and wet states [14]. Specifically, this article reveals that *Chaetopterus* tubes share many parallels with, if not inspire, the design and fabrication process of conventional, man-made composite pipes and tubes.

In terms of general design, *Chaetopterus* tubes vary greatly in size, and both the diameter and the length are enlarged during growth [10]. In length, tubes can be up to 50 cm long from orifice to orifice, with vertical arms protruding above the seafloor up to 22 cm long [10]. Notably, the tube ends are tapered (Fig. 1a, b) so that the diameter of the two openings (ranging from 0.1 to 0.5 cm) is smaller than

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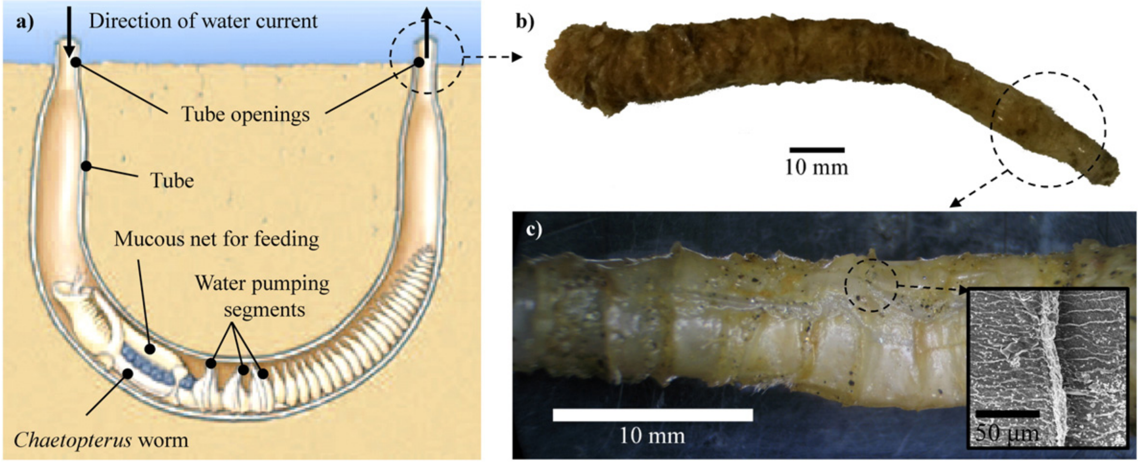

Fig. 1. a) Schematic, adapted from [32], of a *Chaetopterus* worm pumping water within its U-shaped tube for feeding using an elongated mucous bag. b)–c) Views of a terminal section of the leathery tube, distinctly showing b) the wrinkled uneven surface, and c) external annulations, magnified in the inset SEM micrograph.

that of the greater fraction of the buried tube (up to 4 cm) [10]. While the constricted openings may be anti-predatory adaptations [1,7], it may (also) be that the lack of perforated transverse partitions within the *Chaetopterus* tubes, which in many other chaetopterid tubes function as water pressure (and therefore velocity) regulators [3], is here replaced by adopting smaller diameters at the tube openings. By the venturi or jet effect, the speed and pressure of the water current would be higher and lower, respectively, in the constricted orifices compared to the larger diameter body [3,7]. In fact, Brown [7] has verified this, having measured the velocity at the orifice to be 5.5 times greater than that in the body of the *Chaetopterus* tubes. The high intake and discharge velocity may be important in i) capturing zooplankton for food into the tube, ii) ejecting excrements, clogging material and biofouling out of the tube, and iii) preventing sessile organisms from settling near the orifice and obstructing the water current.

It is intriguing to find that the *Chaetopterus* marine worm is using a form of an industrial 'venturi scrubber' design, rather than perforated transverse partitions, for fluid flow regulation and particle (*i.e.* food and waste) collection and disposal. While venturi devices have been used for centuries to measure fluid flow in pipes, venturi scrubbers are also the most commonly used wet-scrubbing system in the modern piping industry due to their high particle collection efficiency [15–17]. In industry, such wet-scrubbing systems are typically evaluated against 'baghouse' fabric filters and electrostatic precipitators [15,17]. Notably, 'baghouse' fabric filters are analogous to both the perforated transverse partitions found in many chaetopterid tubes (but not those of *C. variopedatus*), and the mucous filter net used by most chaetopterid worms for feeding (Fig. 1a). Interestingly, we find that the worms use similar engineering design principles and solutions as we use in industry for the problem of particle collection in a fluid-flowing pipe.

Like all polychaete tubes, *Chaetopterus* tubes are biocomposite structures with a self-secreted organic matrix phase. However, *Chaetopterus* tubes are one of the few polychaete tubes, alongside vestimentiferan tubes [13,18], that utilise high aspect ratio, self-secreted fibres [11], rather than bio-mineralised crystals (as in many serpulids [19]), and/or gathered minerals and inorganic particles such as sand (as in many sabellariids [20]), as the reinforcement phase. Here, we investigate the complex oriented fibre reinforced composite structure of *Chaetopterus* tubes and subsequently argue that it may not only enable fast tube production and repair, but also impart structural integrity to the tubes [14]. These lessons are shown to be relevant to current technologies in man-made fibre-reinforced composite pipes.

2. Material and methods

2.1. Tube collection

All the *Chaetopterus* tubes used in this study originated from the La Jolla submarine canyon in San Diego, California, as described elsewhere [21]. The *Chaetopterus* species in Southern California is often described as *C. variopedatus* however recent molecular phylogeny studies indicate otherwise (Rouse, unpublished manuscript). Therefore, the worms are referred to as *Chaetopterus* sp. here.

Bundles of tubes were hand collected by scuba at 20 to 30 m depth in Spring 2013, transported in seawater to the Marine Biology Experimental Aquarium Facility at Scripps Institution of Oceanography and kept in circulating seawater at ambient temperature. The worms were left to grow in their tubes with no additional food, and a majority of the tubes rapidly showed new growth indicated by a much paler coloration on the tubes. Sections of tubes (from the freshly grown tip but also from older sections) were then carefully cut-off using a scalpel blade and shipped to the Department of Zoology at the University of Oxford, in Falcon tubes with 70% ethanol. Once received, the tube sections were kept in cold (7 °C) seawater.

This study was conducted in accordance to the Animals (Scientific Procedures) Act 1986 of the UK, and following standard animal maintenance and manipulations ethics code of the University of California.

2.2. Optical and electron microscopy

Optical micrographs of *Chaetopterus* tubes were captured with an Olympus SZ40 microscope, equipped with a Canon PC1200 camera, to examine the general structure of the tubes.

The detailed surface morphology and ultrastructure of the *Chaetopterus* tubes was examined using a JCM-5000 NeoScope (JEOL) scanning electron microscope (SEM), at an acceleration voltage of 10 kV under high vacuum. Sample preparation prior to SEM observation included drying and equilibrating of the tube in ambient conditions for 48 h, followed by sputter coating with gold/palladium (Au/Pd) alloy. In particular, the inner and outer surfaces of the tubes, as well as fractured edges and ends were analysed. Images were taken along the same tube sample and across five different tubes samples.

2.2.1. Orientation analysis

Studies on the orientation distribution of the tube microstructure were perforce (a limitation of the technology) restricted to assessing the orientation of filaments on the surface of a layer. A total of twenty-five SEM images, including micrographs i) along the same