

Ultrastructure of the coenecium of *Cephalodiscus* (Hemichordata: Pterobranchia)

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Abstract: The ultrastructure of the coenecia of *Cephalodiscus* (*Cephalodiscus*) *hodgsoni* Ridewood, 1907, *Cephalodiscus* (*Idiothecia*) *nigrescens* Lankester, 1905, and *Cephalodiscus* (*Orthoecus*) *densus* Andersson, 1907 was characterized using light microscopy, transmission electron microscopy, and scanning electron microscopy. The coenecium of *Cephalodiscus* is composed of layers of coenecial material of variable thickness laid down one upon the next and separated by sheets. Thick fusellar-like layers (up to 160 μm thick) and thin cortical-like layers (down to 15 nm thick) are present, but do not form two distinct components. Instead, a continuum exists in the thickness and shape of these layers. At the ultrastructural level, both fusellar-like and cortical-like layers are composed of thin (16–23 nm) long and straight fibrils, similar to the fibrils described in extant *Rhabdopleura* Allman, 1869. In *C. densus*, fibrils in the outer secondary deposits show a parallel arrangement, similar to the arrangement of fibrils in the graptolite eucortex. Although similarities in the shape and arrangement of growth increments between *Cephalodiscus*, *Rhabdopleura*, and graptolites probably reflect homologous zooidal behaviors and secretion mechanisms, differences at the ultrastructural level show that fibril types and fibril arrangement can evolve independently from larger scale features of the coenecium.

Key words: coenecium, *Cephalodiscus*, cortex, fuselli, Pterobranchia, pterobranchs, ultrastructure.

Résumé : L'ultrastructure des coenécies de *Cephalodiscus* (*Cephalodiscus*) *hodgsoni* Ridewood, 1907, *Cephalodiscus* (*Idiothecia*) *nigrescens* Lankester, 1905 et *Cephalodiscus* (*Orthoecus*) *densus* Andersson, 1907 a été caractérisée par microscopie optique et par microscopie électronique à transmission et à balayage. La coenécie de *Cephalodiscus* est composée de couches de matériel coenécial d'épaisseur variable déposées l'une sur l'autre et séparées par des films. D'épaisses couches (épaisseur maximum de 160 μm) d'apparence fusellaire et de minces couches (épaisseur minimum de 15 nm) d'apparence corticale sont présentes, mais ne constituent pas deux éléments distincts. Il s'agit plutôt d'un continuum d'épaisseurs et de formes de ces couches. À l'échelle de l'ultrastructure, les couches d'apparence fusellaire et les couches d'apparence corticale sont toutes deux composées de longues et minces (16–23 nm) fibrilles droites semblables aux fibrilles décrites chez le taxon moderne *Rhabdopleura* Allman, 1869. Chez *C. densus*, les fibrilles dans les dépôts secondaires externes présentent une disposition parallèle semblable à la disposition des fibrilles dans l'eucortex des graptolithes. Si les similitudes sur le plan de la forme et de la disposition des incréments de croissance chez *Cephalodiscus*, *Rhabdopleura* et les graptolithes reflètent probablement des comportements zooïdaux et des mécanismes de sécrétion homologues, des différences à l'échelle de l'ultrastructure indiquent que les types de fibrilles et la disposition de ces dernières peuvent évoluer de manière indépendante à partir de plus grands éléments de la coenécie.

Mots-clés : coenécie, *Cephalodiscus*, cortex, fusellus, Pterobranchia, ptérobranches, ultrastructure.

[Traduit par la Rédaction]

Introduction

Members of the hemichordate class Pterobranchia are colonial or pseudocolonial zooids that secrete a sclerotized, collagenous domicile called a coenecium (Towe and Urbanek 1972; Armstrong et al. 1984) using their cephalic shield. There are few observations on the actual method of tube building, but what appears to happen is that the cephalic shield releases a jelly-like material into the fold of the dorsal and ventral cephalic shield. This mass is spread out on to the previously existing coenecial material by a rocking and stroking motion of the cephalic shield (Dilly 1986, 1988). Pterobranchs are closely related to the graptolites, an extinct

group of colonial benthic and pelagic organisms known only from the fossilized remains of their rhabdosome, a collagenous external domicile that shares structural similarities with the pterobranch coenecium. Pterobranchia comprise two main subgroups, the cephalodiscids (*Cephalodiscidae* and *Eocephalodiscidae*) and the rhabdopleurids (*Rhabdopleuridae*). The morphology of the coenecium forms the basis of pterobranch taxonomy and morphological phylogenetic studies (Bulman 1970; Markham 1971; Rickards and Durman 2006), provides the data for graptolite paleontology, and allows inferences of graptolite zooidal features (Rigby 1994; Rigby and Sudbury 1995). Zooidal characters are often insufficient to discriminate between closely related species of pter-

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obranch (van der Horst 1939; Hyman 1959) and are, with one exception (Hou et al. 2011), completely unknown among graptolites.

Similarities between *Rhabdopleura* Allman, 1869 and graptolites include the condition that individuals in the colony are serially budded in an organized pattern and retain a connection by a stolon system throughout their lifespan (Kozłowski 1949, 1966). The coenecium of *Rhabdopleura* and the graptolite rhabdosome share structural similarities. They are united by the possession of a sclerotized vesicle called prosicula where the larva undergoes metamorphosis (Stebbing 1970; Kozłowski 1971; Dilly 1985; Sato et al. 2008). In addition, the increments that form the bulk of the rhabdopleurid coenecium and the graptolite rhabdosome, called fuselli, possess a similar structure and arrangement. Fuselli on the creeping tubes are half rings that form a regular zigzag pattern and those on the upright tubes are similar or regularly arranged full rings (Kozłowski 1966). The shared presence of these morphological characters among rhabdopleurids and graptolites indicate that these two taxa form a monophyletic group to the exclusion of cephalodiscids (Mitchell et al. 2012). The alternative hypotheses, which posit that Pterobranchia is a monophyletic sister group to the graptolites (Bulman 1970) or that cephalodiscids are more closely related to graptolites than to rhabdopleurids (Rickards and Durman 2006), requires the secondary loss of all these characters in the cephalodiscid lineage.

The graptolite rhabdosome typically comprises two types of growth increments, forming two distinct components: the primarily secreted fuselli and secondarily secreted cortex. These two components (typically referred to as fusellar and cortical “tissues” in the graptolite literature) have been defined based on two different sets of criteria. First, they have been defined on the sequence of secretion events during the astogeny of the coenecium (primary versus secondary deposits) and based on their microstructure (i.e., the shape, size, and relative arrangement of growth increments) (Kozłowski 1949). Fuselli are secreted first and form the bulk of the coenecium (Kozłowski 1949), and cortex is laid down secondarily and is formed by the cumulative addition of thin layers of coenecial material (Kozłowski 1949) called cortical bandages (after their typical ribbon-like appearance under scanning electron microscopy (SEM)) (Andres 1977, 1980; Crowther 1978). Ectocortex is secreted on the outside of the fuselli and endocortex on the inside. Subsequently, as ultrastructural data became available in graptolites, the criteria used to define fuselli and cortex were extended to include the size, shape, and arrangement of the collagen fibrils. The fuselli and cortex of graptolites show consistently distinct patterns of collagen fibrils. Fuselli enclose a fabric typically composed of fusellar fibrils that are characteristically wavy, branching and anastomosing, forming a spongy three-dimensional meshwork (Urbanek and Towe 1974). Fusellar fibril diameters vary between 60 and 110 nm (Mierzejewski and Kulicki 2003) (Table 1). The cortex is composed of sheets that are separated by an electron-lucent material that typically contains cortical fibrils that are straight, thick (100 nm – 1 µm) and arranged in parallel (referred to as a eucortex) (Table 1). However, exceptions exist: graptolite secondary deposits may not possess cortical fibrils, but are still referred to as cortical. In the graptolite paracortex, cortical fibrils are

absent and intersheet material is composed of a tightly packed meshwork of ill-defined fibrous material, while pseudocortex lacks fibrils altogether (Urbanek and Mierzejewski 1984). Additionally, cortical fibrils may be found in fuselli (Mierzejewski and Kulicki 2001, 2003).

Ultrastructural studies on the coenecium of rhabdopleurids have yielded contradictory results. Transmission electron microscopic (TEM) observations on extant *Rhabdopleura* showed that the coenecium was only composed of fuselli, and that the fusellar fabric was made of thin and long fibrils randomly oriented (Dilly 1971). More recent observations using SEM demonstrated that the coenecium of fossil rhabdopleurids and extant *Rhabdopleura* comprises graptolite-like fusellar and cortical fibrils, as well as a graptolite-like organization with well-defined fuselli overlaid with inner cortical deposits comparable to the graptolite eucortex, paracortex, and pseudocortex (Mierzejewski and Kulicki 2001, 2003). Based on these observations, the authors have suggested that the Pterobranchia and Graptolithina should be regarded as members of a single class Graptolithoidea. However, this merging has been done without taking into account the pterobranch genus *Cephalodiscus* M’Intosh, 1882.

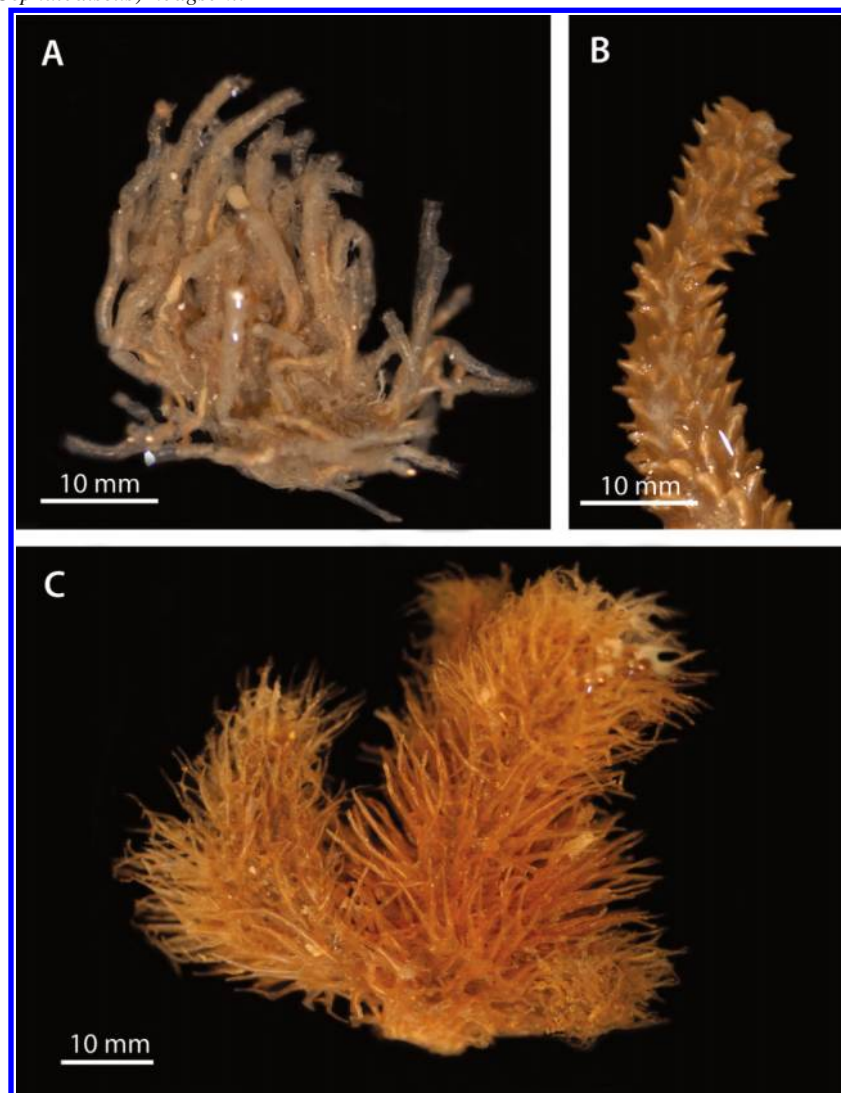
The presence of cortex in *Cephalodiscus* is debated. The thin layers of secondary deposits at the surface of the coenecium of extant cephalodiscids have been interpreted as cortical by Kozłowski (1966). The presence of cortical bandages in *Cephalodiscus* has also been acknowledged by Crowther (1978) and the secretion mechanisms used by *Cephalodiscus* zooids to secrete their secondary deposits have been used to provide a model of cortex secretion by graptolites (Crowther 1978). Dilly (1993; plate IV) interpreted the thin layers at the surface of the spines of *Cephalodiscus graptolitoideus* Dilly, 1993 as cortical. However, differences at the ultrastructural level with the typical graptolite eucortex led other authors to the idea that the coenecium of *Cephalodiscus* lacks a cortical component (Urbanek 1976). What is known of the coenecial ultrastructure of extant *Cephalodiscus* is limited to a poorly preserved specimen of *Cephalodiscus (Cephalodiscus) hodgsoni* Ridewood, 1906 (formerly known as *Cephalodiscus inaequatus* Andersson 1907) (Urbanek 1976), one unidentified species of the subgenus *Orthoecus* (Andres 1980), and the spines of *C. graptolitoideus* (Dilly 1993). These studies show that the coenecium of *Cephalodiscus* is made of layers, separated by sheets, that contain very thin straight fibrils (19–22 nm) randomly oriented and loosely dispersed in an electron-lucent matrix. Because these fibrils resemble the thin, randomly oriented fibrils found in the fuselli of *Rhabdopleura* by Dilly (1971), the irregularly shaped growth increments of *Cephalodiscus* have been regarded as homologous to fuselli (Urbanek 1976). However, unpublished observations of P.N. Dilly, A. Urbanek, and P. Mierzejewski indicate the presence of fibrils arranged in a parallel or subparallel arrangement in *Cephalodiscus (Orthoecus) solidus* Andersson, 1907, similar to the parallel arrangement of fibrils typical of graptolite eucortex (Mierzejewski and Kulicki 2001).

As *Cephalodiscus* is the sister group to the clade that comprises *Rhabdopleura* and Graptolithina (Mitchell et al. 2012), any understanding of *Cephalodiscus* coenecial features would be of great interest. To this end, we set out to characterize the organization of the coenecium layers and fibril types of a representative species of each of the three major *Cephalodiscus*

Table 1. Distribution of fibril types in the Pterobranchia and Graptolithina.

Fibril type	Diameter	Shape	Organization	Taxon	Localization	References
Fusellar	60–110 nm	Wavy, branching, anastomosing	Random	Graptolithina	Fuselli, paracortex	Mierzejewski and Kulicki 2001, 2003
Cortical	100 nm – 1 μ m	Straight	Parallel	Graptolithina	Eucortex	Mierzejewski and Kulicki 2001, 2003
		Straight or slightly bent	Parallel	Rhabdopleurids	Fusellar collar, eucortex	
?	4–30 nm	Straight	Random	<i>Rhabdopleura</i>	Fuselli	Dilly 1971
?	16–23 nm	Straight	Random	<i>Cephalodiscus</i>	Secondary deposits	Present study
			Parallel	<i>Cephalodiscus (Orthoecus)</i>	Secondary deposits	
?	120–280 nm	Straight or bent	Random	<i>Cephalodiscus hodgsoni</i>	?	Present study

Note: A question mark in the first column indicates that the fibril type or localization is not clearly defined.

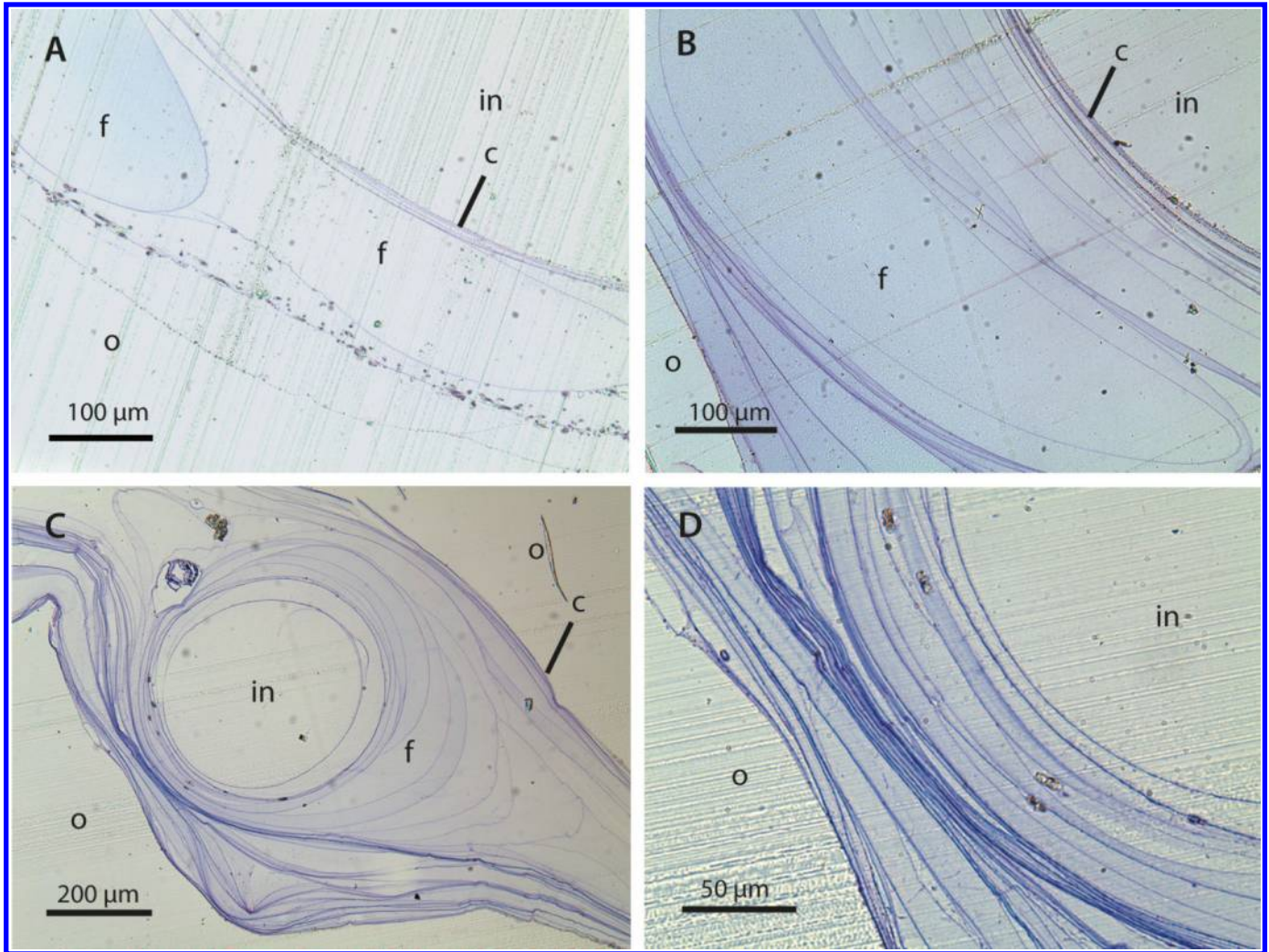
Fig. 1. Diversity of coenecial morphology in *Cephalodiscus*. (A) *Cephalodiscus (Orthoecus) densus*. (B) *Cephalodiscus (Idiothecia) nigrescens*. (C) *Cephalodiscus (Cephalodiscus) hodgsoni*.

subgenera: *Cephalodiscus (Orthoecus) densus* Andersson, 1907, *Cephalodiscus (Cephalodiscus) hodgsoni*, and *Cephalodiscus (Idiothecia) nigrescens* Lankester, 1905, using light microscopy, TEM, and SEM. Based on these data, we address the issue of the homology between the growth increments of *Cephalodiscus* and the fuselli and cortex of *Rhabdopleura* and Graptolithina, and infer the plesiomorphic state of the common ancestor to pterobranchs and graptolites.

Materials and methods

Fragments of coenecium from three specimens identified as *C. (O.) densus* (Fig. 1A), *C. (I.) nigrescens* (Fig. 1B), and *C. (C.) hodgsoni* (Fig. 1C) were obtained from the pterobranch collection at the National Museum of Natural History (Smithsonian), Washington, D.C. Specimens were stored in 70% ethanol, but the original fixation method is unknown.

Fig. 2. Light micrographs of transverse sections through the tube walls of *Cephalodiscus (Orthoecus) densus* (A), *Cephalodiscus (Idiothecia) nigrescens* (B), and *Cephalodiscus (Cephalodiscus) hodgsoni* (C, D). c, cortical-like layers; f, fusellar-like layers; in, inside of tube; o, outside of tube.



We have studied only one sample from each species because these samples are extremely rare and our methods are destructive. The species identification of samples was determined by comparing the coenecia with the taxonomic literature (for *C. (I.) nigrescens*: Lankester 1905; Ridewood 1907, 1918a, 1918b; John 1931; Markham 1971; for *C. (O.) densus*: Andersson 1907; Ridewood 1918a, 1918b; John 1931; Markham 1971; for *C. (C.) hodgsoni*: Andersson 1907; Ridewood 1907, 1918a, 1918b; Harmer and Ridewood 1913; John 1931; Johnston and Muirhead 1951; Markham 1971). Regrettably, the collection locations of our Smithsonian specimens are unknown. Publications from the early 1900s indicate that specimens of *C. (O.) densus* have been collected at 66°S, 94°E; 64°S, 56°W; 62°S, 65°W; Palmer Archipelago; McMurdo Sound; Graham Land; Queen Mary Land; Kerguelen Islands; specimens of *C. (I.) nigrescens* at 77°S, 166°E; 66°S, 94°E; Jenny Island; Queen Mary Land; Coulman Island; McMurdo Sound; Palmer Archipelago; and specimens of *C. (Cephalodiscus) hodgsoni* at 66°S, E 94°E; S 78°S, E 197°E; S 64°S, E 97°E; 64°S, 56°W; 52°S, 55°W; Adélie Land; South Georgia; South Orkneys; Palmer Archipelago; McMurdo Sound; Kaiser Wilhelm II Land; Queen

Mary Land; in other words, from deep water in the Southern Ocean and its associated seas bordering Antarctica (in van der Horst 1939). We can safely assume that our samples also come from the Southern Ocean.

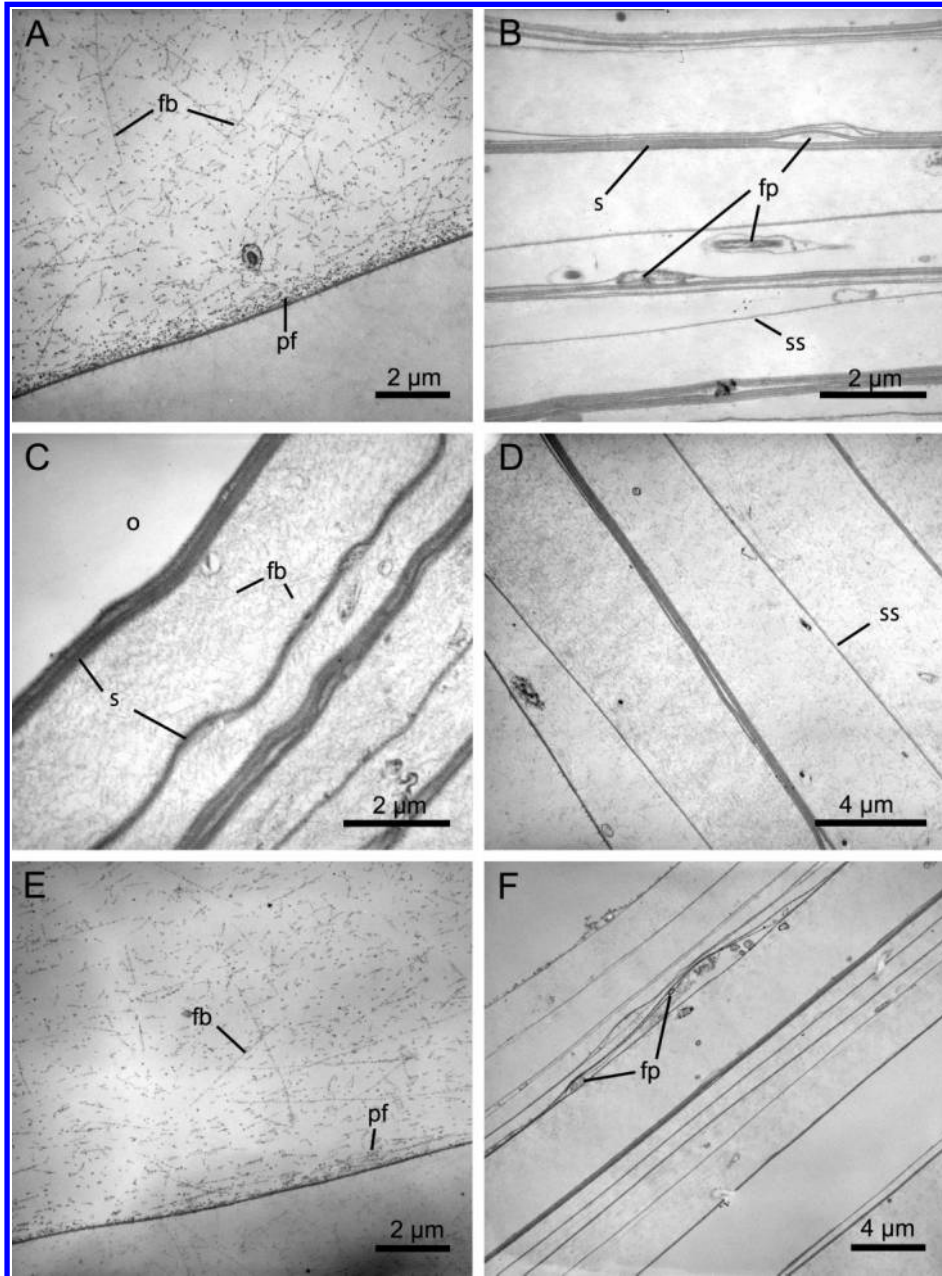
Light microscopy

Pieces of coenecia were dissected, fixed in Karnovsky's solution (5% glutaraldehyde and 4% paraformaldehyde) in 0.1 mol/L phosphate buffer at pH 7.3 for 2 h, rinsed in phosphate buffer and postfixed in 1% osmium tetroxide in 0.1 mol/L phosphate buffer at pH 7.3 for 1 h. Samples were then dehydrated in a graded series of ethanol solutions and embedded in Araldite (SPI supplies, West Chester, Pennsylvania). Semi-thin transverse sections of tubes were cut on an ultramicrotome, mounted on microscope slides, stained with toluidine blue, and observed with an Olympus BX-51 microscope.

Transmission electron microscopy

Pieces of coenecia were treated as described above. Ultrathin sections were cut on an ultramicrotome, mounted on

Fig. 3. Transmission electron micrographs of the coenecium of *Cephalodiscus* (*Cephalodiscus*) *hodgsoni* and *Cephalodiscus* (*Idiothecia*) *nigrescens*. (A, B) Transverse sections through the walls of the coenecium of *C. (C.) hodgsoni*. (C, D) Transverse sections through a spine of *C. (C.) hodgsoni*. (E, F) Transverse sections through the walls of the coenecium of *C. (I.) nigrescens*. fb, fibrils; fp, foreign particles; pf, parallel fibrils; s, sheet; ss, single sheet.



copper or nickel grids, and stained with phosphotungstic acid for 5 min (*C. (C.) hodgsoni*, *C. (I.) nigrescens*), or with uranyl acetate (*C. (O.) densus*) for 30 min. Sections were observed with a Jeol JEM 100-S microscope. Fifty fibrils were measured from one sample of each species.

Scanning electron microscopy

Pieces of coenecia were dissected, fixed in 4% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer at pH 7.4 and postfixed in 1% osmium tetroxide in 0.1 mol/L sodium cacodylate buffer. They were then dehydrated through a graded series of ethanol solutions, followed by critical point drying. After sputter coating with an alloy of gold–palladium, the

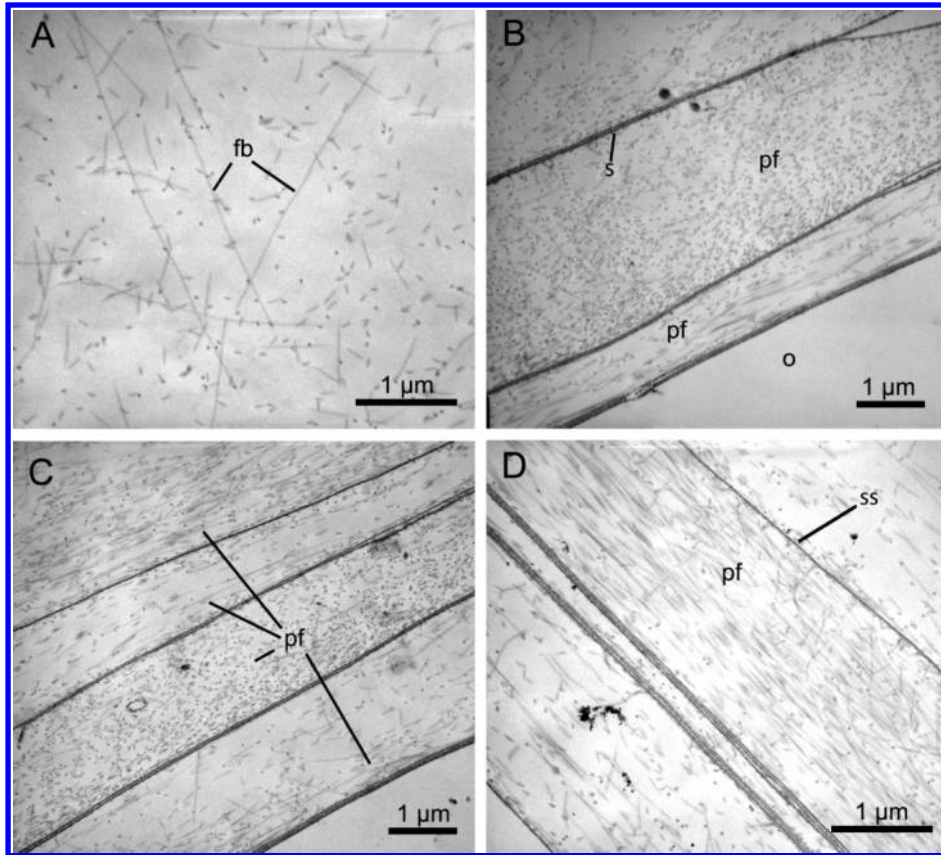
specimens were examined with a FEI Quanta 200–3D scanning electron microscope.

Results

Light microscopy

The walls of the coenecium of *C. (O.) densus* (Fig. 2A), *C. (I.) nigrescens* (Fig. 2B), and *C. (C.) hodgsoni* (Figs. 2C, 2D) are composed of layers of coenecial material of variable thickness overlaid one upon the next. Thick fusellar-like layers (up to 160 μm thick in *C. (O.) densus*, 150 μm in *C. (I.) nigrescens*, and 135 μm in *C. (C.) hodgsoni*) and thin cortical-like layers (see TEM section for measurements) are

Fig. 4. Transmission electron micrographs of transverse sections through the walls of the coenecium of *Cephalodiscus (Orthoecus) densus*. (A) Randomly oriented fibrils. (B, C, D) Outer layers of the coenecium, showing a parallel arrangement of fibrils. fb, fibrils; pf, parallel fibrils; s, sheet; ss, single sheet.



present, but are not as distinct as in other tube-building hemichordates. Instead, a continuum exists in the thickness and shape of these layers, and the coenecial walls are characterized by an irregular laminated organization.

Transmission electron microscopy

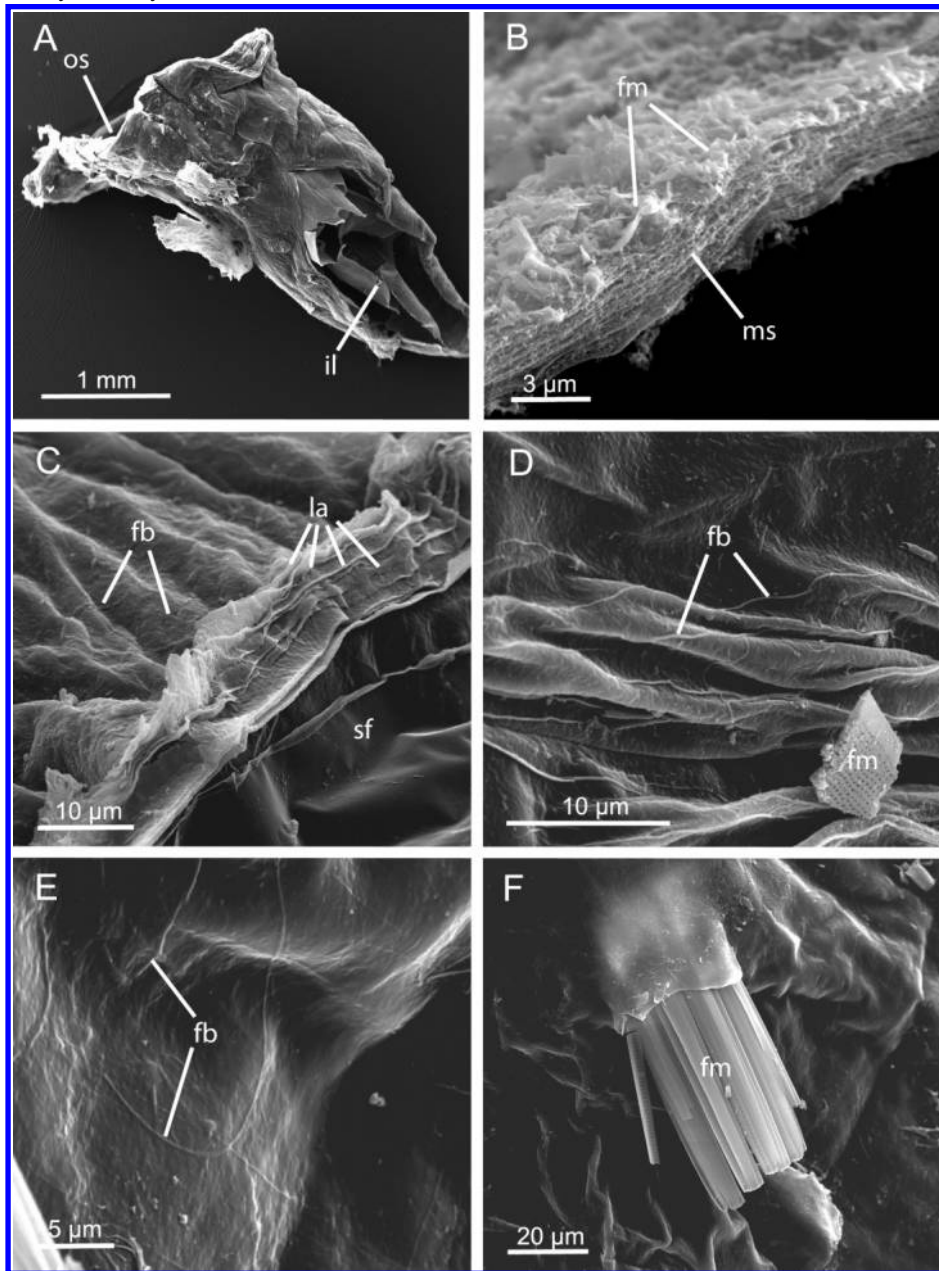
Both the large fusellar-like layers and the thinner cortical-like layers of the coenecium of *C. (C.) hodgsoni*, *C. (I.) nigrescens*, and *C. (O.) densus* are composed of straight fibrils embedded in an electron-lucent matrix (Figs. 3A–3F, 4A–4D). The longest fibril observed was 75 µm long for *C. (C.) hodgsoni*, 58 µm for *C. (I.) nigrescens* and 70 µm for *C. (O.) densus*. The minimal length of fibrils could not be evaluated because the smallest fibrils on TEM micrographs represent transverse sections. The diameter (mean ± SD) of fibrils were 23 ± 3.5 nm ($N = 50$) for *C. (I.) nigrescens*, 21 ± 3.8 nm ($N = 50$) for *C. (C.) hodgsoni*, and 16 ± 3.3 nm ($N = 50$) for *C. (O.) densus* (Table 1). This fibrillar fabric is arranged in layers of irregular thickness, separated by conspicuous electron-dense lines, interpreted here as sheets (Figs. 3A–3F, 4A–4D). A continuum exists in the thickness of layers: the distance between two sheets varies from approximately 15 nm (in all three species) to more than 100 µm (the thickest layers were measured on light micrographs, see above). These sheets occurred either individually (Figs. 3B, 3D, 4D) or in groups of 2–12 sheets. Closely set sheets can separate to enclose foreign particles (Figs. 3B, 3F). One or several sheets always delineated the inner and

outer layers of the tubes. The orientation of fibrils within each layer was variable. In some places, their orientation was random (Figs. 3A, 3C, 3E, 4A), whereas in others, it was organized. In the region closest to the outer sheet, they were typically arranged parallel to the sheet (Figs. 3A, 3E). In these regions, fibrils were more numerous and more closely packed. In *C. (O.) densus*, the outermost layers of the coenecium showed a clear pattern of parallel arrangement of the fibrils. In each layer, fibrils run parallel to each other, but the orientation of fibrils was different between adjacent layers (Figs. 4B, 4C, 4D).

Scanning electron microscopy

In the three species studied, the outer surfaces of the coenecium were usually featureless and smooth (Fig. 5A). This smooth texture probably corresponds to the sheet fabric covering the growth increments. The layered arrangement of the growth increments was visible where the walls of the coenecium were torn (Figs. 5A, 5B, 5C). As seen with TEM, the whole coenecium was composed of a superposition of these thin layers. In certain regions, where the sheet fabric was peeled apart, the fibrillar material was exposed, showing fibrils in the same diameter range as those observed with TEM (Figs. 5C, 5D, 5E). In cross-sections of the walls, the three-dimensional arrangement of these fibrils was visible, showing the presence of a spongy meshwork (Fig. 5B). In *C. (C.) hodgsoni*, much thicker fibrils (120–280 nm, 170 ± 40 nm (mean ± SD), $N = 10$) were visible on the outer sur-

Fig. 5. Scanning electron micrographs of the coenecium of *Cephalodiscus*. (A) Individual tube of *Cephalodiscus* (*Idiothecia*) *nigrescens*. (B) Cross-section through layers of the wall of *C. (I.) nigrescens*. (C) Outer wall of the coenecium of *Cephalodiscus* (*Cephalodiscus*) *hodgsoni* showing the layered organization of the coenecium. (D, E) Outer walls of the coenecium of *C. (C.) hodgsoni* showing thick fibrils. (F) Outer wall of *C. (C.) hodgsoni* showing the inclusion of foreign material between successive layers of the coenecium. fb, fibrils; fm, foreign material; il, inner layer; la, layers; os, ostium; ms, meshwork of fibrils; sf, sheet fabric.



face of the coenecium (Table 1, Figs. 5D, 5E). These fibrils were not arranged in bundles and showed no clear sign of a parallel orientation. The frequent occurrence of foreign material embedded between adjacent layers (Fig. 5F) suggests that the layers are deposited successively one upon the next.

Discussion

The aim of this study was to address the question of the homology of the growth increments of *Cephalodiscus* with the two main components of the graptolite and rhabdopleurid coenecium: the fuselli and the cortex. To address this issue,

comparisons at both the microstructural and ultrastructural level have been made.

Microstructure

Walls of the *Cephalodiscus* coenecium are composed entirely of layers of variable thickness overlaid one upon the next and delineated by sheets. Fusellar-like units (vaguely annular) are present and secondary deposits are ubiquitous, but there is no clear differentiation between these two components, as a continuum exists in the shape and thickness of these layers. These observations are compatible with two interpretations. The first one is that the common ancestor of

pterobranchs and graptolites was able to secrete fusellar-like growth increments and to thicken them with thin trails of laminated secondary deposits, but in an irregular fashion as seen in *Cephalodiscus*. In the lineage leading to *Rhabdopleura* and graptolites, the differentiation between these two components became more marked as fusellus shape became more regular. The alternative interpretation is that well-defined fuselli and cortical bandages are plesiomorphic to (Pterobranchia + Graptolithina), and that this regular mode of growth secondarily became more irregular in the cephalodiscid lineage.

Ultrastructure

In the three species under consideration, TEM observations revealed that intersheet fibrillar material contains only one type of fibril (the homology of these fibrils with rhabdopleurid and graptolite fibrils is discussed below). In *C. (C.) hodgsoni* and *C. (I.) nigrescens*, fibrils are arranged randomly throughout the coenecium, showing an absence of differentiation between primary and secondary deposits at the ultrastructural level. This random arrangement of fibrils is similar to the arrangement of fibrils in the graptolite fuselli. A differentiation between primary and secondary deposits is only evident in *C. (O.) densus*, where fibrils of the outer layers of the coenecium are arranged in parallel. This parallel arrangement is similar to the arrangement of fibrils in the graptolite eucortex, but the fibrils themselves are much thinner. This finding can be interpreted in two different ways. First, the parallel arrangement in the secondary deposits may have evolved independently in *Cephalodiscus* and graptolites. An identical type of organization was reported from *C. (O.) solidus* (unpublished data mentioned in Mierzejewski and Kulicki 2001), and thus may be restricted in *Cephalodiscus* to the subgenus *Orthoecus*. Alternatively, the parallel organization of fibrils in outer secondary deposits could be plesiomorphic to a clade that includes Pterobranchia and Graptolithina. This hypothesis implies that fibril organization could be conserved during the evolution of new fibril types.

Homology of fibril types

One type of fibril was found in the coenecium of *Cephalodiscus* with TEM, but a second thicker fibril type was found with SEM. As reported by Urbanek (1976) and Andres (1980), these fibrils are very thin, long, and straight. They are similar in size and shape to the fibrils found in the fuselli of *Rhabdopleura* by Dilly (1971) using TEM. However, they differ from the fibrils found with SEM in recent and fossil rhabdopleurids by Mierzejewski and Kulicki (2001, 2003), who did not address the issue of the discrepancy of their results with previous observations. Dilly's observations, as well as ours, may be artifacts caused by fixation method. However, glutaraldehyde fixation is a standard method for imaging collagen and is not known to cause alterations in fibril size and shape. Moreover, similar fibrils were described by Urbanek (1976) who did not use aldehyde fixation. An alternative hypothesis would be that the typical graptolite-like pattern is the result of taphonomic changes. However, this also seems unlikely because the same fibril pattern was found in living *Rhabdopleura* (Mierzejewski and Kulicki 2003). The most probable explanation is that the fibril characterizations of both Dilly and Mierzejewski and Kulicki were cor-

rect, but incomplete because they did not employ both SEM and TEM. In our view, it seems most likely that *Rhabdopleura* secretes both fibril types. Supporting this hypothesis is the fact that our SEM observations revealed larger fibrils that we did not detect with TEM. This issue remains an obstacle to interpreting the evolution of fibril types in Pterobranchia, and until this problem is solved, we provisionally accept both sets of data as valid. In this context, the long thin fibrils of *Cephalodiscus* and *Rhabdopleura* may be plesiomorphic to the clade that comprises Pterobranchia and Graptolithina, and the fusellar and cortical fibrils characteristic of extinct graptolites are a synapomorphy of a clade that includes rhabdopleurids and Graptolithina.

The homology of *Cephalodiscus* growth increments with the rhabdopleurid and graptolite fuselli and cortex

Our observations show that the coenecium of *Cephalodiscus* is composed of primary growth increments and secondary deposits that are similar (although more irregular and less well-differentiated) in their microstructure to the graptolite and rhabdopleurid fusellar and cortical components, respectively. These similarities presumably result from conserved zooid behavioral patterns and secretion mechanisms and can be considered homologous. Differences at the ultrastructural level, however, show that fibril organization and probably fibril types can vary within this conserved pattern of tube-building. For these reasons, we propose that fibril type and organization should not be used as a preferred criterion over other morphological characters in the determination of tissue homology, but that the homology of units at each level of organization (i.e., microstructure and ultrastructure) should be treated independently. This reasoning was applied implicitly by Urbanek and Mierzejewski (1984) who regard pseudocortex and paracortex as cortical despite the absence of cortical fibrils arranged in parallel in these tissues. In this way, *Cephalodiscus* secondary deposits can be regarded as a new type of cortex.

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