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Ultrastructure of the pseudocnidae of the palaeonemerteans *Cephalothrix* cf. *rufifrons* and *Carinomella lactea* and an assessment of their phylogenetic utility

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Abstract

The ultrastructure of the pseudocnidae (filament-core rhabdoids) of the palaeonemerteans *Cephalothrix* cf. *rufifrons* and *Carinomella lactea* is described and the phylogenetic utility of these organelles evaluated. Pseudocnidae of *Cephalothrix* cf. *rufifrons* are clavate structures, measuring 3–5 µm in length with a bulbous lateral process present near their midregion, whereas those of *Carinomella lactea* are rod-shaped structures, measuring 2–3 µm in length and lack a lateral process. In both species, these structures exhibit a somewhat electron-lucent cortex, an electron-dense medulla and a distinct filament-like core, extending from the apex of the pseudocnida toward its base. The pseudocnidae are situated within pseudocnida-forming cells that constitute a portion of the glandular proboscis epithelium and are oriented parallel to the apical basal axis of the cell. No evidence of core extrusion was observed in these species. Comparative structural analyses confirm that these secretory granules are neither rhabdite homologues nor cleptocnidae. Position and ultrastructure of palaeonemertan pseudocnidae support the hypothesis that they are homologous to those of heteronemerteans, and pseudocnidae are interpreted as a provisional synapomorphy of the Anopla, as suggested in previous studies. However, recent molecular phylogenies of nemerteans do not support anoplans monophyly, necessitating alternative interpretations of pseudocnida evolution, namely, that these structures were present in the nemertean common ancestor and subsequently lost in the hoplonemerteans and *Carinoma* or that they evolved independently in the Pilidiophora and a *Cephalothricidae*+*Tubulamidae* clade. A rigorous explanation of pseudocnida evolution awaits a simultaneous analysis of molecular and morphological characters inclusive of pseudocnidae.

Keywords: *Nemerteans, ultrastructure, morphology, systematics, phylogenetics*

Introduction

The prominent synapomorphy of the Nemertea is an eversible proboscis apparatus that functions principally in prey capture (see McDermott and Roe 1985). The glandular

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epithelium of the proboscis contains a variety of secretory cells that synthesize products involved in subduing prey (Jennings and Gibson 1969; Stricker and Cloney 1983). These include cells that produce prey-immobilizing toxins (see Kem 1985; Stricker and Cloney 1983), those that secrete substances facilitating adhesion of prey to the proboscis (see Stricker and Cloney 1983), and those that elaborate rod- or club-shaped structures with an internal filament presumably involved in gripping prey (e.g. Bürger 1895; Jennings and Gibson 1969). These structures have been variously termed nematocysts (e.g. Hubrecht 1887, as cited in Bürger 1895:58), pseudocnidae (Martin 1914), rhabdites (Gontcharoff 1957; Hyman 1959:739; Ling 1971) and barbs (Jennings and Gibson 1969). These conspicuous gland-cell products have been reported at the light microscopical level in numerous heteronemerteans, including, *Micrura purpurea* Dalyell, 1853, *Lineus geniculatus* (= *Notospermus geniculatus*) Delle Chiaje, 1828, *Cerebratulus urticans* Müller, 1854 (Bürger 1895), *Zygeupolia rubens* Coe, 1895 (Thompson 1901), *Paralineus elisabethae* (Schütz, 1912) and many palaeonemerteans, including, among others, species of *Hubrechtella* (Hylbom 1957; Gibson 1979), *Cephalothrix* (Wijnhoff 1910; Gerner 1969; Jennings and Gibson 1969; J. L. Norenburg and C. Santos, pers. commun.) and *Carinomella lactea* (Coe 1905). Ultrastructural examinations of these rod-shaped or clavate bodies have been limited to the heteronemerteans *Lineus ruber* Müller, 1774 (Gontcharoff 1957; Ling 1971), *Lineus gesserensis* Müller, 1780 (Anadón 1976), *Zygeupolia rubens* (Turbeville 1991), *Riseriellus occultus* Rogers, Junoy, Gibson and Thorpe, 1993 (Montalvo et al. 1998) and the palaeonemertean *Tubulanus* cf. *pellucidus* Coe, 1895 (Turbeville 1991). Hereinafter, these structures in nemerteans will be referred to collectively as pseudocnidae, which represent a class of rhabdoid, following terminology of Smith et al. (1982).

Detailed comparative data for palaeonemertean pseudocnidae are lacking, precluding the assumption that these structures are homologous among anoplan nemerteans. I examined the ultrastructure of pseudocnidae of the *Cephalothrix* cf. *rufifrons* Johnston, 1837 and *Carinomella lactea* Coe, 1905 to clarify the organization of these structures in palaeonemerteans and to enhance evaluation of their phylogenetic utility. A comparative assessment confirms that these structures are neither rhabdite homologues nor cleptocnidae, but rather a nemertean apomorphy and, furthermore, indicates that they are potentially informative for reconstructing the intraphyletic relationships of the major nemertean taxa as previously suggested based on ultrastructural data from four heteronemerteans and limited data for a single palaeonemertean (Turbeville 1991; Montalvo et al. 1998).

Materials and methods

Specimens of *Cephalothrix* cf. *rufifrons* were collected from a rock jetty on the coast at Saint Augustine, Florida by R. S. Fox and E. E. Ruppert. Specimens of *Carinomella lactea* were collected from fine-medium sediments near the mouth of North Inlet, near Georgetown, South Carolina, by the author. Identification of the former species is provisional and based on the descriptions of Johnston (1837) and Wijnhoff (1910, 1913).

For transmission electron microscopy (TEM), specimens relaxed in MgCl₂ isotonic to seawater were immersed in 2.5% glutaraldehyde in 0.4 M Millonig's phosphate buffer (pH 7.4), cut into small pieces and allowed to fix for 1–2 h at room temperature. Prior to postfixation, tissue pieces were rinsed in Millonig's phosphate buffer wash. Postfixation was for 1 h at room temperature in either 2% OsO₄ buffered with Millonig's phosphate buffer or in 1% OsO₄ in 0.7 M NaCl and 0.4 M Millonig's phosphate buffer.

One specimen of *Carinomella lactea* was fixed in 4% formaldehyde–1% glutaraldehyde in 0.1 M phosphate buffer with a trace of CaCl_2 for several hours at 5°C (S. Tyler, pers. comm.). Tissue pieces were subsequently rinsed briefly with 0.1 M phosphate buffer and allowed to remain overnight in fresh buffer at 5°C. Tissue specimens were postfixed in 1% OsO_4 in phosphate buffer for 1 h at room temperature. Following postfixation, all specimens were dehydrated through an ethanol series, immersed in propylene oxide and embedded in Polybed 812 epoxy resin.

Semiserial or representative transverse thin sections of the proboscis or entire worms were cut with a diamond knife on either an LKB Ultratome Nova or Sorvall Porter-Blum MT-2B ultramicrotome. Sections were obtained from four specimens of *Cephalothrix* cf. *rufifrons* and two of *Carinomella lactea*. Thin sections were mounted on uncoated, thin-bar hexagonal grids, stained with aqueous uranyl acetate and Reynolds lead citrate and observed with a Philips EM 300 or Zeiss EM9 S2 electron microscope.

Results

Proboscis organization

The proboscis is composed of the following tissue layers: (1) an inner squamous epithelium (adjacent to the rhynchocoel fluid), (2) a subepithelial circular muscle layer (observed only in *C. lactea*), (3) an extracellular matrix layer (ECM), (4) a longitudinal muscle layer, (5) a circular muscle layer, (6) a thin discontinuous ECM layer (inconspicuous in *C. cf. rufifrons*), (7) a nerve plexus, and (8) a pseudostratified glandular epithelium (Figures 1, 2A and 4A). This glandular epithelium is exposed to the external environment when the proboscis is everted.

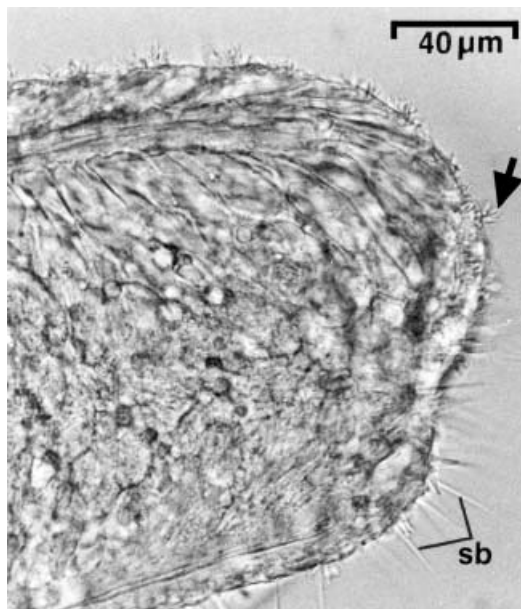


Figure 1. Light micrograph of the everted proboscis of *Cephalothrix* cf. *rufifrons* revealing apices of pseudocnidae (arrow). Note also the sensory bristles (sb).

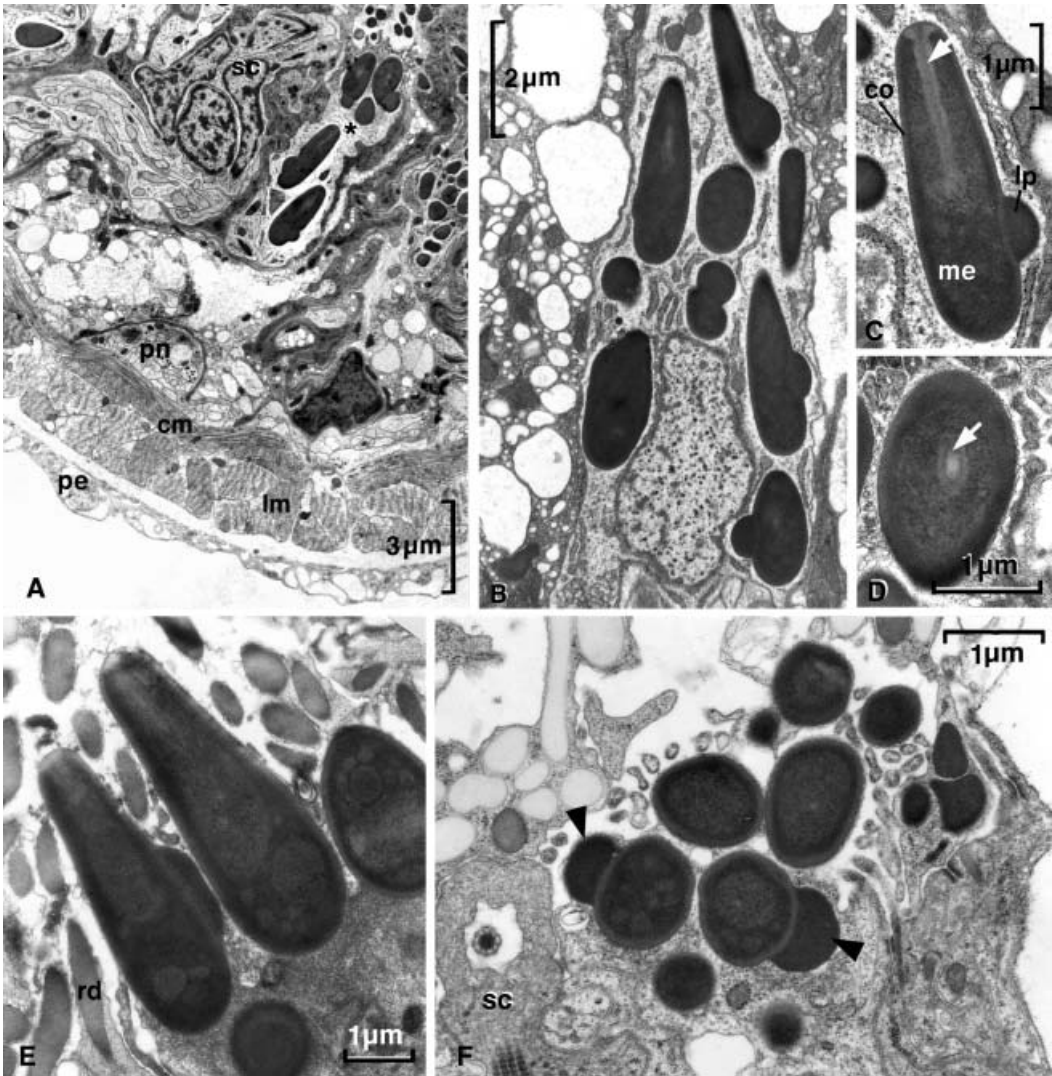


Figure 2. Transmission electron micrographs of the middle proboscis of *Cephalothrix cf. rufifrons*. (A) Survey micrograph of a cross section of the middle proboscis. Asterisk (*) indicates a pseudocnida-forming cell. (B) Pseudocnida-forming cell revealing the nucleus, RER and several pseudocnidae. (C) Longitudinal section of pseudocnida. Arrow indicates the filament core. (D) Cross-section of a pseudocnida. Arrow indicates core. (E) Section of an everted proboscis. Apices of the pseudocnidae extend into the lumen. (F) Cross-sections of bases of pseudocnidae. Arrowheads indicate the lateral processes. Also note the sensory cilium of the adjacent sensory cell (sc). Abbreviations: cm, circular muscle; co, cortex; lm, longitudinal muscle; lp, lateral process; me, medulla; pe, proboscis peritoneum; pn, proboscis nerve; rd, fusiform rhabdoid; sc, sensory cell.

The proboscis glandular epithelium of both species consists of a variety of gland cell types and monociliated cells that are presumed to be sensory. The cilia of these sensory cells are surrounded by stout microvilli (stereovilli) and together with the cilium they form a sensory bristle (Figures 1, 2A, F and 4C). Similar cells have been recently examined in detail for the heteronemertean *Riseriellus occultus* by (Montalvo et al. 1996). The histology of the entire proboscis has been described for *Cephalothrix cf. rufifrons* by Wijnhoff (1910) and for *Carinomella lactea* by Coe (1905).

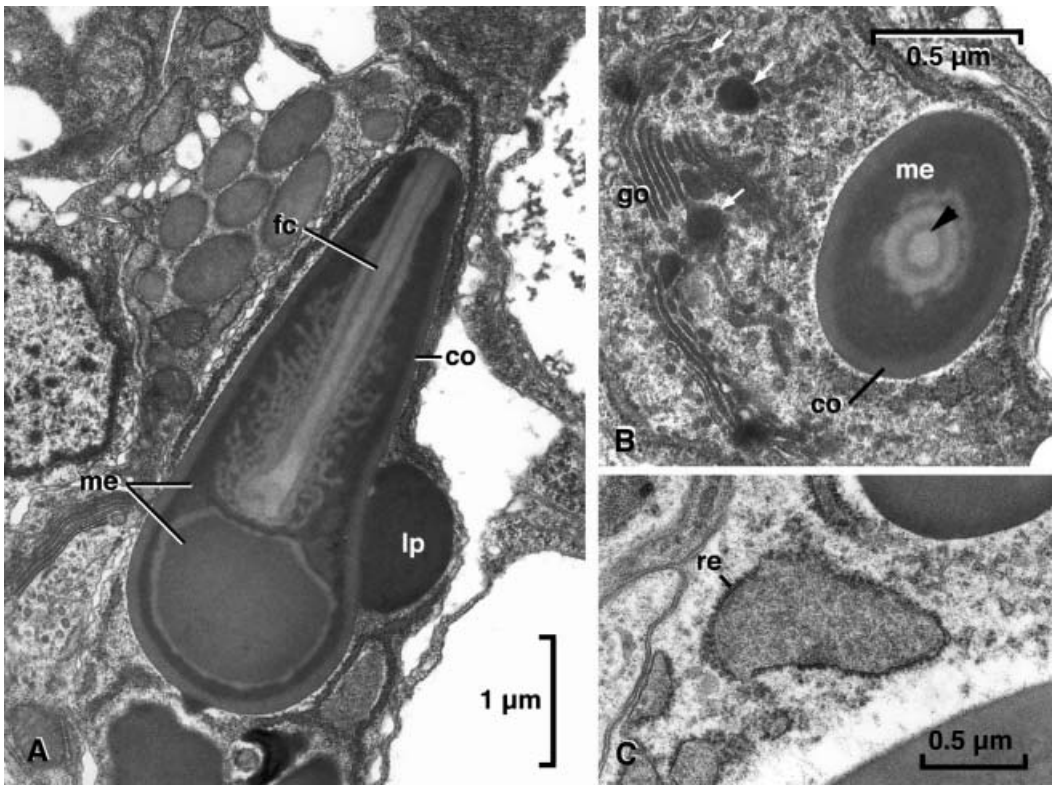


Figure 3. Transmission electron micrographs of *Cephalothrix cf. rufifrons* pseudocnidae and pseudocnida-forming cells. (A) Longitudinal section of a mature pseudocnida. Note the filament core (fc) and the lateral process (lp). (B) Cross-section of a pseudocnida. Note the Golgi complex in the cell cytoplasm and the putative pseudocnida precursor material (arrows). (C) RER (re) containing flocculent material in a pseudocnida-forming cell. Abbreviations: co, cortex; fc, filament core; go, Golgi complex; lp, lateral process; me, medulla; re, rough endoplasmic reticulum (RER).

Pseudocnidae are rod- or club-shaped secretory bodies occurring in clusters within pseudocnida-forming secretory cells that constitute a portion of the glandular epithelium. These structures are electron-dense and possess a central, tubular core resembling an eversible filament as found in nematocysts (Figures 2–6). These distinct granules will be detailed separately below for each species.

Cephalothrix cf. rufifrons

Pseudocnidae of this palaeonemertean are club-shaped (clavate) structures ranging in length from 3–5 μm. The base of the secretory body possesses a lateral bulbous process that, together with the expanded base, may function to anchor the secretory structure in the cell cytoplasm (Figures 2 and 3). *Cephalothrix cf. rufifrons* pseudocnidae exhibit an outer moderately electron-lucent, unilaminar, homogenous cortex surrounding a subcortical electron-dense zone (medulla) and a centrally situated filament-like, tubular core of moderate electron density (Figures 2, 3 and 6). The tubular core, measuring 2–3 μm, extends proximally from the apex of the rhabdoid and terminates about 1.5 μm from the base of the granule. The medulla of the enlarged, bulbous base corresponds in electron

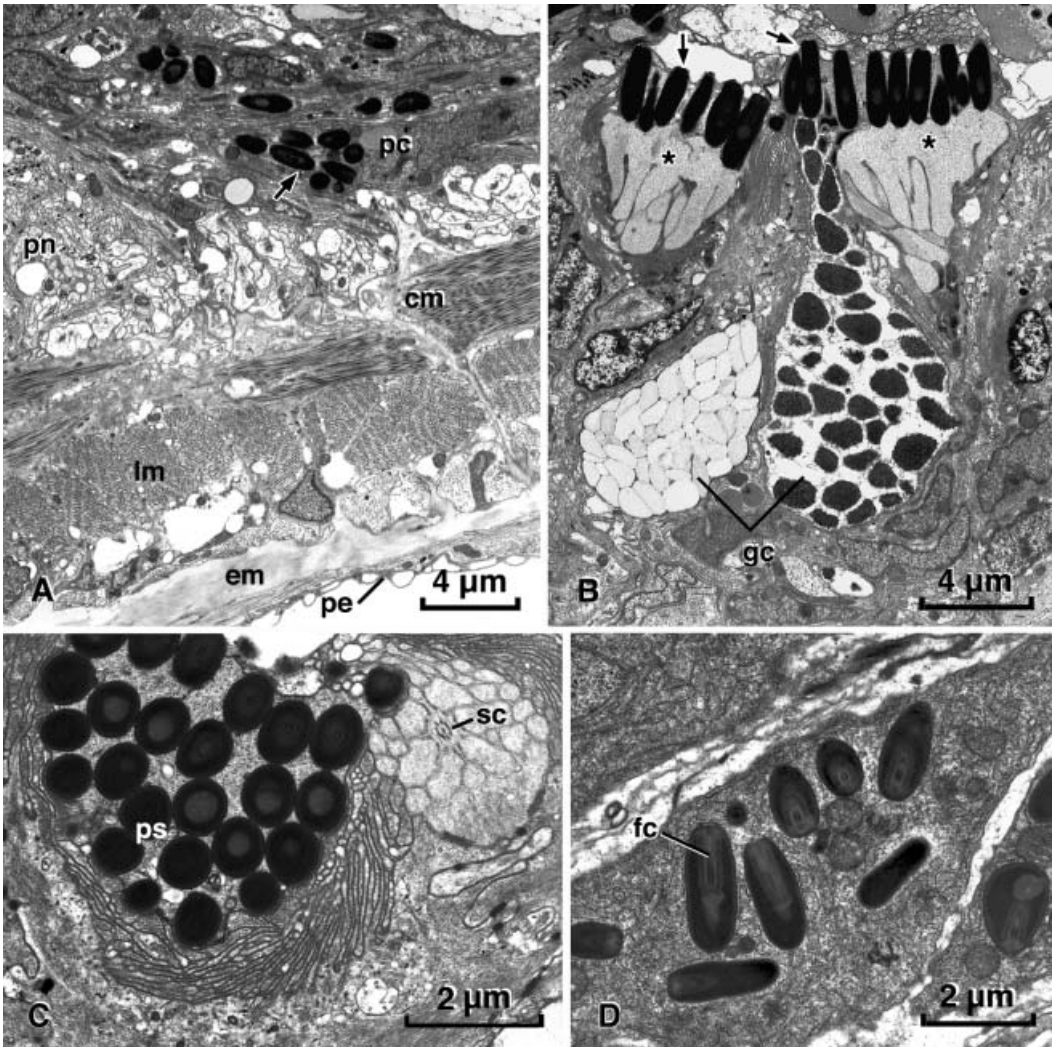


Figure 4. Cross-sections of the proboscis of *Carinomella lactea*. (A) Survey transmission electron micrograph of the proboscis. Note the pseudocnida-forming cell containing pseudocnidae (arrow). (B) Low-power micrograph revealing two groups of pseudocnidae (arrows) resting on secretions (*) of underlying gland cells. (C) Cross section of a group of pseudocnidae (ps) and a sensory bristle of an adjacent sensory cell. (D) Low-power micrograph of longitudinal sections of pseudocnidae. Note the filament core (fc). Abbreviations: cm, circular muscle; em, extracellular matrix; fc, filament core; gc, gland cell; lm, longitudinal muscle; pc, pseudocnida-forming cell; pe, proboscis peritoneum; pn, proboscis nerve; ps, pseudocnida; sc, sensory cell cilium.

density to that of the outer cortex and is sometimes fringed by a more electron lucent layer (Figure 3A). In some pseudocnidae, the electron density of the basal medulla is less uniform. In these pseudocnidae, electron-lucent patches surrounded by a matrix of greater electron density characterize the basal medulla, giving it a mottled appearance (Figure 2E). Several pseudocnidae occur in groups within a single cell and they are typically oriented parallel to the apical basal axis of the cell. A reliable estimate of the average number of pseudocnidae per cell was difficult to obtain, but as many as eight pseudocnida profiles have been observed in a single cell in representative sections (Figure 2B). Extrusion of the filament-like core was not observed in living or preserved specimens (Figures 1–3).

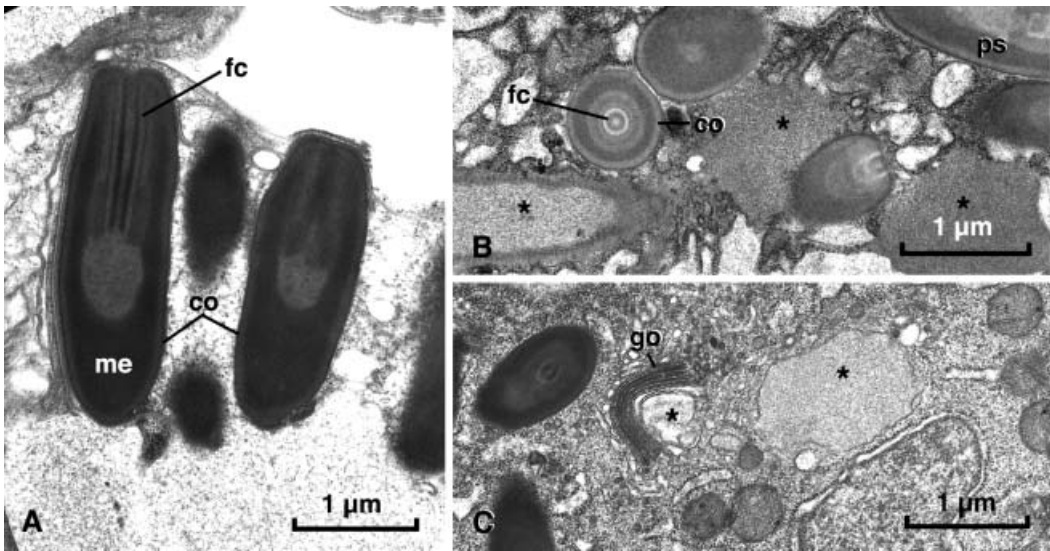


Figure 5. Transmission electron micrographs of *Carinomella lactea* pseudocnidae and pseudocnida-forming cells. (A) Longitudinal section of pseudocnidae. Note the medulla (me) cortex (co) and filament core (fc). (B) Cross section of a pseudocnida. Putative pseudocnida precursors are present in the cell cytoplasm (*). (C) Pseudocnida-forming cell revealing Golgi complex (go) and pseudocnida precursor material (*). Abbreviations: co, cortex; fc, filament core; go, Golgi complex; me, medulla; ps, pseudocnida.

Pseudocnida-forming cells are characterized by a nucleus containing a large nucleolus, prominent Golgi complexes, a large complement of RER and a few mitochondria (Figures 2B, C and 3B, C). Electron-lucent flocculent material is often present within the cisternae of the RER (Figure 3C), but it is unclear if this material is incorporated into developing pseudocnidae. Membrane-bound electron-dense granules associated with the *trans* or maturing face of the Golgi complex are common and may be pseudocnida precursors (Figure 3B). Further stages in the formation of pseudocnidae have not been elucidated.

Carinomella lactea

The pseudocnidae of this species are rod-shaped structures, measuring 2–3 μm in length and about 1 μm in diameter with a rounded base and a flattened apex (Figures 4–6). The secretory bodies possess an outer homogenous somewhat electron-lucent cortex, a subcortical layer (medulla) of greater electron density and an inner filament-like core of variable electron density (Figures 4D, 5A, B and 6). The tubular, filament-like core is separated from an inner surrounding area of moderately electron-lucent material (Figure 5B). The filament originates at the apex of the rhabdoid and extends towards its base, terminating in the expanded electron-lucent area close to the midregion (Figure 5A). The filaments typically measure about 1.25 μm in length. In contrast to the pseudocnidae of *C. rufifrons*, those of *C. lactea* lack a lateral bulbous process.

Pseudocnida forming-cells contain a prominent nucleus, abundant RER, conspicuous Golgi complexes and mitochondria (Figure 5C). A flocculent material was occasionally observed in the cisternae of the ER and may represent pseudocnida precursor material. In some cells secretory bodies with an outer fibrous, somewhat striated cortex and

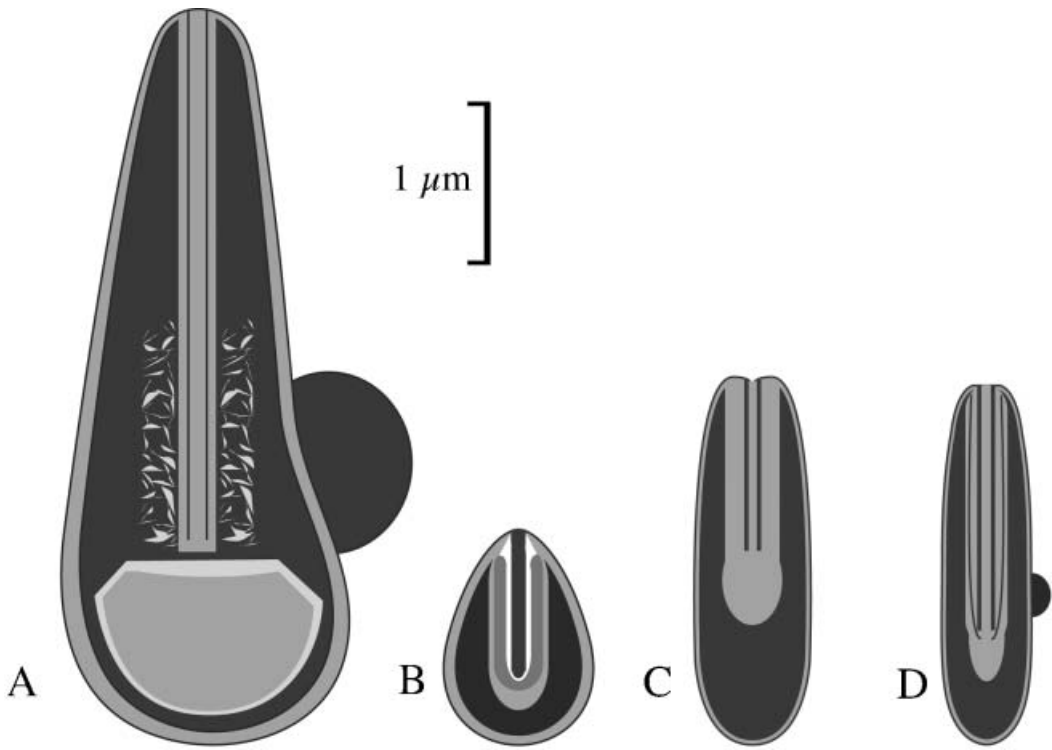


Figure 6. Schematic longitudinal TEM sections of nemertean pseudocnidae. (A) *Cephalothrix* cf. *rufifrons*. (B) *Tubulanus* cf. *pellucidus*. (C) *Carinomella lactea*. (D) *Zygeupolia rubens*. (A) and (C) drawn from data presented herein. (B) and (D) drawn from micrographs in Turbeville (1991) and unpublished data. Scale is approximate.

an inner fibrous medulla were present (Figure 5B). These structures may be pseudocnidae at an early stage of differentiation, but this could not be unequivocally confirmed despite extensive observation. Putative mature pseudocnidae are organized in bundles of 20 or more near the cell apex and are oriented parallel to the apical-basal axis of the cell (Figures 4B and 5A). Bases of mature pseudocnidae rest on a homogenous secretion produced by underlying gland cells (Figures 4B and 5A).

Discussion

Comparative morphology

Pseudocnidae have been interpreted as platyhelminth rhabdite homologues by a number of investigators (e.g. Gontcharoff 1957; Ling 1971), but the structure of rhabdites differs markedly from that of nemertean pseudocnidae. Unlike pseudocnidae, turbellarian rhabdites are of uniform diameter ($\sim 1 \mu\text{m}$) and are composed of a striated, lamellate cortex and a lamellated, homogenous or granular medulla. A filament core is also lacking (Smith et al. 1982; Rieger et al. 1991). Although the cortex of putative immature pseudocnidae of *C. lactea* appears to be faintly striated (Figure 5B), it does not remain so, and the cortex is never lamellated. Clearly, the structural data do not support the interpretation of these structures as rhabdite homologues (see also Turbeville 1991, 2002), and they can be classified, along with other fusiform, rod- or club-shaped secretory

products, as a type of rhabdoid (filament-core rhabdoid) to indicate the distinction for ectodermal secretory products (see Smith et al. 1982 for terminology and also Stricker and Cavey 1988 for nemertean epidermal rhabdoids).

Resemblance of pseudocnidae to platyhelminth extrusomes, such as proseriate platyhelminth paracnids, is also superficial. Eversible paracnids consist of a muscle cell and a secretory cell forming a tube that is everted by contraction of the associated muscle (Sopott-Ehlers 1981). The other recognized paracnid type is comprised of a single bulb-shaped secretory cell that contains numerous membrane-bound granules, and it lacks an eversible tube or filament (Sopott-Ehlers 1985).

Cnidarian nematocysts, which represent one type of true cnida, also exhibit a substructure unlike that of nemertean pseudocnidae. Nematocysts consist of a hollow, fluid-filled capsule containing an inverted, typically coiled, hollow tube ("thread"). The fluid matrix of the capsule contains protein toxins and ions (see Watson and Mire-Thibodeaux 1994). The mature capsule wall may be homogenous (e.g. Watson and Mariscal 1984; Hausmann and Holstein 1985) or serrated in appearance (Schmidt and Moraw 1982) and consists of two or three layers (cf. Schmidt and Moraw 1982; Watson and Mariscal 1984; Yanagihara et al. 2002). The nematocyst thread substructure varies among taxa, but it is hollow, unlike the tubular core of the nemertean pseudocnidae. Hubrecht (1887, as cited in Bürger 1895:58) apparently considered the pseudocnidae to be nematocyst homologues, but other early investigators either implicitly or explicitly indicated that these were uniquely nemertean structures rather than cleptocnidae (e.g. Bürger 1895; Martin 1914).

The pseudocnidae of all nemerteans investigated thus far with TEM exhibit similar morphology. They possess an outer homogenous cortical layer, an inner medulla and a central tubular core originating at the apex, resembling an inverted filament (Figures 2–6; Gontcharoff 1957; Ling 1971; Anadón 1976; Turbeville 1991; Montalvo et al. 1998). Only the electron density of these layers varies, and this variability may be attributable to the different fixatives employed by each investigator (see Montalvo et al. 1998) or other factors, such as section thickness, staining properties and maturation stage of the granule. Shared similarity in position (all are situated within pseudocnida-forming cells of the proboscis glandular epithelium) and structure support the hypothesis these rhabdoids are homologous among nemerteans. Outgroup comparison further suggests that these structures represent an evolutionary novelty of Nemertea.

Such secretory granules, first termed pseudocnidae by Martin (1914), have been reported in a number of hetero- and palaeonemertean species at the level of light microscopy. Some of these structures closely resemble the pseudocnidae that have been examined with TEM (e.g. Bürger 1895 for *Micrura*: plate 10, Figures 1 and 15a; Schütz 1912 for *Paralineus elisabethae*: plate 8, Figure 17; Norenburg 1993 for *Riserius*), whereas others appear morphologically distinct. In his description of meiofaunal cephalothricids, Gerner (1969) distinguished both rod-shaped "rhabdites" (=rhabdoids), measuring 3–8 µm in length and 1 µm in width, which are strikingly similar to rod-shaped pseudocnidae, and two types of pseudocnidae. Type "A" pseudocnidae consist of a bulb-shaped intracellular capsule (4–11 µm in length by 3–4 µm in width) containing an eversible filament, whereas type "B" pseudocnidae lack a capsule and are composed of a filament coiled within an intracellular space. Wijnhoff (1910) reported both pseudocnidae, which she termed nematocysts ("Nesselelemente"), and "rhabdites" in the proboscis of *C. cf. rufifrons*. However, her figures, though difficult to interpret, suggest that the pseudocnidae she described differ in organization from those described herein for *C. cf. rufifrons*.

Although some minor variation in *C. cf. rufifrons* pseudocnida structure is apparent (compare Figure 2C and Figure 3A), I have not observed other nematocyst-like structures in the proboscis of live or fixed specimens examined in this study. The structures described for *C. cf. rufifrons* most closely resemble the A-type pseudocnidae described by Gerner (1969) for meiofaunal cephalothricids. Jennings and Gibson (1969) referred to proboscis barbs with bulbous proximal ends in their analysis of the proboscides of *Cephalothrix bioculata* Örsted, 1843 and *Cephalothrix linearis* Rathke, 1799, and their figures suggest that these correspond to pseudocnidae.

Reported structural diversity and inconsistent use of terminology are likely attributable in part to different states of pseudocnida differentiation (see Gerner 1969), the extent of observational rigor and variable staining properties of the secretory granules. For example Schütz (1912:129) stated that he misidentified the pseudocnidae, which he termed nematocysts (“Nesselkapseln”), of the heteronemertean *Paralíneus elisabethae* as unstained “rhabdites” in his preliminary study of this species (Schütz 1911). Bürger (1895:263) also mentioned the marked similarity of pseudocnidae, which he referred to as nematocysts (“Nesselkapseln”), to large rhabdites in the heteronemertean *Lineus geniculatus*. Although additional comparative analyses of nemertean rhabdoids and pseudocnidae formation from a wide sample of taxa will be necessary to clarify the actual extent of their structural diversity, it is reasonable to assume that the filament-containing clavate and rod-like structures described with light microscopy for other hetero- and palaeonemerteans are homologous to those described herein.

Function

The present analysis provides no new insight into the function of these structures; thus, their precise role in prey capture remains conjectural. Jennings and Gibson (1969) observed that the barbs of *Cephalothrix bioculata* and *Cephalothrix linearis*, which protrude from the proboscis epithelium, pierce the body wall of the prey organism and speculated that this action might facilitate entry of paralyzing toxins. However, substantiating evidence for this mode of toxin delivery is lacking (see McDermott and Roe 1985). Jennings and Gibson (1969) further proposed that the pseudocnidae may prevent prey from slipping from the proboscis, and this is consistent with the views of Montalvo et al. (1998) for the pseudocnidae of the heteronemertean *Riseriellus occultus*.

Light micrographs of the everted proboscis of living *C. cf. rufifrons* revealed that the rhabdoids of this species protrude from the surface of the epithelium (Figure 1), as do those of *Rhamphogordius sanguineus* (unpublished observations), but interaction of the proboscis with prey was not examined. Evidence from light microscopy for pseudocnida core-extrusion was presented by a number of investigators, including Bürger (1895), Schütz (1912), Martin (1914) and Gerner (1969), and TEM evidence was provided by Ling (1971) and Anadón (1976). Core extrusion was not observed for these structures in *C. cf. rufifrons*, *C. lactea* (this paper), *Tubulanus cf. pellucidus* (Turbeville 1991) or in the heteronemerteans *Zygeupolia rubens* (Turbeville 1991), and *Riseriellus occultus* (Montalvo et al. 1998). Complementary studies utilizing live specimens will be necessary to fully explain the function of these structures.

It is important to note in the context of function that in *C. lactea* (Figures 4B and 5A), and *Riseriellus occultus* (Montalvo et al. 1998) bases of mature pseudocnidae rest on a homogenous secretion produced by adjacent gland cells, and, as Montalvo et al. (1998) suggested, this secretory product likely serves to anchor the pseudocnidae to the proboscis

surface after they move out of the gland cell neck. Schütz's observations (1912: Figure 33) imply that a similar situation occurs in the heteronemertean *Paralineus elisabethae*. In contrast, the pseudocnidae of *C. cf. rufifrons* appear to be anchored exclusively in the forming-cell cytoplasm.

Implications for nemertean phylogeny

The filament-core rhabdoids described in this paper represent a potentially informative character for inference of higher-order relationships of nemerteans. Similarity in position and structure of pseudocnidae support the hypothesis that they are homologous among palaeo- and heteronemerteans (see page 975), and these secretory bodies are restricted to

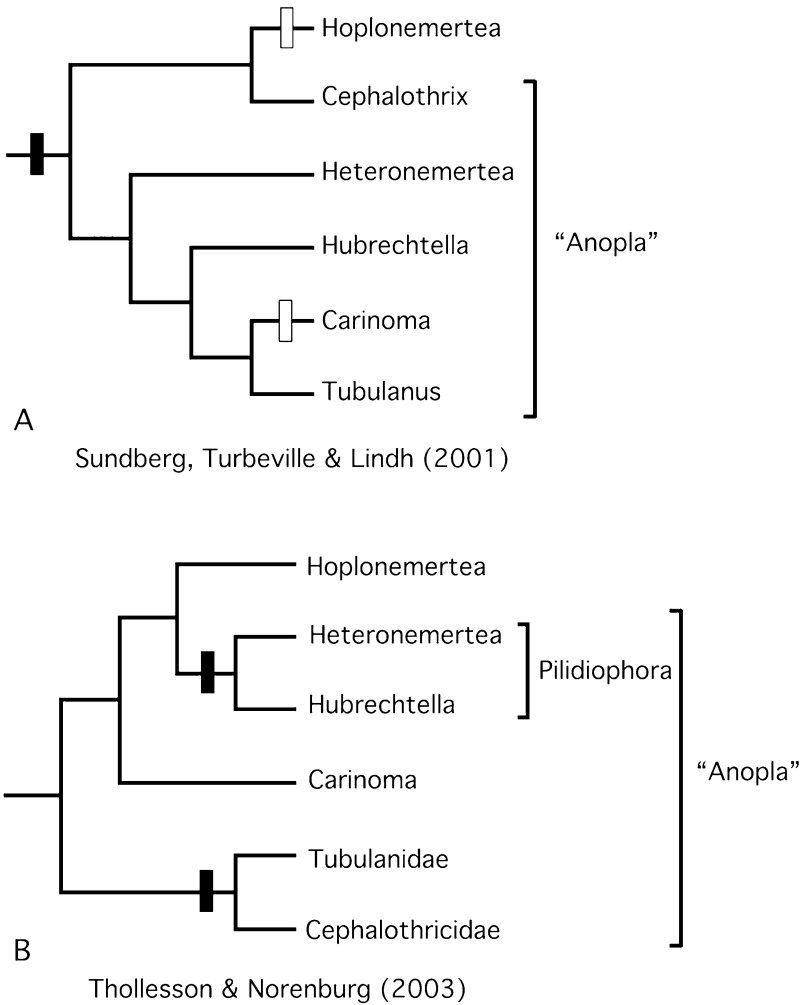


Figure 7. Molecular phylogenies of nemerteans. (A) Phylogeny inferred from 18S rDNA sequences. (B) Phylogeny inferred from a simultaneous analysis of partial 28S rRNA, H3, 16S rRNA and COI gene sequences. Phylogeny "A" implies that pseudocnidae were present in the common ancestor of nemerteans and subsequently lost in two lineages. Phylogeny "B" implies independent evolution of pseudocnidae. Open boxes represent character losses and solid boxes represent character acquisitions.

these nemertean taxa. Based on outgroup comparison, pseudocnidae are interpreted as a provisional synapomorphy of an anoplan clade, as previously suggested by Turbeville (1991) and Montalvo et al. (1998). Preliminary analyses of molecular data do not, however, support anoplan monophyly, thus contradicting this primary synapomorphy statement. The 18S rDNA sequence analysis of Sundberg et al. (2001) implies that this character was present in the stem lineage of the Nemertea and was lost in *Carinoma* and the hoplonemertean clade (Figure 7A). In contrast, the combined analysis of partial sequences of four genes (28S rRNA, H3, 16S rRNA, COI) from a larger sample of nemertean diversity by Thollessen and Norenburg (2003) suggests that pseudonidae evolved independently, once in the stem lineage of the Tubulanidae+Cephalothricidae clade and once in the stem lineage of the Pilidiophora (Figure 7B). The best estimate of nemertean phylogeny awaits simultaneous analyses of all available evidence. An extensive cladistic analysis of nemertean phylogeny based on morphology and molecular data is ongoing (J. L. Norenburg, pers. comm.) and will provide a more critical test of pseudocnida evolution.

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