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Proboscis apparatus: What do we know (and not know) about nemerteans?

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ABSTRACT: This paper provides an overview of recent data on the morphology and functions of the proboscis in nemerteans. It is still unclear whether rhynchocoel and proboscis are synapomorphies of the phylum Nemertea and whether rhynchocoel is homologous to coelom in other Spiralia. The proboscis initially had a structure similar to the body wall, which is typical of the class Palaeonemertea. However, subsequently, the proboscis evolved in two directions: (1) the development of bilateral symmetry in palaeonemerteans from the family Cephalotrichellidae and Pilidiophora (except some Valenciniidae species) and (2) the development of radial symmetry in Hoplonemertea. Pilidiophorans are characterized by a wide variety of proboscis structures, while hoplonemerteans have the most diverse rhynchocoel morphology. The emergence of the stylet apparatus in hoplonemerteans led to the loss of diagonal musculature and pseudocnidae in the proboscis, as well as to a decrease in the number of the family of peptide toxins.
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KEY WORDS: proboscis, rhynchocoel, coelom, endothelium, pseudocnidae, armature, evolution.

Хоботной аппарат: что нам известно (и не известно) о немертинах?

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РЕЗЮМЕ: Данная работа посвящена обзору морфологии и функционированию хоботного аппарата немертин. До сих пор нет однозначного представления является ли наличие ринхоцеля и хобота синапоморфиями типа Nemertea и гомологичен ли ринхоцель целому других Spiralia. Первоначально хобот немертин имел строение сходное со стенкой тела, что типично для представителей класса Palaeonemertea. Однако в дальнейшем хобот эволюционировал в двух направления: (1) развитие билатеральной симметрии у палеонемертин из семейства Cephalotrichellidae и у Pilidiophora (за исключением некоторых Valenciniidae) и (2) формирование радиальной симметрии у представителей Hoplonemertea. Если пилидиофоры характеризуются большим разнообразием в организации хобота, то у гоплонемертин наблюдается

Devoted to memory of Claus Nielsen.

высокое разнообразие в морфологии ринхоцеля. Появление стилетного аппарата у гоплонемертин привело к потери диагональной мускулатуры и псевдокнид в хоботе, а также к уменьшению числа семейств пептидных токсинов, которые используются при нападении на добычу.

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КЛЮЧЕВЫЕ СЛОВА: хобот, ринхоцель, целом, эндотелий, псевдокниды, вооружение, эволюция.

Introduction

The proboscis apparatus, a formation consisting of two general components, the rhynchocoel (or proboscis sheath) and the proboscis proper, is specific for Nemertea (Fig. 1A). With contraction of the rhynchocoel walls, the proboscis is everted via rhynchopore and is inverted back by the retractor muscle. The proboscis is an organ of attack and defense; in some nemerteans (primarily terrestrial and semiterrestrial ones) (Fig. 1B), it is also used for locomotion (Gibson, 1972; Moore, Gibson, 1981). The rhynchocoel, in addition to being a proboscis reservoir, performs a supporting function, especially well expressed in benthic polystiliferous nemerteans (Chernyshev, 2011). The nemertean brain surrounds the rhynchocoel like a ring, but not the foregut as in many other Spiralia. In most nemerteans, one or two vessels of the circulatory system enter the rhynchocoel or even the proboscis (Kajihara, 2010; Chernyshev, 2011). In most hoplonemerteans, the mouth merges with the rhynchopore to form the rhynchostomadaeum or the atrium. All the above facts suggest that the proboscis apparatus is an essential component in the ‘Bauplan’ of nemerteans. Thus, another name of the phylum Nemertea is Rhynchocoela, which is preferred by some authors (Hyman, 1951). On the basis of proboscis structure, nemerteans were divided into two groups: Anopla (unarmed nemerteans) and Enopla (armed nemerteans). To date, a high classification has been adopted that divides nemerteans into three classes and seven orders: Palaeonemertea (orders Carinomiformes, Archinemertea, and Tubulaniformes), Pilidiophora (orders Hubrechtiiformes and Heteronemertea), and Hoplonemertea (orders Polystilifera and Monostilifera) (Chernyshev, 2021).

The only known nemertean species that lacks the proboscis apparatus is the enigmatic *Arhynchonemertes axi* described from New Zealand (Riser, 1988). Its systematic position remains unresolved: it is either a representative of an ancient branch of nemerteans that have not yet acquired the proboscis or a specialized nemertean that has lost its proboscis and rhynchocoel (Riser, 1989). If the former assumption is correct, then the proboscis apparatus is not a synapomorphy of Nemertea. However, even if the molecular phylogenetic analysis shows the basal position of *A. axi*, it still does not answer the question as to whether ancestors of this nemertean had the proboscis apparatus.

In the present review, we consider the most debatable issues of the morphology and functions of the proboscis apparatus. These are, first, the origin of the proboscis apparatus and also the evolution of its musculature, nervous system, and some epithelial structures.

Do nemerteans have true coelom?

The proboscis apparatus was well studied at the light microscopy level in the late 19th century. The magnificent drawings of the proboscis, especially the stylet apparatus, in the books by McIntosh (1873–1894) and Bürger (1895, 1897–1907), still remain unsurpassed. A number of studies with electron microscopy of the proboscis were published in the second half of the 20th century (Ling, 1971; Stricker, Cloney, 1983; Stricker, 1985; Montalvo *et al.*, 1996, 1998; Junoy *et al.*, 2000). The most important were the comparative analyses of microscopic anatomies of nemerteans from different groups, based on which the authors concluded that the nemertean rhynchocoel is a coelomic cavity (Turbeville, Ruppert, 1985; Turbeville, 1991). The statement that the rhynchocoel is a coelom was made by Hyman (1951) and Starobogatov (1983), but Turbeville & Rupert (1985) showed that the endothelium of the rhynchocoel and proboscis has all the morphological features of coelothelium.

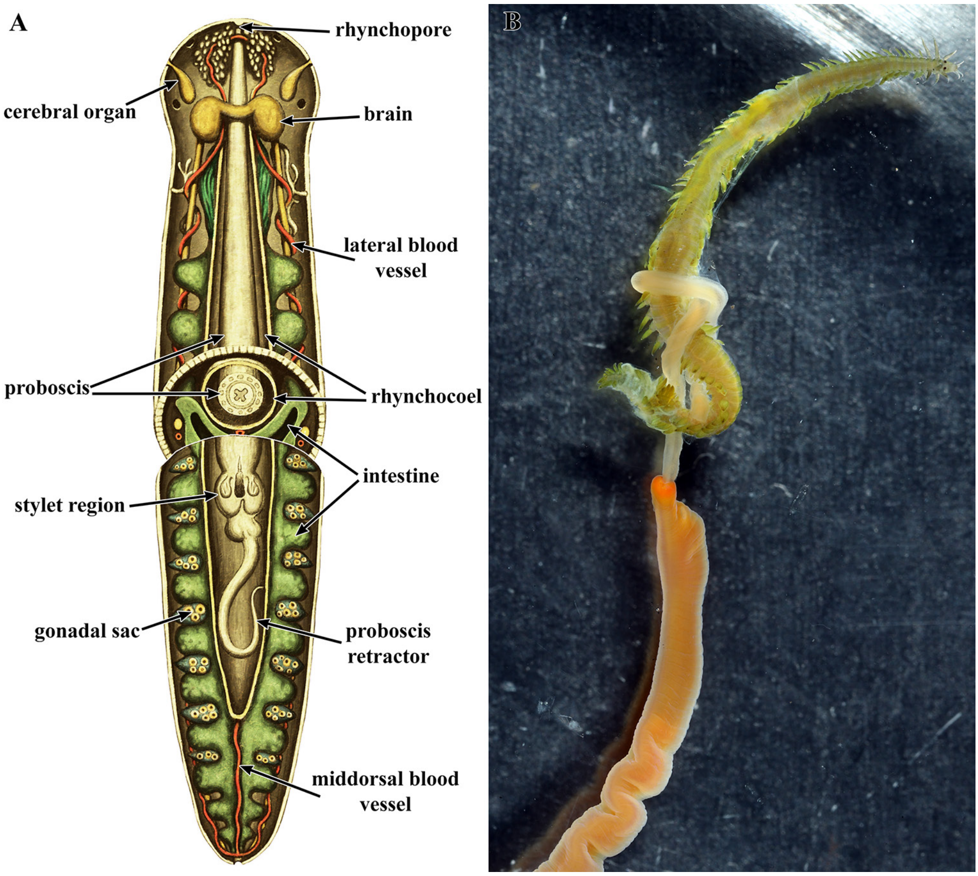

Fig. 1. Nemerteans with inverted (A) and everted (B) proboscis apparatus. A — diagram of internal morphology of a hoplonemertean (drawn by Oleg Dobrovolsky); B — palaeonemertean *Cephalothrix* cf. *simula* with everted proboscis attacking a polychaete.

Turbeville & Rupert (1985) considered not only the rhynchocoel but also the circulatory system and gonadal sacs as derivatives of coelom. This interpretation has not found wide support (especially as regards gonadal sacs), although it has changed the opinion about nemerteans as exclusively parenchymatous worms (however, in many Russian universities, nemerteans are still considered as acoelomic invertebrates).

Ax (1996) insisted on the origin of the coelom (rhynchocoel and blood vessels) in nemerteans independent from that in Spiralia (he did not attribute nemerteans to Spiralia). Nielsen (2001) expressed a similar opinion by placing nemerteans closer to Platyhelminthes. Subsequently, he (Nielsen, 2012) tended to assume that blood vessels can be interpreted as modified coelomic cavities, but rhynchocoel should unambiguously be considered as an independently originated secondary body cavity. Malakhov & Bogomolova (2016), in contrast, stated that rhynchocoel is the same homologue of coelom as blood vessels. Chernyshev (1999, 2011) proposed a hypothetical scenario for the transformation of the paired coelom into the rhynchocoel, blood vessels, and gonadal sacs in the nemertean ancestor. It opposed the hypothesis by Starobogatov (1983) who considered the rhynchocoel homologous to the unpaired acrocoelom (head coelom), and the gonadal funnels homologous to the paired trunk coeloms.

The question as to whether the rhynchocoel is homologous to the true coelom of other Spiralia or not remains open. It is also unclear which one was formed earlier, the proboscis or the rhynchocoel. Wijnhoff (1914) and Senz (1997)

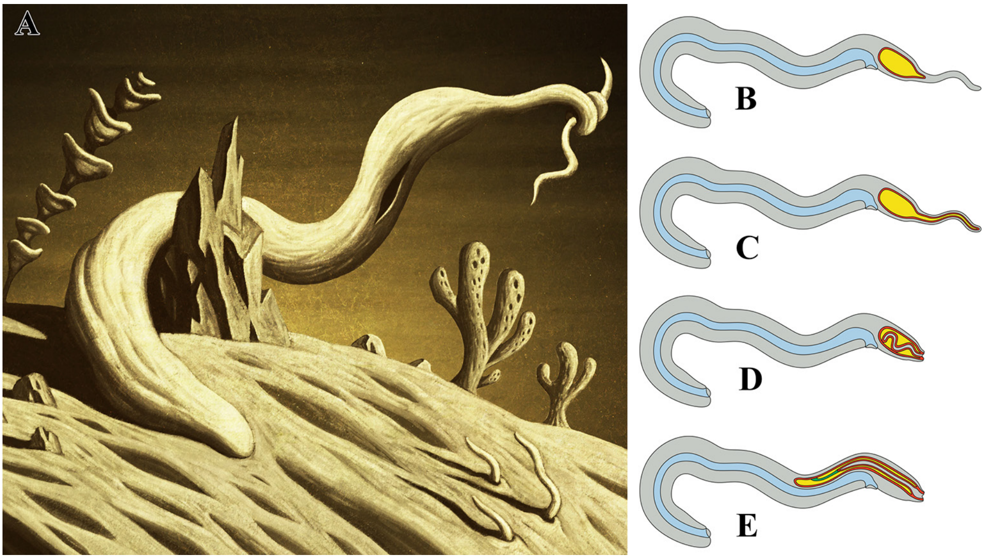

Fig. 2. Origin and early evolution of Nemertea. A — hypothetical ancestor of nemerteans (drawn by Oleg Dobrovolsky); B–E — diagrams of presumptive origin of proboscis apparatus (yellow — rhynchocoel, red — endothelium; blue — digestive tract, green — muscle-retractor).

suggested the proboscis as an invagination of the anterior end of the head that was formed first, and then the rhynchocoel appeared around it. This hypothesis does not explain why the coelomic epithelium lines both the rhynchocoel and the inverted proboscis. Another hypothesis seems more plausible: the elongated anterior end of the head in nemertean ancestors served to capture food (Fig. 2A) and subsequently began to invert into the already existing coelomic cavity (the future rhynchocoel) (Chernyshev, 2011). As a result, the proboscis became lined inside by the coelomic epithelium of this cavity (Fig. 2B–E). It is still impossible to confirm the correctness of such a pattern, and it remains purely hypothetical.

The endothelium of the rhynchocoel and proboscis has cilia characteristic of coelothelium. These are mostly rare and short, but in some nemerteans they can be long and numerous (Magarlamov, Chernyshev, 2015) (Fig. 3D, E). The endothelium structures in the proboscis and the rhynchocoel differ. The proboscis endothelium is pseudostratified coelothelium, where peritoneal cells overlap myocytes that form the outer (endothelial) circular musculature (Magarlamov, Chernyshev, 2015). Initially, myocytes are few in number and scattered, and can only be found in ultrathin sections or by confocal laser scanning microscopy (CLSM) with phalloidin labeling (Fig. 3A). However, in many nemerteans, myocytes are numerous and arranged into several layers (Fig. 3B); in this case, outer circular musculature becomes clearly visible in histological sections. This has created an erroneous impression that some of nemerteans have the endothelial musculature in the proboscis while others do not.

The endothelium of the rhynchocoel is true peritoneum, and myocytes are separated from the endothelium by basal lamina and extracellular matrix (Fig. 3C). If we assume that myoepithelium is the initial state for coelothelium (Kuzmina *et al.*, 2018), then the proboscis endothelium has retained more archaic features than the rhynchocoel endothelium.

The proboscis morphology as a reflection of the anterior body end

While the origin of the rhynchocoel remains unclear, there are almost no disagreements as regards the origin of the proboscis: it is a modified anterior outgrowth of the head inverted inside. As Hyman (1951) noted, this explains why the structure of the nemertean proboscis repeats the structure of the nemertean's body wall. Is it so? Seventy years ago, the locations of the muscular

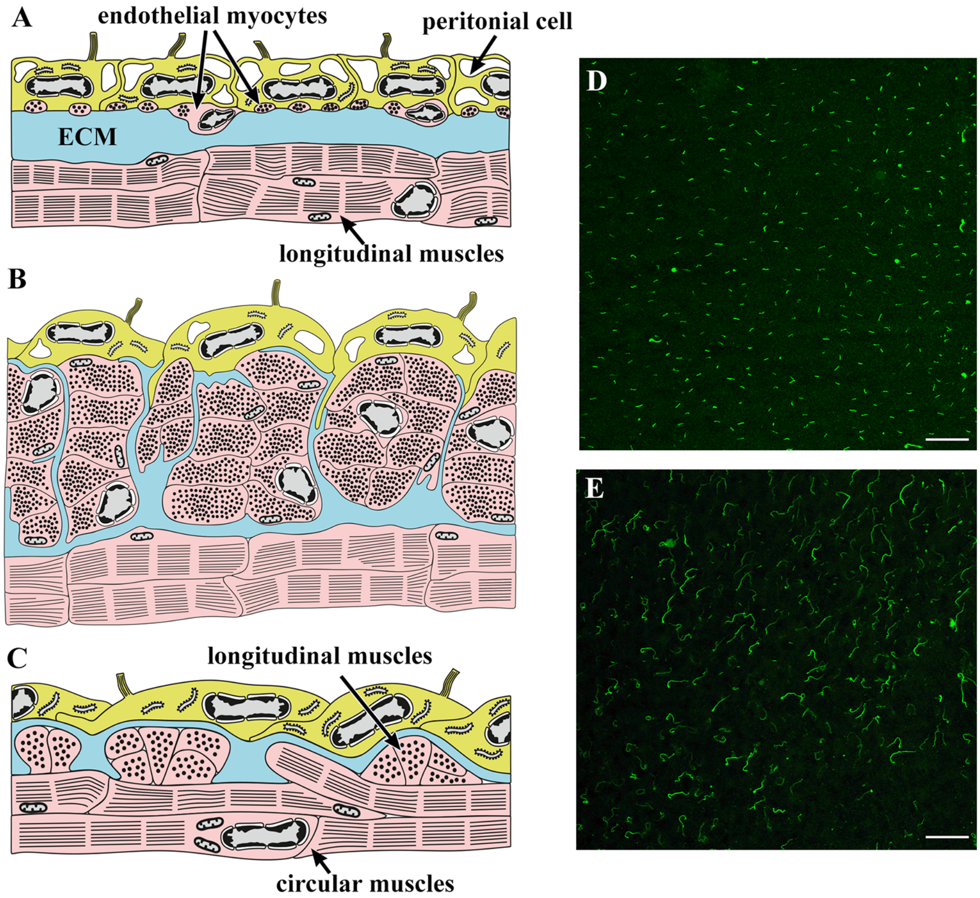

Fig. 3. Endothelium of proboscis apparatus in nemerteans. A — diagram of proboscis endothelium of *Cephalothrix* cf. *simula*, longitudinal section; B — diagram of proboscis endothelium in *Tortus tokmakovae*, longitudinal section; C — diagram of rhynchocoel endothelium in *Hubrechtella juliae*, transverse section; D, E — CLSM micrographs of proboscis endothelium labeled with α-tubulin antibodies (D — *Baseodiscus* cf. *princeps*; E — *Cerebratulus* sp.). Scale — 10 μm.

layers of the proboscis and the body wall were examined only in histological sections. The CLSM and phalloidin labeling methods have revealed new details in the structure of both the proboscis and the body walls in nemerteans (Chernyshev, 2010, 2011, 2015). The proboscis structure fully repeats the structure of the body wall only in palaeonemerteans that have four layers of musculature (outer circular, diagonal, longitudinal, and inner circular) and a pair of intraepithelial or subepithelial nerve cords both in the proboscis and in the body wall (Fig. 4A, B). The exception is representatives of the order Archinemertea whose diagonal musculature is located under the outer circular musculature in the proboscis and under the epidermis in the body wall (Chernyshev, Kajihara, 2019). Furthermore, the proboscis of archinemerteans *Cephalotrichella* (Fig. 5A) and *Balionemertes* has the outer longitudinal musculature that is absent from the body wall (Chernyshev, 2015; Chernyshev, Kajihara, 2019).

In a great number of species in the class Pilidiophora, both the proboscis and the body wall have the outer longitudinal musculature (Fig. 4C). However, there is no complete similarity here because the diagonal musculature in the proboscis is located between the outer circular and inner longitudinal musculatures (Fig. 4C), while the diagonal musculature in heteronemerteans is

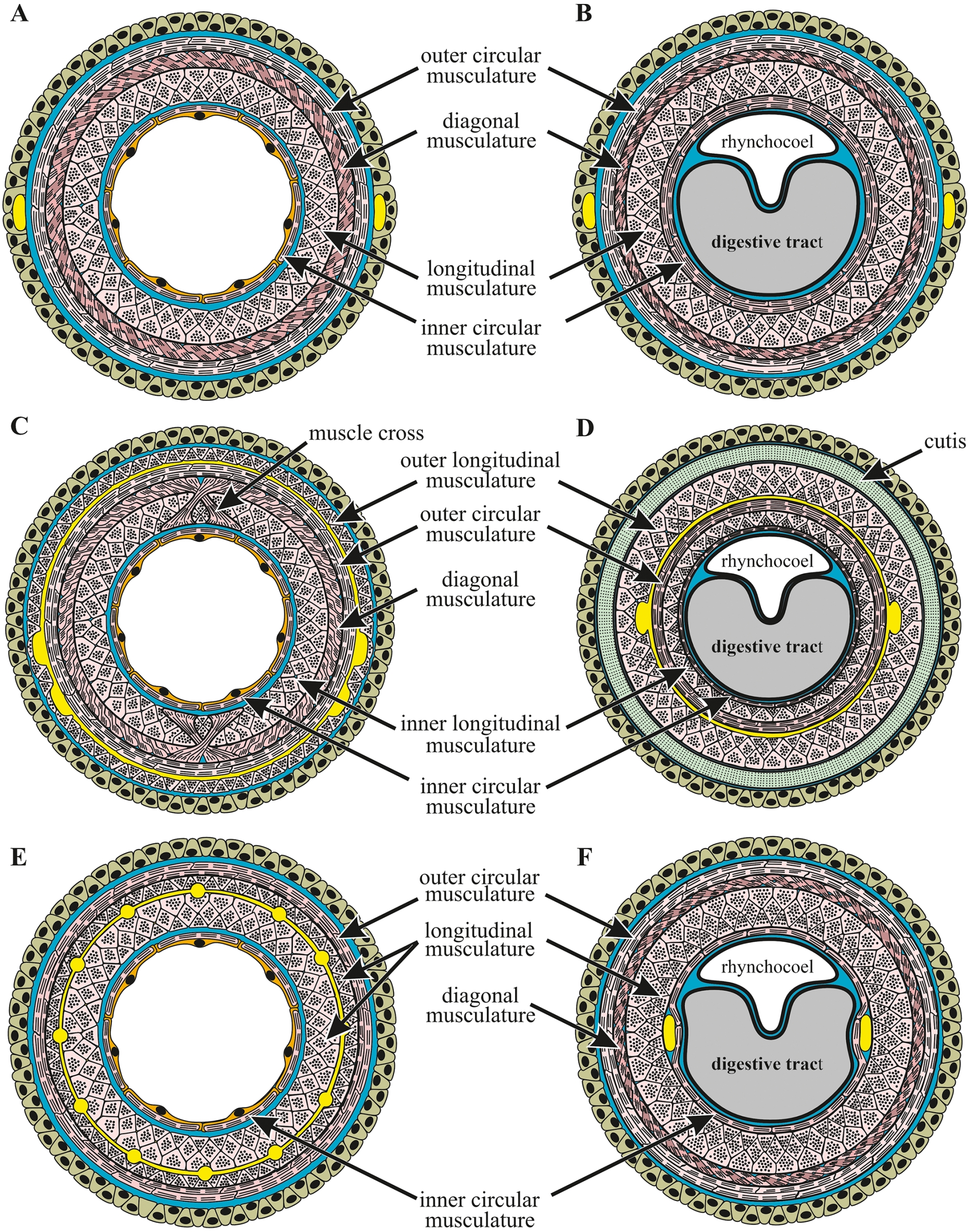

Fig. 4. Diagrams of transverse sections of everted proboscis (A, C, E) and body (B, D, F). A, B — Palaeonemertea; C, D — Heteronemertea; E, F — Hoplonemertea.

located in the cutis, and also between the outer longitudinal and outer circular musculatures of the body wall in some species (Chernyshev, 2011; Hookabe, Kajihara, 2020). Only hubrechtiids and the heteronemertean *Archimicrura ignae* have the body diagonal musculature with a plesiomorphic position between the outer circular and inner longitudinal muscle layers (Schwartz, Norenburg, 2005; Chernyshev, 2010). Many pilidiophorans lack the outer longitudinal musculature in the proboscis: in hubrechtiids, this lack is primary, while in heteronemerteans, it is,

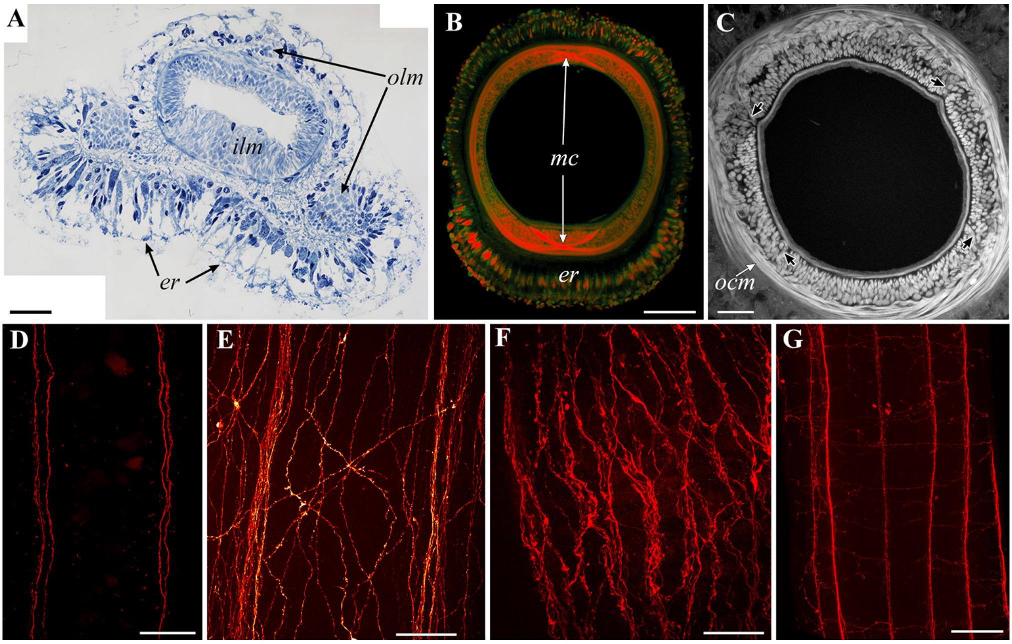

Fig. 5. Light (A) and CLSM (B–G) micrographs of proboscis labeled with phalloidin and 5-HT antibodies. A — *Cephalotrichella echinicola* (transverse section); B — *Lineus viridis* (transverse section); C — *Carinoma* sp. (transverse section, dark arrows show muscle crosses); D — *Cephalothrix* cf. *simula* (longitudinal section); E — *Cerebratulus* sp. (longitudinal section); F — *Baseodiscus* sp. (longitudinal section); G — *Kurilonemertes dilutebasisae* (longitudinal section).
Abbreviations: er — epithelial ridge, ilm — inner longitudinal musculature, mc — muscle crosses, ocm — outer circular musculature. Scale: A, C — 50 µm, B, D–G — 100 µm.

apparently, secondary. In species of the genera *Hubrechtella* and *Baseodiscus*, the proboscis diagonal musculature is located between the inner circular and longitudinal musculatures, while the outer circular musculature is absent, i.e., the proboscis musculature differs significantly from the body wall musculature (Chernyshev *et al*., 2013). A total of at least eight different variants of arrangement of the muscular layers in the proboscis have been recorded among Pilidiophora, which is twice as many as in the other two classes combined (Chernyshev, 2015).

Another feature of the proboscis musculature in pilidiophorans is the presence of one or two muscle crosses, connected to the diagonal musculature (Fig. 5B) (Chernyshev, 2010, 2015). Muscle crosses are present in most heteronemerteans and recently have been described in *Hubrechtella ijimai* (Kajihara, 2006; Chernyshev *et al*., 2017). In the body wall of some of heteronemerteans, a dorsal "cross" is present between the outer circular muscle layer and the rhynchocoel musculature. The dorsal and ventral muscle crosses are characteristic of the body wall in many palaeonemerteans which, however, lack any similar structures in their proboscises, except those in *Carinoma* (Fig. 5C) and *Parahubrechtia* (see Chernyshev, 2010; Chernyshev *et al*., 2017).

In nemerteans of the class Hoplonemertea, the proboscis repeats the body wall to an even lesser extent: the proboscis lacks diagonal musculature, and several nerve cords are oriented radially in the longitudinal musculature (Fig. 3E, F). Thus, the musculature structure and the location of the nervous system in the anterior part of the proboscis in hoplonemerteans is very conserved, with, however, some deviations in *Malacobdella* and *Ototyphlonemertes valentinae* (Magarlamov, Chernyshev, 2010; Chernyshev, 2015).

The hoplonemerteans are characterized by a diversity of rhynchocoel morphologies. In a number of hoplonemerteans, the rhynchocoel has lateral, ventral, or dorsal pouches; in species of the genus *Uniporus*, the lateral pouches are branched. At least nine different variants of the rhynchocoel muscular wall structure have

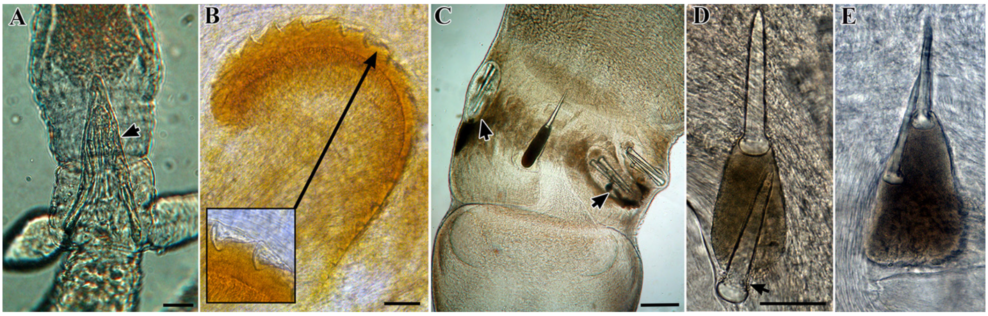

Fig. 6. Proboscis armature. A — *Callinera* sp. (arrow shows Kajihara's stylet); B — polystiliferous armature of *Drepanophoridae* sp. (inset shows stylets at high magnification); C — monostiliferous armature of *Amphiporus* sp. (arrows show accessory stylets); D — monostiliferous armature of *Nipponnemertes* cf. *rubella* (arrow shows accessory stylet inside basis); E — "bistiliferous" armature of *Cratenemertidae* sp. IZ-45644. Scale: A, B, D — 50 µm, C — 100 µm.

been distinguished within the class (vs. only four variants found in palaeonemerteans and pilidiophorans), and five, where the circular musculature is transformed into the spiral one, are unique for pelagic nemerteans (Norenburg, Roe, 1998; Chernyshev, 2011; Chernyshev, Polyakova, 2018, 2019). There is still no consensus as to which arrangement of the rhynchocoel musculature is primary for Hoplonemertea: from the outer circular and inner longitudinal musculatures as in a sister group, Pilidiophora, or from the intertwined longitudinal and circular muscles as in benthic Polystilifera (see Kajihara, 2021). Some authors suggested that initially the rhynchocoel wall should have also reflected the body wall, i.e., consisted of separate layers (Wijnhoff, 1914; Senz, 1997).

The proboscis nervous system in palaeonemerteans consists of two nerve cords located intraepithelially or subepithelially, repeating the nervous system of the body wall (Figs 4A, B; 5D). Heteronemerteans usually have two or more pairs of main nerve cords that are randomly connected via secondary nerves, forming a nerve plexus with a pronounced bilateral symmetry (Figs 4C; 5E). In *Baseodiscus*, *Sonnenemertes*, and *Hubrechtella*, the nerve plexus of the proboscis lost its bilateral symmetry (Fig. 5F) and became similar to the proboscis nervous system of hoplonemertean *Malacobdella* (Magarlamov *et al.*, 2011; Chernyshev *et al.*, 2013; Chernyshev, Polyakova, 2018). In hoplonemerteans, seven to 36 nerve cords embedded in the longitudinal musculature and numerous connectives between them form a more or less ordered radially symmetrical nerve plexus (Fig. 5G) (Chernyshev, 2011; Magarlamov *et al.*, 2011) (except *Ototyphlonemertes valentinae* with its secondarily bilaterally symmetrical proboscis — see Chernyshev, 2015).

Proboscis armature

The main distinguishing feature of the hoplonemertean proboscis is armature, which is also found, however, in some of Palaeonemertea and Pilidiophora. The trowel-like armature is present in the proboscis of palaeonemerteans of the genus *Callinera* (Kajihara, 2006; Chernyshev, 2011, 2015) (Fig. 6A). It was first reconstructed and described by Hiroshi Kajihara and, therefore, we suggest referring to it as Kajihara's stylet. This structure is apparently composed of chitin and is flexible. Functions of the Kajihara's stylet remain unknown, but, when the proboscis is everted, it is located subapically and can potentially cause wounds to prey. An armature consisting of a multitude of hooked parastylets has been found in the proboscis of the heteronemertean *Heteroenopleus enigmaticus* (Wern, 1998). Thus, there are armed "unarmed" nemerteans. Moreover, there are also unarmed hoplonemerteans such as species of the genus *Malacobdella* that completely lost the stylet apparatus.

The true stylet apparatus is present only in hoplonemerteans and is located in the middle proboscis chamber. Two types of this apparatus are distinguished: polystiliferous (with numerous short stylets on a falciform basis) (Fig. 6B) and monostiliferous (with a single long stylet on the longitudinally stretched basis) (Fig. 6C).

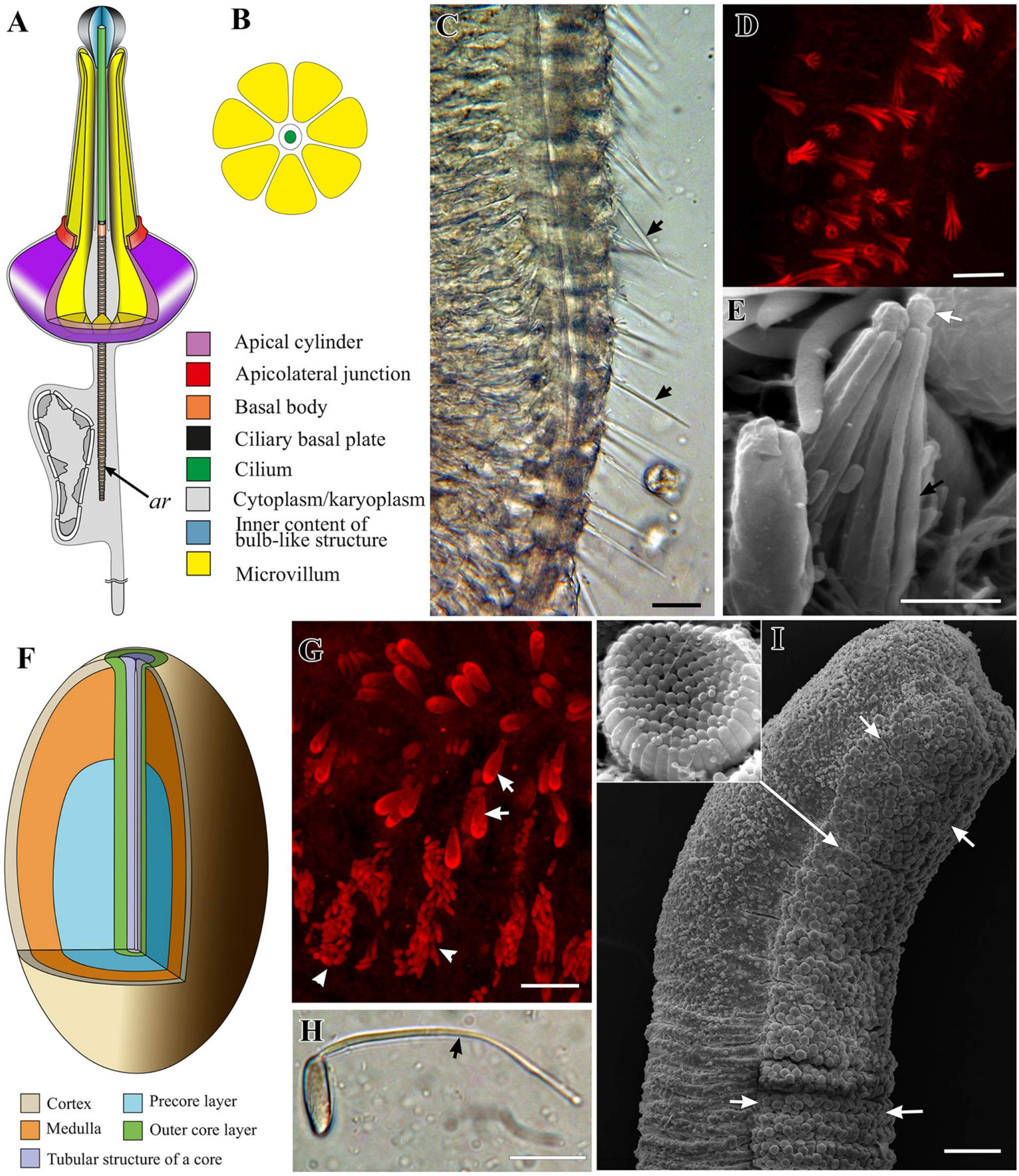

Fig. 7. Monociliated sensory cells (A–E) and pseudocnidae (F–I). A — diagram of monociliated sensory cell of *Lineus viridis*; B — diagram of transverse section of the collar part; C — fragment of everted proboscis in *Cephalothrix* cf. *simula* with monociliated sensory cells (arrows show sensory processes); D — CLSM micrograph of proboscis epithelium in *Nipponomicrura uchidai* labeled with phalloidin (with collar microvilli visible); E — SEM micrograph of proboscis epithelium of monociliated sensory cells in *Hubrechtella juliae* (white arrow shows bulb-like structure, black arrow shows microvilli); F — diagram of pseudocnida structure in *Lineus viridis*; G — CLSM micrograph of proboscis epithelium in *Cephalothrix* cf. *simula* with large (arrows) and small (arrowheads) pseudocnidae (autofluorescence); H — pseudocnida of *Hubrechtella juliae* with extruded core (arrow); I — SEM micrograph of everted proboscis in *Micrura kulikovae* showing epithelial ridge (arrows) with pseudocnida clusters (inset shows pseudocnidae cluster at high magnification). Abbreviation: ar — axial rootlet. Scale: C, D, G, H — 10 μm, E — 2 μm, I — 50 μm.

Judging by some features, the polystiliferous apparatus is organized simpler than the monostiliferous one: the diaphragm is absent, the bulb region is weakly pronounced and has a common musculature (not crisscrossed as that in monostiliferous nemerteans) (Chernyshev, 2015). However, the polystiliferous apparatus acts completely differently: the basis turns 90° during the attack and its short stylets make incisions to prey's tissues (Chernyshev, 2011, 2015, personal observations). Due to the very short stylets, the wounds cannot be deep, thus, meaning that the choice of prey is limited to invertebrates with thin integuments, which polystiliferous nemerteans, apparently, ingest whole[1]. The monostiliferous apparatus causes deep punctures in prey's tissues. This has allowed a transition to suctorial feeding on crustaceans with thick chitinous cuticles. Based on this difference, a hypothesis was proposed about the independent origins of the polystiliferous and monostiliferous apparatuses (Crandall, 2001; Chernyshev, 2011). In this regard, monostiliferous epipelagic nemerteans *Korotkevitschia* and *Achoronemertes*, whose armature consists only of accessory stylets and lacks the basis, are of certain interest (Crandall, Gibson, 1998; Chernyshev, 2005). This state may be initial for Hoplonemertea (see Chernyshev, 2011). However, an assumption has been expressed that these epipelagic nemerteans are giant long-swimming "larvae" of some benthic cratenemerteans (Chernyshev, Polyakova, 2019).

The armature of cratenemerteans is also monostiliferous, but the morphology of the stylet apparatus can be considered even more primitive than that in Eumonostilifera (Chernyshev, 2011, 2015). First, the basis in Cratenemertidae is always shorter than the stylets (Fig. 6D) which are relatively small in size compared to the large body of some cratenemertids. In some of cratenemertids, the accessory stylet is located within the basis (Berg, 1972; Korotkevich, 1983; Chernyshev, 2011; Chernyshev, Polyakova, 2022) (Fig. 6D), and this can be considered as a way to strengthen the armature. The numerous accessory stylets are arranged randomly and greatly vary in length (Chernyshev, 2011). In the enigmatic Cratenemertidae sp. IZ-45644 (which probably belongs to the genus *Akrostomum*), the monostiliferous stylet apparatus has two stylets on the basis (Fig. 6E) (Chernyshev, Polyakova 2019). It may be an anomaly but may also turn out to be a "polystiliferous character" in the monostiliferous armature. Unfortunately, the stylet apparatus of the phylogenetically close *Uniporus* has not been described.

Proboscis epithelium

According to the hypothesis explaining the origin of the proboscis from the anterior cephalic process, the glandular epithelium of the proboscis is a modified epidermis but with a number of characteristic features. The first one is the lack of true ciliated cells that are numerous in epidermis. This is quite natural because ciliated cells of the epidermis provide movements of the worm, and the proboscis is not involved in this any way (the use of the proboscis for movement in terrestrial hoplonemerteans is secondary and is associated only with musculature). Nevertheless, cilia in the proboscis are present only on sensory cells.

The second feature is the monociliated sensory cells that are found much more frequently in the proboscis than in the epidermis, which indicates the proboscis as also a tactile organ (Fig. 7C). Each sensory process of such cells bears a single cilium with a thickening at the top and has a cone-like collar of microvilli (Fig. 7A, B, E) with positive response to actin (Fig. 7D). These cells are particularly numerous in palaeonemerteans, pilidiophorans, and polystiliferous hoplonemerteans, the groups characterized by macrophagia (ingestion of whole prey). It is likely that the emergence of monostiliferous armature and the subsequent transition to suctorial feeding led to a reduction in the number of these cells, because, with such a feeding method, the proboscis' contact with the prey is significantly reduced. The structure of sensory cells is specific to each order. For example, heteronemerteans have an apical cylinder (Fig. 7A), which is absent from sensory cells of other nemerteans (Magarlamov, Chernyshev, 2022). Polystiliferous nemerteans also have less specialized multiciliated sensory organs (Magarlamov, Chernyshev, 2022).

The third feature is the presence of pseudocnidae that resemble cnidae in Cnidaria but are organized completely differently (Magarlamov *et al.*, 2021). Each pseudocnida is from 0.5 to

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[1] The feeding behavior of polystiliferous nemerteans has not been studied to date. However, as Coe (1926: 92) wrote, *Drepanophorus* species feed on large polychaetes, which suggests only macrophagy.

100 μm in size and consists of several layers, including the extruded core (Fig. 7F, H). There is usually only one type of pseudocnidae in the proboscis, but some nemerteans (e.g., species of the genus *Cephalothrix*) may have two types, large and small (Fig. 7G) (Magarlamov *et al*., 2018). The mechanism of core extrusion has not yet been fully understood, because extruded pseudocnidae are very rare and are generally unknown for most of the studied species. Apparently, pseudocnidae, like cnidae, can either damage prey's integument or attach to it (Magarlamov *et al*., 2021). Pseudocnidae are a synapomorphy of Nemertea, but they disappear in hoplonemerteans, and this should be associated with the emergence of armature in this group.

The diversity of proboscis glandular cells in different nemertean classes has been studied extremely insufficiently, which does not allow conducting a comparative analysis, as was done for epidermis glands (Norenburg, 1985). Nevertheless, we may already state that there are more types of glands in the proboscis than in the epidermis. In palaeonemerteans and, especially, pilidiophorans, the epithelium structures on the dorsal and ventral halves of the proboscis differ; Cephalotrichellidae and heteronemerteans frequently have a glandular ridge (Fig. 7I) that, apparently, contacts the prey (Chernyshev, 2015; Magarlamov *et al*., 2021). In hoplonemerteans, the proboscis epithelium does not have dorsoventral differentiation and consists of variously shaped papillae.

Undoubtedly, one of the major functions of the proboscis glandular system is the release of toxins to paralyze or kill prey. A great variety of toxins have been identified in nemerteans, but, for them, it is usually unknown whether these are contained in the proboscis (but see Verdes *et al*., 2022) and in which glands exactly (the exception is tetrodotoxin, see Malykin *et al*., 2021). The use of bioinformatics analysis methods has shown the highest variety of peptide toxin families in Pilidiophora and Palaeonemertea, 77 and 42 toxin families, respectively; only 30 toxin families have been identified in Hoplonemertea (Kuznetsov *et al*., 2025). The stylet apparatus provides more efficient and quick entry of toxins to the prey's body, and this is apparently a factor that has caused the decrease in the number of toxins in hoplonemerteans. As a result, the diversity of glands in the proboscis epithelium of hoplonemerteans may be lower than in the proboscis of palaeo- and heteronemerteans, but this hypothesis needs to be confirmed by analyzing a larger number of species.

General considerations on the proboscis evolution

When considering the evolutionary changes of the proboscis apparatus, one can find that it originally repeated the body wall structure (more precisely, the cephalic end) and had the biradial symmetry as in the vast majority of palaeonemerteans (except Cephalotrichellidae). The proboscis epithelium likely had a dorso-ventral differentiation because we find it in basal palaeonemerteans (*Carinoma* and *Carinina*) and hubrechtiids. Initially, the proboscis apparently had the following structure: glandular epithelium without papillae and ridges but with numerous monociliated sensory cells and pseudocnidae; two intra- or subepithelial longitudinal nerve cords; layers of outer circular, diagonal, and longitudinal musculature; and endothelium with scattered myocytes forming a very thin inner circular musculature. The diagonal musculature is required to wrap the proboscis around prey.

In the class Pilidiophora, we observe the greatest diversity of proboscis structures. Only this class comprises nemerteans with a branched proboscis, which appeared at least twice (Hookabe *et al*., 2021). It exhibits significantly more variants for arrangement of muscle layers in the proboscis than other classes. In Heteronemertea, the proboscis acquires the bilateral symmetry due to the location of the nervous system, the diagonal musculatures in the right and left halves differing in direction, the ventral and dorsal muscle crosses differing in size, often the presence of a glandular ridge, and the intermittent appearance of the outer longitudinal musculature (Chernyshev, 2015). It is likely that the bilateral morphology of the musculature and the presence of the muscle crosses allow wrapping the proboscis around the prey more efficiently and holding it firmly. The exception is *Baseodiscus* and *Sonnenemertes* whose proboscis is polyradially symmetrical. In species of these two genera, pseudocnidae and sensory cells disappear in the proboscis. The explanations for this disappearance should undoubtedly be sought in the feeding habits of these heteronemerteans. However, to date, it is only known that *Sonnenemertes cantelli* apparently feeds on sipunculids (Chernyshev, Polyakova, 2018).

In Hoplonemertea, pseudocnidae and dorsoventral differentiation of the proboscis epithelium were also lost, and the proboscis acquired the polyradial symmetry. However, such changes in these nemerteans were caused by a different hunting strategy: puncture with the stylet apparatus and injection of proteolytic toxins into the wound. The stylet region of the proboscis is always bilaterally symmetrical. The diagonal musculature has been identified only in the very specialized interstitial *Ototyphlonemertes valentinae* (Chernyshev, 2015). We have not managed to find sensory cells in many of monostiliferous nemerteans (Magarlamov, Chernyshev, 2022).

With only a single type of arrangement of proboscis musculature (except *Ototyphlonemertes valentinae*), Hoplonemertea has the most diverse rhynchocoel structures. In this class, there are significantly more variants of arrangement of muscles in the rhynchocoel wall than in other classes. A variety of rhynchocoel pouches are also found here. This may probably be explained as follows: for hoplonemerteans, only the completely everted proboscis is required when hunting (other nemerteans can capture prey even with a partially everted proboscis), with the stylet apparatus appearing at the end of the proboscis. However, the proboscis in some of hoplonemerteans (*Carcinonemertes*, many species of *Ototyphlonemertes*, and symbiotic *Oerstedia actinophila* and *O. sofiae*) may be very short (1–3 mm long) but has a developed (and, therefore, functioning) stylet apparatus and associated musculature (Chernyshev, 2015; Chernyshev, Kuznetsov, 2024). The stylet apparatus was completely lost only in *Malacobdella* species (but a rudimentary middle chamber is present, see Magarlamov, Chernyshev, 2010), and the proboscis was no longer involved in the process of food capture (*Malacobdella* filter out plankton using the voluminous foregut). As a result, the proboscis has acquired its unique structural features: the intertwined longitudinal musculature, the embedded epithelial glands, and the irregular nerve plexus (Magarlamov, Chernyshev, 2010).

In conclusion, we would like to highlight three of the most urgent problems concerning the nemertean proboscis to address:

Is the lack of proboscis and rhynchocoel in *Arhynchonemertes axi* a plesiomorphic state or the proboscis was completely lost in this nemertean? To answer this question, it is necessary to determine the phylogenetic position of *A. axi* and elucidate whether any rudiments of the proboscis or rhynchocoel occur in the ontogeny of this nemertean.

Is the nemertean rhynchocoel homologous to the coelom of other Spiralia? The formulation of this problem makes sense if problem (1) is not clearly addressed or it is proven that *A. axi* lost its proboscis. Unfortunately, almost nothing is known as to how the rhynchocoel wall is laid down in the nemertean embryogenesis and whether its formation is associated with the anterior part of the circulatory system. Such studies are expected to provide a key to answering this question.

Did the monostiliferous armature originate from the polystiliferous armature or the two types of stylet apparatus arose independently? This question is unlikely to be answered soon, but the stylet apparatus of the *Uniporus* species and the formation of the polystiliferous armature definitely need further study.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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References

Ax P. 1996. Multicellular animals: a new approach to the phylogenetic order in nature. Vol. I. Berlin: Springer-Verlag. 225 p.

Berg G. 1972. Studies on *Nipponnemertes* Friedrich, 1968 (Nemertini, Hoplonemertini) // Zool. Scripta. Vol.1. No.5. P.211–225.

Bürger O. 1895. Die Nemertinen des Golfes von Neapel und der angrenzenden Meeres — Abschnitte // Fauna und Flora des Golfes von Neapel (Bd. 22). Verlag von R. Friedländer & Sohn. 743 S.

Bürger O. 1897–1907. Nemertini (Schnurwürmer) // Klassen und Ordnungen des Tier-Reichs, Vol. 4. Leipzig: C.F. Winter'sche Verlagshandlung. 542 S.

Chernyshev A.V. 1999. Origin and early evolution of nemertines // Russ. J. Mar. Biol. Vol.25. No.2. P.206–208.

Chernyshev A.V. 2005. Redescription of *Korotkevitschia pelagica* (Korotkevitsch, 1961) (Enopla: Hoplonemertea: Cratenemertea), a pelagic nemertean from Antarctica // Zootaxa. Vol.862. P.1–14. https://doi.org/10.11646/zootaxa.862.1.1

Chernyshev A.V. 2010. Confocal laser scanning microscopy analysis of the phalloidin-labelled musculature in nemerteans // J. Nat. Hist. Vol.44. No.37. P.2287–2302. https://doi.org/10.1080/00222933.2010.504890

Chernyshev A.V. 2011.Comparative morphology, systematics and phylogeny of the nemerteans. Vladivostok: Dalnauka. 309 p. [In Russian]

Chernyshev A.V. 2015. CLSM analysis of the phalloidin-stained muscle system of the nemertean proboscis and rhynchocoel // Zool. Sci. Vol.32. No.6. P.547–560. https://doi.org/10.2108/zs140267

Chernyshev A.V. 2021. An updated classification of the phylum Nemertea // Invert. Zool. Vol.18. No.3. P.188–196. https://doi.org/10.15298/invertzool.18.3.01

Chernyshev A.V., Kajihara H. 2019.Comparative muscular morphology in Archinemertea (Nemertea: Palaeonemertea) // Zoomorphology. Vol.138. No.2. P.193–207. https://doi.org/10.1007/s00435-019-00440-w

Chernyshev A.V., Kuznetsov V.G. 2024. A description of two new deep-sea nemerteans from North Pacific with reconsideration of the genus *Oerstedia* (Hoplonemertea, Oerstediidae) // Deep Res Part II Top Stud Oceanogr. Vol.216. Art.105390. https://doi.org/10.1016/j.dsr2.2024.105390

Chernyshev A.V., Magarlamov T.Yu., Turbeville J.M. 2013. Morphology of the proboscis of Hubrechtella juliae (nemertea, pilidiophora): implications for pilidiophoran monophyly // J. Morphol. Vol.274. No.12. P.1397–1414. https://doi.org/10.1002/jmor.20189

Chernyshev A.V., Polyakova N.E. 2018. Nemerteans from deep-sea expedition SokhoBio with description of *Uniporus alisae* sp. nov. (Hoplonemertea: Reptantia s.l.) from the Sea of Okhotsk // Deep. Res. Part II. Vol.154. P.121–139. https://doi.org/10.1016/j.dsr2.2017.09.022

Chernyshev A.V., Polyakova N.E. 2019. Nemerteans from the deep-sea expedition KuramBio II with descriptions of three new hoplonemerteans from the Kuril-Kamchatka Trench // Prog. Oceanogr. Vol.178. 102148. https://doi.org/10.1016/j.pocean.2019.102148

Chernyshev A.V., Polyakova N.E. 2022. Nemerteans collected in the Bering Sea during the research cruises aboard the R/V Akademik M.A. Lavrentyev in 2016, 2018, and 2021 with an analysis of deep-sea heteronemertean and hoplonemertean species // Deep Sea Res. II. Vol.199. Art.105081. https://doi.org/10.1016/j.dsr2.2022.105081

Chernyshev A.V., Sun S.-C., Polyakova N.E., Shen C.-Y. 2017. First records of *Hubrechtella ijimai* (Nemertea, Hubrechtiiformes) from Korea and China // Check List. Vol.13. No.4. P.107–111. https://doi.org/10.15560/13.4.107

Coe W.R. 1926. The pelagic nemerteans // Mem. Mus. Comp. Zool. Harv. Vol.49. P.1244.

Crandall F.B. 2001. A cladistic view of the Monostilifera (Hoplonemertea) with interwoven rhynchocoel musculature: A preliminary assessment // Hydrobiologia. Vol.456. P.87–110. https://doi.org/10.1023/A:1013031900458

Crandall F.B., Gibson R. 1998. A second genus of pelagic Cratenemertidae (Nemertea, Hoplonemertea) // Hydrobiologia. Vol.365. P.173–198. https://doi.org/10.1023/A:1003107332635.

Gibson R. 1972. Nemerteans. London: Hutchinson. 224 p.

Hookabe N., Kajihara H. 2020. Taxonomic reappraisal of *Lineus longifissus* auct. (Nemertea: Pilidiophora) from Japan for the first time in 122 years // Zool. Sci. Vol.37. P.467–475. https://doi.org/10.2108/zs200001

Hookabe N., Xu C. M., Tsuyuki A., Jimi N., Sun S.-C., Kajihara H. 2021. A new nemertean with a branched proboscis, *Gorgonorhynchus citrinus* sp. nov. (Nemertea: Pilidiophora), with molecular systematics of the genus // Invertebr. Syst. Vol.35. No.3. P.350–359. https://doi.org/10.1071/IS20057.

Hyman L.H. 1951. The invertebrates: Platyhelminthes and Rhynchocoela // E.J. Boell (ed.). The acoelomate Bilateria. New York: McGraw-Hill. 550 p.

Junoy J., Montalvo S., Roldán C., García-Corrales P. 2000. Ultrastructural study of the bacillary, granular and mucoid proboscidial gland cells of *Riseriellus occultus* (Nemertini, Heteronemertini) // Acta Zool. Vol.81. P.235–242. https://doi.org/10.1046/j.1463-6395.2000.00053.x

Kajihara H. 2006. Four palaeonemerteans (Nemertea: Anopla) from a tidal flat in middle Honshu, Japan // Zootaxa. Vol.1163. P.1–47. https://doi.org/10.11646/zootaxa.1163.1.1

Kajihara H. 2010. Rhynchocoel vessel in Cephalotrichidae (Nemertea: Palaeonemertea) // J. Nat. Hist. Vol.44. P.2321–2329. https://doi.org/10.1080/00222933.2010.504892

Kajihara H. 2021. Higher classification of the monostilifera (Nemertea: Hoplonemertea) // Zootaxa. Vol.4920. No.2. P.151–199. https://doi.org/10.11646/zootaxa.4920.2.1.

Korotkevich V.S. 1983. [On the stylets of nemerteans of a new species of *Cratenemertes* (Hoplonemertini, Amphiporidae) from Antarctica] // Antarktika, Doklady komissii AN SSSR. Vol.22. P.137–143 [in Russian].

Kuzmina T.V., Temereva E.N., Malakhov V.V. 2018. Ultrastructure of the lophophoral coelomic lining in the brachiopod *Hemithiris psittacea*: functional and evolutionary significance // Zoomorphology. Vol.137. No.2. P.257–272. https://doi.org/10.1007/s00435-018-0397-8

Kuznetsov V.G., Melnikova D.I., Shabelnikov S.V., Magarlamov T.Y. 2025. Proteotranscriptomic profiling of the toxic mucus of *Kulikovia alborostrata* (Pilidiophora, Nemertea) // Toxins (Basel). Vol.15. No.1. Art.5. https://doi.org/10.3390/toxins17010005

Ling E.A. 1971. The proboscis apparatus of the nemertine *Lineus ruber* // Philos. Trans. R. Soc. London B. Vol.262. P.1–22.

Magarlamov T.Yu., Chernyshev A.V. 2010. Ultrastructural study of the proboscis of *Malacobdella grossa* (Nemertea: Hoplonemertea) // J. Nat. Hist. Vol.44. No.37. P.2349–2361. https://doi.org/10.1080/00222933.2010.504894

Magarlamov T. Yu., Chernyshev A.V. 2015. Structure of the proboscis endothelium in Nemertea // Zool. Sci. Vol.32. No.6. P.561–566. https://doi.org/10.2108/zs140269

Magarlamov T.Yu., Chernyshev A.V. 2022. Proboscis sensory cells in Nemertea: comparative morphology and phylogenetic implications // Zool. J. Linn. Soc. Vol.196. No.1. P.453–478. https://doi.org/10.1093/zoolinnean/zlab106

Magarlamov T.Yu., Chernyshev A.V., Turbeville J.M. 2011. Morphology of the proboscis of some nemerteans from

Vietnamese and Russian coastal waters // K.A. Lutaenko (ed.). Coastal Marine Biodiversity and Bioresources of Vietnam and Adjacent Areas to the South China Sea. Nha-Trang, Vietnam: Dalnauka. P.48–52.

Magarlamov T.Yu., Chernyshev A.V., Turbeville J.M. 2018. Pseudocnidae of archinemerteans (Nemertea, Palaeonemertea) and their implications for nemertean systematics // J. Morphol. Vol.279. No10. P.1444–1454. https://doi.org/10.1002/jmor.20881

Magarlamov T.Yu., Turbeville J.M., Chernyshev A.V. 2021. Pseudocnidae of ribbon worms (Nemertea): ultrastructure, maturation, and functional morphology // PeerJ. Vol.9. P.1–51. https://doi.org/10.7717/peerj.10912

Malakhov V.V., Bogomolova E. 2016. [A new look on the structure and origin of metazoan body cavity] // Priroda. Vol.11. P.33–37 [in Russian].

Malykin G.V., Chernyshev A.V., Magarlamov T.Yu. 2021. Intrabody tetrodotoxin distribution and possible hypothesis for its migration in ribbon worms *Cephalothrix* cf. *simula* (Palaeonemertea, Nemertea) // Mar. Drugs. Vol.19. No.9. P.1–17. https://doi.org/10.3390/md19090494

McIntosh W.C. 1873–1894. Part I. The nemerteans. A monograph of the British marine annelids. London: Ray Society. 218 p.

Montalvo S., Junoy J., Roldán C., García-Corrales P. 1996. Ultrastructural study of sensory cells of the proboscidial glandular epithelium of *Riseriellus occultus* (Nemertea, Heteronemertea) // J. Morph. Vol.229. No.1. P.83–96. https://doi.org/10.1002/(SICI)1097-4687(199607)229:1<83::AID-JMOR5>3.0.CO;2-Q

Montalvo S., Roldán C., Junoy J., García-Corrales P. 1998. Ultrastructural study of two glandular systems in the proboscidial glandular epithelium of *Riseriellus occultus* (Nemertea, Heteronemertea) // Zoomorphology. Vol.117. No.4. P.247–257. https://doi.org/10.1007/s004350050049

Moore J., Gibson R. 1981. The Geonemertes problem (Nemertea) // J. Zool. London. Vol.194. P.175–201. https://doi.org/10.1111/j.1469-7998.1981.tb05768.x.

Nielsen C. 2001. Animal evolution: interrelationships of the living phyla. New York: Oxford University Press. 563 p.

Nielsen C. 2012. Animal Evolution: Interrelationships of the living phyla. New York: Oxford University Press. 414 p.

Norenburg J.L. 1985. Structure of the nemertine integument with consideration of its ecological and phylogenetic significance // Amer. Zool. Vol.25. No.1. P.37–51. https://doi.org/10.1093/icb/25.1.37.

Norenburg J. L., Roe P. 1998. Observations on musculature in pelagic nemerteans and on pseudostriated muscle in nemerteans // Hydrobiologia. Vol.365. P.109–120. https://doi.org/ 10.1023/A:1003105804893.

Riser N.W. 1988. *Arhynchonemertes axi* gen. n., sp. n. (Nemertinea) — an insight into basic acoelomate bilaterian organology // Prog. Zool. Vol.36. P.367–373.

Riser N.W. 1989. Speciation and time-relationships of the nemertines to the acoelomate metazoan bilateria // Bull. Mar. Sci. Vol.45. No.2. P.531–538.

Senz W. 1997. Über Organisation und stammesgeschichte der Nemertinen — eine untersuchung basierend auf kritischen uberlegungen zur theorie der stammesgeschichtsforschung // Sitzungsber. Österr. Akad. Wissensch. Abt. I. Mathem.-Naturwiss. Kl. Bd.204. S.3–38.

Stricker S.A. 1985. The stylet apparatus of monostiliferous hoplonemerteans // Integr. Comp. Biol. Vol.25. P.87–97. https://doi.org/10.1093/icb/25.1.87.

Stricker S.A., Cloney R.A. 1983. The ultarstructure of venom-producing cells in *Paranemertes peregrina* (Nemertea, Hoplonemertea) // J. Morphol. Vol.177. P.89–107.

Schwartz M.L., Norenburg J.L. 2005. Three new species of *Micrura* (Nemertea: Heteronemertea) and a new type of heteronemertean larva from the Caribbean Sea // Carib. J. Sci. Vol.41. P.528–543.

Starobogatov Ya.I. 1983. [Brachiocoelic (hydrocoelic) hypothesis of origin of the coelom] // Trudy Zool. Inst. AN SSSR. Vol.109. P.111–122 [in Russian].

Turbeville J.M. 1991. Nemertinea // B.J. Bogitsh (ed). Platyhelminthes and Nemertinea. W. Harrison (ed). Microscopic Anatomy of Invertebrates. New York: Wiley-Liss. P.285–328.

Turbeville J.M., Ruppert E.E. 1985. Comparative ultrastructure and the evolution of nemertines // Amer. Zool. Vol.25. No.1. P.53–71. https://doi.org/10.1093/icb/25.1.53.

Verdes A., Taboada S., Hamilton B.R., Undheim E.A.B. 2022. Evolution, expression patterns and distribution of novel ribbon worm predatory and defensive toxins // Mol. Biol. Evol. Vol.39. No.5. Art.msac096. https://doi.org/10.1093/molbev/msac096

Wern J.O. 1998. An enigmatic heteronemertean from the Gulf of Mexico // Hydrobiologia. Vol.365. P.135–147. https://doi.org/10.1023/A:1003194913071

Wijnhoff G. 1914. The proboscidian system in nemertines // Q. J. Microsc. Sci. Vol.60. P.273–312.

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