

Ultrastructure, Biology, and Phylogenetic Relationships of Kinorhyncha¹

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SYNOPSIS. The article summarizes current knowledge mainly about the (functional) morphology and ultrastructure, but also about the biology, development, and evolution of the Kinorhyncha. The Kinorhyncha are microscopic, bilaterally symmetrical, exclusively free-living, benthic, marine animals and ecologically part of the meiofauna. They occur throughout the world from the intertidal to the deep sea, generally in sediments but sometimes associated with plants or other animals. From adult stages 141 species are known, but 38 species have been described from juvenile stages. The trunk is arranged into 11 segments as evidenced by cuticular plates, sensory spots, setae or spines, nervous system, musculature, and subcuticular glands. The ultrastructure of several organ systems and the postembryonic development are known for very few species. Almost no data are available about the embryology and only a single gene has been sequenced for a single species. The phylogenetic relationships within Kinorhyncha are unresolved. Priapulida, Loricifera, and Kinorhyncha are grouped together as Scalidophora, but arguments are found for every possible sister-group relationship within this taxon. The recently published Ecdysozoa hypothesis suggests a closer relationship of the Scalidophora, Nematoda, Nematomorpha, Tardigrada, Onychophora, and Arthropoda.

INTRODUCTION

Since the last compilation on the ultrastructure (Kristensen and Higgins, 1991), numerous studies have provided different views of morphology and phylogenetic relationships both within Kinorhyncha and of Kinorhyncha to other Bilateria (Nebelsick, 1992a, b, 1993; Adrianov *et al.*, 1993; Adrianov and Malakhov, 1994, 1996, 1999a; Neuhaus, 1994, 1995, 1997; Neuhaus *et al.*, 1996, 1997b; Wallace *et al.*, 1996; G^oOrdóñez *et al.*, 2000; Müller and Schmidt-Rhaesa, 2002). In addition, the traditional view of a taxon Articulata consisting of Annelida, Tardigrada, Onychophora, and Arthropoda (see Schmidt-Rhaesa *et al.*, 1998) has been recently challenged by the Ecdysozoa hypothesis uniting all moulting animals such as Tardigrada, Onychophora, Arthropoda, Nematoda, Nematomorpha, Kinorhyncha, and Priapulida (Aguinaldo *et al.*, 1997). Therefore, this review summarizes the new information and provides additional data on these topics and other aspects of kinorhynch biology.

The Kinorhyncha is a group of exclusively free-living, marine, meiofaunal, benthic animals up to 1.03 mm long. They occur throughout the world, generally in sediments but sometimes associated with algae, sponges, or other invertebrates usually themselves closely associated with sediment. They are found from intertidal (Zelinka, 1928) to abyssal zones at least as deep as 5,300 m (Meadows *et al.*, 1994) interstitially in usually the upper few millimeters (mud) or centimeters (sand) of the sediment layer. At depths of 8–30 m along the coast of the North Adriatic, Kinorhyncha occupies the top, well oxygenated, soft silt sedi-

ment layer (Vidaković, 1984). In the Pacific deep sea around 5,200 m depth, the animals inhabit the upper, oxygenated centimeter of the sediment where the number of microorganisms is highest and decreases exponentially with increasing sediment depth (Meadows *et al.*, 1994). At a deep-sea location in Japan (depth 1,450 m), kinorhynchs occur only temporarily below the oxygenated sediment layer; the animals react positively to seasonal food supply and migrate some 5 mm into upper layers (Shimanaga *et al.*, 2000). Most species have been described from muddy substrates, but kinorhynchs also inhabit sandy biotopes such as the mid-tide level of exposed beaches at depths of 10–60 cm (*Cateria gerlachi*, *C. styx*, and *Echinoderes nybakkeni*: Higgins, 1968, 1986). *Echinoderes coulli* withstands salinities reaching from 12–42‰ for at least a short time in intertidal flats of North Carolina (Horn, 1978). In an 18-mo study, Rao and Satapathy (1996) found that unidentified species of *Echinoderes* and *Pycnophyes* survive salinities of 5.7–20.3‰, pH of 7.64–9.50 and temperatures up to 33.5°C in a lagoon in India.

Kinorhynchs may reach densities of 45 animals per 10 cm² in shallow waters of the Antarctic and densities of 1–10 animals per 10 cm² in the deep sea (Dinet, 1979; Vanhove *et al.*, 1995). Most studies concentrate on the upper 100 m of the continental shelf, and no record of a kinorhynch from below 500 m depth identified to species level has been published yet. Our unpublished observations from both the Atlantic and the Pacific Ocean suggest a high diversity of Kinorhyncha in the deep sea, nearly all species being new to science. However, such species can be assigned to known genera from the continental shelf.

The Kinorhyncha is divided into the two subgroups, Cyclorhagida and Homalorhagida, containing

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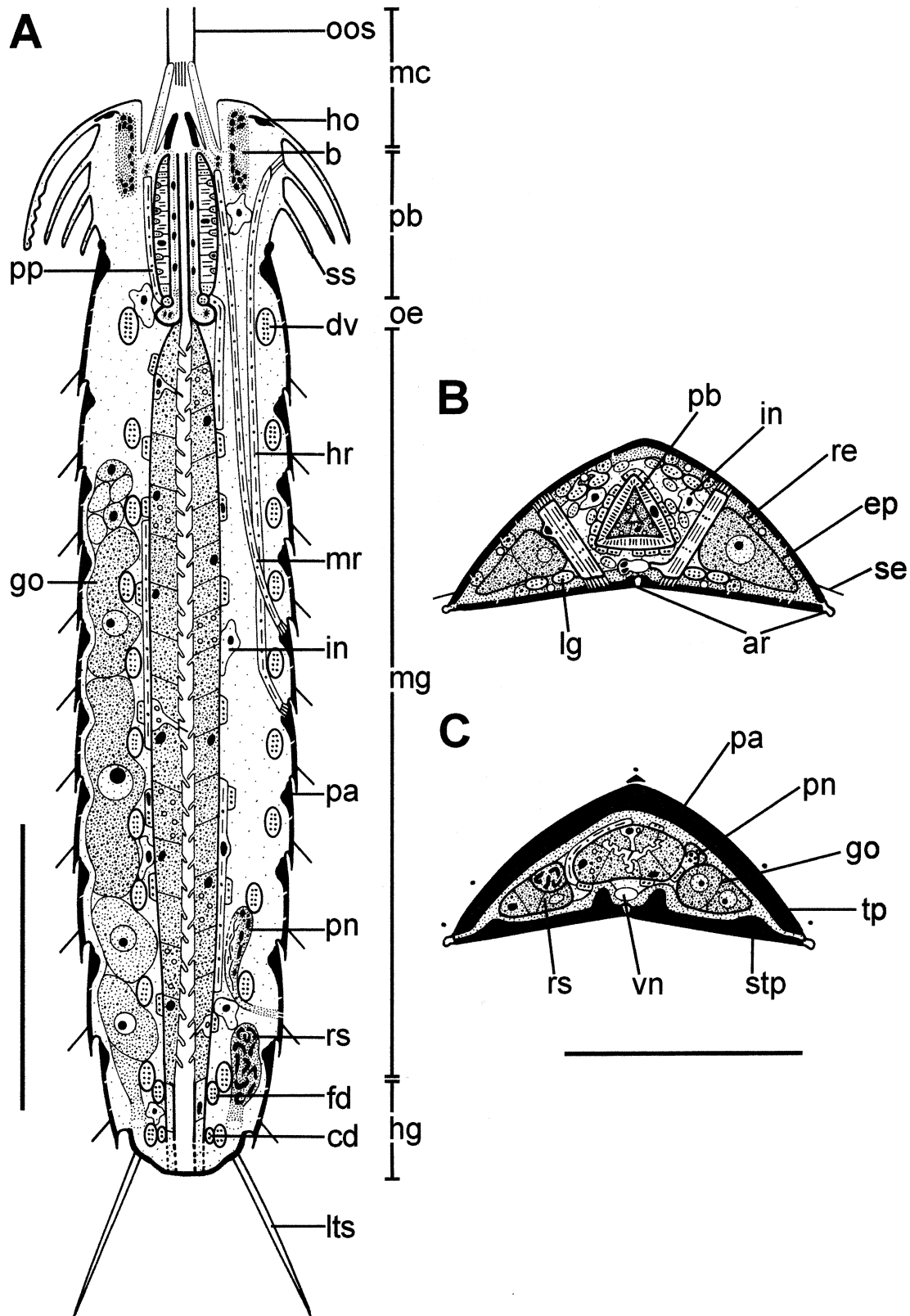


FIG. 1. Morphology based on female *Pycnophyes dentatus* (Homalorhagida). **A.** Longitudinal section of specimen with protruded head. **B–C.** Cross-sections through trunk, drawn for specimen with withdrawn introvert. Inner oral styles not shown. Epidermis not drawn cellularly. **B.** Segment 4. **C.** Combined cross-section through pachycyclus of segment 9 (right side) and of segment 10 (left side). Scale bar in **A** 200 μm, in **B–C** 100 μm. After Neuhaus, 1994.

141 species described from adult life history stages and 38 species described from juvenile stages, all grouped in 15 genera (Bauer-Nebelsick, 1996; Pardos *et al.*, 1998; Adrianov and Malakhov, 1999a). Zelinka (1928) and others introduced new genus names for species described from juvenile stages of species of known genera, although these scientists were aware of the life-history relationships. Higgins (1983) extensively synonymized species described from juveniles. The species names based on descriptions of juvenile stages are available and valid in the sense of the International Code of Zoological Nomenclature, so currently 179 species are recognized. No formal classification is presented in this review, because hierarchical assignments in classification systems cannot be defined on an objective basis. Several classification schemes are summarized and discussed by Adrianov and Malakhov (1996).

EXTERNAL GROSS ANATOMY

The kinorhynch body is divided into 3 regions: head (=introvert), very short neck, and trunk (Figs. 1A, 2A). Introvert and neck have often been considered as a "segment" comparable to the 11 trunk segments (Kristensen and Higgins, 1991). Trunk segmentation is apparent externally with cuticular plates and spines and in the internal organs such as longitudinal and dorsoventral muscles, central nervous system, glands, and sensory structures (Zelinka, 1928; Kristensen and Higgins, 1991; Neuhaus, 1994; Adrianov and Malakhov, 1994). However, these segmental characters do not appear in the head and neck region at all, and extension of the term "segmentation" to the neck and head is therefore not justified.

The head bears 5–7 rings of posteriorly directed, sensory-locomotory spinose appendages (=scalids) (Figs. 1A, 2A, 3A). The 54–93 scalids are arranged regularly in circles of 10–20; succeeding circles show a staggered arrangement. Scalids of the first 6 rings consist of two elements, a broad basal part and a narrower spinose distal part (Brown, 1989; Higgins, 1990; Nebelsick, 1993; Neuhaus, 1995).

The neck possesses up to 16 cuticular plates (=placids) (Fig. 3A) which may be barely distinguishable from the first trunk segment (Higgins, 1990). These plates act solely or in combination with specialized trunk plates (*Semnoderes*, *Sphenoderes*) to close off the withdrawn head (Zelinka, 1928; Higgins, 1969a, 1983).

In cross-section, the trunk may appear round, broadly oval (Fig. 2B, C), subtriangular, triangular (Fig. 1B, C), or narrowly oval (laterally compressed). The cuticle is organized into one to several plates, beyond trunk segment 2 or 3 in a single dorsal (=tergal) plate, and two ventral (=sternal) plates (Zelinka, 1928; Higgins, 1968, 1969b). Borders between cuticular dorsal and ventral plates may not be recognizable in the SEM in species with a thin cuticle such as *Zelinkaderes floridensis* (Fig. 3B; Higgins, 1990). In those species with a thick cuticle (*e.g.*, species of *Echinoderes*, *Pycno-*

phyes, and *Kinorhynchus*), a distinct ball-and-socket articulation usually exists at the anterior margin of the plates of most segments. The cuticle of the anterior margin of each segment is thickened towards the interior of the animal in order to form a pachycyclus for the attachment of longitudinal muscles (Figs. 1A, C, 2A, 4A; Zelinka, 1928; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994).

INTEGUMENT

A cuticle ensheathes the entire animal including foregut, hindgut, tubules of the protonephridial nephropore, and tubes of the sensory spots (Neuhaus, 1993). The cuticle of each sternal and tergal plate is composed of a chitinous basal layer which appears to be finely granular at the TEM level (Fig. 4A–C), and a monolamellar, membrane-like epicuticle (Neuhaus *et al.*, 1996). Thin fibrillar material may occur below the basal layer in some species especially in flexible areas. Stronger fibres connect the sternal and tergal plates laterally as well as anteriorly and posteriorly in many species (Fig. 4A–C; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994). In the matrix embedding the thin and stronger fibres, chitin may be localized too (Fig. 4B, C; Neuhaus *et al.*, 1996). To demonstrate chitin in a specific cuticular layer (Fig. 4B, C), the lectin-gold labelling technique was used; it previously detected chitin in the body cuticle of Loricifera, Priapulida, and juvenile Nematomorpha, and in the pharyngeal cuticle of nematodes (Saldarriaga *et al.*, 1995; Neuhaus *et al.*, 1996, 1997a, b; Lemburg, 1998).

With increasing age of the adult individual, the fine-granular layer of the cuticle as well as the pachycycli increase in thickness and ball-and-socket articulations become more prominent and turn increasingly yellow-brown (tanning?) in species of *Echinoderes* and especially of *Pycnophyes* and *Kinorhynchus*. The epidermis is cellular and does not possess any locomotory cilia (Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994).

At least two types of integumental gland cells seem to exist in several species of *Echinoderes*, *Pycnophyes*, and *Kinorhynchus*, and they are arranged in species-specific patterns. These gland cells open to the outside via a convoluted duct system or through a single pore (Nebelsick, 1992b; Adrianov and Malakhov, 1994; G^aOrdóñez *et al.*, 2000). A sensory cell with or without a modified cilium may be associated with a gland cell. A gland cell is often associated with a sensory spot (Fig. 3C), a cuspidate spine, a seta (Fig. 3C), or an adhesive spine in segment 4 of male *Pycnophyes* and *Kinorhynchus* (Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994).

NERVOUS SYSTEM AND SENSE ORGANS

The central nervous system consists of a circumenteric brain (Figs. 1A, 2A, D) and several longitudinal nerve cords in the trunk (Figs. 1B, C, 2B, C) which are connected generally by two commissures per trunk segment (Kristensen and Higgins, 1991; Nebelsick,

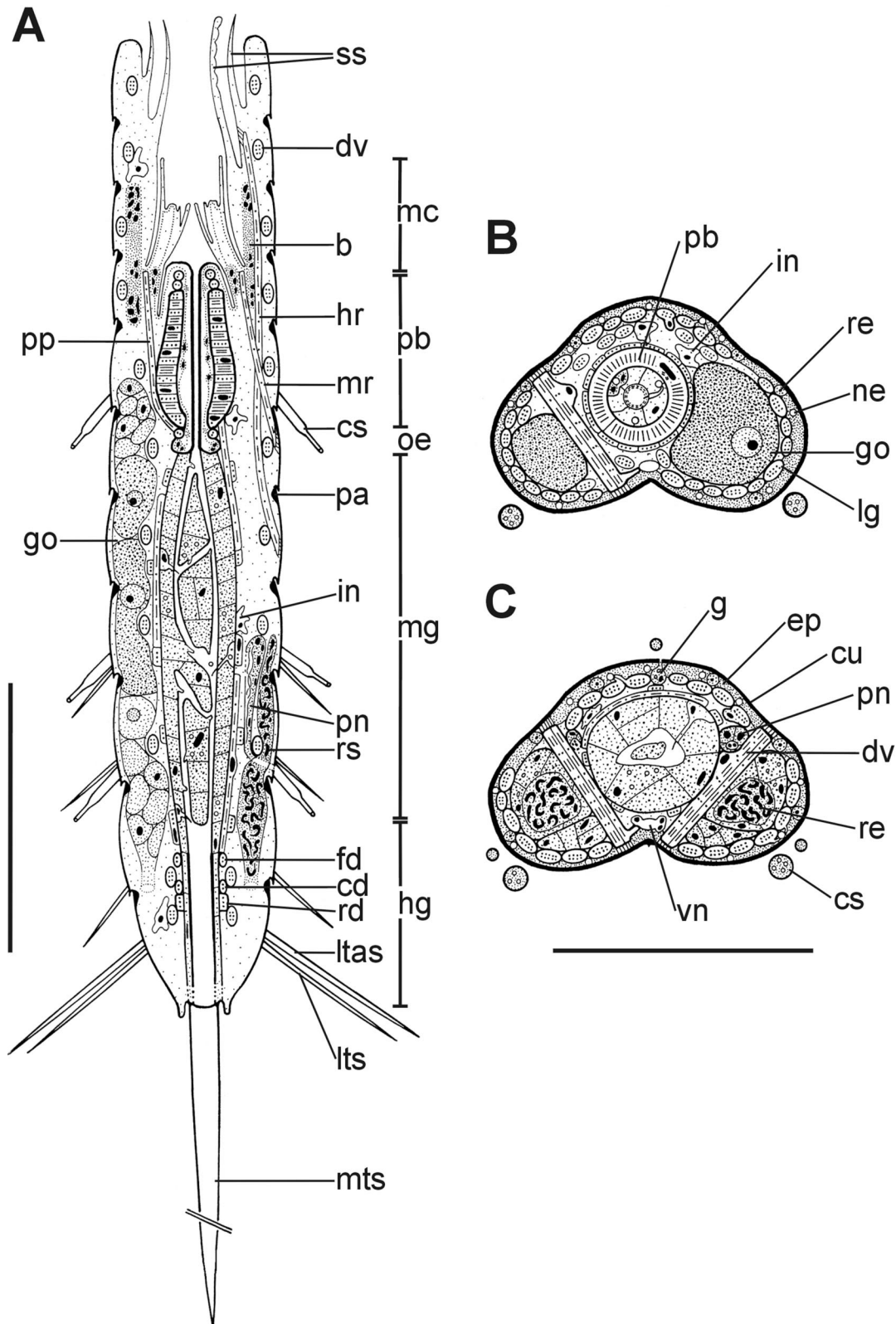


FIG. 2. Morphology based on female *Zelinkaderes floridensis* (Cyclorhagida). **A.** Longitudinal section through entire specimen with withdrawn head. **B., C.** Cross-sections through trunk in segment 5 (**B**) and in segment 9 (**C**), respectively. Epidermis not drawn cellularly in **A–C**. **D.** Longitudinal section through mouth cone, brain, and anterior pharynx. Nervous system and epithelia not drawn cellularly. Note innervation of pharynx through mouth cone. Arrow heads mark extracellular matrix separating inner and outer mouth cone epithelium. Numbers refer to inner oral styles of rings 1–4. Arrows point to subapical opening of oral styles. Scale bar in **A–C** 100 μm , in **D** 20 μm . After Neuhaus, 1994.

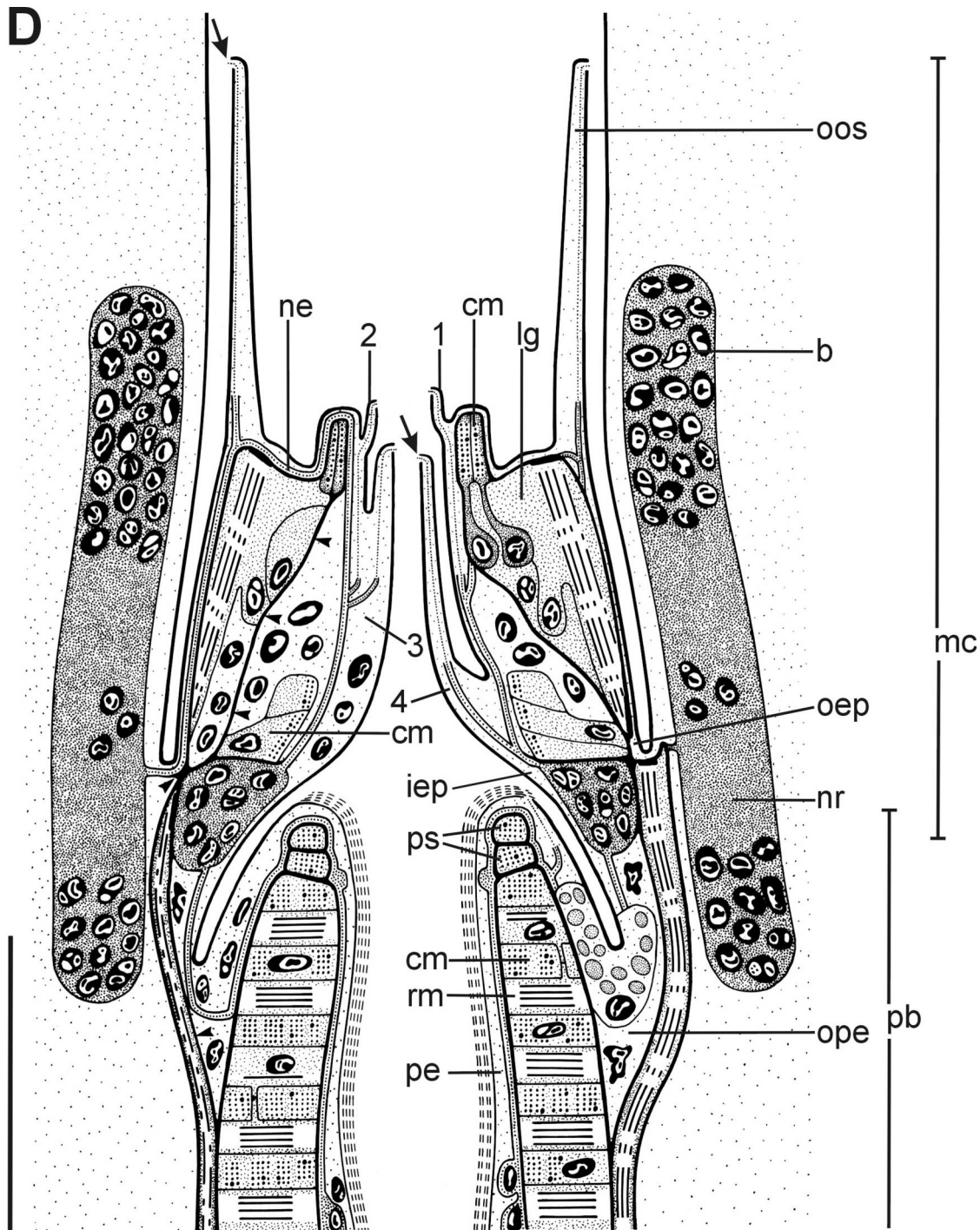


FIG. 2. Continued.

1993). The brain is organized into a 10-lobed forebrain with numerous perikarya, a midbrain with few perikarya but abundant neuropile, and a hindbrain also with numerous perikarya (Fig. 2D; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994; Neuhaus, 1994). In *Echinoderes capitatus*, 10 nerve cords extend from the forebrain and innervate the introvert, neck, and trunk. The nerves fuse to two subdorsal, two sublateral, and one midventral nerve strand from trunk

segment 2 on (Nebelsick, 1993). The perikarya of the unpaired ventral nerve cord (Fig. 4D) are distributed over almost the entire length of a trunk segment. In *Zelinkaderes floridensis*, 12 longitudinal nerve cords seem to exist (Fig. 2B, C; Neuhaus, 1994), whereas 8 nerves have been reported for *Pycnophyes greenlandicus* and *P. kielensis* (Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994) and 7 for *P. dentatus* (Fig. 1B; Neuhaus, 1994).

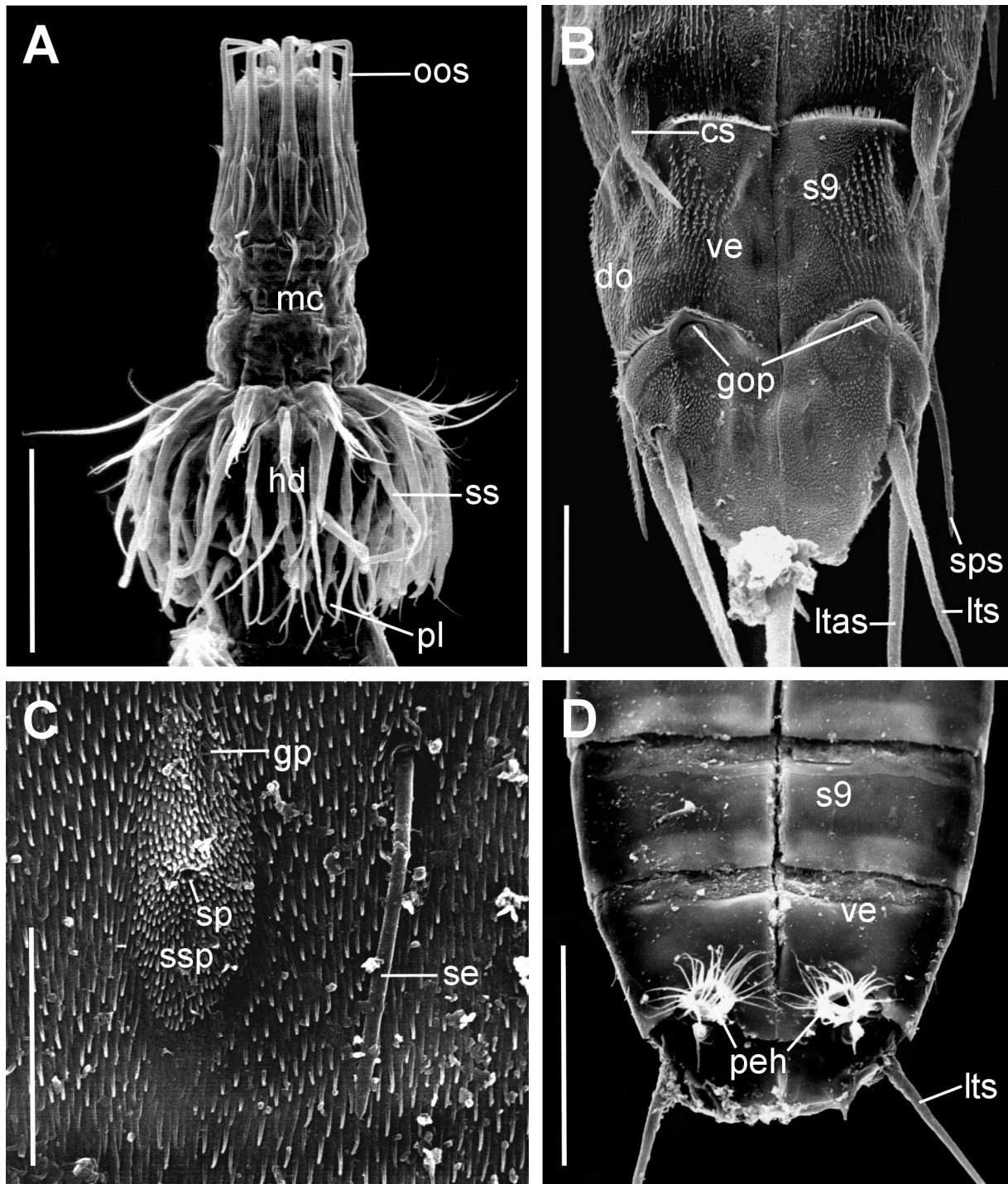


FIG. 3. SEM. **A.** Head of *Antygomonas* sp. with far extended mouth cone. **B.** Ventral view of last trunk segments of female *Zelinkaderes floridensis*. Notice regular spinose and cuspidate spines, gonopores, and lack of obvious border between ventral and dorsal cuticular plates. **C.** Sensory spot and seta of *Pycnophyes greenlandicus*. Notice gland pore and sensory pore. **D.** Ventral view of posterior trunk segments of *P. greenlandicus* with cuticular hairs surrounding male gonopores. Scale bar in **A**, **D** 50 μm , in **B** 20 μm , and in **C** 10 μm .

The posterior midbrain sends 9 nerve cords frontally to the outer oral styles. From there the nerves turn caudally to the basal mouth cone nerve ring (*Z. floridensis*, *E. capitatus*) or to the nerve ring in the outer pharyngeal epithelium (*P. kielensis*, *P. dentatus*). The nerves continue over the anterior pharyngeal muscle

bulb into the inner pharyngeal epithelium (Fig. 2D) until they end in the esophageal epithelium (Nebelsick, 1993; Adrianov and Malakhov, 1994; Neuhaus, 1994). The alimentary canal is not innervated via the ventral nervous system as has been claimed earlier (Kristensen and Higgins, 1991).

No nerve cells have been traced to innervate the longitudinal and circular muscle cells of the midgut. Therefore, it is assumed that this musculature acts autonomously; however, the muscles may be triggered to some degree by presumably sensorimotor cells in the midgut epithelium (Neuhaus, 1994).

Scalids, in addition to their function in locomotion, may also be mechano- and chemoreceptive. A scalid contains up to 10 monociliary sensory cells which communicate with the exterior by way of a distal pore (Adrianov and Malakhov, 1994). Spiniscalids may contain a cell with piles of membranes and a porous cuticle. Possibly, this cell releases some secretions (Brown, 1989; Kristensen and Higgins, 1991).

A sensory cell with or without cilium is associated with each cuticular spine, seta, and gland cell of the trunk. All species of Kinorhyncha show a species-specific pattern of sensory spots on the trunk. On the surface of the cuticle, each round to oval sensory spot exhibits numerous cuticular micropapillae around one or two pores (Fig. 3C) through which a cilium may jut out (Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994; Neuhaus, 1995); below, one to few monociliary sensory cells occur; their cilia are surrounded by a circle of 9 microvilli containing electron-dense fibrils. At least for *E. capitatus*, an additional sheath cell has been reported (Nebelsick, 1992a). Gland cells seem to be associated with sensory spots in *Pycnophyes* and *Kinorhynchus* (Fig. 3C; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994). The sensory spots of juvenile stages of *P. kielensis* appear as papillae which elevate slightly above the surface of the trunk cuticle. Fewer cuticular micropapillae than in the adult are also characteristic of these juvenile organs (Neuhaus, 1993; Adrianov and Malakhov, 1994). Adult *Kinorhynchus yushini* possess sensory spots with a reduced number of cuticular micropapillae and have been named "flosculi" by Adrianov and Malakhov (1994) although a detailed reconstruction at the TEM level for comparison with the flosculi of Loricifera (Kristensen, 1991) and Priapulida (Lemburg, 1995) is missing; Priapulida and/or Loricifera are certainly most closely related to Kinorhyncha (see Phylogenetic relationships below).

In *Pycnophyes dentatus*, *P. kielensis*, *P. greenlandicus*, and *Kinorhynchus phyllotropis*, one pair of cephalic sensory organs is located at the base of the first-ring head scalids (Fig. 1A). Each organ consists of one enveloping cell and one receptor cell. The latter possesses a single, modified cilium which extends into the lumen of the sensory organ. The cilium swells and branches into multiple processes (Neuhaus, 1997). Pigmented eye spots with lenses have been reported for several species of *Echinoderes*, but pigmentation disappears during fixation of specimens (Zelinka, 1928). However, TEM results are missing entirely.

MUSCULATURE

The segmental cuticular plates are moved by sets of segmentally arranged dorsal and ventral longitudinal

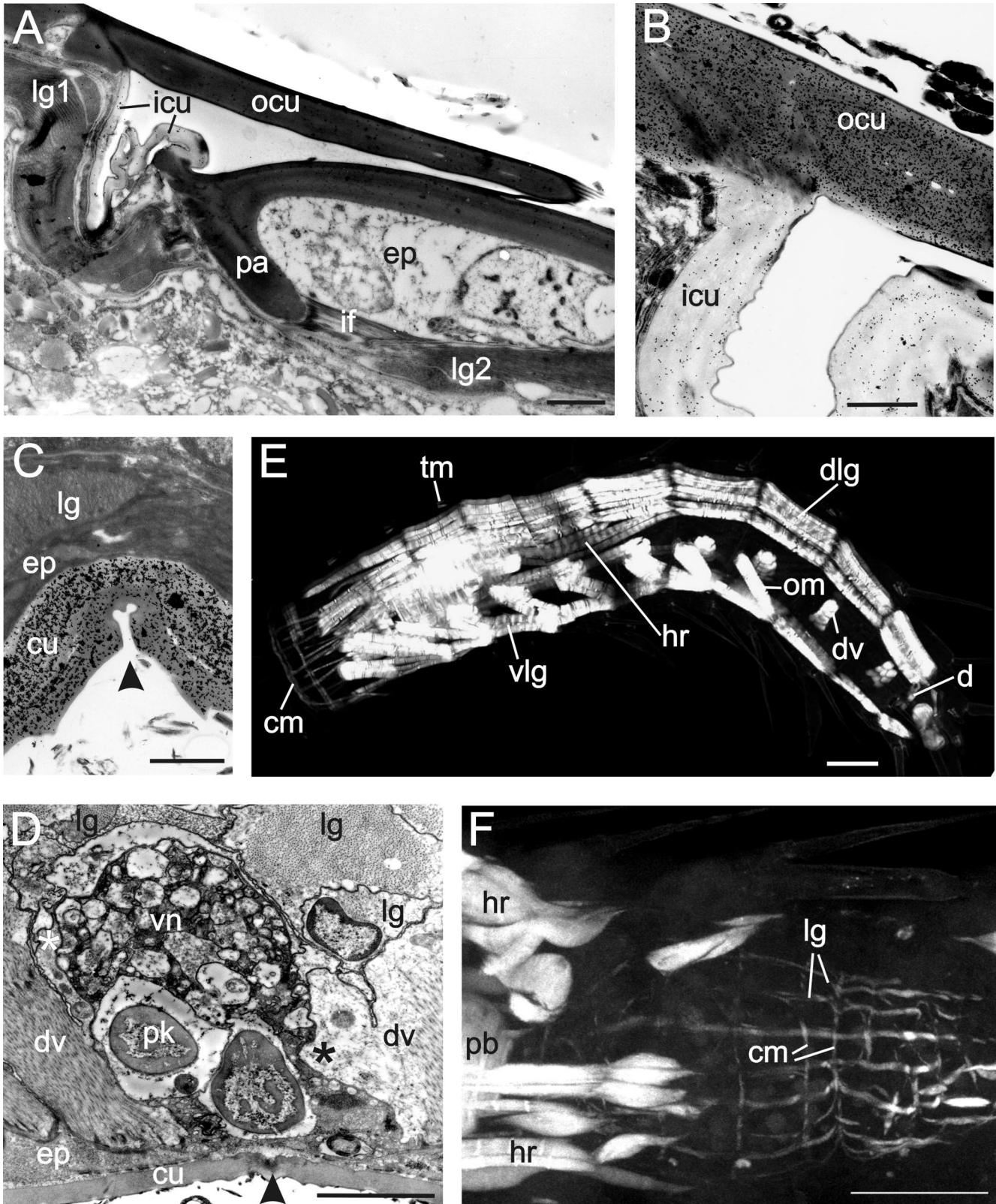
muscles, oblique muscles (only in Cyclorhagida), and dorsoventral muscles (Fig. 4E; Müller and Schmidt-Rhaesa, 2002). In *Zelinkaderes floridensis*, the longitudinal muscles seem to be distributed regularly in the periphery of a cross-section (Fig. 2B, C), whereas these muscles concentrate in bundles in species of *Echinoderes*, *Pycnophyes*, and *Kinorhynchus* (Fig. 1B, C; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994; Neuhaus, 1994). Mouth cone retractor muscle cells extend between the mouth cone and posterior trunk segments (Figs. 1A, 2A). Head retractor muscle cells stretch between the base of head scalids and posterior trunk segments (Figs. 1A, 2A, 4E); they pass the periphery of the brain via extremely elongated, epidermal cells containing intermediate filaments (=tanocytes) (Kristensen and Higgins, 1991; Neuhaus, 1994). Circular muscles occur in association with the placids of the neck region (Fig. 4E; Müller and Schmidt-Rhaesa, 2002) and in the mouth cone (Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994; Neuhaus, 1994). One pair of small trapezoidal muscles is reported for each middorsal spine of *Antygomonas* sp. (Fig. 4E; Müller and Schmidt-Rhaesa, 2002).

All muscle cells connecting to the cuticle attach via desmosomes, epidermal intermediate filaments, hemidesmosomes and thin filaments into the cuticle (Fig. 4A). The longitudinal and dorsoventral muscles appear cross-striated with isolated z-elements. This arrangement allows supercontraction of the muscle cells by letting the thick filaments pass the z-elements (Nyholm and Nyholm, 1976). Dorsoventral, longitudinal, at least some circular and dilatator muscles send cell processes towards nerve cells (Figs. 1B, 2B, C, 4D; Neuhaus, 1994) and not *vice versa* as found in most invertebrate groups.

Kinorhynch locomotion is facilitated by alternative action of dorsoventral muscles and head and mouth cone retractor muscles. Contraction of dorsoventral muscles diminishes the space between dorsal and ventral cuticular trunk plates, increases the pressure in the small but liquid-filled body cavity, and last but not least protrudes the head. Slight contraction of the dorsal and ventral longitudinal muscles and the stiff cuticle overlapping the intersegmental cuticle from anterior (Fig. 4A, B) may prevent the flexible cuticle between subsequent segments from bulging outward by the increased hydrostatic pressure. Consequently, the abundant head scalids move forward, plow backward through the water and interstices surrounding the animal, and therefore propel the animal forward. At the minute size of a kinorhynch and its very quick movement of the introvert, the water has to be imagined more as a viscous mass than as a thin fluid. Finally, the head and mouth cone retractors, respectively, withdraw the head and mouth cone back into the trunk, and the cycle can start again.

DIGESTIVE SYSTEM

The alimentary canal is divided into a cuticular foregut (with the mouth cone, pharyngeal crown in many



species, pharyngeal bulb, and esophagus), the non-ciliated midgut with microvilli, and a cuticularized hindgut (Figs. 1A, 2A). The digestive system is cellular throughout (Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994; Neuhaus, 1994).

The mouth cone bears one ring of 9 outer oral styles (Fig. 3A) and 3–4 rings of 5 inner oral styles (Fig. 2D; Brown, 1989; Nebelsick, 1993; Neuhaus, 1994). Both outer and inner oral styles possess monociliary sensory cells which contact the outside via terminal pores. One or 2 circular muscle cells allow constriction of the anterior mouth cone lumen. Each outer oral style of at least *Zelinkaderes floridensis* can be manipulated with the help of 2 longitudinal muscle cells (Fig. 2D). Each basal inner oral style is connected with a circular muscle cell which allows limited movement of styles (Nebelsick, 1993; Neuhaus, 1994). The anterior mouth cone of *Pycnophyes kielensis*, *P. dentatus*, and *Kinorhynchus phyllotropis* possesses longitudinal cuticular rods which probably serve as filtering system for bacteria (Brown, 1989; Neuhaus, 1994). Species such as *Z. floridensis* do not exhibit a cuticular weir and seem to feed on diatoms (Neuhaus, 1994). *Echinoderes* seems to feed on diatoms in three ways: algae are selectively sucked in by the pharynx or collected between the head scalids and then ingested by the pharynx or stripped off the lateroterminal spines between the head scalids and taken up afterwards (Adrianov and Malakhov, 1994).

In order to facilitate feeding, the sucking pharynx may be protruded by a set of pharynx protractor muscle cells while the head is pushed out of the trunk. The protractors extend from the basal mouth cone to the caudal end of the pharynx (Figs. 1A, 2A). The pharyngeal lumen is triradiate in species of *Pycnophyes* and *Kinorhynchus* (Fig. 1B), but round (Fig. 2B), oval, or 9-lobed in the Cyclorhagida. Muscular sphincters may exist anteriorly or posteriorly in the pharyngeal bulb. Two layers constitute the pharynx of the Kinorhyncha: an outer layer of circular and radial musculature surrounds the inner epithelium (Fig. 2D; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994; Neuhaus, 1994). Circular and radial muscle cells are arranged like a roll of coins with a central hole (=the pharyngeal lumen) and alternate in the pharynx of *Z. floridensis* (Fig. 2D), whereas circular muscle cells occur only in the periphery of the muscle bulb of *Pycnophyes kielensis* and *P. dentatus*. Monociliary receptor cells and gland cells are embedded into the

pharyngeal epithelium. An additional set of 10, usually monociliary gland cells opens anteriorly and outside of the pharyngeal bulb into the mouth cone lumen (Neuhaus, 1994).

The existence of “salivary glands” or “pancreatic glands” has not been confirmed by TEM investigations (Neuhaus, 1994). The midgut is composed more than 95% of heavily interdigitated epithelial cells which probably have an absorptive function. Based on TEM observations, Adrianov *et al.* (1993) report epithelial cells of the midgut of *P. kielensis* to contain bacteria as well as osmiophilic granules possibly filled with sulfur; therefore, the authors claim the bacteria to represent endosymbiotic, chemolithoautotroph sulfur oxidizer. However, neither have the bacteria been tested to possess the enzymes for sulfur oxidizing, nor have the electron-dense granules been checked for inclusion of sulfur, nor has the actual concentration of H₂S been measured in the exact sediment layer from which the kinorhynchs originate. Therefore, it cannot be excluded that the “endosymbiotic bacteria” represent ingested and partly digested bacteria.

Few gland cells and at least 2–5 monociliary receptor cells are interspersed with the epithelial cells. The receptor cells may be sensorimotor in function and may influence the net of longitudinal and circular muscle cells surrounding the midgut (Fig. 4F; Müller and Schmidt-Rhaesa, 2002) in order to facilitate peristalsis. The receptor cells may even assist in defecation, since some of them are in very close contact with the dilator muscle cells of the hindgut (Neuhaus, 1994). The midgut lumen may branch irregularly (*Z. floridensis*) or be triradiate (*Echinoderes aquilonius*, species of *Pycnophyes*; Fig. 1C).

The lumen of the hindgut may be triradiate (*E. aquilonius*) or a transverse broad slit (*Z. floridensis*, *Kinorhynchus phyllotropis*, *P. kielensis*, and *P. dentatus*) (Kristensen and Higgins, 1991; Neuhaus, 1994). Seven dilator muscle cells attach to the rectal epithelium (Figs. 1A, 2A, partly seen in 4E). During defecation, these muscles probably dilate the rectal lumen against the body pressure. Adrianov and Malakhov (1994: Fig. 86 A, B) report a “peritrophic membrane” in the anterior hindgut. However from their TEM images, the authors of this review conclude that the “peritrophic membrane” in fact represents the anteriormost part of the hindgut cuticle which at this point lacks the basal fine-granular cuticular layer that exists more posteriorly (*cf.* Neuhaus, 1994).

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FIG. 4. **A–D.** TEM longitudinal sections (**A, B**) and cross-sections (**C, D**). Originals B. Neuhaus. **E, F.** Confocal laser scanning microscope images of muscles labelled with phalloidin. Courtesy of M. Müller, Osnabrück. **A.** *Echinoderes* sp., two subsequent segments with dorsal longitudinal muscles attaching to pachyclus anteriorly and posteriorly. **B.** *Pycnophyes dentatus*, dorsal cuticular plate and intersegmental cuticle. **C.** *Paracentrophyes praedictus*, midventral connection (arrowhead) between the sternal plates of a trunk segment. Black dots in **B** and **C** are gold particles conjugated to the lectin wheat germ agglutinin (WGA). WGA binds specifically to N-acetyl-D-glucosamine and its polymers such as chitin. Control incubations (not shown) reveal that labelling results from binding to chitin. **D.** *Zelinkaderes floridensis*, midventral connection (arrowhead) between sternal plates and ventral longitudinal nerve cord. Asterisks mark cell processes of dorsoventral muscle cells to nerve cells. **E, F.** *Antygomonas* sp. **E.** Lateral view of the muscle system. **F.** Muscle net surrounding the midgut. Scale bar in **A–C** 1 μ m, in **D** 2 μ m, and in **E–F** 25 μ m.

Apicomplexa parasitize the gut of *Kinorhynchus yushini* and *Z. floridensis* (Adrianov *et al.*, 1993; Neuhaus, 1994).

REPRODUCTIVE SYSTEM

Sexes are separate, and asexual reproduction has never been demonstrated. Only males of some species show primary external sexual dimorphic characters (Higgins, 1974; Adrianov and Malakhov, 1999b; Neuhaus, 1999). In *Paracentrophyes praedictus*, *P. quadridentatus* and *Neocentrophyes intermedius*, two pairs of regular spines of the last trunk segments are probably transformed to penile spines. Two pairs of penile spines together with penial bristles (Fig. 3D) are assumed to have evolved *de novo* ventrally in species of *Pycnophyes* and *Kinorhynchus*. In *Echinoderes*, one pair of regular spines of the last trunk segment is transformed to penile spines, whereas two pairs may have evolved *de novo* laterally. Due to differences in position and morphology, penile spines are suspected to have been modified for reproduction several times independently (Neuhaus, 1999).

Secondary external dimorphic characters are found in the last two trunk segments. Middorsal, lateral or laterodorsal spines appear flexible in males of many Cyclorhagida, *Paracentrophyes praedictus*, *P. quadridentatus* and *Neocentrophyes intermedius* (Zelinka, 1928; Higgins, 1969b, 1983) but are rigid or missing in females. Male *Pycnophyes* and *Kinorhynchus* show one pair of weakly cuticularized (adhesive?) tubules on the 2nd trunk segment. Male and female of the same species may exhibit a different arrangement and number of sensory organs or tubules. Female gonopores (Fig. 3B) are more strongly sclerotized in Cyclorhagida than in Homalorhagida (Fig. 3D) but often more difficult to observe in males of both groups (Higgins, 1983, 1990).

One pair of saccate gonads lies between the dorsoventral muscles and the epidermis (Figs. 1A, 2A; Higgins, 1974). Spermatogonia at the anterior end of the male gonad differentiate to mature sperm cells in the caudal part of the gonad via spermatocytes and spermatids (Nyholm and Nyholm, 1983; Adrianov and Malakhov, 1999b). Mature spermatozoa are cigar-shaped and measure up to $\frac{1}{4}$ of the body length. In *Pycnophyes flaveolatus*, *P. communis*, *P. kielensis* and *Kinorhynchus phyllotropis*, a central elongated nucleus is surrounded by 3 types of vesicles. Opposite to the vesicles, tubes with a central electron-dense filament run along the entire length of each spermatozoon. An acrosomal structure is missing. One type of vesicle derives from mitochondria and may serve as a storage organelle of the female, the zygote, or for movements of the spermatozoon. The latter hypothesis is supported by the observations that a middle piece is lacking and that locomotory activity is limited to the anterior third of the cell. Whereas in *P. flaveolatus* and *P. communis* a short cilium with a $9 \times 2 + 2$ axoneme seems to originate at the posterior end of the mature sperm (Nyholm and Nyholm, 1983), Adrianov and Malakhov

(1999b) report for *P. kielensis* that the cilium starts at the anterior end accompanying the elongated sperm cell over its entire length.

In females, a pair of receptacula seminales joins the gonoducts from dorsally close to the genital aperture at the border of the 12th and 13th segment (Figs. 1A, 2A). Duct cells exhibit numerous microvilli anteriorly but a thin cuticular lining posteriorly. Epithelial cells envelop the receptacula and seem to surround the gonads in some species, whereas extracellular matrix alone covers the gonads in other species (Neuhaus, 1999). Ultrastructural observations are limited to *Zelinkaderes floridensis*, *Echinoderes aquilonius*, *P. flaveolatus*, *P. communis*, *P. kielensis*, and *K. phyllotropis* (Brown, 1983; Nyholm and Nyholm, 1983; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994; own unpublished observations).

A single, immense oocyte with numerous yolk vesicles and an enormous nucleus and nucleolus develops in the centre of each ovary. Smaller cells frontal and caudal of the giant oocyte are interpreted as immature germ cells which are probably resorbed during maturation of the gonad in *P. flaveolatus* and *P. communis* (see Nyholm and Nyholm, 1983). Sperm cells in the receptaculum seminis differ considerably from sperm in what has been called spermatophore (see below) and testis. In the receptaculum, 20–30 irregularly shaped sperm cells exhibit condensed or uncondensed, polymorphic nuclei, vesicles are smaller and fewer, and membrane-bound tubes each with a central electron-dense filament occur in the periphery of the cells. Nyholm (1977) considered these dramatic changes in the anatomy of sperm cells in *P. communis* “morphological hermaphroditism.” However, wall cells of the receptaculum may have been misinterpreted as spermatogonia which were assumed to differentiate to the sperm cells in the lumen of the receptaculum (Kristensen and Higgins, 1991); the authors of this review follow Kristensen and Higgins’s interpretation.

Fertilization is supposed to be internal, but data are quite scarce (Higgins, 1974; Needham, 1989). Penile spines of all species of *Echinoderes*, *Pycnophyes*, and *Kinorhynchus* appear to be rigid. These spines contain epidermal cells and ciliary sensory cells but no duct; muscles do not attach to them (Adrianov and Malakhov, 1999b; Neuhaus, 1999). Possibly, penile spines are inserted into the female gonopores by contraction of the trunk’s dorsoventral muscles, which increases the internal body pressure. In this way, the spines are assumed to keep the genital apertures open and to anchor males and females together. However, the flexible spines of *Paracentrophyes praedictus*, *P. quadridentatus* and *Neocentrophyes intermedius* may be exclusively sensory during copulation (Neuhaus, 1999).

Females of *Pycnophyes* and *Kinorhynchus* are often found with a brownish, mucous mass at their posterior end. The mass is usually covered by detritus and seems to contain one or two spherical bodies filled with about 140 intertwined spermatozoa and about the same number of spermatids. Therefore, this mass has been in-

terpreted as a spermatophore (Brown, 1983; Kristensen and Higgins, 1991).

Copulation has been observed only once, in *P. kielensis* by the first author. Here, the ventral posterior ends of male and female are directed towards each other with the heads of the animals facing opposite directions. A brownish mucous mass surrounds the posterior ends. From this observation, it is concluded that the spermatophore indeed originates from the substance secreted during copulation (Neuhaus, 1999).

EXCRETORY SYSTEM

The excretory system consists of one pair of protonephridia located dorsolaterally to the gut in trunk segments 8–9 (Figs. 1A, 2A). The protonephridia open laterally on trunk segment 9 through pores in the body cuticle (Horn, 1978; Neuhaus, 1988; Kristensen and Hay-Schmidt, 1989). In *Echinoderes aquilonius*, a protonephridium consists of 3 terminal cells each with 2 cilia, a single, long, non-ciliated canal cell, and a nephridiopore cell with many microvilli. Long microvilli filled with thin fibres constitute the common filtration area of all terminal cells (Kristensen and Hay-Schmidt, 1989). In *Pycnophyes kielensis*, a protonephridium is composed of 22 terminal cells each with two cilia, two biciliary canal cells, and a single, non-ciliated nephridiopore cell. The peripheral cytoplasmic walls of all terminal cells contain narrow, longitudinal openings and build up the compound filter with a common filtration area. The first juvenile stage of *P. kielensis* has a protonephridium with only three terminal cells, one canal cell, and one nephridiopore cell. Both in juvenile and adult *P. kielensis*, two or three accessory cells each with a modified cilium penetrate into the nephridiopore cell. Presumably, such cells are sensory in function (Neuhaus, 1988). Only 11 terminal cells, two canal cells, and one nephridiopore cell comprise the protonephridia of adult *P. greenlandicus* (Kristensen and Higgins, 1991).

BODY CAVITY

The spacious body cavity between the digestive system and the epidermis described by previous authors (Zelinka, 1928) represents an artifact of inappropriate fixation. The inner organs lie closely together, but fluid-filled gaps do exist especially in the anterior trunk segments. Numerous polymorphous amoebocytes occur in the interstices between the inner organs. The amoebocytes show electron-dense vesicles (Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994; Neuhaus, 1994). Whereas Adrianov and Malakhov (1994) suspect the electron-dense material in the body cavity to represent respiratory pigments, Neuhaus (1994) assumes such pigments in the osmiophilic vesicles of the amoebocytes. Extracellular matrix surrounds each inner organ and probably allows smooth gliding of the organs against each other while the animal moves or feeds. Surprisingly, Adrianov and Malakhov (1999a, p. 237) name the muscle net of the midgut “an incomplete myoepithelial lining” and the

amoebocytes “coelomocytes.” Such terminology is usually applied to taxa with a coelom or with a reduced coelom (e.g., Schmidt-Rhaesa *et al.*, 1998). However, the Russian authors do not indicate in the English text or in the English figure legends that they assume the body cavity of kinorhynchs to represent a reduced coelom.

DEVELOPMENT

The life history of kinorhynchs includes a series of six juvenile stages, which has been documented for *Zelinkaderes floridensis*, *Echinoderes bookhouti*, *Paracentrophyes praedictus*, *Pycnophyes kielensis*, *P. dentatus*, *P. beaufortensis*, and *Kinorhynchus phyllotropis* (Higgins, 1974, 1990; Brown, 1985; Neuhaus, 1993, 1995). In *Z. floridensis* and *Antygomonas oreas*, moulting has been observed in the adult (Higgins, 1990; Bauer-Nebelsick, 1996). Juveniles emerge from the egg with 9 of the adult 11 trunk segments well-defined. The remaining two segments are introduced in a subcaudal growth zone at various life history stages in different species. Also, the number of head scalids increases during ontogeny. Each scalid appears as a spinose anlage (=protoscalid) and differentiates in the next stage. Four rings of scalids develop in a subfrontal growth zone between the 1st and 2nd ring scalids of the 1st juvenile stage so that these 2nd ring scalids later become the 6th ring scalids (Neuhaus, 1995). The cuticle of juvenile stages is thin and weakly sclerotized, and sternal and tergal plates are barely recognizable in the light microscope (Higgins, 1974, 1990; Neuhaus, 1993, 1995; Adrianov and Malakhov, 1994).

PHYLOGENETIC RELATIONSHIPS

The phylogenetic relationships within the Kinorhyncha are far from being resolved. Characters substantiating the monophyly of Kinorhyncha are (1) the external and internal segmentation of the trunk (cuticular plates, paired lateral and unpaired middorsal spines, sensory spots, gland cells, nervous system, musculature); (2) the mouth cone as a ring-like epidermal fold around the pharynx with inner and outer oral styles, circular musculature, and basal nerve ring; and (3) the 7 dilators of the hindgut (Neuhaus, 1994). The latter publication also lists the characters in the ground pattern of the Kinorhyncha based on a phylogenetic analysis sensu Hennig (1966). Very similar characters have been considered to belong to the “morphological prototype” or “Ur-Kinorhyncha” by Adrianov and Malakhov (1996, p. 26; 1999a, p. 240). In addition, these authors “suggest evolutionary lines of primitive (plesiomorphic) and advanced (apomorphic) states of the main morphological characteristics” (Adrianov and Malakhov, 1996, p. 26; 1999a, p. 240). However, some of their evaluations of character state polarity are not at all clear.

Prior assumptions of radiation in Kinorhyncha by neoteny are dubious. *Cateria styx*, *C. gerlachi*, *Paracentrophyes praedictus*, *P. quadridentatus*, and *Neo-*

centrophyes intermedius have been regarded as “neotenus” due to their thin, juvenile-like cuticle with little morphological differentiation (e.g., Higgins, 1968; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1994, 1999a). Were this true, the above mentioned species should mature at an earlier developmental stage than the remaining species of Kinorhyncha. However *P. praedictus* clearly possesses 6 juvenile stages (Neuhaus, 1995) as in all other studied kinorhynch species (see above). This makes radiation by neoteny very unlikely, and it is rejected. Alternatively, species with a thin cuticle, and without overly distinct cuticular dorsal and ventral trunk plates may have inherited this character from the last common ancestor of the Kinorhyncha. Also, thickening of the cuticle may have occurred several times independently within Kinorhyncha, because species of the most closely related groups Priapulida and/or Loricifera (see below) do not possess a segmented trunk or subdivision of trunk segments into ventral and dorsal plates. A more detailed argumentation is given by Neuhaus (1995), but see also Adrianov and Malakhov (1996, 1999a).

Loricifera, Priapulida, and Kinorhyncha are unequivocally assumed to be most closely related and are, therefore, united in one group (Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus, 1994; Nielsen, 1995; Wallace *et al.*, 1996; Schmidt-Rhaesa *et al.*, 1998), named Scalidophora first by Lemburg (1995). Adrianov and Malakhov (1994, 1995, 1996, 1999a) postulate Nematomorpha to be grouped together with Priapulida, Loricifera, and Kinorhyncha in a taxon Cephalorhyncha. The following characters (Neuhaus, 1994) support the monophyly of the Scalidophora: (1) introvert with scalids, scalid arrangement staggered, scalids short and spinose and triradiate in cross-section, at least during the ontogeny, scalids with ciliary receptors; (2) introvert with inner and outer retractor muscles, muscles attaching via tancytes (=extremely elongated epidermal cells with intermediate filaments); (3) compound filter of protonephridia built by two or more terminal cells; (4) basally thickened, cuspidate spines; and (5) sensory organs (flosculi, sensory spots) with external cuticular micropapillae surrounding a central pore, few ciliary receptors, each receptor with 7–9 microvilli.

Conflicting evidence exists for every one of the three possible sistergroup relationships within the Scalidophora (Neuhaus, 1994). Characters supporting monophyly of a group Loricifera + Kinorhyncha: (1) scalids elongate and with articulation site between at least 2 elements; (2) trichoscalids with 2–3 basal plates; and (3) one pair of elongated, lateral cuspidate spines in the middle of the trunk at least during ontogeny. Characters supporting monophyly of a group Priapulida + Loricifera: (1) body divided into: introvert—neck region (concertina-like)—trunk with lorica (at least larval); and (2) urogenital system?. Characters supporting monophyly of a group Priapulida + Kinorhyncha: (1) pharynx 2-layered with inner epitheli-

um and outer muscle bulb, radial and ring muscles alternating; (2) pharynx protractor muscles; and (3) cilia of protonephridial terminal cells without circumciliary microvilli. No preference is given to any of the three alternating phylogenetic hypothesis mentioned above, because morphological and developmental data in all three taxa are not sufficient for a sound analysis.

Kinorhyncha have been included in the taxa Aschelminthes, Nematelminthes, Pseudocoelomata, and Cycloneuralia containing varying combinations and groups of Acanthocephala, Rotifera, Gastrotricha, Nematoda, Nematomorpha, Priapulida, and Loricifera (cf., discussions in Ruppert, 1991; Adrianov and Malakhov, 1995; Nielsen, 1995; Wallace *et al.*, 1996). From their analysis of 18S ribosomal DNA sequences, Aguinaldo *et al.* (1997) first suggested a taxon Ecdysozoa composed of all the moulting animals: Tardigrada, Onychophora, Arthropoda, Nematoda, Nematomorpha, Kinorhyncha, and Priapulida. Based on the same DNA sequence, Aleshin *et al.* (1998) conclude that Ecdysozoa is paraphyletic, whereas recent studies on hox genes and anti-horseradish peroxidase immunoreactivity in the central nervous system support the hypothesis of a taxon Ecdysozoa (Rosa *et al.*, 1999; Haase *et al.*, 2001). However, none of these molecular studies includes data from Loricifera and only a single gene has been sequenced from a single species of Kinorhyncha (*Pycnophyes kielensis*); Rosa *et al.* (1999) and Haase *et al.* (2001) considered Priapulida but not Loricifera and Kinorhyncha in their investigations. Morphological characters in congruence with the Ecdysozoa concept are (1) moulting of cuticle, induced by ecdysteroid hormones, (2) loss of locomotory cilia, (3) chitinous endocuticle, and (4) secretion of epicuticle by the tips of epidermal microvilli (cf., Schmidt-Rhaesa *et al.*, 1998).

Unfortunately, ecdysteroid hormones as one of the potentially strongest evidences for the Ecdysozoa hypothesis have not yet been reported for Tardigrada, Onychophora, Nematomorpha, Priapulida, and Kinorhyncha. The Ecdysozoa concept questions the traditional view of a taxon Articulata consisting of Annelida, Tardigrada, Onychophora, and Arthropoda (cf., discussion in Schmidt-Rhaesa *et al.*, 1998) and therefore evokes an ongoing debate about the phylogenetic relationships of Acanthocephala, Rotatoria, Gastrotricha, Nematoda, Nematomorpha, Priapulida, Kinorhyncha, and Loricifera (e.g., Wägele *et al.*, 1999; Zrzavý, 2001; Wägele and Misof, 2001).

ABBREVIATIONS

ar—articulation between cuticular plates of trunk; b—brain; cd—caudal dilatator; cm—circular muscle; cs—cuspidate spine; cu—cuticle; d—dilatator muscle of hindgut; dlg—dorsal longitudinal muscle; do—dorsal cuticular plate; dv—dorsoventral muscle; ep—epidermis; fd—frontal dilatator; g—gland cell; go—gonad; gop—gonopore; gp—gland pore; he—head; hg—hindgut; ho—head sensory organ; hr—head retractor; icu—intersegmental cuticle; iep—inner mouth cone

epithelium; if—intermediate filaments; in—interstitial cell; lg—longitudinal muscle; lts—lateroterminal accessory spine; lts—lateroterminal spine; mc—mouth cone; mg—midgut; mr—mouth cone retractor; mts—midterminal spine; ne—nerve cord; nr—nerve ring; ocu—overlapping cuticle of anterior segment; oe—oesophagus; oep—outer mouth cone epithelium; om—oblique muscle; oos—outer oral style; ope—outer pharyngeal epithelium; pa—pachycyclus; pb—pharyngeal bulb; pe—pharyngeal epithelium; peh—penial hairs surrounding male gonopore; pk—perikaryon of nerve cell; pl—placid of neck; pn—protonephridium; pp—pharynx protractor; ps—praepharyngeal sphincter; rd—circular dilatator; re—receptor cell; rm—radial muscle cell; rs—receptaculum seminis; se—seta; sp—sensory pore; sps—spinose spine; ss—spinoscalid; ssp—sensory spot; stp—sternal cuticular plate of trunk; s9—trunk segment 9; tm—trapezoidal muscle; tp—tergal cuticular plate of trunk; ve—ventral cuticular plate; vlg—ventral longitudinal muscle; vn—ventral nerve cord.

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