

Fine Structure of the Integumentary Cuticles and Alimentary Tissues of Pycnophyid Kinorhynchs *Pycnophyes oshoroensis* and *Kinorhynchus yushini* (Kinorhyncha, Homalorhagida)

Hirose, Euichi

Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus

Yamasaki, Hiroshi

Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus

<https://hdl.handle.net/2324/4773142>

出版情報 : Zoological Science. 32 (4), pp.389-395, 2015-08-01. Zoological Society of Japan
バージョン :
権利関係 : (c) 2015 Zoological Society of Japan



**Fine Structure of the Integumentary Cuticles and Alimentary Tissues of
Pycnophyid Kinorhynchs *Pycnophyes oshoroensis* and *Kinorhynchus yushini*
(Kinorhyncha, Homalorhagida)**

Author(s): Euichi Hirose and Hiroshi Yamasaki

Source: Zoological Science, 32(4):389-395.

Published By: Zoological Society of Japan

DOI: <http://dx.doi.org/10.2108/zs150021>

URL: <http://www.bioone.org/doi/full/10.2108/zs150021>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Fine Structure of the Integumentary Cuticles and Alimentary Tissues of Pycnophyid Kinorhynchs *Pycnophyes oshoroensis* and *Kinorhynchus yushini* (Kinorhyncha, Homalorhagida)

Euichi Hirose* and Hiroshi Yamasaki

Department of Chemistry, Biology and Marine Science, Faculty of Science,
University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

Integumentary and alimentary tissues were ultrastructurally examined in two pycnophyid kinorhynchs, *Pycnophyes oshoroensis* and *Kinorhynchus yushini*, to elucidate some aspects of their ecology. The body is entirely enveloped by an epicuticle layer with no gaps between cuticle plates and joints. The cuticular layer has a structure dense enough to prevent invasion by foreign organisms. The cuticular surface is overlaid by a mucus layer that may form a hydrophilic surface. The alimentary contents were heterogeneous, probably including some cellular components, such as chloroplast-like structures. Kinorhynchs likely break down food particles in the pharyngeal bulb by pressing it between the cuticulated epithelia. The pharyngeal crown was located in front of the pharyngeal bulb and had a thick wall with a striated sub-structure. Contraction of the pharyngeal bulb probably increases the internal pressure of the pharyngeal crown; this may be one reason for the thick wall of the pharyngeal crown. Nutrients appear to be taken up by midgut epithelial cells through both absorption via microvilli and endocytosis. Additionally, sperm tails in the testis of *P. oshoroensis* have unusual axonemes; i.e., an 18+9+2 pattern.

Key words: cuticle, mucus layer, pharyngeal crown, pharyngeal bulb, midgut contents, unusual axoneme

INTRODUCTION

Species of the phylum Kinorhyncha are marine, tiny (less than 1.3 mm), benthic animals with a body composed of a head, neck, and 11 trunk segments. To date, about 220 kinorhynch species have been identified worldwide and are classified into two orders, 10 families and 23 genera (Neuhaus, 2013; Dal Zotto et al., 2013; Sánchez et al., 2014). Kinorhynchs occur in various marine sediments; e.g., mud, fine sand and coarse shell sand, and are distributed in the intertidal zone to the abyssal depths from the polar to the equatorial regions. Along with harpacticoids and nematodes, kinorhynchs are one of the major metazoan groups in the marine meiobenthic environment (Jensen, 1983; Herman and Dahms, 1992; Santos et al., 2009; Grzelak and Kotwicki, 2012). Due to their abundance, they are thought to be significant in the understanding of marine ecosystems. However, the life of kinorhynchs is poorly understood, probably because of their very small body size and habitat. For instance, we rarely know what they eat and what they are eaten by, while kinorhynchs are often supposed to feed on detritus and bacteria (see Neuhaus, 1994) and there are a few records of kinorhynchs in alimentary systems of other benthic animals (see Martorelli and Higgins, 2004).

Tissue morphology is fundamental to understand the life of organisms. Integumentary tissues provide information about the life of the organism in its habitat, as it is a barrier and interface between the internal tissues and external environment. The integument of kinorhynchs and some other meiobenthic invertebrates may be unique among aquatic invertebrates because of their wettability: the body surface of kinorhynchs and some other meiobenthic invertebrates is exceptionally hydrophobic compared to other aquatic invertebrates, which generally have hydrophilic bodies (Cloney and Harrison, 1996). The alimentary tissue and its contents should provide data on dietary habit: what and how they eat, and whether they feed selectively or indiscriminately.

The fine structures of kinorhynchs have been reported for several species, including *Echinoderes aquilonius*, *E. cantabricus*, *E. capitatus*, *E. dujardini*, *E. hispanicus*, *Kinorhynchus phyllotropis*, *Pycnophyes communis*, *P. dentatus*, *P. flaveolatus*, *P. greenlandicus*, *P. kielensis* and *Zelinkaderes floridensis* (Merriman and Corwin, 1973; Nyholm and Nyholm, 1976, 1982; Neuhaus, 1988, 1994, 1997; Brown, 1989; Kristensen and Hay-Schmidt, 1989; Kristensen and Higgins, 1991; Nebelsick, 1992a, b, 1993; G^oOrdóñez et al., 2000). Considering that various kinorhynchs are widely distributed in diverse geographic areas and depths, accumulation of morphological information is important to better understand the kinorhynch life. We describe the fine structures of the integumentary and alimentary tissues of two pycnophyid kinorhynchs, *Pycnophyes oshoroensis* and *Kinorhynchus yushini*, which inhabit sandy mud in shallow

* Corresponding author. Tel. : +81-98-895-8880;
Fax : +81-98-895-8576;
E-mail: euichi@sci.u-ryukyu.ac.jp
doi:10.2108/zs150021

waters around the northwest Pacific.

MATERIALS AND METHODS

Sandy mud about 13 m below the sea surface was sampled in Oshoro Bay (Otaru, Hokkaido, Japan: 43°12'45.84"N, 140°51'21.88"E) on 6 October 2012, using the Ekman bottom sampler. Adults of *Pycnophyes oshoroensis* and *Kinorhynchus yushini* were collected by means of the bubbling and blot method (Higgins, 1988; Sørensen and Pardos, 2008). We had to maintain the specimens for a few days in filtered seawater with some other meiofaunal metazoans, such as nematodes, harpacticoid copepods, ostracods and tanaids, until we transferred and sorted the specimens in the laboratory. The kinorhynch specimens were selected under a stereomicroscope and fixed with 2.5% glutaraldehyde-0.45 M sucrose-0.1 M sodium cacodylate (pH 7.4). They were rinsed with 0.1 M cacodylate-0.45 M sucrose and post-fixed for 1.5 h in 1% osmium tetroxide-0.1 M cacodylate. The specimens were then dehydrated with ethanol, cleared with *n*-butyl glycidyl ether and embedded in epoxy resin (Agar Low Viscosity Resin, Agar Scientific Ltd.). Four adults of each species were sectioned: thick sections were stained with toluidine blue for light microscopy. Thin sections were stained with uranyl acetate and lead citrate, and examined in a transmission electron microscope (JEM1011, JEOL).

The terminology used in this study mainly followed that of Neuhaus (2013).

RESULTS

General morphology

The kinorhynch body consists of three parts: a head composed of a mouth cone with oral styles and introvert with scalids, a neck, and 11 trunk segments. The head is retractable into the body as shown in Fig. 1. In both species, segment 1 had four cuticular plates: one tergal plate covering the dorsal and both lateral sides of the body and three sternal plates covering the ventral side. Each of the following trunk segments had three cuticular plates: one tergal plate covering the dorsal and lateral sides and two sternal plates covering the ventral side. The cuticular plates were connected via joints to one another (arrowheads in Fig. 1C–F). In the toluidine blue stained sections, the cuticular plates are light blue, while the joints are dark blue (Fig. 1C–F). The alimentary tract was a simple, straight tube running down the center of the body. The alimentary system was comprised of a mouth cone, pharyngeal crown, pharyngeal bulb,

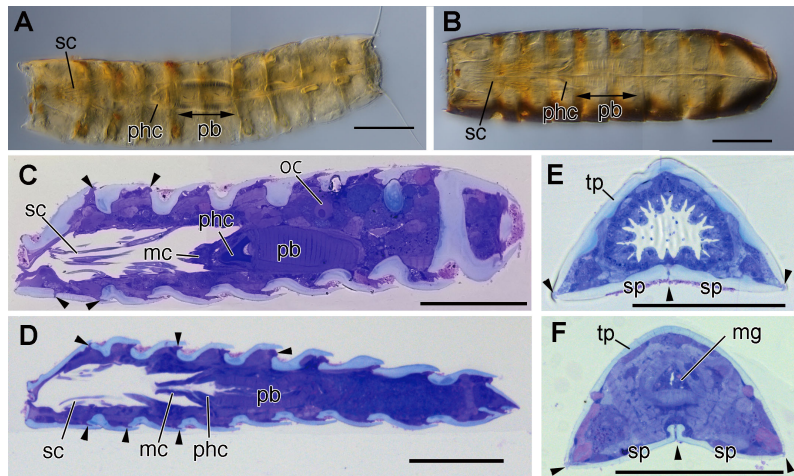


Fig. 1. Light micrographs of *Pycnophyes oshoroensis* (A, C, E) and *Kinorhynchus yushini* (B, D, F). Whole individuals (dorsal view) (A, B) and resin sections stained with toluidine blue (C–F). The heads face leftward in (A–D). (C, D): semi-sagittal sections. (E, F): cross-sections of the anterior and posterior parts of the trunk, respectively. Arrowheads indicate some of the joints between cuticular plates. jo, joint; mc, mouth cone; mg, midgut; oc, oocyte; pb, pharyngeal bulb; phc, pharyngeal crown; sc, scalids; sp, sternal plate; tp, tergal plate. Scale bars, 0.1 mm.

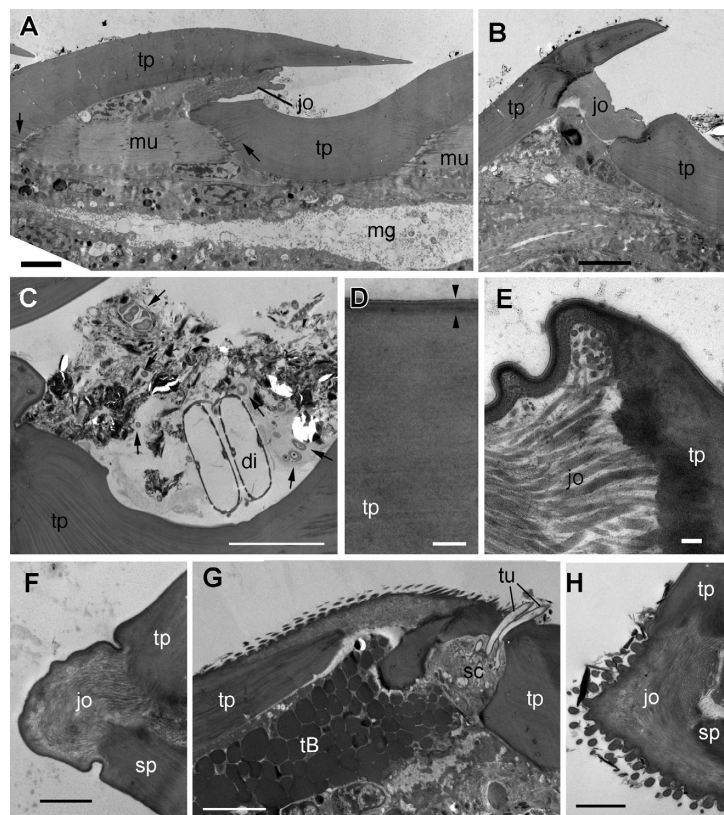


Fig. 2. Cuticular plates of the trunk of *Pycnophyes oshoroensis* (A, C–H) and *Kinorhynchus yushini* (B). (A, B). Semi-sagittal sections of tergal plates and joints in the posterior part of the trunk (A) and the first and second segments (B). Muscles (mu) rostrally attach to the plates with cuticular attachment fibers (arrows). (C) Cuticle surface of the tergal plate and muscles containing various materials, including bacterial cells (arrows) and diatoms (di). (D) Enlargement of the outer part of the cuticle. The epicuticle layer was comprised of thin, electron-dense layers (facing arrowheads). (E) Enlargement of the joint and tergal plate. There is no disconnect between the epicuticle layer between the joint and plate. (F) Cross-section of the joint between tergal and sternal plates of the first segment. (G) Sensory spot at the posterior part of the trunk. (H) Cuticular protrusions of the joint surface between tergal and sternal plates. jo, joint; mg, midgut; sc, sensory cell; sp, sternal plate; tB, type B cell; tp, tergal plate, tu, tubular hair. Scale bars: 5 μm in (A–C, G); 0.1 μm in (D, E); 1 μm in (F, H).

midgut, and hindgut, whereas we did not examine the hindgut ultrastructurally. (Fig. 1A–D).

Trunk cuticle

Cuticular plates were homogeneous, moderately electron-dense structures with a stratified sub-structure, with each plate connected by joints (Fig. 2A–C, F). Muscles also connected the plates antero-posteriorly (Fig. 2A). We found no considerable differentiation of the plates among the trunk segments. The surface of the cuticular plates was often covered with a mucus-like layer that densely contained unstructured debris, bacteria, diatoms, and so on (Fig. 2C). In closer observations, the matrix of the cuticular plate had a dense structure and the outermost part of the plate, i.e., epicuticle, is an electron-dense layer of 50–100 nm thick (Fig. 2D). The joint was comprised of a moderately electron-dense fibrous matrix and an electron-dense epicuticle that was continuous with the epicuticle of the cuticular plate (Fig. 2E, F). The fibrous matrices appeared to be in direct contact with the cuticular plate matrices (Fig. 2E).

The sensory spot was composed of three regions: matrix similar to the joint matrix, normal cuticular plate region and sensory cell. Spiny projections covered the epicuticle of the sensory spot (Fig. 2G). The projections were about 0.2 μm in diameter and about 2 μm in length. A sensory cell protruding tubular hairs, about 0.5 μm in diameter, was embedded in the cuticular plate at the posterior part of the sensory spot (Fig. 2G).

Minute cuticular protrusions about 0.1–0.3 μm in diameter densely covered the surface of the joint between the tergal and sternal plates (Fig. 2H), while the protrusion were not found in the first segment of the trunk (Fig. 2F).

Gland cells

There were two types of gland cells (type A and type B) beneath the cuticular plates (Figs. 2G, 3). In both types, small vesicles occupy the bulk of the cytoplasm. In type A cells (Fig. 3A, C), the vesicles contain fibrous materials that form electron-dense tubes about 50 nm in diameter in *P. oshoroensis* (Fig. 3B), and those contain fuzzy materials that do not form organized structures in *K. yushini* (Fig. 3D). In type B cells (Figs. 2G, 3E, 3F), vesicles were filled with homogeneous, electron-dense material in both species.

Alimentary elements

The apical part of the retracted introvert formed a mouth cone with the mouth opening at the apical tip (Fig. 4A, B). There were many, deep folds of weir on the inner wall of the mouth cone in *P. oshoroensis* (Fig. 4C). The mouth cone was followed by a pharyngeal crown with a thick wall that was well-stained by toluidine blue, especially in *P. oshoroensis* (Figs. 1C–D, 4A, –B). In our specimens, the lumen of the pharyngeal crown was filled with ingested materials in *P. oshoroensis* (Fig. 4A, D), while it was empty in *K. yushini*. The cuticular wall of the pharyngeal crown had a checkered sub-

structure (Fig. 4D and inset). The base of the pharyngeal crown attached directly to the apical end of the pharyngeal bulb, which consists of inner pharyngeal epithelium and muscle cells (Fig. 4A, B). The pharyngeal epithelium had a cuticular surface emerging the tufts of cuticular lamellae (Fig. 4E, F). In the examined specimens, the lumen of the pharyngeal bulb was a narrow tube (2 μm or less in diameter) and contained few materials other than the cuticular lamellae extending from the cuticle of the pharyngeal epithelium (Fig. 4F).

The contents of the lumen of the pharyngeal crown were heterogeneous. Clear vesicles of various sizes (from a few micrometers to 0.5 μm in diameter) occupied a major part of the contents and often contained smaller vesicles of 0.1- μm diameter or less (Fig. 4G). Moderately electron-dense material enveloped by irregularly shaped membranes appeared similar to cellular fragments and a stack of membranous structures was reminiscent of a chloroplast and/or a cyanobacterial cell (Fig. 4H). Occasionally, we found an aggregate of rods (ca. 0.1 μm in diameter) enveloped by concentric membranes (Fig. 4I). We did not find any unstructured debris.

The midgut lumen was not crowded by ingested material in the examined specimens (Fig. 5A–C). The midgut con-

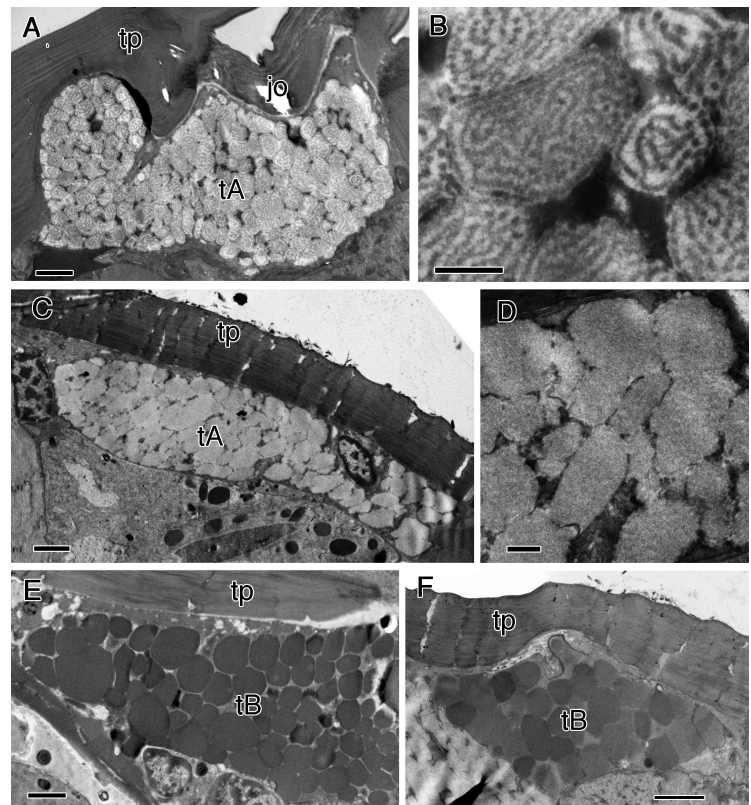


Fig. 3. Gland cells in *Pycnophyes oshoroensis* (A, B, E) and *Kinorhynch yushini* (C, D, F). (A) Type A cell beneath the joint between the segments in the anterior trunk (sagittal section). (B) Enlargement of the granular contents of the type A cell (cross-section). (C) Type A cell (cross-section). (D) Enlargement of the granular contents of the type A cell. (E) Type B cell beneath the sensory spot in the posterior part of the trunk (sagittal section). (F) Type B cell (cross-section). jo, joint; tA, type A cell; tB, type B cell; tp, tergal plate. Scale bars: 2 μm in (A, C, F); 0.5 μm in (B, D).

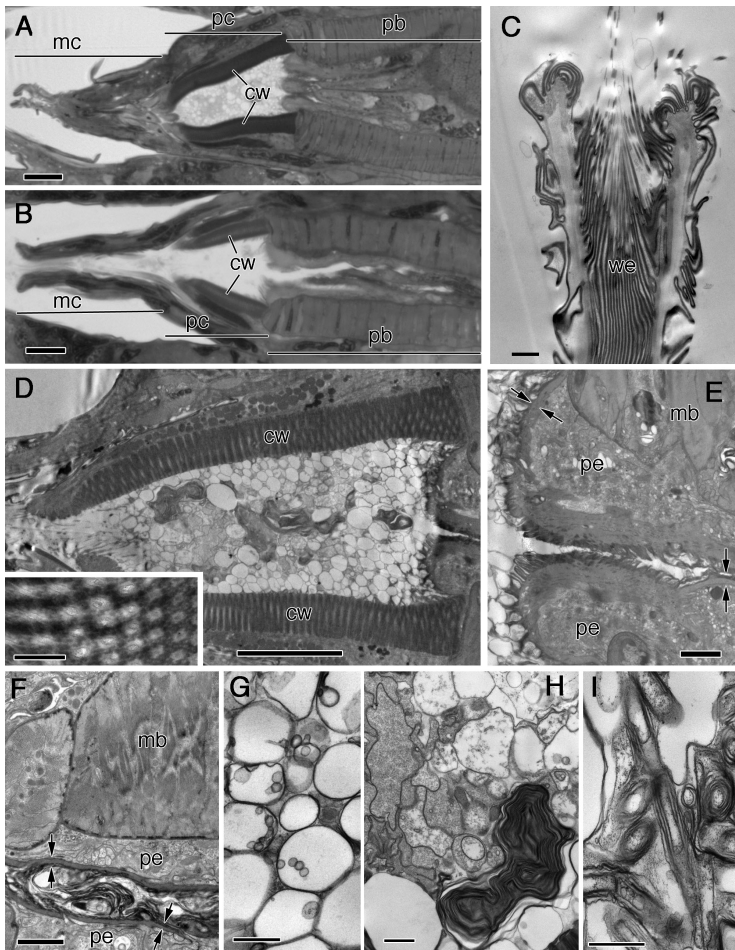


Fig. 4. Mouth and pharyngeal parts in *Pycnophyes oshoroensis* (A, C–I) and *Kinorhynchus yushini* (B). (A, B) Histological sections stained with toluidine blue. C, Transverse section of the mouth cone. (D) Pharyngeal crown and enlargement of the crown wall (inset). (E) Anterior opening of the pharyngeal bulb. (F) Muscles and tract of the pharyngeal bulb. Inner surface of the tract wall emerges cuticular lamellae. (G–I), Contents in the lumen of the pharyngeal crown. Facing arrows indicate the cuticular layer of the pharyngeal epithelium. cw, pharyngeal crown wall; mb, muscle of pharyngeal bulb; mc, mouth cone; pb, pharyngeal bulb; pc, pharyngeal crown; pe, inner pharyngeal epithelium; we, weir. Scale bars: 10 μ m in (A, B, D); 1 μ m in (C), inset of (D, H); 2 μ m in (E, F); 0.5 μ m in (G, I).

tents were heterogeneous, consisting mainly of clear vesicles, and multi-vesicular materials that were similar in structure to the contents of the pharyngeal crown. The midgut lumen was lined with epithelial cells with microvilli. In the anterior part of the midgut, the epithelial cells were cuboidal bearing dense microvilli (Fig. 5A), whereas epithelial cells were squamous, with the microvilli density decreasing towards the middle/posterior part (Fig. 5B–C). There were phagosome-like vacuoles in the epithelial cells and the contents were similar in structure to those in the midgut lumen (arrows in Fig. 5A, C).

Testis

The testis was observed in *P. oshoroensis* (Fig. 5D). Sperm cells at some developmental stages were apparently mixed in the testis. Some developing sperm had elongated heads in the testis, but it is uncertain whether they were

matured or not. In our sections, we could not find a spermatophore or its precursor. A cross-section of the sperm tail revealed an unusual axoneme; i.e., 18+9+2 pattern (Fig. 5D inset).

DISCUSSION

The cuticle as the outer-most interface

The exoskeleton of kinorhynchs is composed of cuticular plates and joints that interconnect each plate. The entire exoskeleton is a continuous cuticle layer of high electron-density, indicating that this layer may be a physical barrier to invasion by micro-organisms. In live specimens, the cuticular plates appear rigid and the joints appear elastic. This is consistent with the plate matrix having a dense structure and the joint matrix being a fibrous material of moderate electron density. Considering the continuity of cuticular layer among the segments, the kinorhynch trunk can be regarded as a continuous tube with repeats of rigid parts (cuticular plates) and elastic parts (joint).

The cuticle layer is partly covered with mucus containing diatoms, bacteria, unstructured debris, etc. It should be noted that some of the contents in the mucus were contaminated during the incubation in filtered seawater after the animal collection. In the examined species, gland cells were found beneath the plate-junction and are probably involved in mucus layer formation. G^aOrdóñez et al. (2000) described two types of gland cells (type 1 and type 2) in *Echinoderes cantabricus* and *E. hispanicus*; secretory vesicles in type 1 cells contain tubular material and those in type 2 cells contain filamentous material. We found two types of gland cell in *P. oshoroensis* and *K. yushini*. The secretory vesicles of the type A cells in *P. oshoroensis* contain tubular structures that are similar in structure to those in type 1 gland cells, whereas the secretory vesicles of the type A cells in *K. yushini* were filled with filamentous materials that are similar to those in type 2 gland cells. In both species, the secretory vesicles in the type B cells were filled with a homogeneous material. It is uncertain whether the type A cells in *P. oshoroensis* correspond to the type A cells in *K. yushini*, because the contents of the vesicles were different in structure between the two species. Since the ultrastructure of the gland cells remains unstudied in many kinorhynchs, caution should be exercised when classifying gland cells.

The body surface of kinorhynchs is thought to be hydrophobic, because kinorhynchs are easily trapped on the surface of seawater by bubbling a suspension of seawater and mud (Higgins, 1988; Sørensen and Pardos, 2008). According to Cloney and Hansson (1995), "Many interstitial invertebrates that do not swim near the surface are hydrophobic (E. E. Ruppert, personal communication)." The body surface of these animals is thus probably composed of hydrophobic materials. Although kinorhynchs are typical meiobenthic invertebrates, some species are known to inhabit intertidal sediments or tidal flats where they occasionally approach the water surface (e.g., Yamasaki and Fujimoto, 2014;

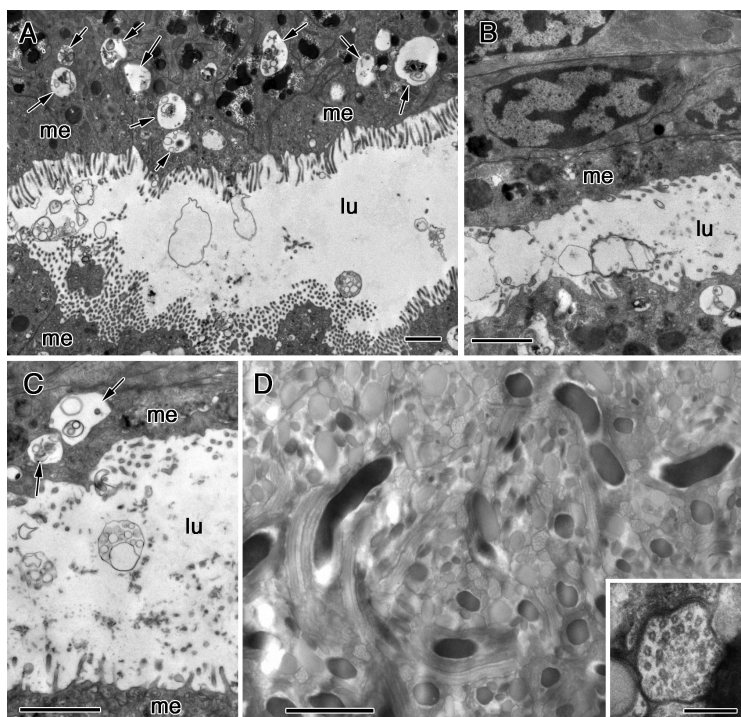


Fig. 5. Midgut of *Pycnophyes oshoroensis*: (A) around the 5th segment; (B) around the 7th segment; (C) around the 8th segment, and testis of *P. oshoroensis* (D). Enlargement of the sperm flagellum (inset in (D)). Arrows indicate phagosome-like vacuoles. lu, midgut lumen; me, midgut epithelium. Scale bars: 2 µm in (A–D); 0.2 µm in inset of (D).

Yamasaki et al., 2014). In these microenvironments, the attachment of bubbles to the body surface would be a potential problem causing unfavorable buoyancy, since the fluctuation of water temperature enhances bubble formation in water. Since more bubbles are able to attach to hydrophobic surfaces (Hirose et al., 2013), kinorhynchs may use a countermeasure to increase wettability of the body surface. This may be a possible function of the mucus layer enveloping the body. To determine this, it will be necessary to measure the wettability of kinorhynchs.

There were spiny projections on the cuticular surface at the sensory spot in *P. oshoroensis* and a sensory cell protruding tubular hairs was embedded in the cuticular plate (Fig. 2G). The spiny projections may protect the sensory cells from abrasion. Similar structures were described for the sensory spot in *Echinoderes capitatus* (Nebelsick, 1992a). Cuticular protrusions (ca. 0.1–0.3 µm in height) are often found on the joint surface between the tergal and sternal plates in *P. oshoroensis* (Fig. 2H). Yamasaki et al. (2012) reported that the lateral margins of the tergal plates are pilose in *P. oshoroensis* and the cuticular protrusions are probably sections of the pilose surface. A pilose surface at the joint between the tergal and sternal plates was also described in *Pycnophyes almansae*, *P. chalgap*, *P. cristatus*, *P. lageria*, *P. pardosi*, *P. smaug* and species of *Centroderes* (Neuhaus et al., 2013, 2014; Sánchez et al., 2013a, b). An array of cuticular protrusions similar in size is known as the comeal nipple array in moth eyes and provides an anti-glare function (e.g., Bernhard, 1967). Similar structures have also been described in various marine invertebrates, such as

annelids (Hausen, 2005), entoprocts (Nielsen and Jespersen, 1997; Iseto and Hirose, 2010), echinoderms (Holland, 1984), copepods (Østergaard and Bresciani, 2000; Hirose and Uyeno, 2014) and ascidians (Hirose et al., 1997). Several functions have been proposed for the nipple array in aquatic and endoparasitic environments, such as anti-reflection (Hirose et al., 2015), lubrication and anti-abrasion (Holland, 1984), and suppression of the host hemocyte activity (Ballarin et al., 2015). In the kinorhynchs, the array of protrusions is found in a limited area on the body surface and its function(s) is unknown. A hydrophilic nipple array has better bubble-repellency than a flat surface (Hirose et al., 2013), but the kinorhynch cuticle is thought to be hydrophobic.

Diet and digestion

The inner wall of the mouth cone was lined with deep, cuticular folds of weir in *P. oshoroensis*. The weir has been found in some kinorhynch taxa including *Pycnophyes*, and it is regarded as a filtering apparatus to exclude particles larger than bacteria (see Neuhaus, 1994). This may indicate that these kinorhynchs selectively feed on bacteria and/or small particles, but majority of the alimentary contents was not so small in *P. oshoroensis*.

The contents of the pharyngeal crown and midgut were heterogeneous and probably included some cellular components, indicating that kinorhynchs may consume some cellular materials. The chloroplast-like structure in the pharyngeal crown was possibly derived from algal cells (Fig. 4H). Since unstructured debris was not found among the alimentary contents, the kinorhynchs studied here may not be simple decomposers non-selectively breaking down organic materials. Although the mucus layer on the body surface usually contains bacterial cells and debris, they were not found in the alimentary contents. This indicates that the kinorhynchs do not use the mucus layer as a feeding apparatus. It is difficult to discuss the kinorhynch diet in detail based on the alimentary tract contents of a few specimens. Further investigation is necessary in more specimens and more species.

The pharyngeal crown has a thick wall with checkered sub-structures, forming a cone-shaped space in front of the pharyngeal bulb. We did not find any structures that would crush the food materials within the pharyngeal crown: this space may be used to stock the food collected by the movement of the introvert with scalds before physical digestion in the pharyngeal bulb. Some kinorhynchs, e.g., *Zelinkaderes floridensis*, are known to lack a pharyngeal crown (Neuhaus, 1994). The function of the pharyngeal crown may be related to the food items and the manner of feeding.

The epithelium lining the pharyngeal bulb has a thick cuticle with tufts of cuticular lamellae, and the thick muscle cells would tightly rub the epithelial cuticles together for physical digestion of the food. The tufts of the epithelial cuticle probably hold the food material. Similar structures, i.e., tufts of cuticle, have been described in other kinorhynchs, such as *Z. floridensis*, *Pycnophyes dentatus*, and *P. kiensis* (Neuhaus, 1994). The contraction of the pharyngeal bulb

probably increases the internal pressure of the pharyngeal crown, which may explain why the pharyngeal crown has a thick wall.

The structures of midgut epithelium in the present species were similar to those in *Z. floridensis*, *P. dentatus*, and *P. kielensis* (Neuhaus, 1994). The midgut epithelium has numerous microvilli, indicating that the epithelial cells absorb nutrients released from the food in the pharyngeal bulb. Uptake of the nutrients via microvilli appears to be carried out mainly in the anterior part of the midgut, because the density of microvilli decreases in the posterior part of the midgut. Moreover, the epithelial cells may directly endocytose the decomposed food particles, as the midgut epithelial cells have phagosome-like vacuoles and the vacuolar contents are similar in structure to the midgut contents. The epithelial cells also contained electron-dense granules that are probably secondary lysosomes, as annotated in Neuhaus (1994). Accordingly, kinorhynch probably ingest nutrients both by absorption via microvilli and endocytosis.

Unusual sperm axoneme

We found an unusual axoneme; i.e., 18+9+2 pattern, in the flagellum of the sperm in the testis of *P. oshoroensis*. This can be thought of as 18 accessory tubules surrounding a 9+2 array. In Kinorhyncha, sperm axoneme structures were described in two *Pycnophyes* species (*P. flaveolatus* and *P. communis*) (Nyholm and Nyholm, 1982, 1983). In these two species, sperm axonemes had a 9+2 pattern along most of the flagellum and an irregular pattern at the basal part of the flagellum. Although the irregular pattern was not described in detail, it seems to be 9+9+2 pattern based on figure 4 of Nyholm and Nyholm (1983). Since we could not find an 18+9+2 pattern in the descriptions by Nyholm and Nyholm (1983), the 18+9+2 pattern of *P. oshoroensis* is probably the first known example in Kinorhyncha. Moreover, Ferraguti and Garbelli (2006) reported unusual axonemes; i.e., 27+9+2 pattern, in the proximal flagellum of sperm in a Priapulida species (*Tubiluchus troglodytes*), which is often regarded as a sister group of Kinorhyncha (Mallatt and Giribet, 2006; Dunn et al., 2008; Sørensen et al., 2008). Unusual sperm axonemes are also known in some other ecdysozoans; the most common structure for the sperm axoneme is the 9+9+2 pattern in hexapods (reviewed in Dallai et al., 2006). Extreme modification of the axoneme pattern has been described in hexapod species and the number of accessory tubules is often identical to that of microtubular doublets. The 9+9+2 patterns in onychophorans and hexapods are regarded as a convergence, because the processes of accessory-tubule formation differ between the two taxa (Dallai and Afzelius, 1993). Although the diversity of axoneme structures may be a key to resolving ecdysozoan phylogeny, little data are available for kinorhynchs. It is important to investigate axoneme structures in other species to confirm whether the presence of accessory tubules in the sperm axoneme is common in kinorhynchs. Furthermore, the development of accessory tubules in kinorhynchs is still uncertain. It is thus difficult to discuss axoneme patterns from the viewpoint of both kinorhynch and ecdysozoan phylogeny at present.

CONCLUSION

The ecology of kinorhynchs is poorly understood, despite them being a major metazoan component of the marine interstitial environment. This fine structure study provides some morphological data, which provides new information about their ecology. An electron-dense cuticular layer envelops the body with no gaps between cuticular plates and joints, probably preventing infection by foreign organisms. The cuticular surface is covered with a mucus layer, which would make the body surface hydrophilic. The alimentary contents were heterogeneous, probably including some cellular components, and kinorhynchs probably break down food particles in the pharyngeal bulb by pressing them between the cuticulated epithelia. The nutrients appear to be taken up by midgut epithelial cells through both absorption via microvilli and endocytosis. However, it is uncertain whether these are common features in other kinorhynchs. Moreover, many kinorhynch species remain to be taxonomically described. It is necessary to examine other kinorhynch species from various habitats to better understand their ecology and roles in ecosystems.

ACKNOWLEDGMENTS

We thank Mr. K. Shibasaki (Oshoro Marine Station, Hokkaido University) for his assistance in collecting specimens. We are also indebted to Dr. M. V. Sørensen, Dr. Fernando Pardos, and the anonymous reviewer for their valuable comments. This study was supported by the "International Research Hub Project for Climate Change and Coral Reef/Island Dynamics" from the University of the Ryukyus.

REFERENCES

- Ballarin L, Franchi N, Gasparini F, Caicci F, Miyauchi A, Hirose E (2015) Suppression of cell-spreading and phagocytic activity on nano-pillared surface: *in vitro* experiment using hemocytes of the colonial ascidian *Botryllus schlosseri*. *Inv Surv J* 12: 82–88
- Bernhard CG (1967) Structural and functional adaptation in a visual system. *Endeavour* 26: 79–84
- Brown R (1989) Morphology and ultrastructure of the sensory appendages of a kinorhynch invertebrate. *Zool Scr* 18: 471–482
- Cloney RA, Hansson LJ (1996) Ascidian larvae: The role of test cells in preventing hydrophobicity. *Acta Zool* 77: 73–78
- Dal Zotto M, Di Domenico M, Garraffoni A, Sørensen MV (2013) *Franciscideres* gen. sp. — a new, highly aberrant kinorhynch genus from Brazil, with an analysis of its phylogenetic position. *Syst Biodivers* 11: 303–321
- Dallai R, Afzelius BA (1993) Development of the accessory tubules of insect sperm flagella. *J Submicr Cytol Path* 25: 499–504
- Dallai R, Lupetti P, Mancarelli C (2006) Unusual axonemes of hexapod spermatozoa. *Int Rev Cytol* 254: 45–99
- Dunn CW, Hejnol A, Matus DW, Pang K, Browne WE, Smith SA, et al. (2008) Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* 452: 745–749
- Ferraguti M, Garbelli C (2006) The spermatozoon of a 'living fossil' *Tubiluchus troglodytes* (Priapulida). *Tissue Cell* 38: 1–6
- G^aOrdóñez D, Pardos F, Benito J (2000) Cuticular structures and epidermal glands of *Echinoderes cantabricus* and *E. hispanicus* (Kinorhyncha, Cyclorhagida) with special reference to their taxonomic value. *J Morphol* 246: 161–178
- Grzelak K, Lech K (2012) Meiofaunal distribution in Hornsund fjord, Spitsbergen. *Polar Biol* 35: 269–280
- Hausen H (2005) Comparative structure of the epidermis in polychaetes (Annelida). *Hydrobiologia* 535/536: 25–35

- Herman RI, Dahms HU (1992) Meiofauna communities along a depth transect off Halley Bay (Weddell Sea – Antarctica). *Polar Biol* 12: 313–320
- Higgins RP (1988) Kinorhyncha. In “Introduction to the Study of Meiofauna” Ed by RP Higgins, H Thiel, Smithsonian Institution Press, Washington, pp 328–331
- Hirose E, Uyeno D (2014) Histopathology of a mesoparasitic hatschekiid copepod *in hospite*: Does *Mihbaicola sakamakii* (Copepoda: Siphonostomatoida: Hatschekiidae) fast within the host fish tissue? *Zool Sci* 31: 546–552
- Hirose E, Lambert G, Kusakabe T, Nishikawa T (1997) Tunic cuticular protrusions in ascidians (Chordata, Tunicata): a perspective of their character-state distribution. *Zool Sci* 14: 683–689
- Hirose E, Mayama H, Miyauchi A (2013) Does the aquatic invertebrate nipple array prevent bubble adhesion? An experiment using nanopillar sheets. *Biol Lett* 9: 20130552
- Hirose E, Sakai D, Shibata T, Nishii J, Mayama H, Miyauchi A, et al. (2015) Does the tunic nipple array serve to camouflage diurnal salps? *J Mar Biol Assoc UK* 95: 1025–1031
- Holland ND (1984) Echinodermata: epidermal cells. In “Biology of the Integument, 1 Invertebrates” Ed by J Bereiter-Hahn, AG Matoltsy, KS Richards, Springer, Berlin, pp 756–774
- Iseto T, Hirose E (2010) Comparative morphology of the foot structure of four genera of Loxosomatidae (Entoprocta): implications for foot functions and taxonomy. *J Morphol* 271: 1185–1196
- Jensen P (1983) Meiofaunal abundance and vertical zonation in a sublittoral soft bottom, with a test of the Haps corer. *Mar Biol* 74: 319–326
- Kristensen RM, Hay-Schmidt A (1989) The protonephridia of the arctic kinorhynch *Echinoderes aquilonius* (Cyclorhagida, Echinoderidae). *Acta Zool* 70: 13–27
- Kristensen RM, Higgins RP (1991) Chapter 10. Kinorhyncha. In “Microscopic anatomy of invertebrates, Vol. 4: Aschelminthes” Ed by FW Harrison, EE Ruppert, Wiley-Liss, New York, pp 377–404
- Mallatt J, Giribet G (2006) Further use of nearly complete 28S and 18S rRNA genes to classify Ecdysozoa: 37 more arthropods and kinorhynch. *Mol Phylogenet Evol* 40: 772–794
- Martorelli S, Higgins RP (2004) Kinorhyncha from the stomach of the shrimp *Pleoticus muelleri* (Bate, 1888) from Comodoro Rivadavia, Argentina. *Zool Anz* 243: 85–98
- Merriman JA, Corwin HO (1973) An electron microscopical examination of *Echinoderes dujardini* Claparède (Kinorhyncha). *Z Morphol Tiere* 76: 227–242
- Nebelsick M (1992a) Sensory spots of *Echinoderes capitatus* (Zelinka, 1928) (Kinorhyncha, Cyclorhagida). *Acta Zool* 73: 185–195
- Nebelsick M (1992b) Ultrastructural investigations of three taxonomic characters in the trunk region of *Echinoderes capitatus* (Kinorhyncha, Cyclorhagida). *Zool Scripta* 21: 335–345
- Nebelsick M (1993) Introvert, mouth cone, and nervous system of *Echinoderes capitatus* (Kinorhyncha, Cyclorhagida) and implications for the phylogenetic relationships of Kinorhyncha. *Zoomorphology* 113: 211–232
- Neuhaus B (1988) Ultrastructure of the protonephridia in *Pycnophyes kielensis* (Kinorhyncha, Homalorhagida). *Zoomorphology* 108: 245–253
- Neuhaus B (1994) Ultrastructure of alimentary canal and body cavity, ground pattern, and phylogenetic relationships of the Kinorhyncha. *Microfauna Mar* 9: 61–156
- Neuhaus B (1997) Ultrastructure of the cephalic sensory organs of adult *Pycnophyes dentatus* and of the first juvenile stage of *P. kielensis* (Kinorhyncha, Homalorhagida). *Zoomorphology* 117: 33–40
- Neuhaus B (2013) Kinorhyncha (=Echinodera). In “Handbook of Zoology. Gastrotricha, Cycloneuralia and Gnathifera. Volume 1: Nematomorpha, Priapulida, Kinorhyncha, Loricifera” Ed by A Schmidt-Rhaesa, De Gruyter, Berlin, pp 181–348
- Neuhaus B, Pardos F, Sørensen MV, Higgins RP (2013) Redescription, morphology, and biogeography of *Centroderes spinosus* (Reinhard, 1881) (Kinorhyncha, Cyclorhagida) from Europe. *Cah Biol Mar* 54: 109–131
- Neuhaus B, Pardos F, Sørensen MV, Higgins RP (2014) New species of *Centroderes* (Kinorhyncha: Cyclorhagida) from the Northwest Atlantic Ocean, life cycle, and ground pattern of the genus. *Zootaxa* 3901: 1–69
- Nielsen C, Jespersen A (1997) Chapter 2. Entoprocta. In “Microscopic anatomy of invertebrates, Vol. 13: Lophophorates, Entoprocta, and Cycliophora” Ed by Harrison FW, Woollacott RM, Wiley-Liss, New York, pp 13–43
- Nyholm KG, Nyholm PG (1976) Ultrastructure of the pharyngeal muscles of homalorhaga Kinorhyncha. *Zoon* 4: 121–130
- Nyholm KG, Nyholm PG (1982) Spermatozoa and spermatogenesis in homalorhaga Kinorhyncha. *J Ultrastruct Res* 78: 1–12
- Nyholm KG, Nyholm PG (1983) Kinorhyncha. In “Reproductive Biology of Invertebrates, Vol. 2, Spermatogenesis and Sperm Function” Ed by Adiyodi KG, Adiyodi RG, Wiley, Chichester, pp 207–220
- Østergaard P, Bresciani J (2000) SEM and TEM study of the integument of *Ophioika* sp. (Crustacea, Copepoda). *J Crust Biol* 20: 674–679
- Sánchez N, Herranz M, Benito J, Pardos F (2013a) *Pycnophyes almansae* sp. nov. and *Pycnophyes lageria* sp. nov., two new homalorhagid kinorhynchs (Kinorhyncha, Homalorhagida) from the Iberian Peninsula, with special focus on introvert features. *Mar Biol Res* 10: 17–36
- Sánchez N, Rho HS, Min W, Kim D, Sørensen MV (2013b) Four new species of *Pycnophyes* (Kinorhyncha: Homalorhagida) from Korea and the East China Sea. *Sci Mar* 77: 353–380
- Sánchez N, Pardos F, Sørensen MV (2014) A new kinorhynch genus, *Myxotophyes* (Kinorhyncha: Homalorhagida), from the Guinea Basin deep-sea, with new data on the family Neocentrophyidae. *Helgol Mar Res* 68: 221–239
- Santos PJP, Botter-Carvalho ML, do Nascimento-Júnior AB, Marinho RGC, Carvalho PVVC, Valença APMC (2009) Response of estuarine meiofauna assemblage to effects of fertilizer enrichment used in the sugar cane monoculture. Pernambuco, Brazil. *Braz J Oceanogr* 57: 43–55
- Sørensen MV, Pardos F (2008) Kinorhynch systematics and biology — an introduction to the study of kinorhynchs, inclusive identification keys to the genera. *Meiofauna Mar* 16: 21–73
- Sørensen MV, Hebsgaard MB, Heiner I, Glenner H, Willerslev E, Kristensen RM (2008) New data from an enigmatic phylum: evidence from molecular sequence data supports a sister-group relationship between Loricifera and Nematomorpha. *J Zool Syst Evol Res* 46: 231–239
- Yamasaki H, Fujimoto S (2014) Two new species in the *Echinoderes coulli* group (Echinoderidae, Cyclorhagida, Kinorhyncha) from the Ryukyu Islands, Japan. *ZooKeys* 382: 27–52
- Yamasaki H, Kajihara H, Mawatari SF (2012) First report of kinorhynchs from Hokkaido, Japan, including a new species of *Pycnophyes* (Pycnophyidae: Homalorhagida). *Zootaxa* 3425: 23–41
- Yamasaki H, Hiruta SF, Kajihara H, Dick MH (2014) Two kinorhynch species (Cyclorhagida, Echinoderidae, *Echinoderes*) show different distribution patterns across Tsugaru Strait, Northern Japan. *Zool Sci* 31: 421–429

(Received February 11, 2015 / Accepted April 11, 2015)