Zootaxa 1163: 1–47 (2006)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)
1163

Four palaeonemerteans (Nemertea: Anopla) from a tidal flat in middle Honshu, Japan

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Abstract

Four species of palaeonemerteans, including *Carinina plecta* sp. nov., *Callinera nishikawai* sp. nov., *Hubrechtella ijimai* comb. nov., and *Hubrechtella kimuraorum* sp. nov., are described from the Pacific coast of Honshu Island, Japan. The genus *Coeia* Takakura, 1922 is considered to be a subjective junior synonym of *Hubrechtella* Bergendal, 1902. *Carinina plecta* sp. nov. can be distinguished from other congeners by possessing a rhynchocoel wall principally composed of interwoven circular and longitudinal muscle fibres, a condition similar to that in another palaeonemertean genus, *Carinoma,* as well as certain members in the Hoplonemertea. *Callinera nishikawai* sp. nov. possesses a remarkable stylet-like apparatus in the proboscis, a feature that has never been reported for any other palaeonemertean. The characteristic 'tail' in *Hubrechtella* (= *Coeia*) *ijimai* comb. nov. can be regarded as a heterochronic retention of the structure that appears only in juveniles of other congeners. *Hubrechtella kimuraorum* sp. nov. has a broad U-shaped blood lacuna in the foregut region before it ramifies into a vascular plexus.

Key words: 3D-reconstruction, DeltaViewer, Bergendal's region, taxonomy

Introduction

Nemerteans in the order Palaeonemertea are exclusively marine benthic dwellers, currently considered to comprise a paraphyletic group on the basis of recent molecular phylogenetic studies (Sundberg *et al.* 2001; Thollesson & Norenburg 2003). Ninety species of palaeonemerteans have previously been described worldwide (Gibson 1995, 1997, 2002; Senz 1997, 2000; Chernyshev 1999, 2002, 2003; Gibson & Sundberg 1999; Cantell 2001), twelve of which have been reported from Japanese waters (Takakura 1898, 1922; Yamaoka 1940; Iwata 1951, 1952, 1954a, b, 1957; Senz 1997; Shimomura *et al.* 2001). The present paper describes three new and one previously described palaeonemertean spe-

Accepted by R. Gibson: 6 Jan. 2006; published: 30 Mar. 2005

cies from a tidal flat at the outlet of Lake Hamanako, a brackish lake in Shizuoka Prefecture, middle Honshu, Japan. This is the first report of nemerteans from this locality.

Material and Methods

Specimens were collected by the author on 31 July and 1 August 2003 from a sandy to muddy tidal flat on Ikarise Island (34º41’04’’N, 137º35’59’’E), located in a channel forming the outlet of Lake Hamanako, Shizuoka Prefecture, Honshu, Japan, to the Pacific Ocean (Figs 1A–D). Nemerteans were dug out of the sediment at ebb tide. Specimens for histological studies were anaesthetized in a $MgCl_2$ solution isotonic to seawater, fixed in Bouin’s fluid for 12 hours, and preserved in 70% EtOH. Later they were dehydrated in 100% EtOH, cleared in xylene, and embedded in paraffin wax (m.p. 56–57 ºC). Sections cut at 6–10 µm thickness were stained with Mallory’s trichrome method (Gibson 1994). Additional material was collected by Dr Taeko Kimura, Mr Sho-ichi Kimura, and Professor Teruaki Nishikawa on 12 May 2002 at the same locality.

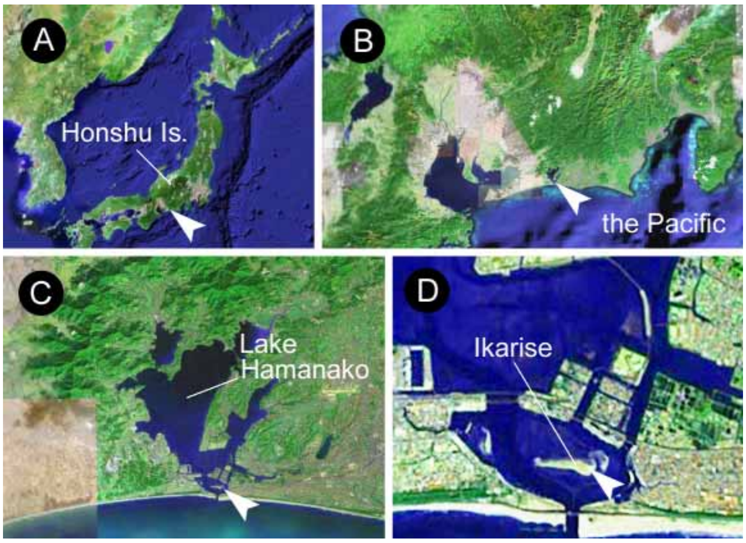

FIGURE 1. A–D, maps showing the sampling locality; arrowheads indicate the sampling site (modified from ©2005 Google Earth images).

The ratio of epidermal thickness to body diameter in the brain and intestinal regions was calculated as the index E,

$$E = (\alpha + \beta + \gamma + \delta) / 2 (A + B)$$

where A and B are, respectively, the maximum width and height of the body in a given cross section on a slide (see Fig. 2); α, β, γ and δ are the thicknesses of the epidermis measured from the basement membrane to the surface of the epidermis on the left, right, dorsal, and ventral sides of the body, respectively. *E* (*b*) and *E* (*i*) represent *E* values in the brain and intestinal regions respectively; *E* (*b*) was calculated at the point where the ventral commissure assumes its maximum thickness, while *E* (*i*) was obtained from a single section randomly selected from the intestinal region of the body.

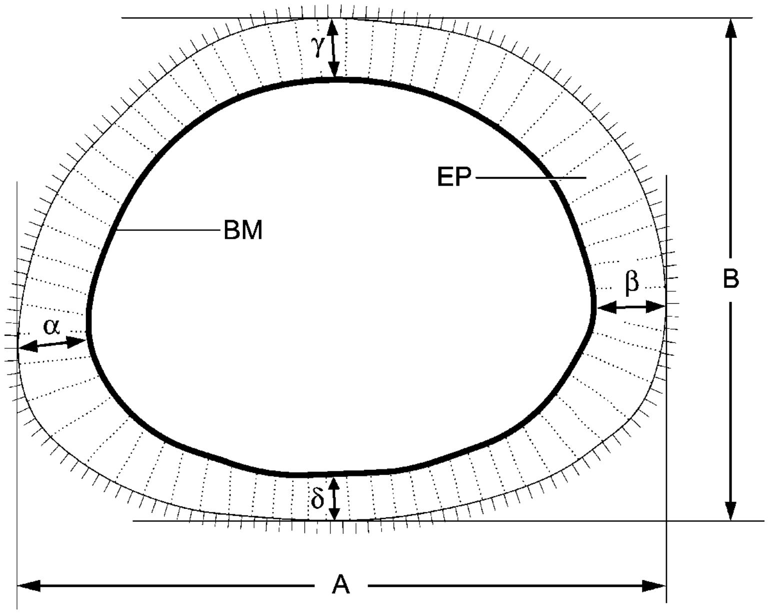

FIGURE 2. Schematic paleonemertean cross section showing the measurements used in calculating the epidermal height to body diameter indices. A, width of the body; B, height of the body; α, β, γ, and δ, the thicknesses of the epidermis on the left, right, dorsal, and ventral sides of the body; BM, basement membrane; EP, epidermis.

Terminology relating to the epidermal glandular cell types in *Hubrechtella* follows the usage of Hylbom (1957).

DeltaViewer 2.0.8 software (Wada *et al.* 2002) was used for the reconstruction of three-dimensional morphology from digital images of serial sections.

All the material used in this study is deposited in the Hokkaido University Museum, Sapporo, Japan, with collection catalogue numbers prefaced by ZIHU.

In the text, the International Commission on Zoological Nomenclature and the International Code for Zoological Nomenclature, respectively, are referred to as 'ICZN' and 'Code'.

Family Tubulanidae Bürger, 1904 (1874)

Carinellidae McIntosh, 1874: 137.
Tubulanidae Bürger, 1904a: 10; Bürger, 1897–1907: 401, 402, 405.

Nomenclatural remarks

In response to Melville's (1986) proposal, ICZN (1988) ruled under Article 40b of the third edition of the Code (ICZN 1985) that the name Tubulanidae has precedence over, but takes the date of, its senior subjective synonym Carinellidae. In his proposal, Melville (1986) stated that the name *Carinella trilineata* "has been regarded as a synonym of *Tubulanus polymorphus* since at least 1905", referring to Bürger (1897–1907), and that the family Tubulanidae "should be cited with the date '1905 (1874)'". The ICZN's ruling, basically following Melville's proposal, states that "the name Tubulanidae Bürger, 1905 (1874) ... is hereby placed on the Official List of Family-Group Names in Zoology".

Bürger's (1897–1907) book was published in six different parts, and Melville (1986) was correct in that the relevant part about the replacement of Carinellidae with Tubulanidae was published in 1905 (pp. 401, 402, 405). However, the name Tubulanidae had already appeared prior to 1905 in Bürger (1904a). Thus the family name should be cited as "Tubulanidae Bürger, 1904 (1874)", with the date of priority being enclosed in parentheses in accordance with Recommendation 40A of the Code (ICZN 1985, 1999).

Genus *Carinina* Hubrecht, 1885

Carinina Hubrecht, 1885: 830.
Procarinina Bergendal, 1902a: 422, synonymised by Hylbom (1957).

Type species

Carinina grata Hubrecht, 1887, by monotypic designation; first mentioned in Hubrecht (1885) but with no specific epithet given.

Diagnosis

Brain and lateral nerve cords situated in epidermis; body-wall musculature consisting of outer circular, diagonal (occasionally), longitudinal, and inner circular muscle layers; longitudinal muscle plate present between rhynchocoel and alimentary canal; rhynchocoel wall consisting only of circular muscle layer in many species, but is occasionally composed of thin inner longitudinal muscle layer and outer layer of interwoven longitudinal and circular muscle fibres; excretory organs with gland-like anterior parts in close connection with lateral blood vessels, nephridial canals running posteriorly from the gland-like portions inside body-wall inner circular muscle layer, at their rear turning at right angles towards body surface to open at an excretory pore; two proboscis nerves extend forward from ventral commissure of brain, entering rhynchodaeum in tip of head; no eyes.

Carinina plecta **sp. nov. (Figs 3–10)**

Diagnosis

Carinina with rhynchocoel wall composed of inner longitudinal muscle layer and outer layer of interwoven circular and longitudinal muscle fibres; proboscis insertion originating only from rhynchocoel inner longitudinal muscle layer; interwoven layer of rhynchocoel wall continues to rhynchodaeum; a pair of rhynchocoel vessels present, communicating with lateral blood vessel via a ladder-like series of transverse connectives.

Etymology

The specific epithet, derived from the Greek *plectos* (= basket), refers to the nature of the rhynchocoel wall primarily composed of interwoven circular and longitudinal muscle fibres.

Material examined

Holotype, female, caudal end missing, ZIHU-3123, 31 July 2003, HK coll.; 6-µm transverse serial sections through the head region, the middle body, and the posterior end of the fragment, 10-µm longitudinal serial sections in the two intervening portions, 116 slides in total.

External features

The body fragment obtained was 13 cm long, 1 mm wide. When alive (Fig. 3A), the nemertean was uniformly a translucent white with no colour pattern; the intestine was light beige and the ovaries tinged with a khaki colour. When the animal was placed in seawater in a Petri dish its movement was very sluggish. The head is wider than the neck, rounded anteriorly, variable in shape during movement; a white-coloured, cylindrical rhynchodaeum could be seen through the epidermis (Fig. 3B).

Body wall, musculature, and parenchyma

Four major types of glandular cells are distinguishable in the epidermis by their staining affinity to the Mallory’s trhichrome method: these are identified as type G1) red granular cells with acid fuchsin positive cytoplasm; type G2) granular cells with Orange G positive cytoplasm, orange in colour with various degrees of chromatic luminosity; type G3) violet granular cells; and type G4) light-blue basophilic mucous cells (Fig. 3C).

Anterior to the brain, the epidermis is 40–80 µm thick and contains all four types of glandular cells situated proximally; glandular cells are sparse at the tip of the head but gradually increasing in number posteriorly. In front of the brain, type G1 glandular cells are predominant mid-ventrally, while the other three types appear over the rest of the circumference of the epidermis (Fig. 3C).

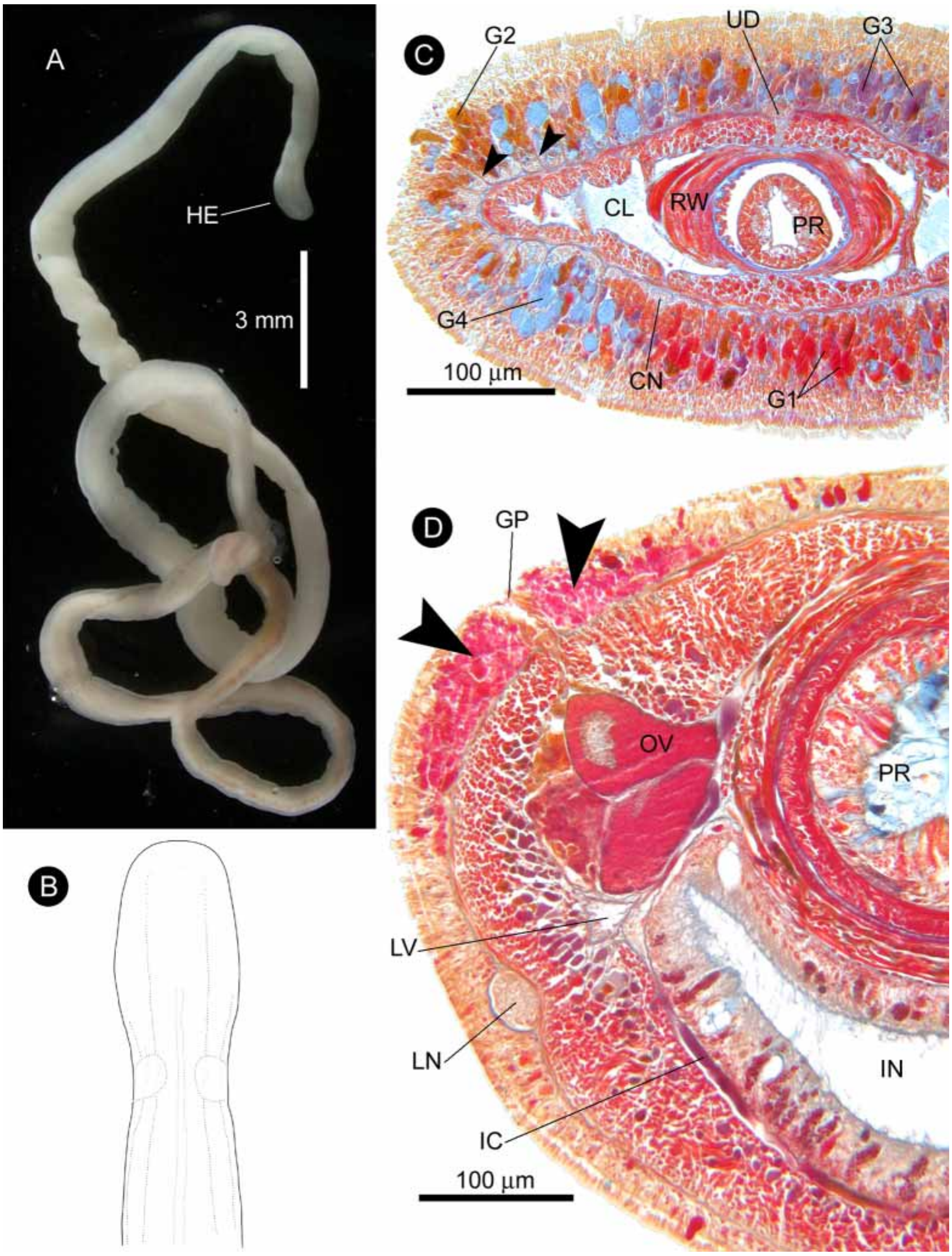

FIGURE 3. *Carinina plecta* sp. nov. Holotype, female, ZIHU-3123. A, photograph of the holotype taken in life; the caudal end of the body is missing. B, the natural shape of the head in creeping mode. C, transverse section through the pre-cerebral region showing the various glandular cell types found in the epidermis; the arrowheads indicate connective tissue processes from the basement membrane. D, transverse section through the intestinal region; note the acidophilic granular accumulation in the epidermis around the gonopore (indicated by the arrowheads). CL, cephalic blood lacuna; CN, cephalic peripheral nerve; G1, acid-fuchsin-positive glandular cell; G2, glandular cell stained with an orange colour; G3, glandular cell stained with a purple colour; G4, basophilic glandular cell; GP, gonopore; HE, head; IC, body-wall inner circular muscle layer; IN, intestine; LN, lateral nerve cord; LV, lateral blood vessel; OV, ovum; PR, proboscis; RW, rhynchocoel wall; UD, upper mid-dorsal nerve.

In the brain region, the epidermal thickness increases up to 130 µm, the glandular cells occupying the proximal 2/3 to 3/4 of the epidermis; type G1 glandular cells form a median mass situated between the ventral cerebral ganglia. In front of the mouth (Fig. 4), this acidophilic glandular mass splits into three lobes, the middle branch leading to the mid-dorsal side of the buccal epithelium and the lateral ones remaining confined to the epidermis; these branches soon disappear into the anterior portion of the mouth opening.

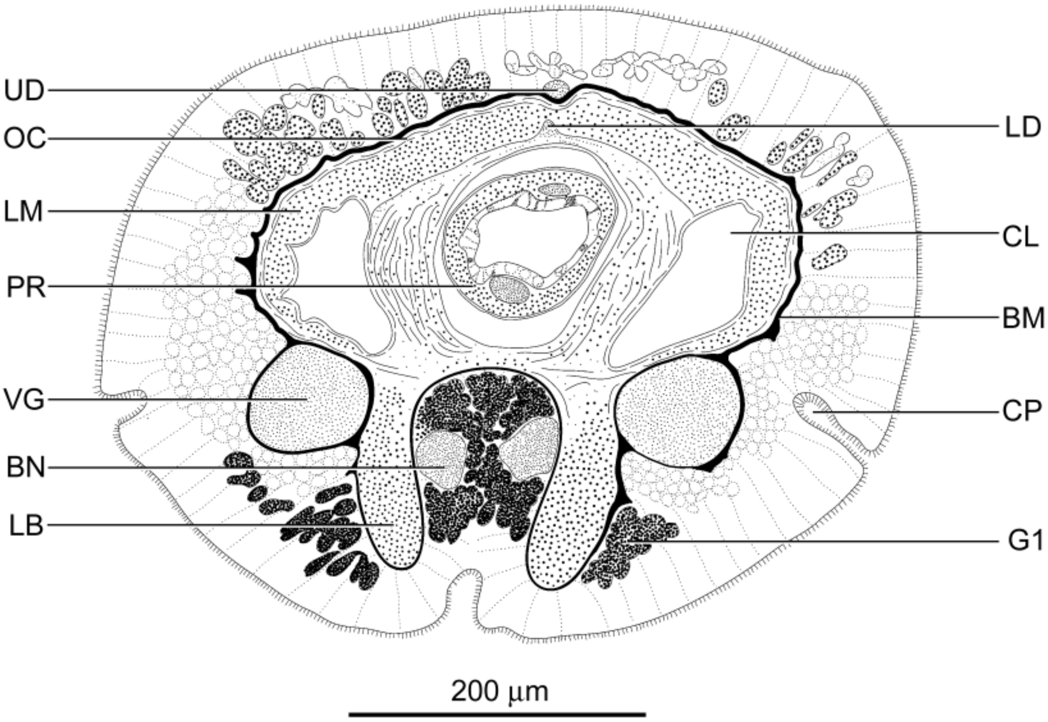

FIGURE 4. *Carinina plecta* sp. nov. Holotype, female, ZIHU-3123. Drawing of a transverse section through just anterior to the mouth. BM, basement membrane; BN, buccal nerve; CL, cephalic blood lacuna; CP, cerebral sensory organ pit; G1, acid-fuchsin-positive glandular cell; LB, longitudinal muscles below horizontal transverse muscles; LD, lower mid-dorsal nerve; LM, body-wall longitudinal muscle layer; OC, body-wall outer circular muscle layer; PR, proboscis; UD, upper mid-dorsal nerve; VG, ventral cerebral ganglion.

In the foregut region, type G4 basophilic mucous cells predominate on all sides of the body and occupy the proximal half of the epidermis, whereas the acidophilic granular glands are scattered and mostly situated in the proximal 1/4 of the epidermis. There is an exclusively acidophilic epidermal region in front of the excretory system. Glandular cells decrease in number posteriorly, but in the intestinal region an acidophilic glandular zone is present around each gonopore (Fig. 3D). *E* (*b*) = 0.25; *E* (*i*) = 0.04.

The epidermal basement membrane (Figs 4, 5) is up to 3 µm thick; connective tissue processes extend from the basement membrane into the epidermis in the brain region without forming a mesh-like network (Fig. 3C); a thin neural layer is present between the epidermis and basement membrane.

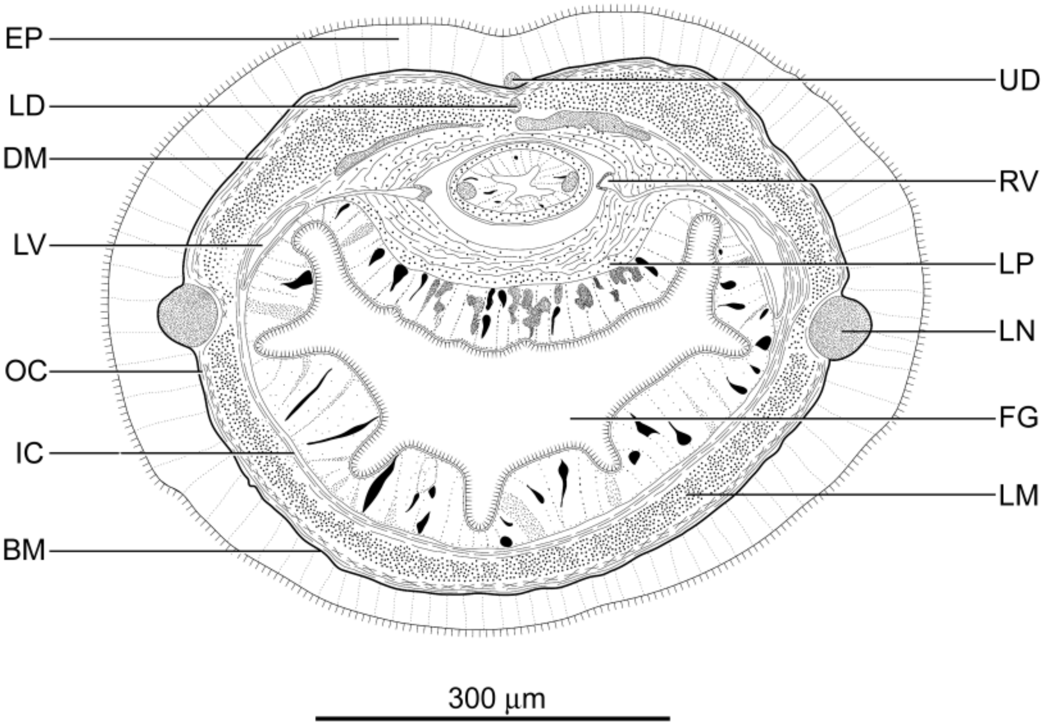

FIGURE 5. *Carinina plecta* sp. nov. Holotype, female, ZIHU-3123. Drawing of a transverse section through the foregut region. BM, basement membrane; DM, diagonal muscle layer; EP, epidermis; FG, foregut; IC, body-wall inner circular muscle layer; LD, lower mid-dorsal nerve; LM, body-wall longitudinal muscle layer; LN, lateral nerve cord; LP, longitudinal muscle plate between rhynchocoel and alimentary canal; LV, lateral blood vessel; OC, body-wall outer circular muscle layer; RV, rhynchocoel vessel; UD, upper mid-dorsal nerve.

Anterior to the mouth, the body-wall musculature consists of outer circular and inner longitudinal muscle layers; a diagonal layer is situated between these two layers in all parts of the body except in the brain region (Fig. 6A). Immediately posterior to the mouth, circular muscles appear lateral to the buccal epithelium and then extend dorsally and ventrally outside the lateral blood vessel. Behind the mouth, these lateral bands of circular muscles meet mid-dorsally and mid-ventrally to form the inner circular muscle layer of the body wall.

Immediately anterior to the ventral cerebral commissure, horizontal transverse muscle fibres appear below the rhynchocoel and above the dorsal side of the body-wall longitudinal muscle layer (Fig. 6B); these horizontal fibres then connect laterally with the outer circular muscle layer of the body wall.

The longitudinal body-wall muscles below these horizontal fibres branch off ventrally from the rest of the longitudinal muscle layer to form a muscle bundle medial to each of the ventral ganglia. Just anterior to the mouth opening, this longitudinal muscle bundle diverges into a branch on each side (Fig. 4). The branches then extend lateral to the buccal cavity as the mouth opens (Fig. 6C).

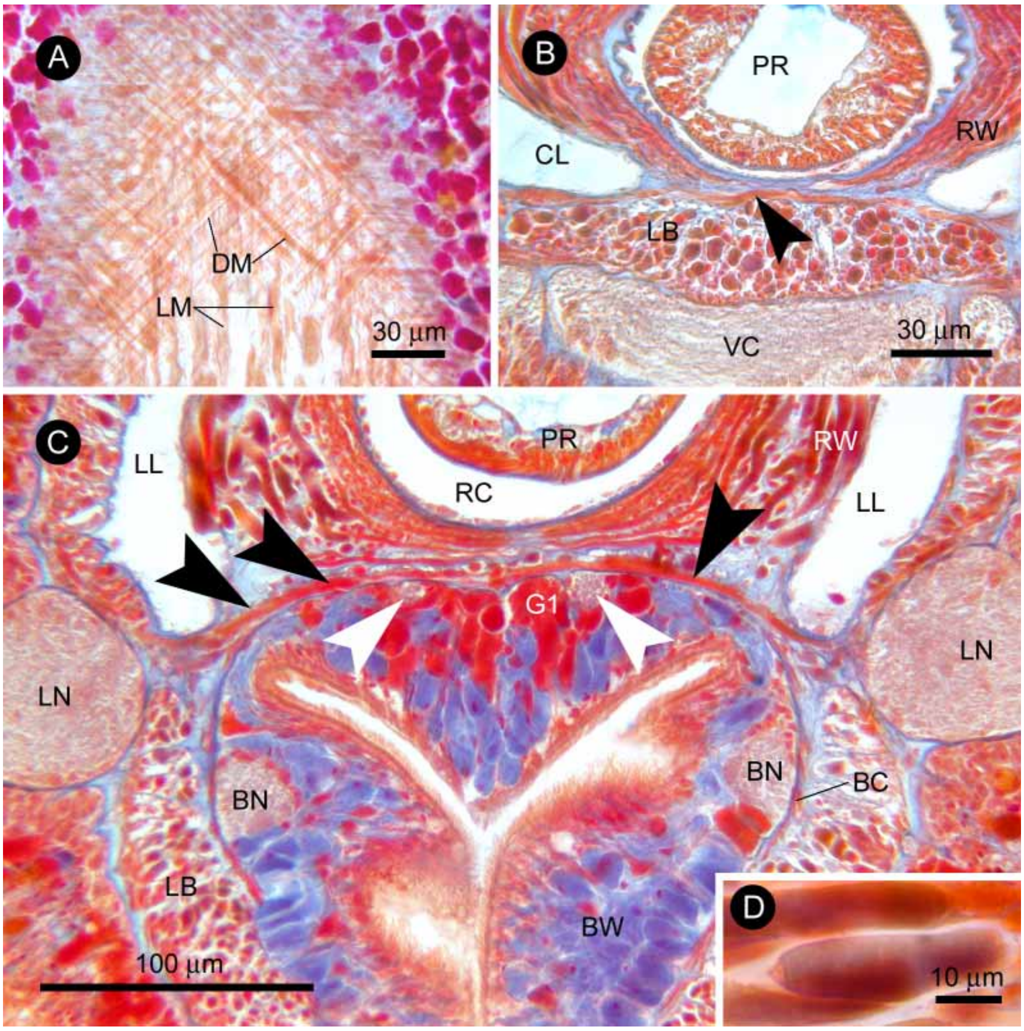

FIGURE 6. *Carinina plecta* sp. nov. Holotype, female, ZIHU-3123. A, tangential section of the body wall showing diagonal muscles (DM). B, transverse section through the anterior portion of the ventral cerebral commissure (VC) showing horizontal transverse muscles below the rhynchocoel (indicated by the arrowhead). C, transverse section through the vicinity of the mouth region; black arrowheads indicate horizontal transverse muscle fibres, white arrowheads indicate branches of the buccal nerves. D, sagittal section through the intestinal region to show one of the aberrantly thick muscle fibres. BC, buccal circular muscle; BN, buccal nerve; BW, buccal wall; CL, cephalic blood lacuna; G1, acid-fuchsin-positive glandular cell; LB, longitudinal muscles below horizontal transverse muscles; LL, lateral blood lacuna; LM, body-wall longitudinal muscle layer; LN, lateral nerve cord; PR, proboscis; RC, rhynchocoel; RW, rhynchocoel wall.

The horizontal transverse muscle fibres beneath the rhynchocoel remain above the buccal cavity in contact with both the circular muscles around the buccal epithelium and the body-wall circular muscle layer (Fig. 6C). These horizontal transverse muscles disappear dorsal to the buccal cavity, and the longitudinal muscles lateral to the buccal cavity fuse with the body-wall longitudinal layer in the dorsal side of the body. A longitudinal muscle plate is present between the rhynchocoel and alimentary canal (Fig. 5), emerging from the region dorsal to the buccal cavity but disappearing in the intestinal region. Unusually thick muscle fibres, up to 10 µm thick, appear in the rhynchocoel wall, body-wall inner circular muscle layer, and proximal portion of the longitudinal muscle layer (Fig. 6D). No dorsoventral musculature was found post-cerebrally.

Parenchymatous connective tissues, though present throughout the body, are not well developed.

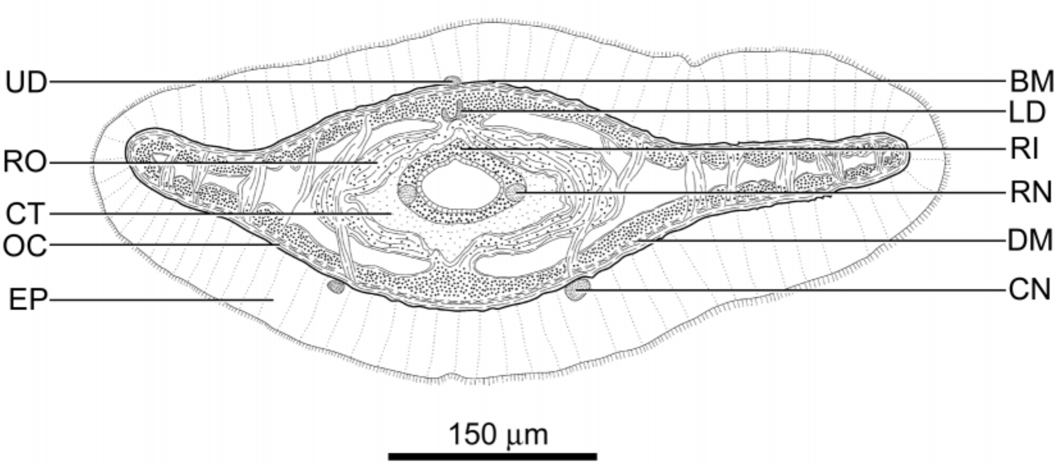

FIGURE 7. *Carinina plecta* sp. nov. Holotype, female, ZIHU-3123. Drawing of a transverse section through the rhynchodaeal region. BM, basement membrane; CN, cephalic peripheral nerve; CT, connective tissue; DM, diagonal muscle layer; EP, epidermis; LD lower mid-dorsal nerve; OC, body-wall outer circular muscle layer; RI, rhynchodaeal inner longitudinal muscle layer; RN, rhynchodaeal nerve; RO, rhynchodaeal outer muscle wall, composed of interwoven circular and longitudinal muscle fibres; UD, upper mid-dorsal nerve.

Proboscis apparatus

The proboscis pore opens mid-ventrally near the tip of the head, leading posteriorly to the rhynchodaeum, which consists of 1) an inner non-ciliated epithelium up to 5–10 µm thick, 2) a longitudinal muscular stratum, 10–20 µm thick, in which a pair of rhynchodaeal nerves is situated, 3) a connective tissue layer 5–30 µm thick, and 4) an outer muscle wall composed of interwoven longitudinal and circular muscle fibres, which leads posteriorly to the rhynchocoel wall. In the region where the rhynchodaeum leads dorsally from the ventral body-wall muscle layer, just behind the proboscis pore, the rhynchodaeum is suspended in the cephalic blood lacuna. It then attaches dorsally and ventrally to the inner surface of the body-wall longitudinal muscle layer; the thickness of the rhynchodaeal outer muscle wall is only a few fibres thick except laterally, where it is up to 40 µm across.

The rhynchocoel wall is composed of an outer layer of interwoven longitudinal and circular muscle fibres, a thin connective tissue layer, a sparse and incomplete inner layer of discontinuous longitudinal muscle fibres, and a fine epithelium. The posterior extent of the rhynchocoel could not be determined, since the body fragment obtained did not contain the posterior end of the rhynchocoel. Just behind the proboscis insertion, the rhynchocoel wall is thin dorsally and ventrally, thicker laterally. Post-cerebrally, the rhynchocoel is generally thinnest dorsally.

The proboscis is inserted pre-cerebrally. Anterior to the ventral cerebral commissure, the connective tissue layer of the rhynchocoel gradually increases in thickness, with its inner surface much folded (Fig. 8A); the inner longitudinal muscle fibres in the rhynchocoel wall increase in number to form a continuous, distinct layer that becomes thicker, up to 7 µm, before it merges into the proboscis insertion (Fig. 8B). No body-wall musculature contributes to the proboscis insertion.

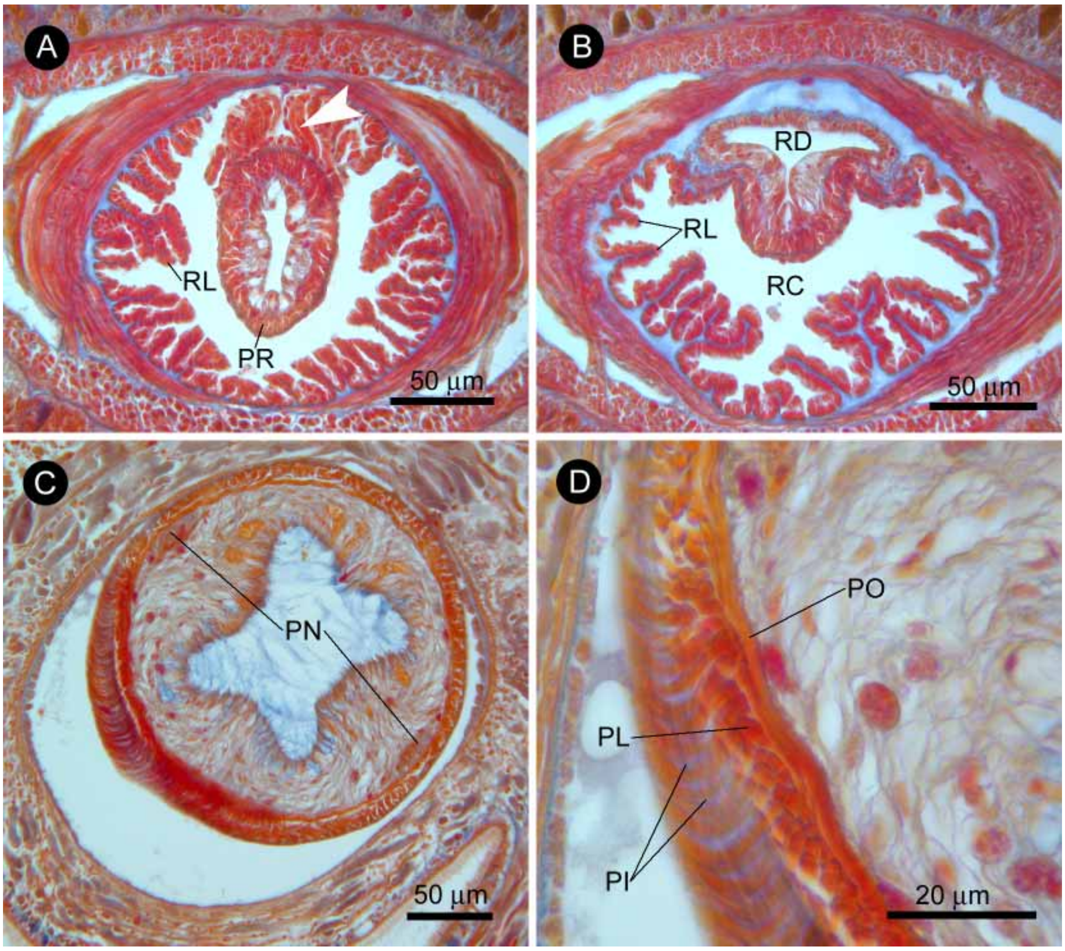

FIGURE 8. *Carinina plecta* sp. nov. Holotype, female, ZIHU-3123. A, transverse section just posterior to the proboscis insertion; arrowhead indicates muscle bundles derived from the rhynchocoel inner longitudinal muscle layer (RL), extending into the proboscis (PR). B, transverse section through the proboscis insertion. C, transverse section through the proboscis. D, higher magnification of C showing the inner circular muscle layer of the proboscis (PI). PL, proboscis longitudinal muscle layer; PN, proboscis nerve; PO, proboscis outer circular muscle layer; RC, rhynchocoel; RD, rhynchodaeum.

The proboscis (Fig. 8C, D) is composed of an outer glandular epithelium, an outer circular muscle layer, a middle longitudinal muscle layer, a connective tissue layer, a delicate inner circular muscle layer and an endothelium; two proboscis nerves are situated between the glandular epithelium and outer circular muscle layer (Fig. 8C). As far as could be observed, the proboscis does not exhibit any significant regional histological differentiation, although the material examined does not contain the posterior end of the organ.

Alimentary canal

The mouth opens just behind the brain. The foregut epithelium is ciliated and deeply folded, up to 90 µm in maximum thickness; basophilic glandular cells stained a bright blue dominate anteriorly, but farther back these are replaced by glandular cells stained purple with Mallory. Posteriorly the foregut epithelium decreases in thickness to 15–20 µm, becomes less folded, and has a greater number of neutrophilic cells. From the junction between the foregut and intestine, a pair of intestinal pouches extends anteriorly for at least 144 µm on both sides of the foregut (Fig. 9A). There are no lateral intestinal diverticula.

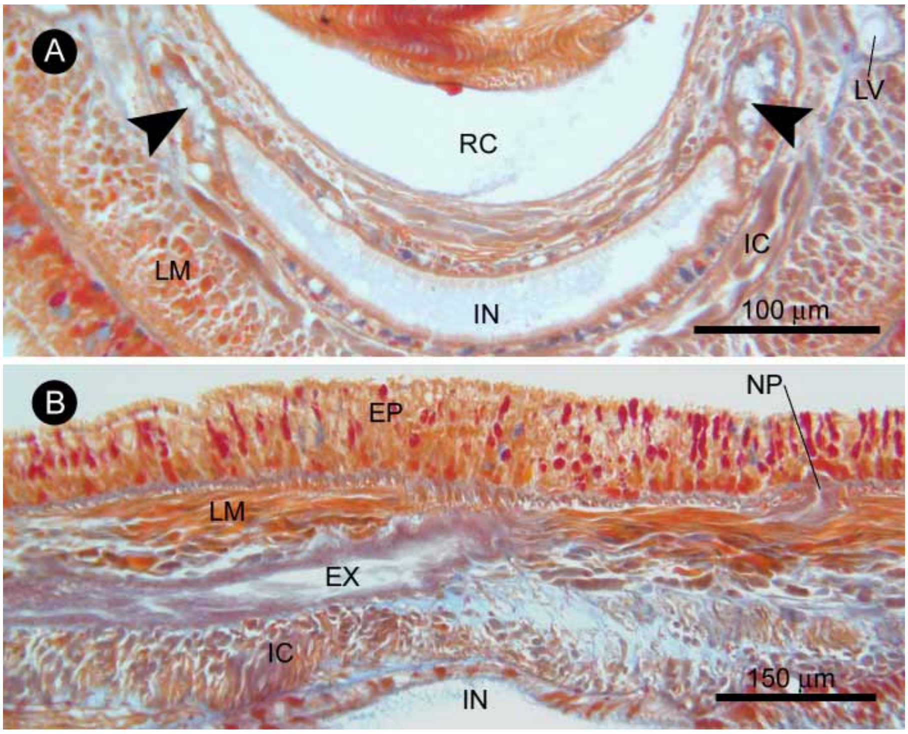

FIGURE 9. *Carinina plecta* sp. nov. Holotype, female, ZIHU-3123. A, transverse section through the junction between the foregut and intestine (IN), showing the intestinal anterior pouches (arrowheads). B, longitudinal section showing the excretory system. EP, epidermis; EX, excretory collecting tubule; IC, body-wall inner circular muscle layer; LM, body-wall longitudinal muscle layer; LV, lateral blood vessel; NP, nephridiopore; RC, rhynchocoel.

Blood system

A pair of cephalic blood lacunae (Fig. 3C) meets over the rhynchodaeal opening; the lacunae also communicate with each other under the rhynchodaeum just after it leaves the ventral part of the body-wall muscle layer. Throughout their length, the cephalic blood lacunae are randomly pierced by dorsoventral muscle bundles, each up to 15 µm thick, that appear to originate from either the body-wall circular or diagonal muscle layers (Fig. 7); these bundles disappear posterior to the brain. The lacunae pass through the cerebral ring along both sides of the rhynchodaeum without changing their diameters. As they traverse the vicinity of the mouth, the lacunae then gradually decrease in diameter, develop distinct walls, and lead into lateral vessels.

Farther back, the lateral blood vessels are situated dorsolateral to the alimentary canal and ventrolateral to the rhynchocoel. They first run inside the body-wall inner circular muscle layer (Fig. 5), but in the posterior foregut region gradually shift peripherally to lie embedded in the circular muscle layer; in the intestinal region they eventually come to lie outside this layer (Fig. 9A).

A pair of rhynchocoel vessels is present in the foregut region; the lateral vessels repeatedly send out branches, at almost right angles, that penetrate the rhynchocoel wall to communicate with the rhynchocoel vessels (Fig. 5); in the holotype, there are 32 of these branches on the right side of the body and 25 on the left. The rhynchocoel vessel is medially exposed to the rhynchocoel lumen and appressed to the rhynchocoel wall, but extends into the wall to connect with branches from the lateral blood vessel (Fig. 10).

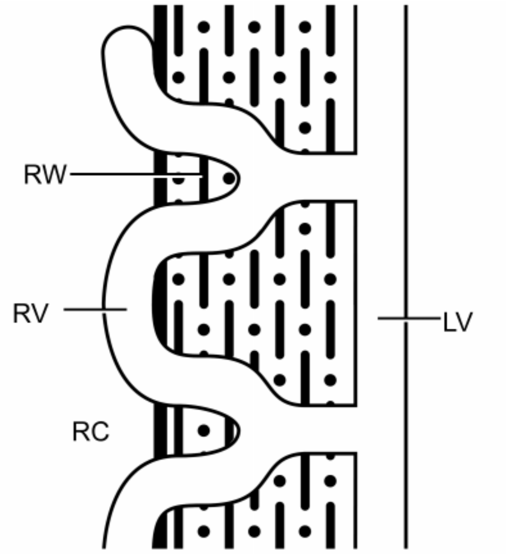

FIGURE 10. *Carinina plecta* sp. nov. Schematic diagram of a longitudinal section through the rhynchocoel wall (RW) in the plane that contains vascular connectives between the rhynchocoel vessel (RV) and the lateral blood vessel (LV), showing the manner how they connect; anterior at top. RC; rhynchocoel.

Nervous system

The brain is situated in the proximally in the epidermis; the inner halves of the lateral nerves are embedded in the body-wall musculature, whereas the outer halves remain in the epidermis (Fig. 5). There are two dorsal cerebral commissures, each about 15 µm thick, and a single ventral one 58 µm thick; the anterior dorsal commissure lies slightly anterior to the ventral commissure. There is an inner, but no outer, neurilemma. Neither neurochords nor neurochord cells were found.

A pair of buccal nerves originates medially from the ventral ganglia just anterior to the mouth, within the mass of the type G1 acid fuchsin-positive glandular cells in the epidermis. They run a short distance posteriorly before uniting into a single trunk; the trunk soon forks, giving rise again to a buccal nerve on each side, each up to 40 µm in diameter, situated laterally on the base of the buccal epithelium (Fig. 4). Each buccal nerve sends off a thin branch, about 10 µm in diameter, which runs upward and posteriorly in the basal part of the buccal epithelium near the dorsal mid-line, the two branches forming a pair (Fig. 6C). The main buccal nerves, situated laterally, then gradually move ventrolaterally and further subdivide into several thin branches near the posterior border of the mouth; these nerves extend posteriorly in the foregut wall, but their ultimate fate could not be traced.

An upper mid-dorsal nerve, originating from the two dorsal cerebral commissures, lies in the basal portion of the epidermis (Figs 4, 5, 7), and extends anteriorly to where the rhynchodaeal outer muscle wall attaches dorsally to the body-wall musculature. The posterior extent of the upper mid-dorsal nerve is uncertain, but it reaches at least to the hind end of the body fragment obtained.

A lower mid-dorsal nerve lies along the dorsal rhynchodaeal (Fig. 7) and rhynchocoel (Fig. 5) walls; it possesses frequent neural connections to the upper mid-dorsal nerve. Pre-cerebrally, the neural connections between the upper and lower mid-dorsal nerves are occasionally forked.

Pre-cerebrally, numerous thick peripheral nerves emerge anteriorly from the cerebral ring and extend between the epidermis and basement membrane; two conspicuous peripheral nerves lie ventrolaterally (Fig. 7), extending to the tip of head, and turning medially to penetrate the rhynchodaeal epithelium, eventually forming the proboscis nerves (Fig. 8C).

Sense organs

The cerebral sense organs are simple, consisting of ciliated pits in the epidermis 50–65 µm deep and up to 10 µm in diameter basally. They are situated laterally to the ventral cerebral ganglia (Fig. 4). Statoliths were not found. Lateral sensory organs are absent. There are no eyes.

Frontal organ and cephalic glands

There are neither a frontal organ nor cephalic glands.

Excretory system

Many details of the excretory system could not be observed, since it lay in the region sectioned longitudinally. The excretory organs are situated in the anterior intestinal region on each side of the body; anteriorly, each organ is composed of a glandular mass, from which a thick collecting tubule, up to 50 µm in diameter, runs posteriorly. At the posterior end, each collecting tubule turns distally at a right angle to form an efferent duct, about 25 µm in diameter, that opens by a single nephridiopore on each side of the body (Fig. 9B). The system extends for over 1.5 mm in length. The collecting tubule on each side lies immediately dorsal to the lateral blood vessel, but whether or not the tubule penetrates the blood vascular wall was unclear from the longitudinal sections.

Reproductive system

The single specimen was female. The paired ovaries are about 180 µm in maximum dimension, situated lateral to the rhynchocoel, immediately above the lateral blood vessels; each ovary contains 3–6 eggs about 80 µm in diameter. The gonoduct leads dorsolaterally into a gonopore in the epidermis; within a radius of about 100 µm from the gonopore, the epidermis contains coarse acidophilic granules that distinguish it from the surrounding epidermis (Fig. 3D).

Systematic remarks

Carinina plecta sp. nov. is anatomically similar to previously described species in the genus that all of have their nervous system situated located in the epidermis, a body-wall musculature composed of inner circular, longitudinal, and outer circular muscle layers, an excretory system with a gland-like anterior portion, and a peculiar course of the origin of the proboscis nerves. In *Carinina plecta* sp. nov., the rhynchocoel wall is principally composed of interwoven longitudinal and circular muscle fibres. This character state has not been reported for previously described species of *Carinina*, in which the rhynchocoel consists only of circular muscles. Hylbom's (1957) generic diagnosis for *Carinina* is here emended to accommodate the type of rhynchocoel wall found in *Carinina plecta* sp. nov., rather than create a monotypic new genus for this species.

The genus *Carinina* currently contains sixteen species. *Carinina plecta* sp. nov. can be distinguished from all previously described species by the suite of characters summarised in Table 1. The description of *Carinina antarctica* Bürger, 1904 is based on a posterior fragment of the body; thus states of the characters tabulated in Table 1 are unknown for this species. *Carinina plecta* sp. nov. differs from *Carinina antarctica* Bürger, 1904 in that the latter has dorsoventral muscles in the intestinal region and a 'lateral sensory line' in the epidermis on each side of the body (Bürger 1904b).

TABLE 1. Comparison of six characters among *Carinina* species. Data compiled from Hubrecht (1887), Bergendal (1902a), Joubin (1902), Bürger (1904b), Nawitzki (1931), Wheeler (1940), Friedrich (1935a, b, 1970), Hylbom (1957), Müller (1965), Korotkevich (1982), Kulikova (1984), Sundberg and Hylbom (1994), Gibson and Sundberg (1999), and Senz (2000).

Taxa	A	B	C	D	E	F
C. antarctica Bürger, 1904[a]	?	?	?	?	?	?
C. arenaria Hylbom, 1957	2	1	0	1	1	0
C. atavia (Bergendal, 1902)	1	1	0	0	1	1
C. buddenbrocki (Friedrich, 1935)	2	?	0	1	1	?
C. burgeri Joubin, 1902[b]	?	?	?	?	1[c]	?
C. coei Hylbom, 1957	2	1	1	1	1	1
C. grata Hubrecht, 1887	2	0	0	0	1	0
C. heterosoma Müller, 1965	1	0	0	1	1	0
C. johnstoni Senz, 2000	1	2	0	0	1	0
C. littorea Korotkevich, 1982	2	1	1	?	?	?
C. mawsoni Wheeler, 1940[d]	1	2	0	?	?	0
C. pacifica Friedrich, 1970	1	0	0	0	?	2
C. poseidoni Friedrich, 1935	2	1	0	1	1	2
C. remanei (Nawitzki, 1931)	1	1	0	1	2	2
C. sinensis Gibson & Sundberg, 1999	1	0	0	0	1	2
C. wijnhoffae Kulikova, 1984	1	1	0	0	1	1
***C. plecta* sp. nov.**	2	0	1	0	2	0

Characters and character states:

A: Mid dorsal nerve(s): (1) upper only; (2) upper and lower.

B: Lateral blood vessels: (0) internal to body-wall inner circular muscle layer anteriorly but ou side these muscles posteriorly; (1)only internal to inner circular muscle layer; (2) only external to the inner circular muscle layer.

C: Rhynchocoel blood vessels: (0) absent; (1) present.

D: Statoliths in cerebral ganglia: (0) absent; (1) present.

E: Dorsal cerebral commissure(s); (1) one; (2) two.

F: Blood system in buccal/foregut region consists of: (0) two lateral vessels; (1) lateral vessels developed as vascular plexus around foregut; (2) four lateral vessels.

[a] Sundberg and Hylbom (1994) regarded the name *Carinina antarctica* as a *nomen dubium*.

[b] Sundberg and Hylbom (1994), Gibson and Sundberg (1999), and Senz (2000) interpret *C. burgeri* as having two dorsal commissures, although Joubin (1902) clearly states “Ce cerveau est pourvu d’une très large commissure dorsale, presque aussi épaisse que les ganglions eux-mêmes”.

[c] Sundberg and Hylbom (1994), Gibson and Sundberg (1999), and Senz (2000) interpret that ocelli are present in *Carinina burgeri*. However, there is no indication of ocelli in Joubin’s original description.

[d] Friedrich (1970), Hylbom (1975), Kulikova (1984), and Sundberg and Hylbom (1994) place this species in *Tubulanus* rather than *Carinina*.

Family Callineridae Bergendal, 1901

Callinereae [sic] Bergendal, 1901: 112. [As a subfamily, correctly Callinerinae]
Callineridae: Chernyshev, 1995: 12; Chernyshev, 2002: 135.

Nomenclatural remarks

The original spelling Callinereae, which was used when Bergendal (1901) established the subfamily, is incorrect according to Article 29.2 (ICZN 1999) and should be corrected as Callinerinae after Article 32.5.3.1 (ICZN 1999). Chernyshev (1995, 2002) raised it to the family rank, attributing the publication date of the family as 1900. However, Bergendal's work was published in two parts (Bergendal 1900a, 1901), and the subfamily was erected in the posterior half (Bergendal 1901). Thus the family-group name should be regarded to be published in 1901.

Genus *Callinera* Bergendal, 1900

Callinera Bergendal, 1900b: 590; 1900c: 313.

Type species

Callinera buergeri Bergendal, 1900, by monotypic designation.

Diagnosis

As given by Hylbom (1957).

Nomenclatural remarks

Bergendal published the name *Callinera buergeri* as a new genus and species in two works, Bergendal (1900b) and Bergendal (1900c); the former, published on 9 May 1900, has precedence over the latter, published on 14 June 1900, according to Article 21.1 of the Code (ICZN 1999). The nominal genus *Callinera* and nominal species *Callinera buergeri* are deemed to have been established in Bergendal (1900b), rather than Bergendal (1900c).

Callinera nishikawai sp. nov. (Figs 11–14)

Diagnosis

A *Callinera* having a proboscis with a stylet-like apparatus; lateral sensory organs present; sub-epidermal glandular cells absent; blood vascular system without a cephalic ventral lacuna, and with a pair of short rhynchocoel vessels; nervous system with single dorsal cerebral commissure and foregut nerves separated anterior to mouth.

Etymology

The specific name is dedicated to one of the collectors of the material, Professor Teruaki Nishikawa, an expert on the taxonomy of sipunculids, echiurans, hemichordates, urochordates, and cephalochordates.

Material examined

Holotype, female, ZIHU-3133, 12 May 2002, TK, SK, and TN coll., serial transverse sections (6 µm thick) of an anterior body fragment about 1.5 cm in length and 0.8–1.0 mm in width, fixed in Bouin's fluid.

External features

The body in a preserved state is pale in colour, without any pattern; the head is tapered, rounded-acute and sharply pointed (Fig. 11A). Cephalic furrows were not found. The intestinal region possesses constrictions at intervals.

Body wall, musculature, and parenchyma

Basophilic glandular cells predominate in the epidermis in the proximal 1/2 to 3/4 of the head anterior to the mouth; acidophilic glandular cells are sparsely distributed distal to the basophilic cells; glandular components are fewer in the extreme anterior tip of the head and also lateral to the brain. Posterior to the mouth, the basophilic glandular cells decrease in abundance; from the stomach region and posteriorly, acidophilic glandular cells predominate before they are abruptly replaced by basophilic granular cells in the intestinal region, at the level where the lateral blood vessel emerges from the body-wall inner circular muscle layer; posteriorly from the posterior end of the rhynchocoel, the basophilic granular cells are again abruptly replaced by Orange-G-positive glandular cells; farther posteriorly, neutrophilic vacuolated cells appear among the Orange-G-positive cells. Precerebrally, the epidermis is 30–60 µm thick; post-cerebrally it is more or less constantly, around 80 µm. $E(b) = 0.13$; $E(i) = 0.06$.

The basement membrane is up to 4 µm thick; processes of connective tissue into the epidermis were not found and the innermost side of the basement membrane does not form a mesh-like structure. There is a thin neuro-fibrous layer between the basement membrane and body-wall outer circular muscle layer (Fig. 11B).

The body-wall musculature consists of an outer circular and an inner longitudinal muscle layer pre-orally (Figs 11C); post-orally, an inner circular muscle layer appears interior to the longitudinal muscle layer (Fig. 12). In the anterior stomach region, these three layers are 10 µm, 150 µm, and 35 µm thick, respectively. The inner circular muscle layer disappears anterior to the region of the rhynchocoel muscular sac. A longitudinal muscle plate is present between the rhynchocoel and alimentary canal and encloses the rhynchocoel except for mid-dorsally, thus appearing to be part of the rhynchocoel wall (Fig. 12). There is a thin diagonal muscle layer between the outer circular and middle longitudinal muscle layers in the post-cerebral region (Figs 11B, 12). Dorsoventral muscles were not found. The inner and outer circular muscle layers are connected to the dorsal side of the body at intervals by muscle fibres through the longitudinal layer (Fig. 12).

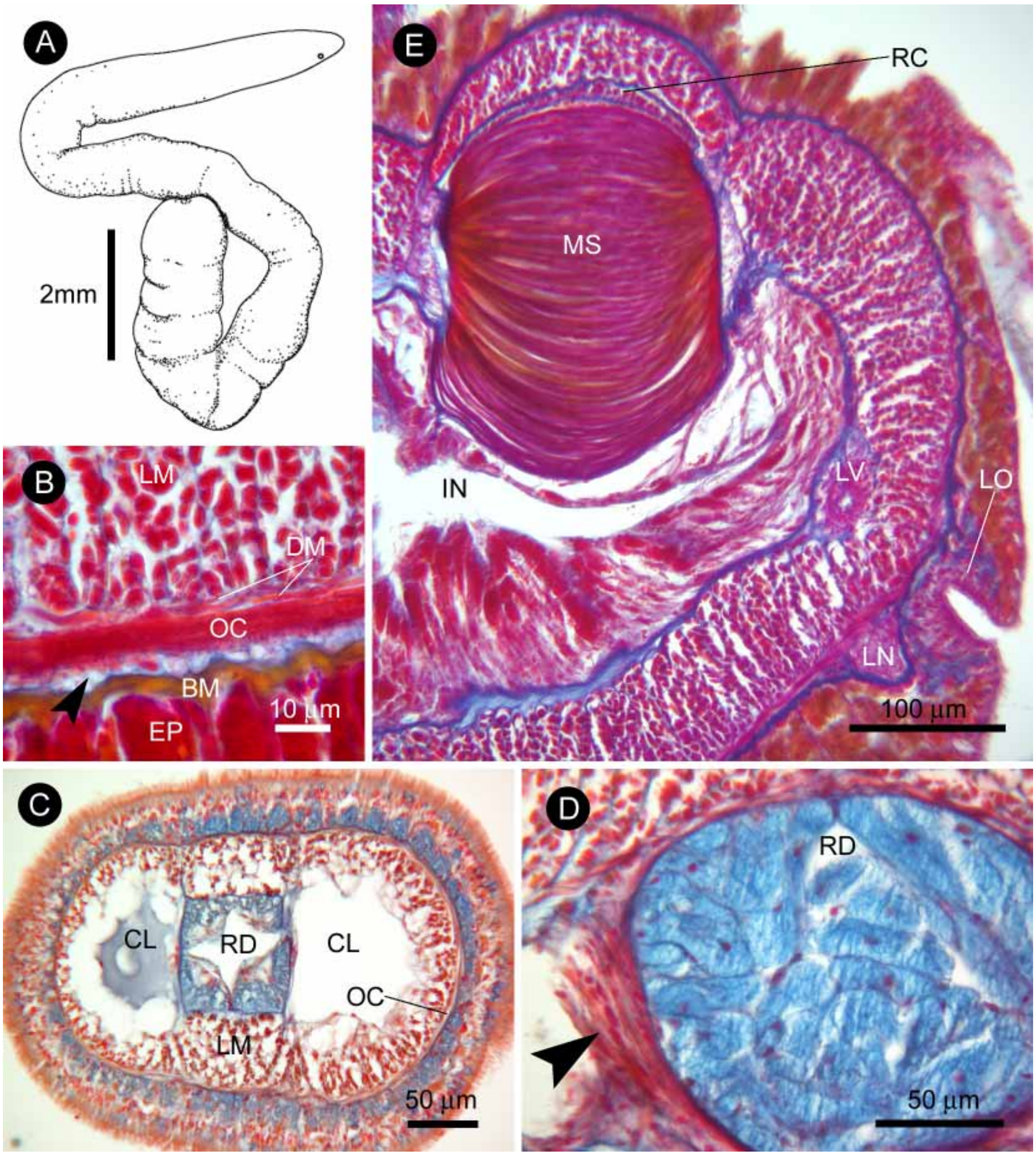

FIGURE 11. *Callinera nishikawai* sp. nov. Holotype, female, ZIHU-3133. A, external appearance of the preserved specimen before serial sectioning. B, transverse section through the intestinal region showing the composition of the body wall; arrowhead indicates the neuro-fibrous layer. C, transverse section through the anterior region of the rhynchodaeum. D, transverse section through the posterior region of the rhynchodaeum showing the sphincter (indicated by the arrowhead). E, transverse section through the intestinal region showing the rhynchocoel muscular sac. BM, basement membrane; CL, cephalic blood lacuna; DM, diagonal muscle layer; EP, epidermis; IN, intestine; LM, body-wall longitudinal muscle layer; LN, lateral nerve cord; LO, lateral sensory organ; LV, lateral blood vessel; MS, rhynchocoelic muscular sac; OC, body-wall outer circular muscle layer; RC, rhynchocoel; RD, rhynchodaeum.

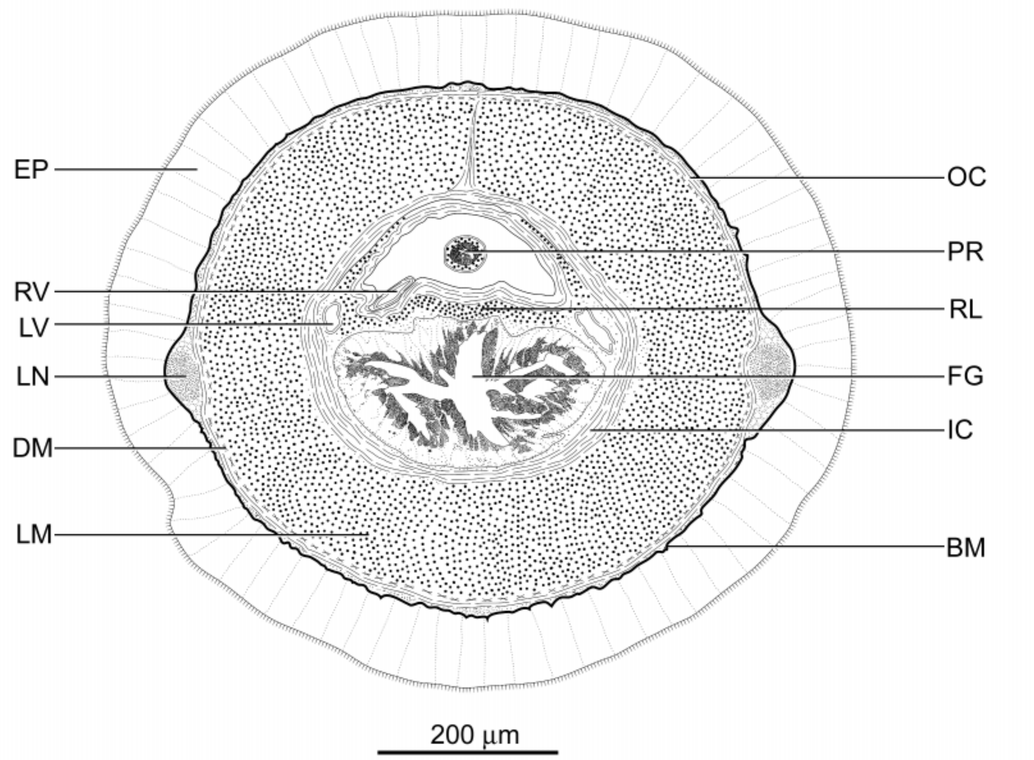

FIGURE 12. *Callinera nishikawai* sp. nov. Holotype, female, ZIHU-3133. Drawing of a transverse section through the posterior foregut region. BM, basement membrane; DM, diagonal muscle layer; EP, epidermis; FG, foregut; IC, body-wall inner circular muscle layer; LM, body-wall longitudinal muscle layer; LN, lateral nerve cord; LV, lateral blood vessel; OC, body-wall outer circular muscle layer; PR, proboscis; RL, rhynchocoel longitudinal muscle layer; RV, rhynchocoel vessel.

A parenchymatous extracellular matrix is well developed between the body-wall inner circular muscle layer and the alimentary canal in the foregut region; posteriorly, it is also well developed inside the body-wall longitudinal muscle layer lateral to the intestine and around the gonads.

Proboscis apparatus

The proboscis pore opens mid-ventrally near the tip of the head, leading backwards to a rhynchodaeum whose epithelium is unciliated but richly glandular, containing basophilic cells throughout its length. The rhynchodaeum is attached dorsally and ventrally to the body-wall longitudinal muscle layer and is flanked by the cephalic blood lacunae, except at its extreme anterior end, where the lacunae meet dorsally. Anteriorly, the rhynchodaeum is square in cross sectional view; the epithelium is thickened at the four corners of the square, so that the lumen of the rhynchodaeum appears cross-shaped. Neutrophilic vacuolated cells occur near the surface of the rhynchodaeal epithelium (Fig. 11C). After passing through the cerebral ring, the rhynchodaeum becomes a rounded tube, with its epithelium enormously thickened to about 100 µm; the vacuolated cells disappear in this region. Anterior to the proboscis insertion, circular muscles are developed around the rhynchodaeum, except dorsally (Fig. 11D).

The rhynchocoel wall is composed of a thin outer longitudinal muscle layer that is dorsally discontinuous, a circular muscle layer, a thin connective tissue layer, a sparse inner longitudinal muscle layer, and a delicate epithelium. The rhynchocoel wall is delicate and thin in the anterior foregut region; more posteriorly, the circular muscle layer is thicker. The rhynchocoel muscular wall terminates near where nephridiopores open on each side of the body in the intestinal region; the rhynchocoel lumen continues posteriorly as a thin-walled chamber, with the proboscis retractor muscle attached to its dorsal wall. The chamber farther back leads into a dorsoventrally compressed canal, the ventral wall of which is connected to a strongly developed muscular sac (Fig. 11E).

The proboscis insertion is situated post-cerebrally. The proboscis, exclusive of the proboscis retractor muscle, is differentiated into five regions. The most anterior region is short, comprising about 13% of the length of the organ (not counting the proboscis retractor muscle), and is composed of a thin outer epithelium with no glandular component, a longitudinal muscle layer arranged in four bands, a connective tissue layer, and a fine endothelium; two proboscis nerves are embedded apically beneath the epithelium in two of the opposing longitudinal muscle bands (Fig. 13A). The second region, also very short and about 11% of the organ's length, is composed of an outer glandular epithelium with both acido- and basophilic glandular cells, an outer circular muscle layer, a thick inner longitudinal muscle layer of four bands, and an outer connective tissue layer (Fig. 13B); the two proboscis nerves lie between the glandular epithelium and the circular muscle layer. The circular muscle layer is powerfully developed in places (Fig. 13C). The third region comprises 21% of the organ's length. It is not radially symmetrical, but rather the epithelium on one side is folded in toward the lumen, forming a concavity that contains an acute triangular cross section of basophilic tissue (Fig. 13D). The fourth region, about 8% of the total length of the organ, contains a previously unknown, stylet-like apparatus (Fig. 13E). The apparatus, 184 µm long and 120 µm in maximum width, is composed of dense, Orange-G-positive tissue; it is acute anteriorly and cross-sectionally elliptical for the anterior 1/4 of its length (Fig. 13F); it is U-shaped in cross section in the middle (Fig. 13G), with the convex base attached to the proboscis epithelium; the apparatus forks posteriorly into two swollen rami that penetrate the proboscis epithelium (Fig. 13H); the rami lie outside the proboscis wall and are exposed to the rhynchocoel lumen (Fig. 13I). The fifth region, to which the proboscis retractor attaches posteriorly, is the longest and comprises 47% of the length of the organ; medially it contains both acido- and basophilic glandular cells (Fig. 13J); its epithelium contains Orange-G-positive substances; the lumen is situated at the periphery of the organ; posteriorly this region is less glandular and the outer longitudinal muscle layer becomes more distinct (Fig. 13K), continuing posteriorly to the proboscis retractor muscle.

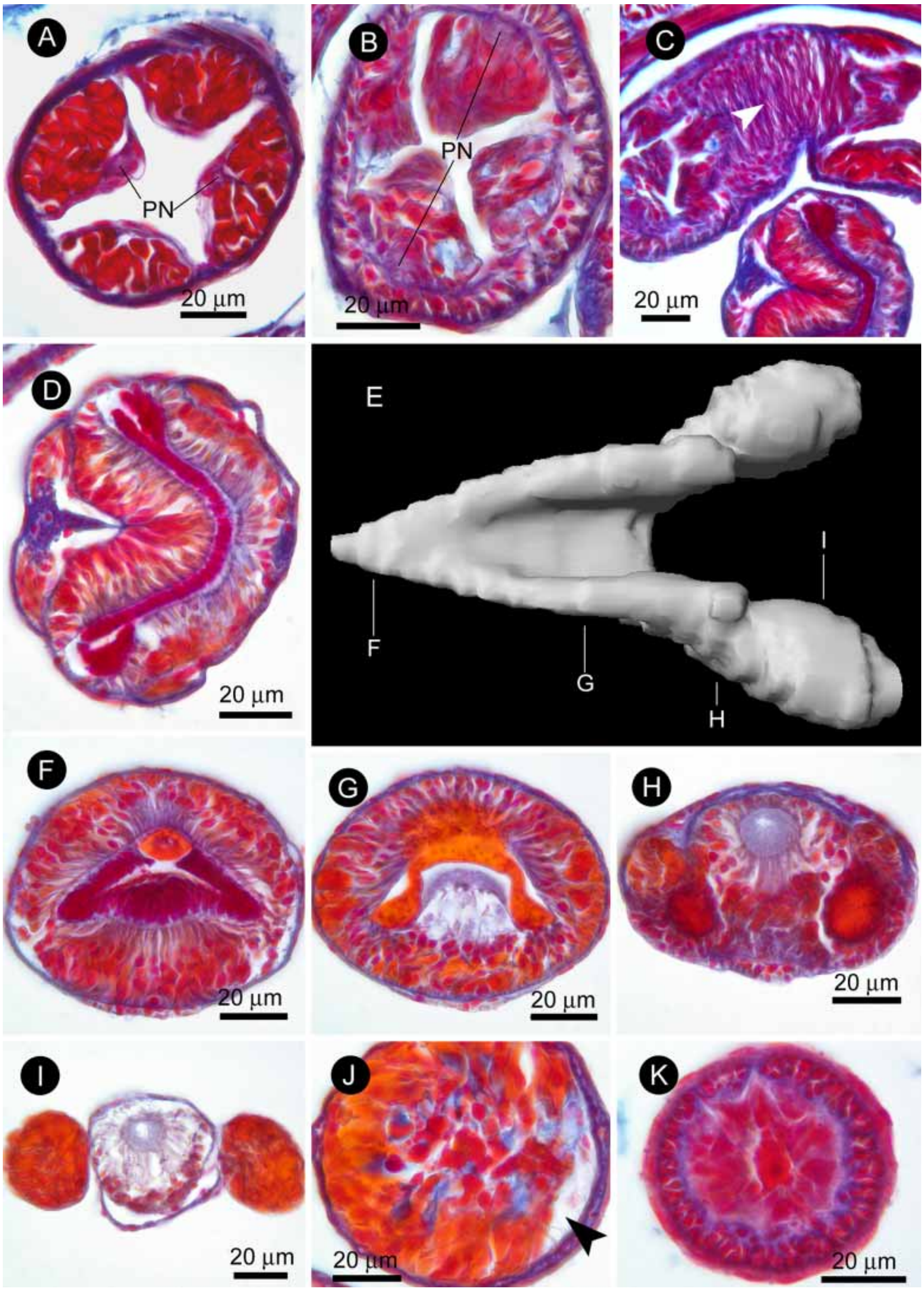

FIGURE 13. *Callinera nishikawai* sp. nov. Holotype, female, ZIHU-3133. A, transverse section through the first region (referred to as Bergendal's region in the present paper) of the proboscis. B, transverse section through the second region of the proboscis. C, tangential section of the proboscis showing the well developed circular muscles, shown by the arrowhead, between the first and second regions of the proboscis. D, transverse section through the third region of the proboscis. E, 3-D reconstruction of the stylet-like apparatus of the proboscis, anterior to left. F–I, anterior-to-posterior transverse sections through the stylet-like apparatus. J, transverse section through the fifth region of the proboscis; arrowhead indicates proboscis lumen. K, transverse section through the posterior portion of the fifth region of the proboscis. PN, proboscis nerve.

Alimentary canal

The mouth is pit shaped in the preserved state, opening just posterior to the brain. It leads to the foregut, whose epithelium is histologically differentiated into two regions, the anterior region about 1 mm long and 1/3 the length of the posterior. In the anterior region, basophilic glandular cells predominate in the epithelium, whereas in the posterior region they are replaced by slender, columnar acidophilic glandular cells. In both regions, the epithelium is densely ciliated and contains neutrophilic vacuolated cells. The junction between the foregut and intestine is located near the rhynchocoel muscular sac; it is marked by a change in the appearance of the surface of the epithelium and the nature of the gland cells: acidophilic granules are densely arranged in a thin layer near the surface of the epithelium in the posterior portion of the foregut, whereas there is no such layer in the intestine. The intestine is surrounded by delicate circular muscle fibres (Fig. 14A), which constrict in places to form a muscular diaphragm (Fig. 14B); the intestinal lumen at the diaphragm is narrowed by a distinct sphincter (Figs 14B, C); there are at least five diaphragms and sphincters in the holotype. No caecum was found in the foregut or intestine.

Blood system

The two lateral cephalic blood lacunae connect with each other above the anterior end of the rhynchodaeum; they run posteriorly alongside the rhynchodaeum, passing through the cerebral ring with no change in diameter, and gradually develop a distinct wall in the vicinity of the mouth, where they lead into lateral blood vessels. The lateral vessels, which become compressed posterior to the proboscis insertion, are situated inside the body-wall inner circular muscle layer, lateral to the rhynchocoel and dorsolateral to the foregut. Just before the posterior foregut region, the lateral blood vessels shift position from inside to outside the body-wall inner circular muscle layer; just anterior to this each vessel sends off a short branch that penetrates the ventrolateral side of the rhynchocoel wall to form paired rhynchocoel vessels (Fig. 12) that soon end blindly. In the intestinal region, the lateral blood vessels are situated lateral to the intestine. No transverse connections between lateral vessels could be found in the foregut and intestinal regions.

Nervous system

The brain and lateral nerves are situated between the epidermal basement membrane and body-wall circular muscle layer; inner and outer neurilemmae are incompletely developed. A dorsal and a ventral commissure, 18 µm and 8 µm thick respectively, lie almost in the same plane. The origin of the buccal nerves could not be traced in detail, but they appear to originate separately medially from the ventral cerebral ganglia, posterior to the ventral cerebral commissure and anterior to the mouth. They do not seem to fuse into a single median trunk before running ventrolateral to the buccal cavity; from here, they lead into a pair of foregut nerves situated ventrolaterally between the basal part of the foregut epithelium and body-wall inner circular muscle layer; their ultimate fate could not be traced. An upper mid-dorsal nerve is present, originating from the dorsal cerebral commissure and situated between the epidermal basement membrane and body-wall outer circular muscle layer; its anterior extent is uncertain; posteriorly, it appears to divide into several branches near the posterior muscular sac of the rhynchocoel. A lower mid-dorsal nerve is not present. Pre-cerebrally, several peripheral nerves extend anteriorly from the dorsal and ventral ganglia.

Sense organs

A pair of lateral sensory organs is situated near the posterior muscular sac of the rhynchocoel, one on each side of the body, consisting of a ciliated furrow, about 10 µm deep and 136–240 µm long, in the epidermis above the lateral nerve cord (Figs 11E, 14F).

There are neither cerebral sense organs nor statoliths. No eyes were found.

Excretory system

The excretory system emerges in the posterior foregut region as a glandular mass inside the lateral blood vessel on each side, appressed to the lateral wall (Fig. 14D); before each glandular mass terminates intravasculary, it gives rise dorsally to a thick-walled collecting tubule running posteriorly. The collecting tubule, about 50 µm in maximum cross-sectional dimension, is situated dorsal to the lateral blood vessel and directly lateral to the body-wall inner circular muscle layer (Fig. 14E); posteriorly it becomes thinner, leading to an efferent duct, about 8 µm in diameter, that turns at a right angle and opens to the exterior via a single dorsolateral nephridiopore on each side of the body (Fig. 14F).

Reproductive system

The single specimen was female. The most anterior ovary appears posterior to the posterior end of the rhynchocoel muscular sac; ovaries are closely attached to each other, situated on dorsolateral side of the body between the intestine and body-wall longitudinal muscle layer; a single gonoduct leads from each ovary to a wide gonopore, up to 40 µm in diameter, opening in the epidermis on the dorsolateral side of the body. The gonopores are arranged longitudinally in a row on each side of the body; the epidermis in the proximal portion of each row contains acidophilic granules (Fig. 14G).

Systematic remarks

Callinera nishikawai sp. nov. is undoubtedly a member of the genus *Callinera*, since it possesses the characteristic muscular sac at the posterior end of the rhynchocoel, which is thought to be a possible synapomorphy for the genus (Sundberg & Hylbom 1994). In addition, the position of the nervous system, between the basement membrane and body-wall outer circular muscle layer, is consistent with all the other congeners.

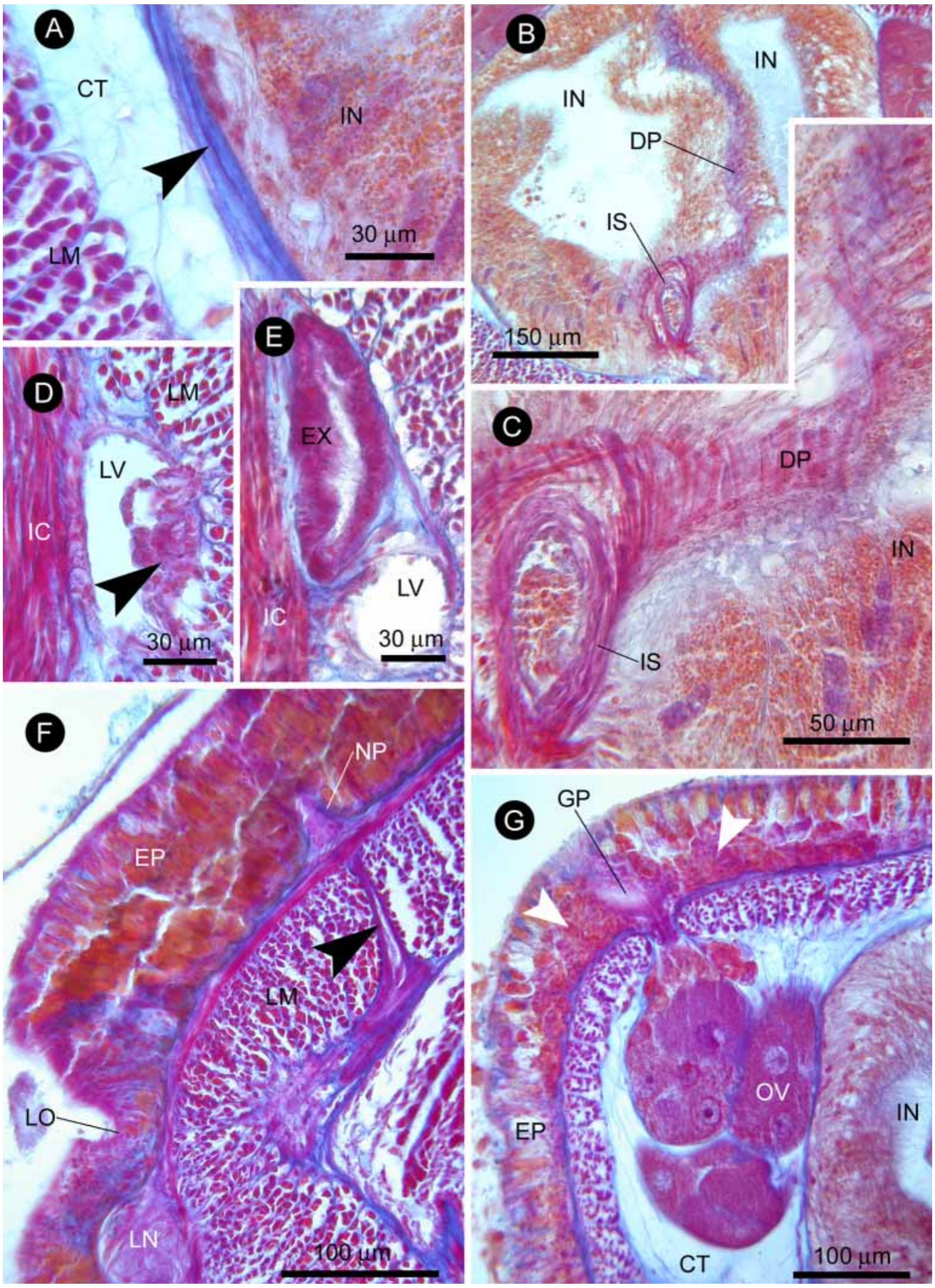

FIGURE 14. *Callinera nishikawai* sp. nov. Holotype, female, ZIHU-3133. A, transverse section through the intestinal region showing the circular muscle layer around the intestine (indicated by the arrowhead). B, transverse section through an intestinal sphincter. C, higher magnification of part of B. D, transverse section showing the nephridial gland in the lateral vessel (indicated by the arrowhead). E, transverse section showing the excretory collecting tubule. F, transverse section through the nephridiopore. G, transverse section through an ovary; arrowheads indicate acidophilic granules around the gonopore. CT, connective tissue; DP, intestinal diaphragm; EP, epidermis; EX, excretory collecting tubule; GP, gonopore; IC, body-wall inner circular muscle layer; IN, intestine; IS, intestinal sphincter; LM, body-wall longitudinal muscle layer; LN, lateral nerve cord; LO, lateral sensory organ; LV, lateral blood vessel; NP, nephridiopore; OV, ovum.

All species of the genus *Callinera* known so far, including *Callinera nishikawai* sp. nov., have a proboscis that is histologically differentiated into multiple regions, though the composition of each region differs from species to species. Among the histologically different regions, however, one is common to all congeners; this region is characterised by longitudinal musculature arranged as four bands, in two of which a pair of proboscis nerves lies beneath a thin, weakly glandular epithelium. This structure was first described and illustrated in detail by Bergendal (1900a) for *Callinera buergeri* Bergendal, 1900. For convenience, I here refer to this part of the proboscis as Bergendal's region. Among the hitherto known seven species of *Callinera*, Bergendal's region is situated immediately posterior to the proboscis insertion in *Callinera bergendali* Gibson & Sundberg, 1999, *Callinera buergeri* Bergendal, 1900, *Callinera grandis* Bergendal, 1903, and *Callinera monensis* Rogers *et al*., 1992, whereas there is an additional region between the proboscis insertion and Bergendal's region in *Callinera blanchardi* Senz, 2000, *Callinera quatrefagesi* Senz, 2000, and *Callinera zhirmunskyi* Chernyshev, 2002.

In the holotype specimen of *Callinera nishikawai* sp. nov., the plane including the proboscis insertion is distorted to lie horizontal to the anteroposterior axis of the body, i.e., perpendicular to the dorsoventral axis; the rhynchocoel thus lies below the rhynchodaeum in the cross section at the proboscis insertion. To what extent this condition reflects the natural morphology is uncertain, although it probably results from the proboscis being pushed forward during fixation. For the same reason, the exact cross-sectional figure of the anteriormost region of the proboscis, i.e., between the proboscis insertion and Bergendal's region, could not be observed. Hence, it is unclear whether or not the proboscis of *Callinera nishikawai* sp. nov. has an additional region in front of Bergendal's region.

In *Callinera nishikawai* sp. nov., the rhynchocoel wall has an incomplete outer longitudinal muscle layer that fails to completely encircle the rhynchocoel circular muscle layer mid-dorsally. This longitudinal layer might be homologous to the longitudinal muscle plate, extending between the rhynchocoel and alimentary canal, found in many species of palaeonemerteans. It is also evident that this layer is also homologous to what Rogers *et al*. (1992) referred to as the body-wall inner longitudinal muscle layer in *Callinera monensis* Rogers *et al*., 1992.

The intestinal diaphragms and sphincters probably correspond positionally to the slightly constricted places found in the posterior part of the specimen in the preserved state. Although these characters have been reported so far only in *Callinera blanchardi* Senz, 2000, due to their fragility they might have been overlooked in other congeners. Thus discretion is advised in regarding these as a reliable taxonomic character.

The characters summarised in Table 2 show that *Callinera nishikawai* sp. nov. is most similar to *Callinera blanchardi* Senz, 2000, especially if the buccal nerves in the present species, which could not be traced in detail in the holotype, turn out to have the same construction as in *Callinera blanchardi* Senz, 2000. In addition to the single differentiating character given in Table 2, *Callinera nishikawai* sp. nov. can be distinguished from *Calli-*

nera blanchardi Senz, 2000 by the presence of rhynchocoel vessels and the enigmatic stylet-like apparatus, which have not been reported in any species of *Callinera*.

TABLE 2. Comparison of six characters among *Callinera* species. Data compiled from Bergendal (1900a, b, c, 1901, 1903), Rogers *et al*. (1992), Gibson and Sundberg (1999), Senz (2000), and Chernyshev (2002).

Taxa	A	B	C	D	E	F
C. bergendali Gibson & Sundberg, 1999	1	0	0	0	1	0
C. blanchardi Senz, 2000	1	0	1	0	1	0
C. buergeri Bergendal, 1900	1	0	1	1	2	0
C. grandis Bergendal, 1903	0	1	1	0	2	1
C. monensis Rogers *et al*., 1992	0	0	0	1	1	0
C. quatrefagesi Senz, 2000	1	0	1	0	2	0
C. zhirmunskyi Chernyshev, 2002	1	0	1	1	2	0
***C. nishikawai* sp. nov.**	1	0	1	0	1	2?

Characters and character states:

A:	Lateral sensory organs: (0) absent; (1) present.
B:	Body-wall longitudinal muscle layer: (0) without; (1) with sub-epidermal glandular cells.
C:	Proboscis: (0) without; (1) with circular muscle layer in region following Bergendal's region.
D:	Cephalic blood vascular system: (0) without; (1) with ventral lacuna.
E:	Dorsal cerebral commissure(s): (1) one; (2) two.
F:	Foregut nerves: (0) fuse to form a single median nerve before branching anterior to mouth, or remaining as two distinct nerves (1) with or (2) without transverse connective.

Family Hubrechtellidae Chernyshev, 2003

Hubrechtellidae Chernyshev, 2003: 370.

Genus *Hubrechtella* Bergendal, 1902

Hubrechtella Bergendal, 1902b: 9.
Coeia Takakura, 1922: 422 **syn. nov.**

Type species

Hubrechtella dubia Bergendal, 1902, by monotypic designation.

Diagnosis

As given by Gibson (1979a).

Systematic remarks

The genus *Coeia* Takakura, 1922 is here considered a subjective junior synonym of *Hubrechtella* Bergendal, 1902. Both genera were recently transferred from Hubrechtidae Bürger, 1892 to a new family, Hubrechtellidae, by Chernyshev (2003). Up until Takakura

(1922) established the monotypic genus *Coeia*, the family Hubrechtidae had contained two genera, *Hubrechtia* Bürger, 1892 and *Hubrechtella* Bergendal, 1902. However, judging from Takakura's (1922) statement that the family contained a single genus, *Hubrechtia*, Takakura was apparently unaware of *Hubrechtella* when he erected *Coeia*.

Takakura (1922) used the following criteria to define *Coeia*: 1) no excretory system, 2) no inner circular muscle layer, 3) no eyes, 4) shallow lateral intestinal diverticula, and 5) a 'tail' region, which lacks both intestine and gonads. The first three characters fully agree with the generic diagnosis of *Hubrechtella*. The fourth character state can be formed by the serial arrangement of gonads, which are situated medially and associated with the intestinal wall; this character might vary intraspecifically, depending upon degree of maturity. The last character, which Takakura (1922) pointed out is similar to the caudal cirrus found in certain heteronemerteans, is known in juveniles of *Hubrechtella dubia* (Cantell 1969); the presence of the 'tail' in adult *Coeia ijimai* can be interpreted as a heterochronic retention of this character.

Hubrechtella ijimai **(Takakura, 1922) comb. nov. (Figs 15–19)**

Coeia ijimai Takakura, 1922

Diagnosis

Hubrechtella with a long 'tail'; body-wall musculature without zigzag fibres; proboscis musculature with inner circular, middle longitudinal, and outer circular layers, with single muscle cross; proboscis epithelium with spherical bodies; mid-dorsal blood vessel penetrating into rhynchocoel; foregut lacunar network present.

Material examined

ZIHU-3122, female, 31 July 2003, HK coll., 55 slides, series of 6-µm transverse sections of a fragment of body containing cephalic tip; ZIHU-3124, female, 1 August 2003, HK coll., 38 + 52 slides, series of 6-µm transverse sections of a complete specimen except middle portion; ZIHU-3126, female, 1 August 2003, HK coll., 39 slides, 8-µm serial transverse sections of a body fragment containing the caudal end.

External features

The body is 3–5 cm long, about 0.7 mm wide, translucent white in colour (Fig. 15A). The head is much more transparent than the following portion, wider than the neck; the rhynchodaeum seen through the epidermis is white, cone shaped, tapering anteriorly. When put in a Petri dish with seawater, the animal showed searching behaviour, frequently swinging the head from side to side. The ovaries are tinged with a greyish colour. There is a transparent 'tail' region that contains neither intestine nor gonads; it tapers posteriorly to end in a pointed tip (Fig. 15B). The tail is at least 10 times as long as the diameter of its most anterior portion.

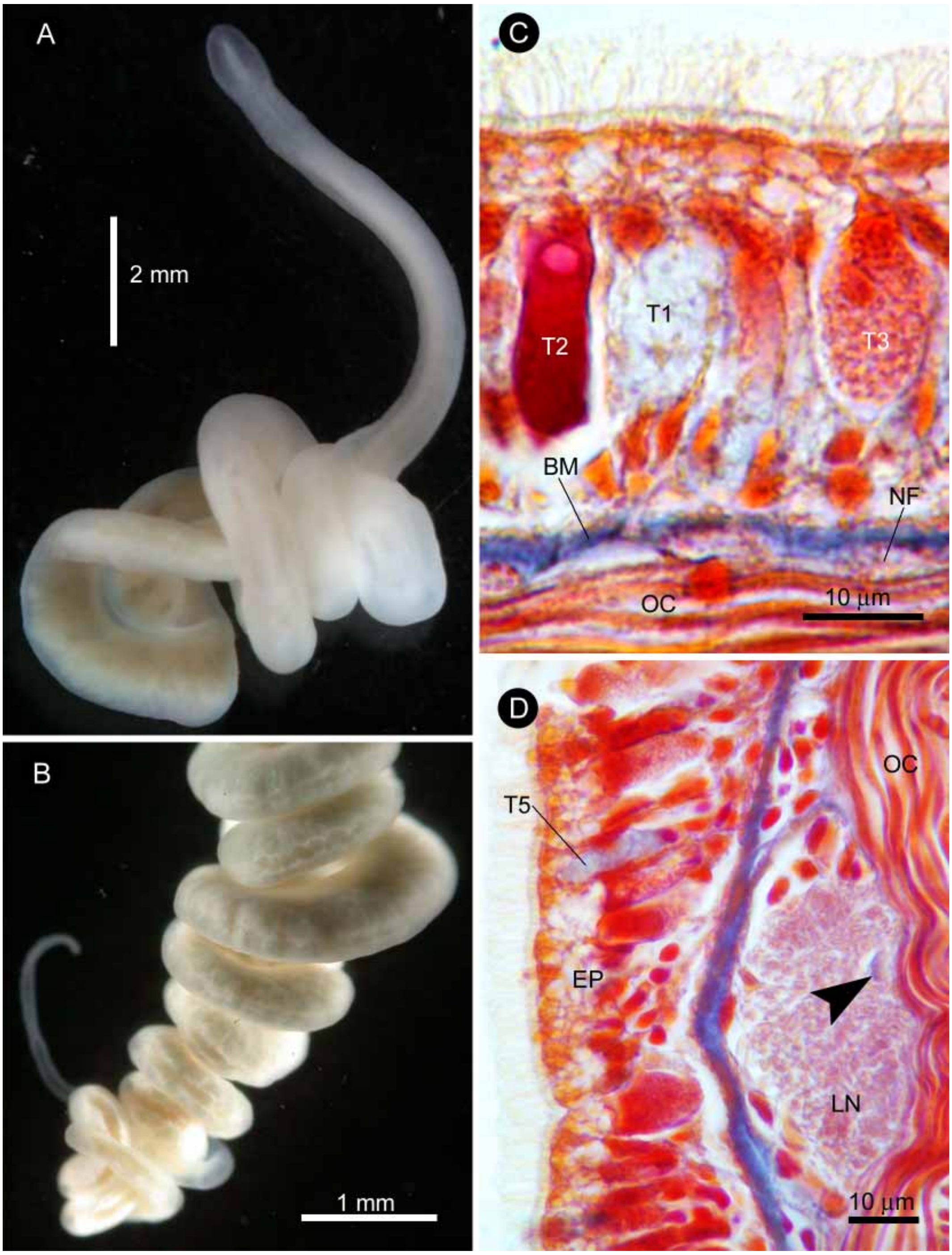

FIGURE 15. *Hubrechtella ijimai* (Takakura, 1922) comb. nov. A, photograph of a body fragment containing the head, taken in life (ZIHU-3122, female). B, photograph of a body fragment containing the tail, taken in life (ZIHU-3123, female). C, transverse section through the anterior intestinal region showing some of the epidermal glandular cell types (ZIHU-3122, female). D, transverse section showing the lateral nerve cord; arrowhead indicates the giant fibre (ZIHU-3122, female). BM, basement membrane; EP, epidermis; LN, lateral nerve cord; NF, neuro-fibrous layer; OC, body-wall outer circular muscle layer; T1, type 1 glandular cell; T2, type 2 glandular cell; T3, type 3 glandular cell; T5, type 5 glandular cell.

Body wall, musculature and parenchyma

The ciliated epidermis (Fig. 16) is up to 30–40 µm thick in the brain region, 45–60 µm thick in the foregut region, reduced posteriorly to 10–15 µm thick in the intestinal region. Type 1 cells are confined to the anterior portion of the foregut-intestine transitional zone (Fig. 15C); type 2 cells predominate throughout the body (Fig. 15C); type 3 cells are distributed post-cerebrally (Fig. 15C); type 4 cells not found; type 5 cells are thinner than type 2 cells, distributed ventrolaterally in the intestinal region (Fig. 15D). *E* (*b*) = 0.07 (ZIHU-3122), 0.11 (ZIHU-3124); *E* (*i*) = 0.04 (ZIHU-3122; the epidermis in the intestinal region was largely sloughed off during fixation or histological preparation in ZIHU-3124 and 3126).

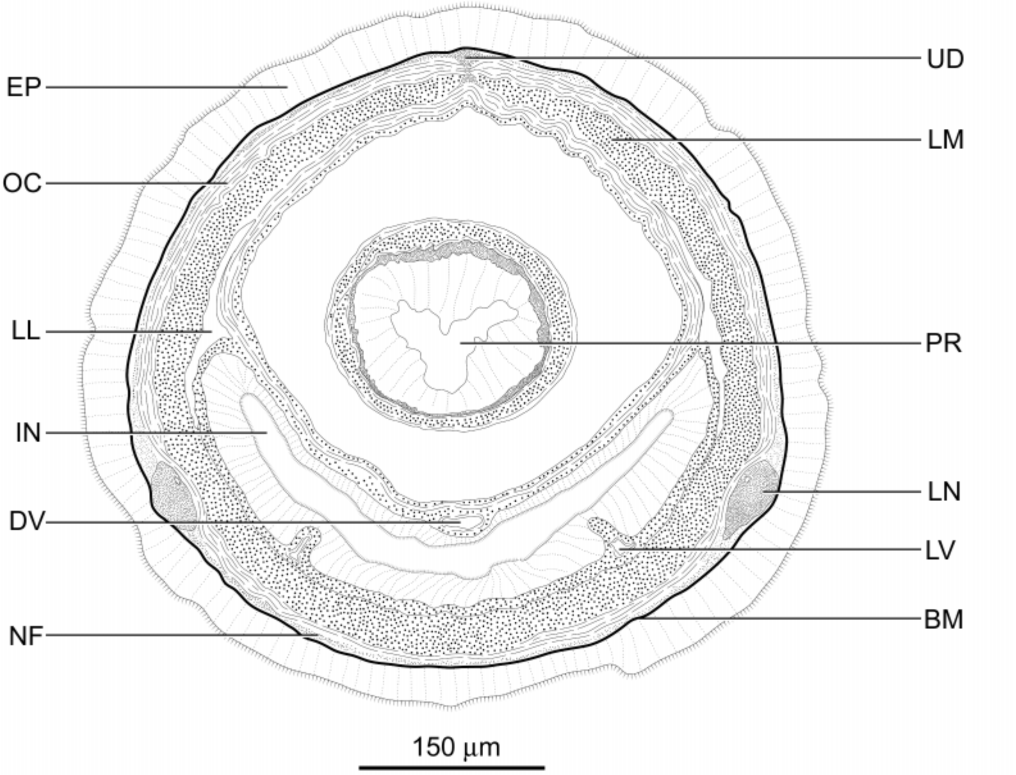

FIGURE 16. *Hubrechtella ijimai* (Takakura, 1922) comb. nov., ZIHU-3122, female, drawing of a transverse section through the anterior intestinal region showing the arrangement of internal organs. BM, basement membrane; DV, mid-dorsal vessel; EP, epidermis; IN, intestine; LL, lateral blood lacuna; LM, body-wall longitudinal muscle layer; LN, lateral nerve cord; LV, lateral blood vessel; NF, neuro-fibrous layer; OC, body-wall outer circular muscle layer; PR, proboscis; UD, upper mid-dorsal nerve.

The basement membrane is best developed in the brain region, where it reaches a thickness of up to 4 µm thick. At the tip of the head, a thin basement membrane lies between the neuro-glandular layer and the body-wall circular muscle layer (Fig. 17A). This basement membrane becomes very inconspicuous in front of the proboscis insertion, while another basement membrane appears between the epidermis and the neuro-glandular layer (Fig. 17B). Post-cerebrally, this basement membrane continues between the epidermis and the neuro-fibrous layer (Fig. 17C); it becomes inverted with the latter in the anterior intestinal region, where the body wall is composed, from the surface inwards, of the epidermis, the neuro-fibrous layer, the basement membrane, and the body-wall musculature (Fig. 17D). In the pre-cerebral region, processes extend from inner to outer basement membranes through the neuro-glandular layer (Fig. 17E); these do not reach the epidermis. A mesh-like structure was not found.

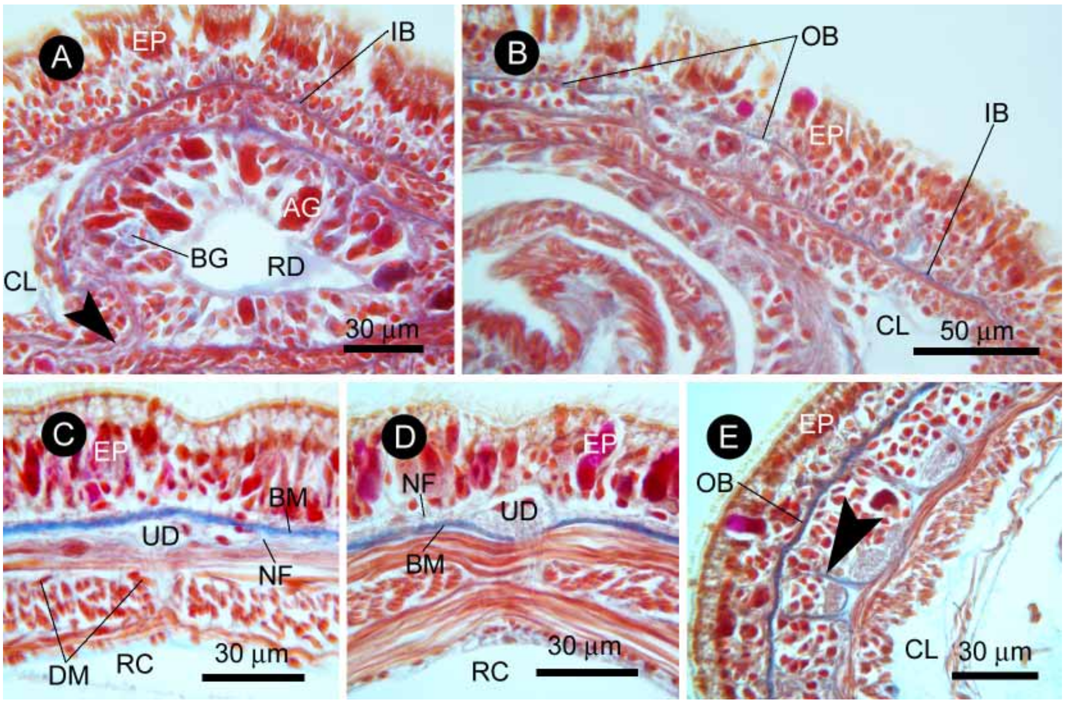

FIGURE 17. *Hubrechtella ijimai* (Takakura, 1922) comb. nov., ZIHU-3122, female. A, transverse section through the pre-cerebral region; arrowhead indicates innervation from the subepidermal neuroglandular layer to the rhynchodaeum (RD). B, transverse section near the proboscis insertion, showing the inner (IB) and outer (OB) basement membranes, the former is about to disappear, while the latter become thicker, posteriorly. C, transverse section through the foregut region showing the arrangement of the neuro-fibrous layer (NF) against the basement membrane (BM). D, transverse section through the intestinal region showing the arrangement of the neuro-fibrous layer (NF) against the basement membrane (BM). E, transverse section through the pre-cerebral region; arrowhead indicates a connective tissue process from the inner to the outer basement membranes. AG, acidophilic glandular cell; BG, basophilic glandular cell; CL, cephalic blood lacuna; DM, diagonal muscle layer; EP, epidermis; RC, rhynchocoel; UD, upper mid-dorsal nerve.

The body-wall musculature consists of an outer circular layer and an inner longitudinal layer, which in the foregut region attain a thickness of 7–15 µm and 12–50 µm, respectively. Zigzag fibres are absent. The diagonal muscle layer is present but not obvious (Fig. 17C). Dorsoventral muscles were not found. Radial muscle fibres connecting the body-wall longitudinal muscle layer and the buccal/foregut wall run through the lumina of the lateral blood lacunae. The foregut and its junction with the intestine are surrounded by a longitudinal muscle layer that is only one or two fibres thick. This splanchnic longitudinal muscle layer is separated from the body-wall longitudinal muscle layer by a thin membrane of connective tissue; the lateral blood lacunae and lateral blood vessels lie outside this splanchnic layer. Posteriorly in the intestinal region, the splanchnic layer disappears except dorsally between the intestine and rhynchocoel, where it remains as a longitudinal muscle plate, terminating anterior to the end of the rhynchocoel.

Parenchymatous connective tissue is inconspicuous throughout the body, except as thin membranes surrounding various organs.

Proboscis apparatus

The rhynchodaeal epithelium is unciliated and shows no significant regional differences in thickness throughout its length; it is generally thinner dorsally and ventrally (20–30 µm thick) than laterally (35–60 µm thick); acidophilic glandular cells predominate, but in one specimen basophilic glandular cells also occur (Fig. 17A). The rhynchodaeum is innervated ventrolaterally on each side by 3–5 nerves from the sub-epidermal neuro-glandular layer; the rhynchodaeal nervous layer is located basal to the glandular epithelium. A definite rhynchodaeal sphincter was not found. A rhynchodaeal caecum is absent; in one specimen, however, a lateral bulge was found on one side, probably caused by contraction during fixation.

The rhynchocoel does not extend to the posterior end of the body. Its wall is composed of separate outer circular and inner longitudinal muscle layers. Posteriorly, it is not developed into a muscular sac. No rhynchocoel caecum was found.

The proboscis insertion is located pre-cerebrally. Four regions can be discerned in the proboscis. The anteriormost region, in retracted position, comprises about 5% of the length of the organ including the proboscis retractor muscle, and is composed of an outer glandular layer, a neural layer, an inner longitudinal muscle layer, an outer circular muscle layer, and an endothelium (Fig. 18A). The second portion, about 22% of the length of the organ, has three muscle layers, including an additional outer circular muscle layer between the neural layer and the longitudinal muscle layer (Fig. 18B). This region is not radially symmetrical, but has a single muscle cross between the inner and outer circular muscle layers (Fig. 18C), although the muscle cross is not always obvious throughout this region. When the muscle cross is viewed in a 12-o’clock position, there are additional glandular masses at 4 and 8 o’clock between the outer glandular layer and the neural layer; these glandular masses contain conspicuous acidophilic spherical bodies up to 10 µm in diameter (Fig. 18B). The third region, about 27% of the length of the organ, is still composed of three muscle layers with a single muscle cross, but lacks the glandular masses containing spherical bodies (Fig. 18D). The last region, about 34% of the length of the organ, has almost the same construction as the first region, but has minute acidophilic spherules near the surface of the epithelium (Fig. 18E). It is followed by the proboscis retractor muscle (Fig.

19A), 12% of the length of the organ, composed entirely of longitudinal muscle fibres; the proboscis retractor muscle posteriorly attaches to the ventral wall of the rhynchocoel.

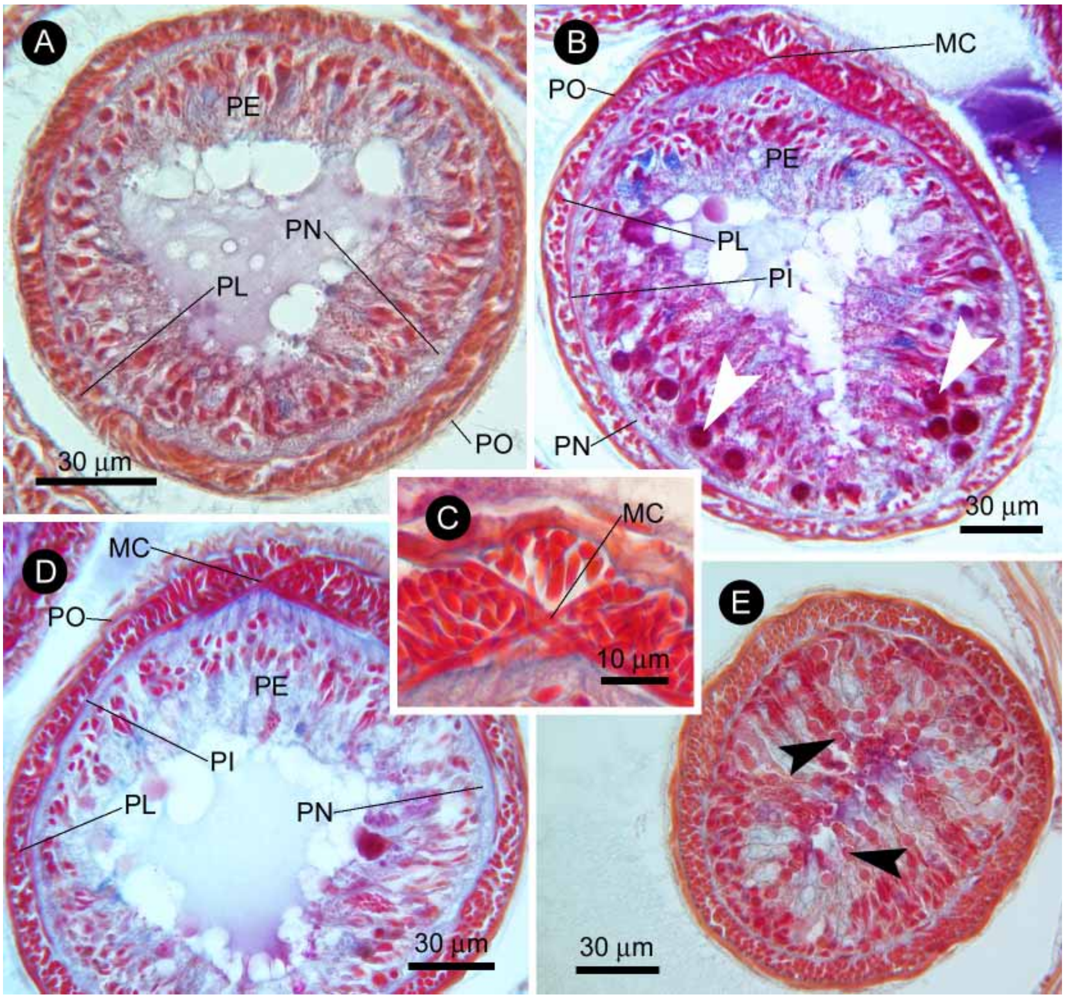

FIGURE 18. *Hubrechtella ijimai* (Takakura, 1922) comb. nov., ZIHU-3122, female. A, transverse section through the first region of the proboscis. B, transverse section through the second region of the proboscis; arrowheads indicate spherical bodies. C, enlargement of the muscle cross in B. D, transverse section through the third region of the proboscis. E, transverse section through the fourth region of the proboscis; arrowheads indicate acidophilic spherules. MC, muscle cross in proboscis; PE, proboscis epithelium; PI, proboscis inner circular muscle layer; PL, proboscis longitudinal muscle layer; PN, nervous layer in proboscis; PO, proboscis outer circular muscle layer.

Alimentary canal

The mouth opens just behind the cerebral sensory organs. The buccal/foregut wall is densely ciliated, with both acido- and basophilic glandular cells; the foregut is about 3 mm long. The intestinal wall contains mainly acidophilic cells, but a small number of basophils cells are also found. The intestine has no lateral diverticula, which, however, appear to be present regionally because the gonads are deeply embedded in the intestinal wall.

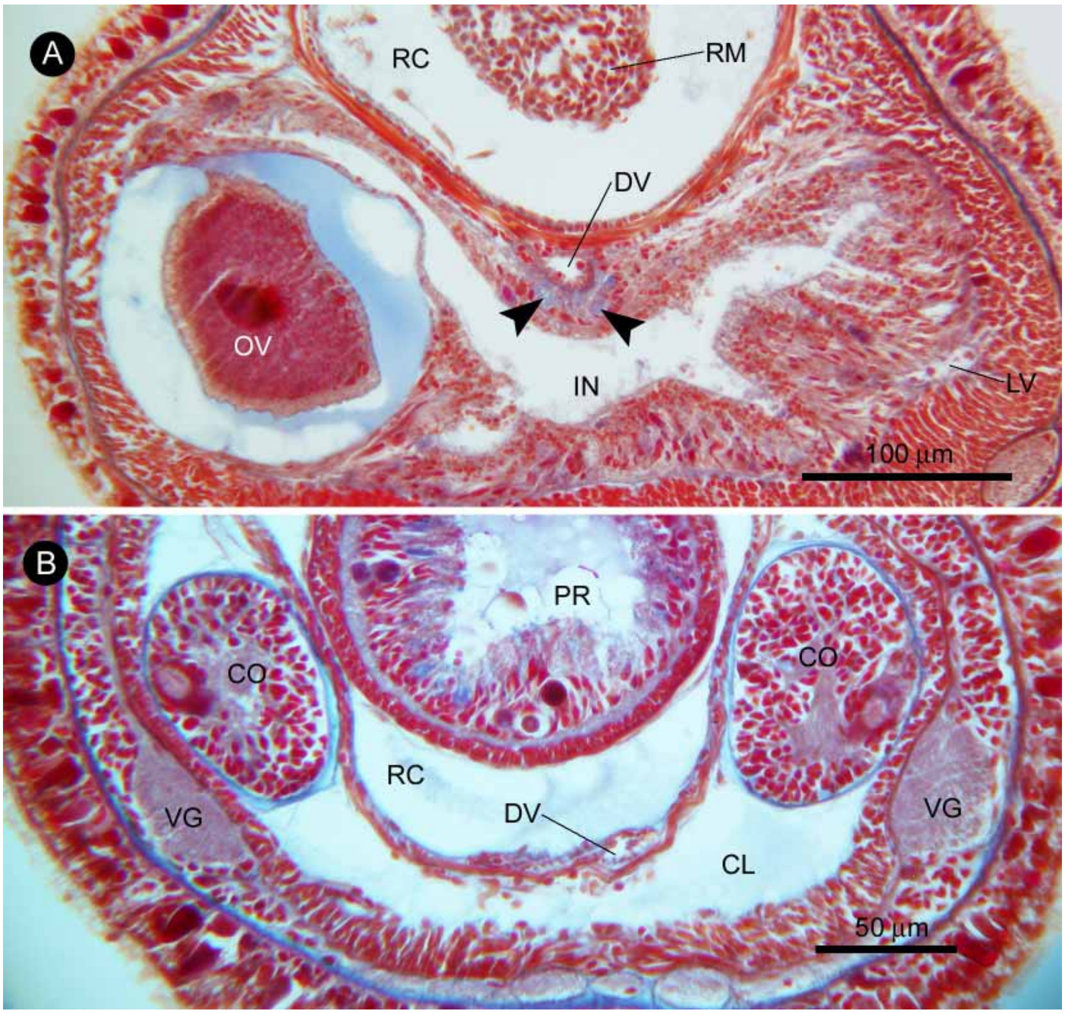

FIGURE 19. *Hubrechtella ijimai* (Takakura, 1922) comb. nov., ZIHU-3122, female. A, transverse section through the intestinal region; arrowheads indicate the basophilic cells in the intestinal wall around the mid-dorsal vessel. B, transverse section through the cerebral sensory organs (CO). CL, cephalic blood lacuna; DV, mid-dorsal vessel; IN, intestine; LV, lateral blood vessel; OV, ovum; PR, proboscis; RC, rhynchocoel; RM, proboscis retractor muscle; VG, ventral ganglion.

Blood system

A pair of lateral cephalic lacunae meets anteriorly above the rhynchodaeal opening. After passing through the cerebral ring, they connect ventrally to form a cross-sectionally U-shaped vessel; a thick-walled mid-dorsal vessel is dorsally branched off from the upper surface of the ventral portion of the U-shaped lacuna to enter the rhynchocoel (Fig. 19B). The U-shaped lacuna, with its two arms surrounding the cerebral sensory organs (Fig.

19B), then divides into two lateral lacunae just anterior to the mouth with the bottom becoming closed between the buccal wall and rhynchocoel. In the foregut region, the thin-walled lacunae lie lateral to the rhynchocoel and above the lateral edges of the foregut, and give rise ventrally to a delicate vascular network that surrounds the foregut laterally, ventrolaterally, and ventrally. In the anterior intestinal region, the vascular network converges posteriorly with a thick-walled lateral blood vessel on each side of the body, while the thin-walled lateral lacunae remain for a short distance, flanked by the rhynchocoel, before ending blindly. The lateral vessels lie ventrolateral to the alimentary canal between the splanchnic and body-wall longitudinal muscle layers. Initially each lateral vessel, together with the bordering splanchnic muscle layer, is distinctly embedded in the gut wall (Fig. 16). Posteriorly, even after the splanchnic muscle layer disappears, the lateral vessels remain in close contact with the intestine.

The mid-dorsal vessel runs inside the rhynchocoel to form a rhynchocoelic villus, then runs down between the rhynchocoel wall and the alimentary canal in the posterior foregut region. Farther backward in the intestinal region, the intestinal wall around the mid-dorsal vessel contains basophilic cells (Fig. 19A).

Pseudometameric transverse connections between the lateral and mid-dorsal vessels in the intestinal region were not found.

Nervous system

The brain and lateral nerve cords are situated between the epidermal basement membrane and the body-wall circular muscle layer. A single dorsal commissure, 22–28 µm thick, lies anteriad to the ventral one, 28–30 µm thick. Dorsal and ventral ganglia are almost the same size. Medially each lateral nerve contains a single giant fibre (Fig. 15D), about 2 µm in diameter, that can be traced forward to a neural cell body, about 5 µm across, located dorsolaterally in the dorsal commissure. The dorsal ganglion slightly forks posteriorly into upper and lower branches, the latter of which innervates the cerebral sensory organ. The upper mid-dorsal nerve originates in the dorsal commissure and extends posteriorly between the epidermal basement membrane and the body-wall outer circular muscle layer (Fig. 17C); farther back, in the anterior portion of the intestine, the mid-dorsal nerve rises to lie between the epidermis and the basement membrane (Fig. 17D); this nerve becomes indistinguishable posterior to the rhynchocoel. The mid-dorsal nerve sends numerous branches downwards to the dorsal side of the rhynchocoel wall; thus a lower mid-dorsal nerve seems to be present, but it is not continuous anteroposteriorly.

Frontal organ and cephalic glands

A frontal organ and cephalic glands are lacking.

Sense organs

On each side of the head there is an epidermal indentation 60 µm long in the anteroposterior axis and 80–100 µm long in the dorsoventral axis, lined with very long (about 20 µm) cilia, but no glandular cells. From each indentation, a ciliated canal leads postero-obliquely inward, narrowing from 40–45 µm to about 30 µm in external diameter (10 µm internal diameter), before turning medioventrally to enter a cerebral sensory organ on its dorsolateral surface 1/3 of the way from its anterior end; the canal runs posteriorly inside the organ without branching for the posterior 5/6 of the length of the organ, then terminates in a blind end. A bundle of nerve fibres, innervated from the ventral branch of the posterior end of the dorsal ganglion, runs along the medial side of the canal. Each cerebral sensory organ is an ovoid mass of neuro-glandular cells, oval in cross section, about 70–80 µm wide by 90–100 µm high, and about 120–150 µm long; it lies in the cephalic blood lacuna (Fig. 19B).

There are neither eyes nor lateral sensory organs.

Excretory system

Not found.

Reproductive system

All the three specimens examined were mature females. The ovaries, up to 170 µm in diameter, each containing a single egg, are embedded in the intestinal wall (Fig. 19A). A gonoduct leads from each ovary, passing above the lateral nerve cord, then opens dorsolaterally in the epidermis.

Systematic remarks

Hubrechtella (=*Coeia*) *ijimai* (Takakura, 1922) comb. nov. has not been redescribed since its original description (Takakura 1922), which provides an account mostly of the features of internal morphology used in the modern diagnosis for the genus *Hubrechtella*, but not of the characters currently used in distinguishing between species placed in this genus. Unfortunately, the type material of *Coeia ijimai* Takakura, 1922 is considered to be lost (Kajihara 2004); thus, it is impossible to identify this species using its internal morphology. Accordingly, I identified my specimens as conspecific with the nominal species *Coeia ijimai* Takakura, 1922 on the basis only of external characters. However, the resemblance of the shape of the head in living material to Takakura's (1922) illustration, and the nearness of my sampling site to the type locality, lend support to the identification of my material as *Coeia ijimai* Takakura, 1922.

One difference between Takakura's description and my material is the size of the body; Takakura's specimens measured over 20 cm in length, while mine do not exceed 5 cm. This difference may be due to the age of the worms. Eventually a neotype must be designated for *Coeia ijimai* Takakura, 1922; however, this will best be done after thorough study of newly collected material from the type locality, or at least closer to it.

The combination of the characters summarised in Table 3 enables *Hubrechtella ijimai*

(Takakura, 1922) comb. nov. to be distinguished from all other species currently placed into *Hubrechtella*.

TABLE 3. Comparison of four characters among *Hubrechtella* species. Data compiled from Bergendal (1902b), Hylbom (1957), Kirsteuer (1967), Senz (1992, 1993, 2000), Gibson (1979a, b, 1997), Gibson and Sundberg (1999), and Chernyshev (2003).

Taxa	A	B	C	D
H. alba Gibson, 1997	0	0	1	1
H. atypica Senz, 1992	0	2	1	0
H. combinata Senz, 1993	?	1	1	0
H. ehrenbergi Senz, 2000	0	1	1	0
H. dubia Bergendal, 1902	0	1	1	1
H. globocystica Senz, 1993	1	2	0	0
H. indica Kirsteuer, 1967	0	1	0	0
H. juliae Chernyshev, 2003	0	1	1	1
H. malabarensis Gibson, 1979	0	1	1	1
H. queenslandica Gibson, 1979	0	1	0	0
H. sarodravayensis Kirsteuer, 1967	0	0	1	0
H. sinimarina Gibson & Sundberg, 1999	0	0	0	1
***H. ijimai* (Takakura, 1922) comb. nov.**	1	2	1	0
***H. kimuraorum* sp. nov.**	1	2	1	0

Characters and character states:

A: Muscle layers in proboscis: (0) two; (1) three.

B: Bodies in proboscis epithelium: (0) lacking; (1) nematocyst-like, rhabditous; (2) spherical.

C: Mid-dorsal blood vessel: (0) does not penetrate the rhynchocoel; (1) penetrates rhynchocoel.

D: Zigzag fibres in body-wall musculature: (0) absent; (1) present.

Hubrechtella kimuraorum sp. nov. (Figs 20–23)

Diagnosis

Hubrechtella with a short tail; body-wall musculature without zigzag fibres; proboscis musculature composed of inner circular, middle longitudinal, and outer circular layers, without muscle cross; proboscis epithelium with spherical bodies; mid-dorsal blood vessel penetrating into rhynchocoel; foregut blood lacunar network present, followed by a broad U-shaped lacuna.

Etymology

The specific name is after Dr Taeko Kimura, a marine ecologist, and Mr Sho-ichi Kimura, a malacologist, who kindly helped me in field sampling.

Material examined

Holotype, ZIHU-3127, male, 1 August 2003, HK coll.: 72 slides, 6-µm serial transverse sections of the body except in the middle portion.

External features

The body is translucent white, about 3 cm long, 0.8 mm wide. The head is more transparent and slightly narrower than the following trunk (Fig. 20); the rhynchodaeum is cone-shaped, tapering posteriorly, visible through the body wall in the precerebral region. Active search behaviour, such as that of in *Hubrechtella ijimai* (Takakura, 1922) comb. nov., was not observed in the living specimen, which held the head rigid while moving, rather than waving it side to side. There is a short, transparent tail region that contains neither intestine nor gonads; it is shorter than twice the width of its basal portion, ending bluntly.

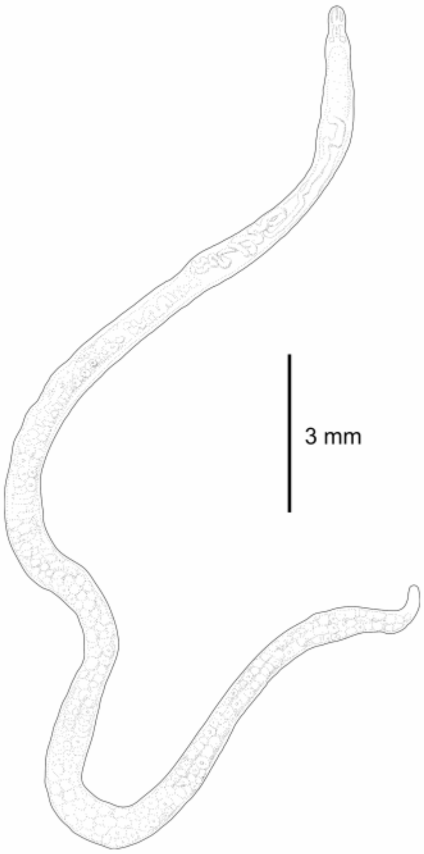

FIGURE 20. *Hubrechtella kimuraorum* sp. nov. Holotype, male, ZIHU-3127, drawing based on a photograph taken in life to show the general shape of the body.

Body wall, musculature and parenchyma

The ciliated epidermis is 20–30 µm thick in the brain region, thickest (up to 50 µm) in the stomach region, and thinnest posteriorly, about 9–10 µm thick in the intestinal region. The distribution of the four types of the glandular cells is similar to that in *Hubrechtella ijimai* (Takakura, 1922) comb. nov.; type 4 cell is similarly absent. *E* (*b*) = 0.09, *E* (*i*) = 0.03.

The basement membrane is about 3–4 µm thick in the brain and foregut regions and about 1 µm thick in the intestinal region. The morphology of the basement membrane in the pre-cerebral region is the same in *Hubrechtella ijimai* (Takakura, 1922) comb. nov. The arrangement of the basement membrane and neuro-fibrous layer also becomes inverted in the anterior intestinal region in this species. A mesh-like structure was not found.

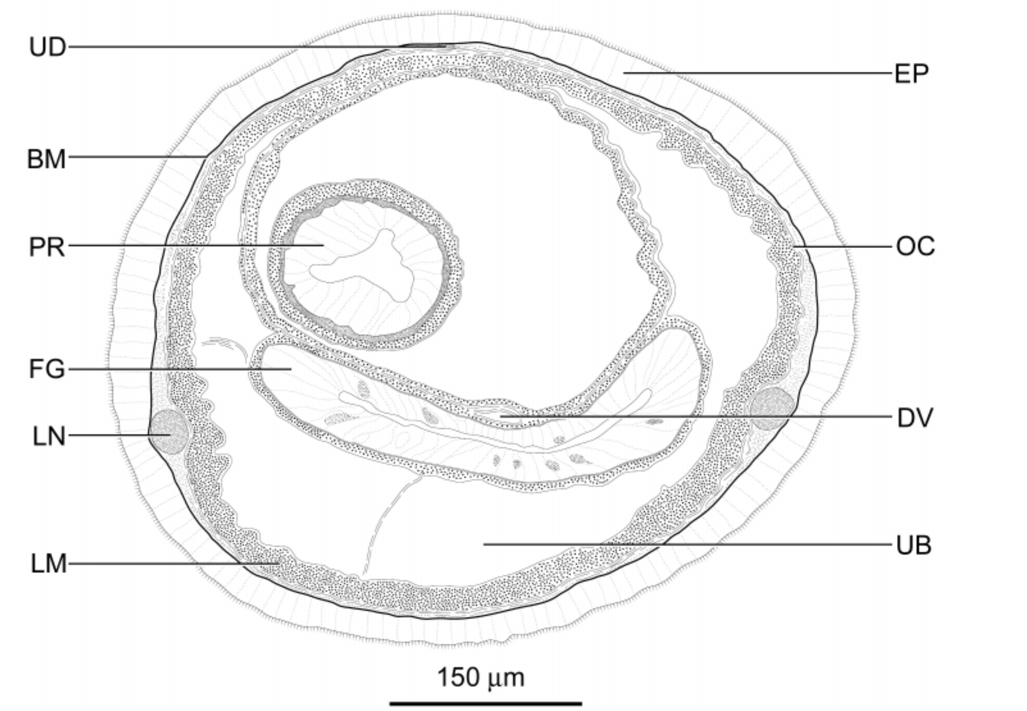

FIGURE 21. *Hubrechtella kimuraorum* sp. nov. Holotype, male, ZIHU-3127, drawing of a transverse section through the foregut region showing the well developed lacunar connection below the alimentary canal. BM, basement membrane; DV, mid-dorsal vessel; EP, epidermis; FG, foregut, LM, body-wall longitudinal muscle layer; LN, lateral nerve cord; OC, body-wall outer circular muscle layer; PR, proboscis; UB, U-shaped blood lacuna below gut; UD, upper mid-dorsal nerve.

The body-wall musculature comprises outer circular and inner longitudinal muscle layers, about 5 µm and 20 µm thick, respectively, in the foregut region (Fig. 21). Zigzag fibres were not found. The diagonal muscle layer is present but not conspicuous. Isolated radial muscle fibres traverse the lumina of the lateral blood lacunae, connecting the body-wall longitudinal muscle layer with the buccal/foregut wall. A longitudinal splanchnic muscle layer surrounds the foregut and anterior intestine (Fig. 21). Farther back, the dorsal portion of the splanchnic layer is retained between the intestine and rhynchocoel as a longitudinal muscle plate that terminates anterior to the end of the rhynchocoel.

Parenchymatous connective tissue is scarcely developed.

Proboscis apparatus

The rhynchodaeum is composed of 1) an unciliated epithelium, up to 40 µm thick, containing acidophilic glandular cells, 2) a thin, amorphous neuro-fibrous layer, 3) a thin connective tissue layer, and 4) a delicate outer circular muscle layer. Six nerves from the subepidermal neuro-glandular layer innervate the rhynchodaeum ventrolaterally on each side anterior to the proboscis insertion (Fig. 22A). No rhynchodaeal sphincter was found.

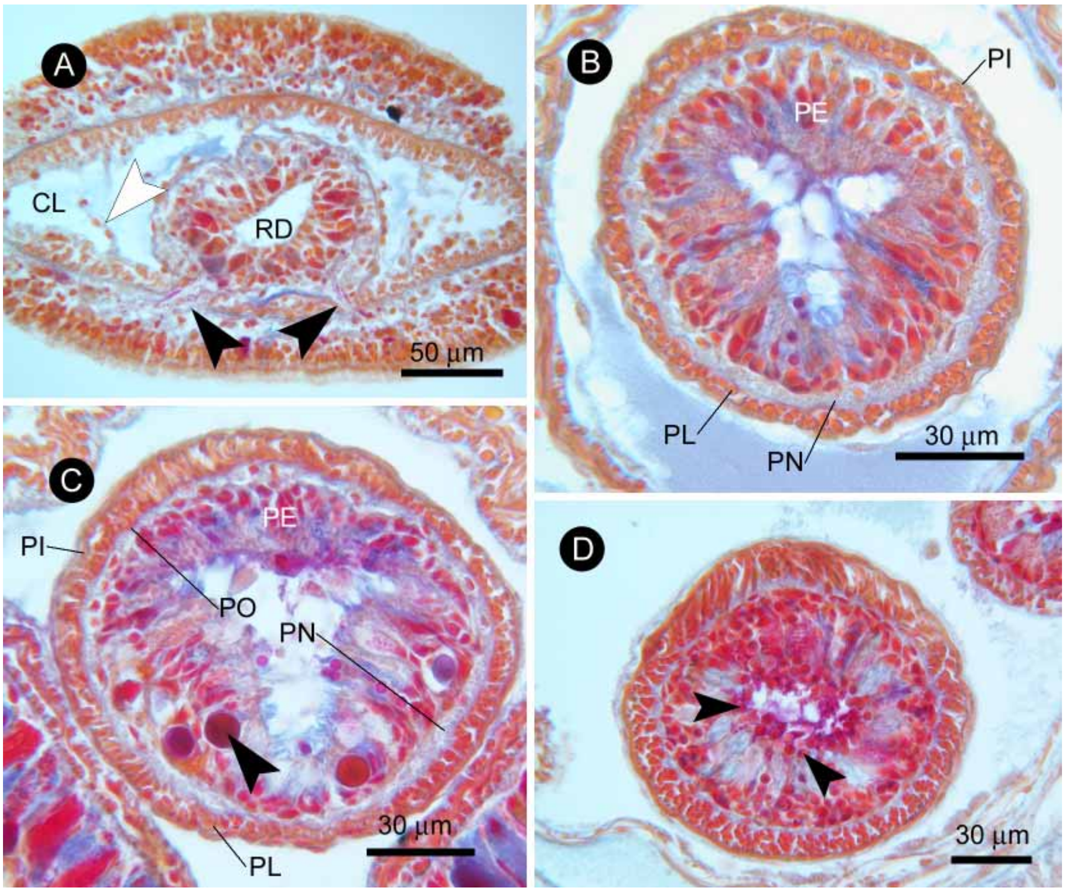

FIGURE 22. *Hubrechtella kimuraorum* sp. nov. Holotype, male, ZIHU-3127. A, transverse section through the pre-cerebral region; white arrowhead indicates a dorsoventral muscle fibre in the cephalic lacuna (CL), black arrowheads indicate innervation from the subepidermal neuro-glandular layer to the rhynchodaeum (RD). B, transverse section through the first region of the proboscis. C, transverse section through the second region of the proboscis; arrowhead indicates an acidophilic spherical body. D, transverse section through the third region of the proboscis; arrowheads indicate acidophilic spherules. PE, proboscis epithelium; PI, proboscis inner circular muscle layer; PL, proboscis longitudinal muscle layer; PN, nervous layer of proboscis; PO, proboscis outer circular muscle layer.

The rhynchocoel does not extend to the posterior end of the body and posteriorly is not developed into a muscular sac. Its wall contains an outer circular and an inner longitudinal muscle layer, separate from one another. There is no rhynchocoel caecum.

The proboscis insertion is located pre-cerebrally. The proboscis is differentiated into three regions. The anteriormost region contains an outer glandular layer, a neural layer, a longitudinal muscle coat, a thin circular muscle layer one-fibre thick, and an endothelium (Fig. 22B). The main component of the epithelium is acidophilic glandular cells, but there are also a few basophilic types. The second, main region is bilaterally symmetrical. A delicate outer circular muscle layer lies between the neural layer and the longitudinal muscle layer (Fig. 22C), but there was no indication of a muscle cross between the inner and outer circular muscle layers. In this region the lumen of the proboscis is T-shaped due to two zones of thickened glandular epithelium. The thickened epithelium basally contains conspicuous spherical acidophilic bodies, up to 10 µm in diameter; on the surface of the epithelium, there are much smaller acidophilic spherules, 2–3 µm in diameter (Fig. 22C). The third region contains small acidophilic spherules on the surface of its epithelium (Fig. 22D); behind it lies the proboscis retractor muscle.

Alimentary canal

The mouth opens close behind the cerebral sensory organs. The buccal/foregut wall reaches 50–60 µm or more in thickness, and is richly glandular and densely ciliated. It contains about equal numbers of basophilic and acidophilic glandular cells. Posteriorly the wall becomes less ciliated. The intestine contains a few basophils. There are no lateral diverticula.

Blood system

Paired lateral cephalic lacunae meet anteriorly above the rhynchodaeal opening. Each lacuna is pierced by isolated bundles of dorsoventral muscle fibres (Fig. 22A). The lacunae extend the full length of the head alongside the rhynchodaeum to enter the cerebral ring, where they meet ventrally and give off a small mid-dorsal branch that enters the rhynchocoel (Fig. 23A). The U-shaped lacuna soon divides into two branches just anterior to the mouth. Post-orally, these two lateral lacunae extend alongside the rhynchocoel. At the junction between the foregut and intestine, the lateral lacunae meet ventrally below the alimentary canal to form a spacious U-shaped lacuna (Fig. 21) that extends for about 1.3 mm. Posteriorly, the lacuna is divided into two lateral branches and a mid-ventral branch (Fig. 23B), which taper posteriorly and connect with one other to form a fine lacunar network (Fig. 23C). Eventually, the network fuses to form a pair of lateral vessels lying ventrolateral to the intestine and extending the remaining length of the body (Fig. 23D).

The mid-dorsal vessel runs inside the rhynchocoel wall as a rhynchocoelic villus, reaching the middle portion of the foregut. The mid-dorsal vessel then emerges from the rhynchocoel to lie appressed to the alimentary canal. As in *Hubrechtella ijimai* (Takakura,

1922) comb. nov., the intestinal wall in the vicinity of the mid-dorsal vessel contains basophilic cells (Fig. 23E).

No pseudometameric transverse vessels connecting the lateral and mid-dorsal vessels were found.

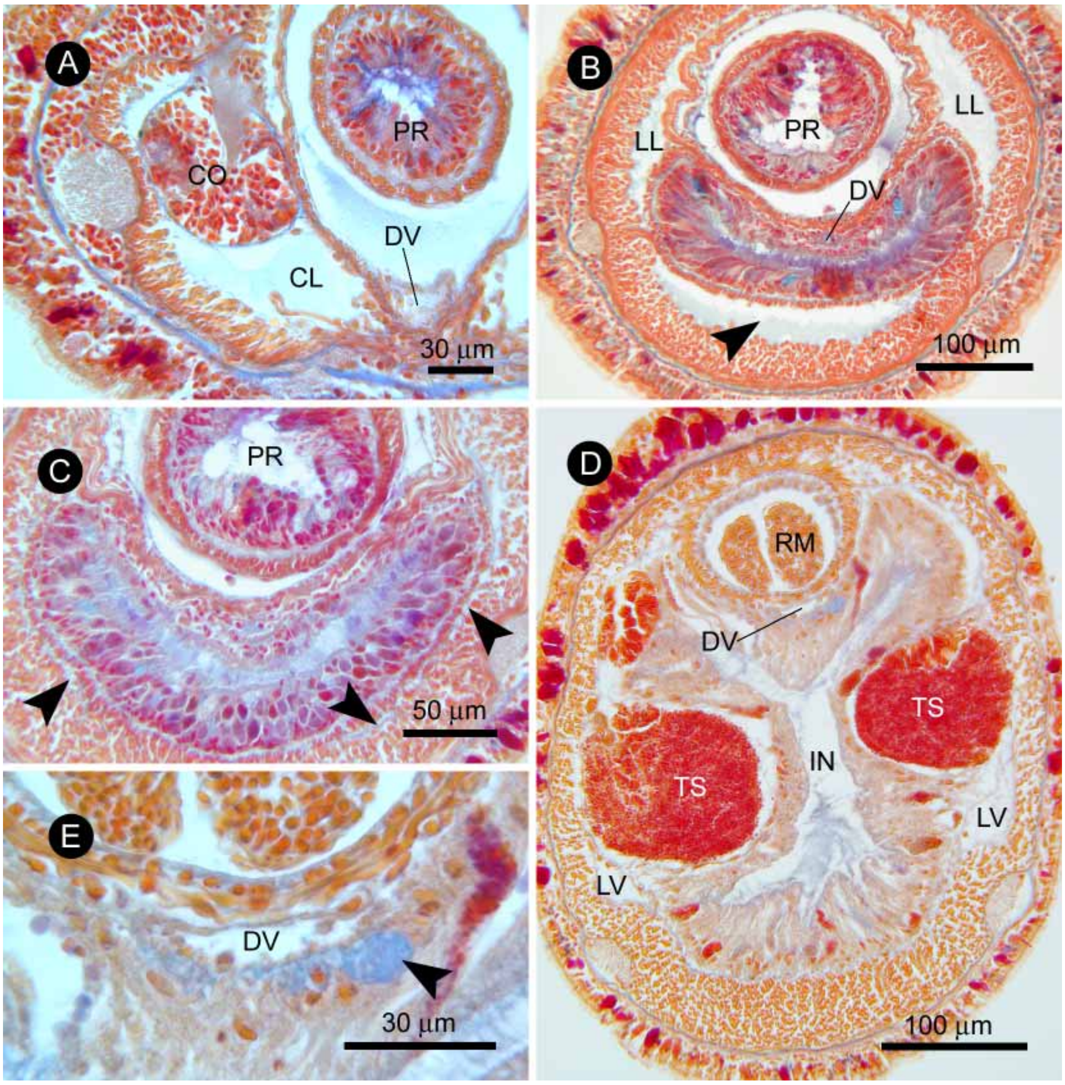

FIGURE 23. *Hubrechtella kimuraorum* sp. nov. Holotype, male, ZIHU-3127. A, transverse section through the cerebral sensory organ (CO). B, transverse section through the foregut region; arrowhead indicates the ventral branch of the lacunar connective. C, transverse section through the foregut region showing the appearance of the lacunar network below the foregut (indicated by the arrowheads). D, transverse section through the intestinal region showing the arrangement of the testes. E, higher magnification of part of D showing basophilic cells (indicated by the arrowhead) in the intestinal wall around the mid-dorsal vessel. CL, cephalic blood lacuna; DV, mid-dorsal vessel; IN, intestine; LL, lateral blood lacuna; LV, lateral blood vessel; PR, proboscis; RM, proboscis retractor muscle; TS, testis.

Nervous system

The arrangement of the nervous system is almost the same as that of *Hubrechtella ijimai* (Takakura, 1922) comb. nov. The lateral nerve cord contains a giant fibre on its medial side. The dorsal and ventral commissures are about 20 µm and 40 µm thick, respectively.

Frontal organ and cephalic glands

There are no frontal organ or cephalic glands.

Sense organs

A ciliated epidermal indentation lies dorsolaterally on each side of the body; it is 60 µm long in the dorsoventral axis and 50 µm long in the anteroposterial axis. The indentation contains no glandular cells, but has cilia about 10 µm long. A ciliated canal 20 µm in maximum external diameter leads from each epidermal indentation to enter the anterolateral side of the cerebral sensory organ, then extends inside the organ for about 70% of the length of the organ before ending blindly. Each cerebral sensory organ is elliptical in cross section (Fig. 23A), 68 µm along the minor axis and 85 µm along the major axis, and 140 µm long. Each sensory organ is innervated from the ventral branch of the posterior end of the dorsal ganglion; the core of nerve fibres in the organ is situated medial to the ciliated canal.

Neither eyes nor lateral sensory organs were found.

Excretory system

Not found.

Reproductive system

The single specimen is male. The paired testes are about 130 µm in maximum diameter, arranged in a single row on each side of the body, deeply embedded in the intestinal wall (Fig. 23D). The anteriormost testis lies anterior to the hind end of the rhynchocoel. A sperm duct, leading from each testis, opens above the lateral nerve cord.

Systematic remarks

Among the known species of *Hubrechtella*, *Hubrechtella kimuraorum* sp. nov. is most similar to *Hubrechtella ijimai* (Takakura, 1922) comb. nov. in terms of the internal morphology. Apart from the characters listed in Table 3, *Hubrechtella kimuraorum* sp. nov. can be distinguished from *Hubrechtella ijimai* (Takakura, 1922) comb. nov. by the absence of the muscle cross between the proboscis inner and outer circular muscle layers; the spacious U-shaped blood lacunar connection below the foregut, before the lacuna branches into a lacunar network; the shape of the head, which is narrower than the trunk in *Hubrechtella kimuraorum* sp. nov., but wider than the trunk in *Hubrechtella ijimai* (Takakura, 1922) comb. nov., and the shape of the tail, which is short in *Hubrechtella kimuraorum* sp. nov. but long in *Hubrechtella ijimai* (Takakura, 1922) comb. nov.

Acknowledgements

I am most grateful to Professor Teruaki Nishikawa for kindly arranging my field trip and offering specimens. Thanks are also due to Dr Taeko Kimura and Mr Sho-ichi Kimura for their support in my field sampling; the staff of the Fisheries Laboratory, Graduate School of Agricultural and Life Sciences, the University of Tokyo, for providing me the laboratory facilities and accommodation during my stay; to Mrs Junko Sato for her assistance in taking the digital photomicrographic images of the slides; to Dr Shin Tochinai for computational assistance; to Mr Andrey V. Grischenko for his help in translating Russian literature; to Dr Frank B. Crandall for bibliographic information; and to Professor Emeritus Ray Gibson and Dr Matthew H. Dick for their comments on an earlier version of the manuscript. This study was partially supported by Grants-in-Aid from the Japan Society for the Promotion of Science (research grant no. 16770059) and Wetlands International Japan.

References

Bergendal, D. (1900a) Studier öfver nemertiner. I. *Callinera bürgeri* Bergendal, en representant för ett afvikande slägte bland Paläonemertinerna. *Lunds Universitets Årsskrift*, 36, 1–47.

Bergendal, D. (1900b) Till kännedomen om de nordiska Nemertinerna. *Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar*, 57, 581–602.

Bergendal, D. (1900c) Über ein Paar sehr eigenthümliche nordische Nemertinen. *Zoologischer Anzeiger*, 23, 313–328.

Bergendal, D. (1901) Studier öfver nemertiner. I. *Callinera bürgeri* Bergendal, en representant för ett afvikande slägte bland Paläonemertinerna. *Lunds Universitets Årsskrift*, 37, 49–116.

Bergendal, D. (1902a) Zur Kenntnis der nordischen Nemertinen. 2. Eine der construierten Urnemertine entsprechende Palaeonemerine aus dem Meere der schwedischen Westküste. *Zoologischer Anzeiger*, 25, 421–432.

Bergendal, D. (1902b) Zur Kenntniss der nordischen Nemertinen. *Bergens Museums Årbog*, 4, 1–22.

Bergendal, D. (1903) Till kännedomen om de nordiska Nemertinerna. 4. Förteckning öfver vid Sveriges vestkust iakttagna Nemertiner. *Arkiv för Zoologi*, 1, 85–156.

Bürger, O. (1892) Zur Systematik der Nemertinenfauna des Golfs von Neapel. *Nachrichten von der Königlichen Gesellschaft der Wissenschaften und der Georg-Augusts-Universität zu Göttingen*, 5, 137–178.

Bürger, O. (1904a) Nemertini. *Das Tierreich*, 20, 1–151.

Bürger, O. (1904b) Nemertinen. *Resultats du voyage du S. Y. Belgica en 1897–1899, Zoologie*, 1–10.

Bürger, O. (1897–1907) Nemertini (Schnurwürmer). *In*: Bronn, H. G. (Ed.), *Klassen und Ordnungen des Tier-Reichs, wissenschaftlich dargestellt in Wort und Bild, Vierter Band, Supplement*. C. F. Winterísche Verlagshandlung, Leipzig, viii + 542 pp. [1897: pp. 1–176; 1898: pp. 177–240; 1899: pp. 241–288; 1903: pp. 289–384; 1905: pp. 385–480; 1907: pp. 481–542]

Cantell, C. -E. (1969) Morphology, development, and biology of the pilidium larvae (Nemertini) from the Swedish west coast. *Zoologiska Bidrag från Uppsala*, 38, 61–111.

Cantell, C. -E. (2001) On the anatomy and taxonomy of *Tubulanus lutescens* n. sp. (Nemertini) from the west coast of Sweden. *Ophelia*, 54, 213–221.

Chernyshev, A. V. (1995) On the higher taxa of the phylum Nemertea with the taxonomic review of the subclass Anopla. *Zoologicheskii Zhurnal*, 74, 7–18. [In Russian with English abstract]

Chernyshev, A. V. (1999) Nemertines of the family Carinomidae (Nemertea, Anopla). 1. Description of *Carinoma uschakovi* sp. n. and *Statolitonemertes sachalinica* Korotkevitsch. *Zoologicheskii Zhurnal*, 78, 1275-1283. [In Russian with English abstract]

Chernyshev, A. V. (2002) A new nemertean, *Callinera zhirmunskyi* sp. n., from the Pacific coast of Canada and a discussion of the taxonomic status of the family Callineridae. *Biologiya Morya*, 28, 143–146.

Chernyshev, A. V. (2003) A new species of the genus *Hubrechtella* (Nemertea, Anopla) from the Sea of Japan and establishment of the family Hubrechtellidae. *Biologiya Morya*, 29, 368–370. [In Russian with English abstract]

Friedrich, H. (1935a) *Carinina poseidoni* n. sp., eine neue Palaeonemertine aus der Nordsee. *Zoologischer Anzeiger*, 109, 64–72.

Friedrich, H. (1935b) Studien zur Morphologie, Systematik und Ökologie der Nemertinen der Kieler Bucht. *Archiv für Naturgeschichte, N. F.*, 4, 293–375.

Friedrich, H. (1970) Nemertinen aus Chile. *Sarsia*, 40, 1–80.

Gibson, R. (1979a) Nemerteans of the Great Barrier Reef. 1. Anopla Palaeonemertea. *Zoological Journal of the Linnean Society*, 65, 305–337.

Gibson, R. (1979b) *Hubrechtella malabarensis* sp. nov. (Palaeonemertea: Hubrechtidae), a new nemertean from Australia. *Zoologischer Anzeiger*, 202, 119–131.

Gibson, R. (1994) *Nemerteans*, Synopses of the British Fauna (New Series), Barnes R S K & Crothers J H (Eds), No. 24 (Second Edition), Field Studies Council, Shrewsbury, 224 pp.

Gibson, R. (1995) Nemertean genera and species of the world: an annotated checklist of original names and description citations, synonyms, current taxonomic status, habitats and recorded zoogeographic distribution. *Journal of Natural History*, 29, 271–562.

Gibson, R. (1997) New benthic nemerteans from Hong Kong. *In*: Morton, B (Ed.), *The Marine Flora and Fauna of Hong Kong and Southern China IV.* Hong Kong University Press, Hong Kong, pp. 9–29.

Gibson, R. (2002) *The Invertebrate Fauna of New Zealand: Nemertea (Ribbon Worms),* National Institute of Water and Atmospheric Research, Wellington, 87 pp.

Gibson, R. & Sundberg, P. (1999) Six new species of palaeonemerteans (Nemertea) from Hong Kong. *Zoological Journal of the Linnean Society*, 125, 151–196.

Hubrecht, A. A. W. (1885) The Nemertea. *Narrativ of the Cruise of H.M.S. Challenger Expedition*, 1, 830–833.

Hubrecht, A. A. W. (1887) Report on the nemertea collected by *H.M.S. Challenger* during the Years 1873–76. *Reports of the Scientific Results of the Voyage of H.M.S. Challenger 1873–1876, Zoology*, 19, 1–150.

Hylbom, R. (1957) Studies on palaeonemerteans of the Gullmar Fiord area (West Coast of Sweden). *Arkiv för Zoologi*, 10, 539–582.

International Commission on Zoological Nomenclature (1985) *International Code of Zoological Nomenclature, Third Edition*, International Trust for Zoological Nomenclature, London, 338 pp.

International Commission on Zoological Nomenclature (1988) Opinion 1486, *Tubulanus* Renier, [1804] and *T. polymorphus* Renier, [1804] (Nemertea): reinstated and made available. *Bulletin of Zoological Nomenclature*, 45, 157–158.

International Commission on Zoological Nomenclature (1999) *International Code of Zoological Nomenclature, Fourth Edition*, International Trust for Zoological Nomenclature, London, 306 pp.

Iwata, F. (1951) Nemerteans in the vicinity of Onomichi. *Journal of the Faculty of Science, Hokkaido University, Series 6, Zoology*, 10, 135–138.

Iwata, F. (1952) Nemertini from the coasts of Kyusyu. *Journal of the Faculty of Science, Hokkaido*

University, Series 6, Zoology, 11, 126–148.

Iwata, F. (1954a) The fauna of Akkeshi Bay. XX. Nemertini in Hokkaido. *Journal of the Faculty of Science, Hokkaido University, Series 6, Zoology*, 12, 1–39.

Iwata, F. (1954b) Some nemerteans from the coasts of the Kii Peninsula. *Publications of the Seto Marine Biological Laboratory*, 4, 33–42.

Iwata, F. (1957) Nemerteans from Sagami Bay. *Publications from the Akkeshi Marine Biological Station*, 7, 1–31.

Joubin, L. (1902) Némertiens. *Expéditions scientifiques du 'Travailleur' et du 'Talisman'*, 4, 181–220.

Kajihara, H. (2004) Usamaro Takakura (1867–1944), Japanese pioneer nemertean researcher. *Archives of Natural History*, 32, 208–213.

Kirsteuer, E. (1967) New marine nemerteans from Nossi Be, Madagascar. *Zoologischer Anzeiger*, 178, 110–122.

Korotkevich, V. S. (1982) Novuie i redkie vidui nemertin (Nemertini) s yuzhnogo shelfa ostrova sakhalin. *Issledovaniya Faunui Morei*, 37, 12–26. [In Russian]

Kulikova, V. I. (1984) Novyi vid nemertiny roda *Carinina* (Palaeonemertini, Tubulanidae) iz belogo morya. *Zoologicheskii zhurnal*, 63, 1092–1095. [In Russian with English abstract]

McIntosh (1873–1874) *A Monograph of the British Annelids. Part I. The Nemerteans*, Ray Society, London, 214 pp. [1873: pp. 1–96, pls I–X; 1874; pp. 97–214, pls XI–XXIII]

Melville, R. V. (1986) *Tubulanus* Renier, [1804] and *T. polymorphus* Renier, [1804] (Polychaeta): proposed reinstatement under the plenary powers. *Bulletin of Zoological Nomenclature*, 43, 112–114.

Müller, G. I. (1965) *Carinina heterosoma* n. sp. Ľi c”teva consideraĘii asupra genului *Carinina* (Vermes, Palaeonemertini). *Hidrobiologia*, 6, 243–257. [In Romanian with German abstract]

Nawitzki, W. (1931) *Procarinina remanei*. Eine neue Paläonemertine der Kieler Förde. *Zoologische Jahrbücher*, 54, 159–234.

Rogers, A. D., Gibson, R. & Thorpe, J. P. (1992) A new species of *Callinera* (Nemertea, Anopla, Palaeonemertea) from the Isle of Man. *Zoologica Scripta*, 21, 119–128.

Senz, W. (1992) *Hubrechtella atypica* sp. n. (Nemertini: Palaeonemertini). *Zoologischer Anzeiger*, 229, 185–190.

Senz, W. (1993) Nemertinen europäischer Küstenbereiche (nebst ergänzenden Angaben zur Anatomie von *Apartonemertes albimaculosa* Wilfert & Gibson, 1974). *Annalen des Naturhistorischen Museums Wien*, 94/95B, 47–145.

Senz, W. (1997) Morphologie und klassifikatorische Position einiger anopler Nemertinen (Nemertini: Anopla). *Annalen des Naturhistorischen Museums in Wien*, 99B, 423–496.

Senz, W. (2000) Neue Nemertinen aus dem Golf von Arabien. 1. Palaeonemertini. *Annalen der Naturhistorischen Museums in Wien*, 102B, 321–373.

Shimomura, M., Kato, T. & Kajihara, H. (2001) Records of some marine invertebrates (nemerteans, asellotes and phyllodocids) from the coast around Otsuchi Bay. *Otsuchi Marine Science*, 26, 46–50.

Sundberg, P. & Hylbom, R. (1994) Phylogeny of the nemertean subclass Palaeonemertea (Anopla, Nemertea). *Cladistics*, 10, 347–402.

Sundberg, P., Turbeville, J. M. & Lindh, S. (2001) Phylogenetic relationships among higher nemertean (Nemertea) taxa inferred from 18S rDNA sequences. *Molecular Phylogenetics and Evolution*, 20, 327–334.

Takakura, U. (1898) Misaki kinbousan himomushi (Nemertini) no bunrui [Classification of the nemerteans of the vicinity of Misaki]. *Zoological Magazine, Tokyo*, 10, 38–44, 116–120, 184–187, 331–337, 424–429. [In Japanese]

Takakura, U. (1922) Honpousan himomushi no ichi shinzoku ni tsukite [On a new genus of nemertean from Japan]. *Zoological Magazine, Tokyo*, 34, 419–422. [In Japanese]

Thollesson, M. & Norenburg, J. L. (2003) Ribbon worm relationships: a phylogeny of the phylum Nemertea. *Proceedings of the Royal Society of London, Series B*, 270, 407–415.

Wada, M., Sugiura, H., Fujihara, S., Ho, Y., Hosoi, E., Isa, T., Yokoyama, I., Nishibori, C., Hagihara, K. & Kubo, A. (2002) *DeltaViewer Project*. DeltaViewer Project Team, Department of Information and Computer Sciences, Nara Women's University, Japan. Available from: http://vivaldi.ics.nara-wu.ac.jp/˜wada/DeltaViewer/ (accessed 16 September 2005)

Wheeler, J. F. G. (1940) Nemerteans of Kerguelen and the southern ocean. *B. A. N. Z. Antarctic Research Expedition Report*, *Series B*, 4, 233–256.

Yamaoka, T. (1940) The fauna of Akkeshi Bay. IX. Nemertini. *Journal of the Faculty of Science, Hokkaido Imperial University, Series 6, Zoology*, 7, 205–263.