

# Potamostoma shizunaiense gen. et sp. nov. (Nemertea: Hoplonemertea: Monostilifera): a New Brackish-Water Nemertean from Japan

Authors: Kajihara, Hiroshi, Gibson, Ray, and Mawatari, Shunsuke F.

Source: Zoological Science, 20(4): 491-500

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.20.491

The BioOne Digital Library (<a href="https://bioone.org/">https://bioone.org/</a>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<a href="https://bioone.org/subscribe">https://bioone.org/subscribe</a>), the BioOne Complete Archive (<a href="https://bioone.org/archive">https://bioone.org/archive</a>), and the BioOne eBooks program offerings ESA eBook Collection (<a href="https://bioone.org/esa-ebooks">https://bioone.org/esa-ebooks</a>) and CSIRO Publishing BioSelect Collection (<a href="https://bioone.org/csiro-ebooks">https://bioone.org/esa-ebooks</a>)

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at <a href="https://www.bioone.org/terms-of-use">www.bioone.org/terms-of-use</a>.

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

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Potamostoma shizunaiense gen. et sp. nov. (Nemertea: Hoplonemertea: Monostilifera): a New Brackish-Water Nemertean from Japan

Hiroshi Kajihara<sup>1\*</sup>, Ray Gibson<sup>2</sup>, and Shunsuke F. Mawatari<sup>1</sup>

<sup>1</sup>Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan <sup>2</sup>School of Biological and Earth Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, U.K.

**ABSTRACT**—*Potamostoma shizunaiense* gen. et sp. nov. (Nemertea: Hoplonemertea: Monostilifera) is described from the mouth of the River Shizunai, Hokkaido, Japan. This genus is readily distinguished from other monostiliferans by an oesophagus opening far anteriorly into the rhynchodaeum, a well developed excretory system extending the whole body length, terminals of the excretory collecting tubules situated between the body wall circular muscle layer and the dermis, and bilobed testes in males.

Key words: Nemertea, excretory system, Hokkaido, the River Shizunai

#### INTRODUCTION

Nemertean worms (phylum Nemertea), with approximately 1150 known species (Gibson, 1995), are typically marine invertebrates but several species are known to occur in brackish waters and some have been able to colonise either freshwater or terrestrial habitats. Some species of brackish-water nemerteans are known to thrive in stable, albeit reduced, salinities. An example is the hoplonemertean Cyanophthalma obscura (Schultze, 1851), which has been found in salt marshes or salt marsh pools on the Atlantic coast of North America (Norenburg, 1986), and is very common in the Gulf of Finland where salinities are below 5-6 psu (Brunberg, 1964). On the other hand, there have been only a few reports on species inhabiting in those environments, where the salinity fluctuates rapidly, like in mangrove swamps, river mouths or brackish-lakes (e.g. Gibson and Qi, 1991; Iwata, 1970; Moore and Gibson, 1981) (Table 1).

During the first author's faunal study around Hokkaido, northern Japan, a hoplonemertean species was obtained at the mouth of the River Shizunai. Subsequent histological study revealed that the combination of morphological characters found in the present nemerteans excludes them from all of the described genera of Monostilifera. The present paper aims to describe the new genus and species of

FAX. +81-11-746-0862. E-mail: kazi@sci.hokudai.ac.jp monostiliferous hoplonemertean inhabiting in salinity down to 2 psu.

Current classifications of nemerteans, particularly above generic level, are in need of thorough taxonomic revision (Gibson, 1985). The traditional taxonomic scheme will change in the future (Sundberg and Gibson, 1995). The new species is not assigned to family for this reason.

# **MATERIAL AND METHODS**

Specimens of the nemerteans were collected from beneath stones on sand at the mouth of the River Shizunai, Hokkaido, Japan (42°20'N, 142°22'E) on 9 May 1996, 16 November 1997, and 24 November 1999. Sampling was carried out at ebb tide when the salinity was about 2 psu. The nemerteans were anaesthetised either in 4% MgCl<sub>2</sub> or MS222, fixed in Bouin's solution for 24hr, embedded in 56–57°C m.p. paraffin wax and sectioned at 6, 7 or 10  $\mu m$ . Most sections were stained by the Mallory trichrome method, but for one slide series haematoxylin and eosin was used. Observations on the stylet apparatus of 11 specimens were made by the squeezing method described by Kirsteuer (1967).

Type material and voucher specimens are deposited in the Zoological Institute, Hokkaido University, Japan (ZIHU).

# Abbreviations used in figures.

**ao** = apical organ; **ap** = anterior pouch of intestinal caecum; **br** = brain; **cg** = cephalic gland; **cm** = body-wall circular muscle layer; **co** = cerebral sensory organ; **cv** = cephalic blood vessel; **de** = dermis; **dm** = dorsoventral muscle; **dv** = mid-dorsal vessel; **ep** = epidermis; **ex** = excretory collecting tubule; **g1** = orange-staining acidophilic gland cell; **g2** = purple-staining basophilic gland cell; **gc** = glandular component of cerebral sensory organ; **ic** = intestinal caecum; **il** = inner layer of body wall longitudinal musculature; **in** = intestine; **Id** = lateral diverticulum of intestinal caecum; **Im** = body-wall longitu-

<sup>\*</sup> Corresponding author: Tel. +81-11-706-2755;

492 H. Kajihara *et al.* 

**Table 1.** Nemertean species recorded from estuarine or other brackish-water habitats.

Species	Comments			
HETERONEMERTEA				
Amniclineus zhujangensis Gibson and Qi, 1991	Found in the lower part of the Pearl River, People's Republic of China, mostly in freshwater conditions (<0.5 psu) but the habitat occasionally reaches a maximum salinity of about 10.9 psu (Gibson and Qi, 1991).			
Hinumanemertes kikuchii lwata, 1970	In mud, Lake Hinuma, Honshu, Japan; the salinity of the lake varies from about 15 psu in summer to about 21 psu in winter (lwata, 1970).			
Micrura baltica Cantell, 1975	From a muddy substratum, Tvären Bay, the Baltic coast of Sweeden; the salinity of the area is usually slightly above 6.5 psu (Bergh, 1973).			
HOPLONEMERTEA MONOSTILIFERA				
Amphiporella baltica Friedrich, 1940	From the south-western Baltic Sea, south of the island of Öland, east of Bornholm (Friedrich, 1940).			
Amphiporus bicoloreus Korotkevich, 1977	Found in intertidal salinities down to about 18 psu in the Kuril Islands, Sea of Okhotsk, Russia (Korotkevich, 1977).			
Amphiporus korschelti Friedrich, 1940	South-western Baltic Sea, west of the island of Bornholm.			
Amphiporus obtusorostris Korotkevich, 1977	Brackish-water intertidal in salinities down to about 18 psu, Kuril Islands, Sea of Okhotsk, Russia (Korotkevich, 1977).			
Arenonemertes minutus Friedrich, 1949	Intertidal in sand, south-western Baltic Sea.			
Communoporus cephalonephridialis (Friedrich, 1940)	From the Baltic Sea, west of the island of Bornholm.			
Communoporus hagmeieri (Friedrich, 1940)	East of Bornholm in the Baltic Sea.			
Communoporus rhynchocoelomicus (Friedrich, 1940)	South of the southern tip of Sweeden, Baltic Sea.			
Cyanophthalma obscura (Schultze, 1851)	In salt marshes or salt marsh pools, Atlantic coast of North America (Norenburg, 1986), Baltic Sea, and the Gulf of Finland in salinities below 5–6 psu.			
Pantinonemertes winsori Moore and Gibson, 1981	In brackish-water mongrove swamps, under the bark or in cavities of rotting fallen mangrove trunks, Queensland, Australia (Moore and Gibson, 1981).			
Proneurotes baltica Friedrich, 1940	Baltic Sea.			
Sacconemertella lutulenta lwata, 1970	In mud, Lake Hinuma, Honshu, Japan, salinity ranging from about 5 psu in summer to 21 psu in winter, occasionally as high as 30 psu at the bottom (lwata, 1970).			
Sacconemertes arenosa Karling, 1933	Western end of the Gulf of Finland, salinity less than 5–6 psu (Karling, 1933).			
Sacconemertopsis belogurovi Chernyshev, 1991	In the upper estuarine region of the River Gladkaya, Russia, at salinities of 1–3 psu (Chernyshev, 1991).			
Sacconemertopsis olivifera lwata, 1970	Lake Hinuma, Honshu, Japan, in mud, salinity range approximately 5–21 psu between summer and winter (Iwata, 1970).			
Tetrastemma tridentata Korotkevich, 1977	Intertidal brackish-water down to salinities of about 18 psu, Kuril Islands, Sea of Okhotsk, Russia (Korotkevich, 1977).			

dinal muscle layer;  $|\mathbf{n}| = |\mathbf{n}|$  lateral nerve cord;  $|\mathbf{v}| = |\mathbf{n}|$  lateral blood vessel;  $|\mathbf{n}| = |\mathbf{n}|$  putative nurse cell;  $|\mathbf{n}| = |\mathbf{n}|$  neuroganglionic component of cerebral sensory organ;  $|\mathbf{o}| = |\mathbf{n}|$  ocellus;  $|\mathbf{o}| = |\mathbf{n}|$  proboscis insertion;  $|\mathbf{p}| = |\mathbf{n}|$  proboscis;  $|\mathbf{p}| = |\mathbf{n}|$  pylorus;  $|\mathbf{r}| = |\mathbf{n}|$  prohocoel;  $|\mathbf{$ 

#### **DESCRIPTION**

#### Potamostoma gen. nov.

Type species: Potamostoma shizunaiense sp. nov.

Etymology: The generic name (neuter) is formed by combining the Greek words potamos (= a river) and stoma (= a mouth) to indicate the habitat in which the animals were found living. The specific epithet is derived from the name of the River Shizunai by adding the Latin adjectival suffix -ense (= belonging to).

Diagnosis: Monostiliferous hoplonemerteans with rhyncho-

coel reaching to posterior end of body; rhynchocoel musculature in two separate layers; proboscis anterior chamber with three muscle layers (outer and inner circular, middle longitudinal); body-wall musculature with diagonal layer, longitudinal layer anteriorly divided by posterior extensions of cephalic glands; pre-cerebral septum absent; cephalic retractor muscles derived from outer portion of longitudinal musculature; oesophagus opening into rhynchodaeum just behind proboscis pore; intestinal caecum with two anterior pouches and lateral diverticula; blood system very well developed, with single vascular plug; cerebral ganglia with neither inner neurilemma nor neurochord cells; lateral nerve cords without accessory nerves but with myofibrillae; peripheral nervous system well developed; single apical organ present, cephalic glands well developed, extending behind brain, sub-muscular glands present; cerebral organs close in front of brain; excretory system well developed, extending

throughout body length, collecting tubule terminals situated between body wall circular muscle layer and dermis; dioecious, testes bilobed.

#### Potamostoma shizunaiense sp. nov.

Material examined: A total of 32 living individuals were collected, of which 11 were examined in vivo by the squeezing



**Fig. 1.** Potamostoma shizunaiense gen. et sp. nov. Photograph of a living specimen, viewed dorsally. Scale bar = 5 mm.

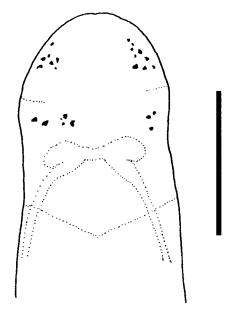
method described by Kirsteuer (1967): 4 specimens, 9 May 1996; 24 specimens, 16 November 1997; 4 specimens, 24 November 1999. Holotype, immature female, complete series of transverse sections, 7  $\mu$ m, 81 slides (ZIHU-2037); paratypes, immature male, transverse sections of anterior body region, 6  $\mu$ m, 39 slides, (ZIHU-1930) and immature female, complete series of transverse sections, 6  $\mu$ m, 84 slides (ZIHU-2040). Nine sectioned voucher specimens are also deposited under ZIHU-1931, -1932, -1933, -2038, -2039, -2041, -2042, -2043, -2044.

Type locality: Mouth of the River Shizunai, Hokkaido, Japan (42°20'N, 142°22'E), under stones on a sandy bottom. Salinity at ebb tide 2 psu.

External features: Body slender, up to 6 cm long and 1.2 mm wide (Fig. 1). Head oval or truncate triangular shape. Eyes arranged in four groups, each containing 2-22 black to dark green pigment-cup ocelli (Fig. 2). White pigment spots on mid-dorsal surface of snout. Two pairs of cephalic furrows present; posterior furrows encircle the body, forming caudally directed V-shape on dorsal surface (Fig. 2). Body gradually tapered posteriorly, ending in bluntly rounded tail.

General body colour yellowish-green to olive, rhynchocoel appearing as yellowish-ochre median dorsal line extending almost to posterior tip. Ovaries in ripening females visible as deep greenish bands arranged pseudometamerically in posterior half of body. Lateral nerve cords also visible through lateral body margins as dark orange lines fringed with whitish dots.

Body wall, musculature and parenchyma: Epidermis ciliated, with maximum thickness of about 38  $\mu$ m in anterior body regions generally thinner posteriorly and in places only 15–



**Fig. 2.** Potamostoma shizunaiense gen. et sp. nov. Enlargement of the head, viewed dorsally, to show the arrangement of the eyes and cephalic furrows. The position of the brain lobes and anterior portions of the lateral nerve cords is also indicated. Scale bar = 1 mm.

20  $\mu m$  tall (Fig. 3). Dermis consistently about 5  $\mu m$  thick throughout body (Fig. 3).

Body wall muscles consisted primarily of outer circular and inner longitudinal layers; circular layer 3-7 µm thick and frequently much thinner than dermis, longitudinal layer varies in thickness from 20-55 µm depending upon local contraction (Fig. 3). In cerebral region longitudinal layer divided into outer and inner zones by posterior extensions of cephalic glands (Fig. 4A). Outer portion, not contributing to proboscis insertion, extended to head tip. Most of inner longitudinal muscle fibres terminated in proboscis insertion, but a few extended anteriorly surrounding rhynchodaeum (Fig. 4B). Cephalic retractor muscles derived from outer portion of longitudinal layer. Thin diagonal muscle layer (Fig. 4C), extending between the two main body wall muscle coats, not enclosed by connective tissue. Dorsoventral muscles moderately well developed in foregut and intestinal regions.

Parenchymatous connective tissues well developed throughout body length, most obvious below rhynchocoel just behind brain.

*Proboscis apparatus*: Proboscis pore, opening ventrally just behind head tip, leading into short ciliated chamber (Fig. 4D, E). Rhynchodaeum with delicate and unciliated epithelium,  $10~\mu m$  in average thickness (Fig. 4E, F). Immediately in front of proboscis insertion, rhynchodaeum surrounded by

sphincter-like layer of circular muscles.

Rhynchocoel reaching almost to posterior tip of body, with wall containing separate inner longitudinal and outer circular muscle layers (Fig. 3). Each layer mostly 2–5  $\mu$ m in thickness, but circular layer in posterior portion of rhynchocoel thickened to about 15  $\mu$ m.

Proboscis slightly shorter than body, white in colour when everted. Larger anterior portion, 30--47% of body diameter when retracted, consisted of papillate epithelium,  $60\text{--}80~\mu\text{m}$  or more tall, containing acidophilic gland cells, distinct connective tissue basement layer, weak layer of outer circular muscles, inner longitudinal muscle stratum 15  $\mu\text{m}$  in maximum thickness, a delicate inner circular muscle layer and a flattened inner lining. Proboscis nerves running in longitudinal muscle layer (Fig. 5A); of 12 sectioned specimens one individual possessed 11 proboscis nerves, one had 12 nerves, and the remainder 13.

Central stylet smooth (Fig. 5B), variable in length, 82–110  $\mu$ m long (mean 93  $\mu$ m); basis 98–158  $\mu$ m long (mean 128  $\mu$ m), 32–54  $\mu$ m wide (mean 45  $\mu$ m) (*in vivo* observation; n=11). Stylet/basis ratio 0.62–0.88 (mean 0.73). Number of accessory stylet pouches 2–4 (mostly 3), each containing 2–4 accessory stylets.

Posterior proboscis chamber about 25–35% of the body diameter, with wall containing basophilic glandular epithelium not forming papillae, outer longitudinal muscle layer

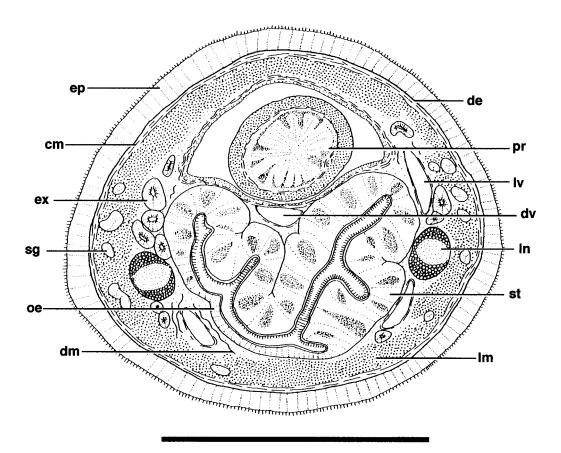
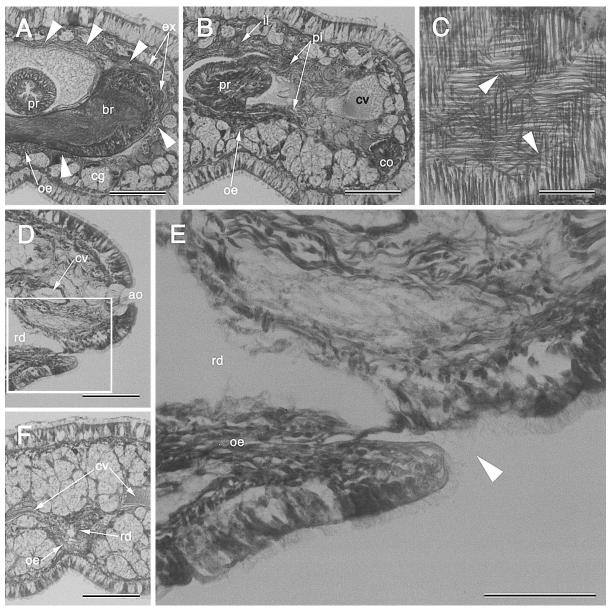


Fig. 3. Potamostoma shizunaiense gen. et sp. nov. Camera lucida drawing of a transverse section to show the organization of the body in the foregut region. Scale bar =  $400 \mu m$ .



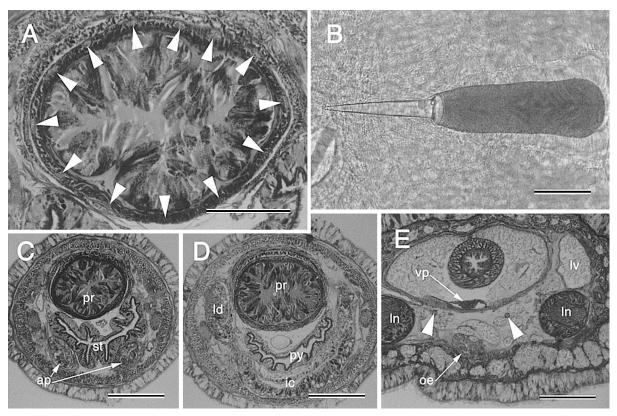
**Fig. 4.** *Potamostoma shizunaiense* gen. et sp. nov. **A.** Transverse section through the brain to show the divided body-wall longitudinal muscle layer. Arrowheads indicate the inner portion of the longitudinal musculature. **B.** Transverse section through the proboscis insertion. **C.** Tangential section through the body wall musculature to show the non-fasciated diagonal fibres, indicated by arrowheads. **D.** Sagittal section through the anterior tip of the head. **E.** Higher magnification of the boxed area in D to show the oesophagus opening (indicated by the arrowhead) from the ventral wall of the rhynchodaeum just behind the proboscis pore. **F.** Transverse section near the tip of the head at the point where the oesophagus is about to branch off from the rhynchodaeum. Scale bars: **A, B, D, F** = 100 μm; **C, E** = 50 μm.

about 5  $\mu m$  thick, thin and indistinct inner circular muscle layer and delicate lining layer. No nerves were distinguished in posterior portion of proboscis.

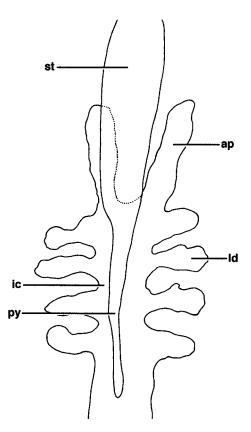
Alimentary canal: Oesophagus moderately thick-walled, opening at ventral wall of rhynchodaeum just behind proboscis pore (Fig. 4D, E). Oesophageal epithelium neither ciliated nor glandular. After passing below ventral cerebral commissure, oesophagus expanded leading to stomach; transitional region between oesophagus and stomach contained violet-staining basophilic gland cells with granular cytoplasm.

Stomach epithelium (Fig. 3, 5C), reaching to 70  $\mu m$  or more in height, dominated by basophilic glands but also contained a few acidophils. Degree of epithelial folding varied among individuals. Stomach gradually narrowed posteriorly, with both epithelial height and gland cell number decreasing posteriorly, leading into narrow pyloric canal (Fig. 5D) opening into dorsal wall of intestine.

Intestinal caecum with a pair of anterior pouches and several pairs of deep lateral diverticula (Fig. 5D, 6): anterior pouches extended forward to middle portion of stomach, not reaching brain. Intestine with distally branched deep lateral



**Fig. 5.** *Potamostoma shizunaiense* gen. et sp. nov. **A.** Transverse section through the anterior portion of the proboscis to show the 13 proboscis nerves, indicated by arrowheads. **B.** Photomicrograph of a typical central stylet and basis. **C.** Transverse section through the posterior region of the stomach. **D.** Transverse section through the pyloric region of the gut. **E.** Transverse section to show the single vascular plug. Foregut nerves are indicated by the arrowheads. Scale bars: **A, B, E** = 100  $\mu$ m; **C, D** = 200  $\mu$ m.



diverticula along whole length. Anus at posterior end of body.

Blood system: Blood vascular system well developed. Pair of cephalic vessels joinning anteriorly by loop running dorsally over front of rhynchodaeum (Fig. 4B, D). Posteriorly, vessels entering cerebral ring. Mid-dorsal vessel arises from one of the lateral vessels. Behind brain three longitudinal vessels, one mid-dorsal and two lateral, extend through remaining body length without connection until meeting posteriorly by supra-intestinal loop. Mid-dorsal vessel, soon after origin, entering ventral rhynchocoel wall forming single large vascular plug up to 55 μm wide and 30 μm tall (Fig. 5E). Blood vessels with distinct walls throughout, up to 60 μm wide anteriorly; gradually narrower in posterior intestinal region.

Nervous system: Brain moderately large, with dorsal and ventral lobes of similar size (Fig. 4A). Dorsal and ventral commissures 20–25  $\mu m$  and 45–50  $\mu m$  thick, respectively. Thin outer neurilemma investing cerebral ganglia, but without inner neurilemma. Lateral nerve cords, lacking accessory nerves and neurochords, meeting posteriorly by supra-intestinal commissure.

**Fig. 6.** Potamostoma shizunaiense gen. et sp. nov. Schematic diagram to show the arrangement of the anterior pouches and lateral diverticula of the intestinal caecum, drawn from living material.

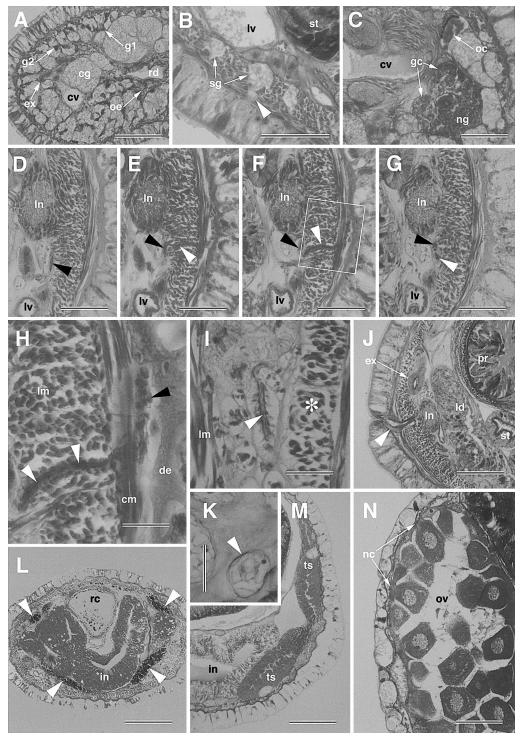


Fig. 7. Potamostoma shizunaiense gen. et sp. nov. A. Transverse section through the pre-cerebral region. B. Transverse section through the stomach region to show the sub-muscular glands, with the aperture of one of the glands indicated by the arrowhead. C. Transverse section to show the appearance of a cerebral sensory organ. D–G. Serial transverse sections through the stomach region to show the excretory collecting tubules, indicated by black arrowheads. White arrowheads in E–G indicate another collecting tubule leading to its terminal region situated between the dermis and body wall circular muscle layer. H. Enlargement of the boxed area shown in F. White arrowheads indicate an excretory tubule leading to its terminal region which is surrounded by small cells (indicated by black arrowhead). I. Longitudinal section to show a putative terminal region of an excretory collecting tubule, indicated by arrowhead. The asterisk indicates the rhynchocoel circular muscle layer. J. Transverse section through the posterior stomach region to show one of the nephridiopores, indicated by the arrowhead. K. Transverse section through the posterior brain region to show one of the sinuses, indicated by the arrowhead, containing unidentified cells, situated in the parenchyma. L. Transverse section through the intestinal region to show the dark pigment granules in immature ovaries (indicated by arrowheads). M. Transverse section through the intestinal region of an immature male with evidence of early spermatogenesis to show the bilobed appearance of a testis. N. Transverse section through the intestinal region of an immature female to show the appearance of a developing ovary. Scale bars: A, J, N = 100 μm; B–G = 50 μm; H = 15 μm; I, K = 20 μm; L, M = 200 μm.

Peripheral nervous system rather well developed. Anteriorly, several cephalic nerves extending from front of both dorsal and ventral brain lobes, innervating proboscis and ocelli mostly. Pair of stout and prominent nerves emerging laterally from dorsal ganglia, leading to neuroganglionic tissues of cerebral sensory organs. Pair of foregut nerves, originating from ventral cerebral ganglia, running posteriorly in parenchyma above oesophagus and stomach (Fig. 5E). *Apical organ and cephalic glands*: Single apical organ, comprising of ciliated pit 35–55  $\mu$ m in diameter and lined by neutrophilic vacuolate epithelium of 10–25  $\mu$ m thick, opening at tip of head (Fig. 4D). Cephalic glands discharging to exterior via apical organ.

Cephalic glands comparatively large, irregularly shaped and vacuolated in appearance, occupying much of cephalic space (Fig. 7A), and extending back behind brain. As well as typical vacuolate basophilic lobules, cephalic glands also contain two other distinct gland types; yellow- to orange-staining acidophils and purple-staining basophils with Mallory's trichrome (Fig. 7A).

Sub-muscular glands: Sub-muscular glands (Fig. 7B) scattered amongst fibres of body-wall longitudinal muscle layer in foregut region on all sides of body. Each gland opening to exterior by own pore.

Sensory organs: 10–70 ocelli, arranged into two groups on each side of head (Fig. 2), 30–40  $\mu$ m in diameter, with dark green to black pigment cup (Fig. 7C). Anterior groups situated near head tip, posterior groups at about level of pro-

boscis insertion.

Cerebral sensory organs (Fig. 7C) comparatively large and situated close below anterolateral margins of cerebral ganglia, opening to exterior via distinct ventrolateral ciliated cerebral canals of about 45  $\mu m$  in diameter, leading inwards from anterior pair of cephalic furrows. Canals surrounded posteriorly by neuroganglionic tissues, flanked above and below by cap-like glandular portions. Cerebral organs elliptical in cross-section, 60  $\mu m$  wide and 200  $\mu m$  tall.

Excretory system: Excretory system very well developed and extending throughout the length of body, including head. Terminal regions of excretory system embedded between dermis and body-wall circular muscle layer (Fig. 7D-H). Terminals surrounded by small but unidentified cells; flame cells not found. Thick-walled (10-15 µm) collecting tubules, 35-45  $\mu m$  or more in diameter, highly convoluted and ramify around rhynchocoel, lateral nerve cords and alimentary canal (Fig. 3). Nuclei of cells forming collecting tubules located close to tubule lumen. Final region of each collecting tubule thick-walled and surrounded by unidentified cells similar to cells around terminal (Fig. 71). Numerous nephridiopores opening laterally (Fig. 7J). In holotype 17 and 15 nephridiopores opening on right and left side of body, respectively. Each nephridiopore leading to distinct collecting tubules and terminals.

Numerous sinuses containing highly vacuolated cells with small and dense nuclei (Fig. 7K) found in parenchyma; each sinus isolated, not opening into either blood vessels or

**Table 2.** Monostiliferous hoplonemertean genera with a bilayered rhynchocoel wall and an oesophagus emerging from the rhynchodaeum close behind the proboscis pore. Data compiled from Friedrich (1940, 1955, 1957), Gibson (1973), Sánchez and Moretto (1988), Senz (1993) and the present study.

Genus	I	II	Ш	IV	V	VI	VII
Arctonemertes <sup>a</sup> Friedrich, 1957	+	0	+	+	0	+	+
Atrionemertes <sup>b</sup> Senz, 1993	0	0	+	+	0	+	0
Communoporus Friedrich, 1955	?	0	?	?	0	+	?
Divanella <sup>c</sup> Gibson, 1973	0	0	+	+	+	+ <sup>d</sup>	?
Koinoporus Sánchez and Moretto, 1988	0	0	0e	0	0	++	O <sup>f</sup>
Potamostoma gen. nov.	+	+	+	+	0	++	+

- I Body-wall musculature with (+) or without (0) diagonal muscle layer.
- II Body-wall longitudinal muscle layer anteriorly divided (+) or not divided (0).
- III Rhynchocoel shorter than body (0) or as long as body (+).
- IV Intestinal caecum present (+) or absent (0).
- V Cerebral sensory organs replaced by neuroglandular complex (+) or with typical structure (0).
- VI Excretory system restricted to foregut region of body (+) or extending throughout body length (++).
- VII Testes in mature males bilobed (+) or simple (0).
- <sup>a</sup> Friedrich (1957: 143) noted that in *Arctonemertes thori* Friedrich, 1957, the rhynchodaeum opens far anteriorly and dorsally to the oesophageal opening. He also stated (1957: 144) that "Der Oesophagus mündet subterminal aus und nimmt ganz vorne das Rhynchodaeum auf". Chernyshev (1998), however, made no mention of the oesophagus-rhynchodaeum relationship in *A. ussuriensis* Chernyshev, 1998.
- <sup>b</sup> Senz (1993) reported that in *Atrionemertes greenlandica* Senz, 1993, there was no oesophagus, the foregut instead opening directly into and atrium.
- <sup>c</sup> Gibson (1973: 802) noted that the rhynchodaeum "opens into the oesophagus close to the common proboscis and oral aperture" in *Divanella* evelinae Gibson, 1973.
- <sup>d</sup> Gibson (1973) recorded that in *D. evelinae* the excretory system was very indistinct and at best poorly developed.
- <sup>e</sup> Sánchez and Moretto (1988: 199) stated that the rhynchocoel "extends up to two-thirds of the total length of the body." in *Koinoporus mapochi* Sánchez and Moretto. 1988.
- <sup>f</sup> K. mapochi is hermaphroditic and possesses ovotestes (Sánchez and Moretto, 1988).

excretory tubules.

Reproductive system: Dioecious. Gonads arranged pseudometamerically, alternating with lateral intestinal diverticula in immature. Gonads in individuals collected in November identifiable to sex; early spermatogenesis or developing ova seen in individuals collected in May. Sexually matured individuals exhibiting colour dimorphism; testes khaki in colour, whereas ovaries deep green. In immature females ovaries filled with dark green pigment granules (Fig. 7L). Testes approaching maturity are dorsoventrally bilobed (Fig. 7M), each lobe about 100  $\mu m$  by 400  $\mu m$  in dimension. Developing ovaries up to about 300  $\mu m$  by 600  $\mu m$ ; each ovary containing at least 30 eggs. Putative nurse cells situated along inner wall of each ovary; some eggs connected to nurse cells by short stalk (Fig. 7N).

Systematic remarks: Comparatively few hoplonemertean genera that have two separate muscle layers in their rhynchocoel wall have been reported in which the oesophagus, as in the present specimens, emerges from the rhynchodaeum far in front of the brain lobes (Table 2). The present new genus closely resembles Arctonemertes Friedrich, 1957, but can be distinguished from the latter by the anteriorly divided body-wall longitudinal musculature and the extensive development of the excretory system. None of the genera listed in Table 2 possesses the anteriorly divided body-wall longitudinal muscle layer, as in the present species; this feature, as well as the position of the terminal portions of the excretory collecting tubules between the dermis and body wall circular muscle layer, together with other characters listed in Table 2, exclude the present species from any of these genera.

# **DISCUSSION**

Nemerteans are thought to have their origin in marine environment. In the majority of marine taxa, the excretory system is restricted in the foregut region of the body. Contrary, in all the terrestrial and freshwater species, as well as in some brackish-water forms, the excretory system is significantly developed throughout the body, regardless of their higher taxonomic ranks, such as class-level. This indicates the presence of an extensively developed excretory system alone cannot always be used as evidence of close common ancestry; lwata (1968, 1970) suggested that the extensive development of the excretory system in brackish-water nemerteans, compared with the much more restricted systems found in marine taxa, has an osmoregulatory function.

Stenohaline marine invertebrates are at best barely able to tolerate the rapid salinity changes characteristic of estuarine conditions (Nybakken, 2001). They are thus generally restricted to salinities of 25 psu or more; most marine nemertean species probably belong in this category and are likely to be found penetrating only into the lower reaches of estuaries. On the other hand, the brackish waters, such as the Baltic and Black Seas where freshwater input exceeds evaporation, are often characterised by much more stable

conditions; invertebrates living in such regions are not subject to the rapid salinity fluctuations associated with estuaries. The Amphiporus and Tetrastemma species, which were reported from Sea of Okhotsk by Korotkevich (1977), are not found in salinities less than 18 psu (Table 1) and their excretory systems are restricted to the foregut region of the body. On the other hand, those euryhaline species, including Amniclineus zhujangensis Gibson and Qi, 1991, Hinumanemertes kikuchii lwata, 1970, Sacconemertella lutulenta Iwata, 1970, Sacconemertopsis belogurovi Chernyshev, 1991, S. olivefera Iwata, 1970, and the present species, which live in salinities fluctuating down to 1-6 psu, possess excretory systems extending throughout most of the body. These findings suggest the critical salinity, where nemerteans have to develop their excretory system throughout the body for survive, would likely to be between 6-18 psu.

### **ACKNOWLEDGEMENTS**

We are grateful to Dr Y. Takashima for bringing to HK's notice the existence of the present specimens, found whilst he was collecting oligochaetes from the mouth of the River Shizunai for a taxonomic investigation. We would also like to thank Dr Janet Moore for criticising and commenting on an earlier version of this paper.

#### **REFERENCES**

- Bergh G (1973) On the distribution and abundance of bottom fauna in Tvären Bay in the Baltic. Zoon 1: 153–171
- Brunberg L (1964) On the nemertean fauna of Danish waters. Ophelia 1: 77–111
- Chernyshev A V (1991) Sacconemertopsis belogurovi sp. nov. (Hoplonemertini, Tetrastemmatidae) novaya solonovatovodnaya nemertina iz yuzhnogo primorya. Biol Nauk 5: 45–49 [In Russian]
- Chernyshev A V (1998) Novuie monostilifernuie nemertinui (Enopla, Monostilifera) iz yaponskogo morya. Zool Zh 77: 397–403 [In Russian]
- Friedrich H (1940) Einige neue Hoplonemertinen aus der Ostsee. Kieler Meeresforsch 3: 233–251
- Friedrich H (1955) Beitrage zu einer Synopsis der Gattungen der Nemertini monostilifera nebst Bestimmungsschlüssel. Z wiss Zool 158: 133–192
- Friedrich H (1957) Beiträge zur Kenntnis der Arktischen Hoplonemertinen. Vidensk Meddr dansk naturh Foren 119: 129–154
- Gibson R (1973) A new littoral hoplonemertean (*Divanella evelinae* gen. et sp. nov.) from the coast of Brazil. Bull Mar Sci 23: 793–810
- Gibson R (1985) The need for a standard approach to taxonomic descriptions of nemerteans. Am Zool 25: 5–14
- 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. J Nat Hist 29: 271–562
- Gibson R, Qi S (1991) A new freshwater heteronemertean from the Zhujiang (Pearl River), People's Republic of China. Hydrobiologia 220: 167–178
- Iwata F (1968) On the circulatory and nephridial organs of Nemertinea and their osmoregulatory function. Proc Jpn Soc Syst Zool 4: 7–9 [In Japanese]
- Iwata F (1970) On the brackish water nemerteans from Japan, provided with special circulatory and nephridial organs useful for osmoregulation. Zool Anz 184: 133–154

500 H. Kajihara et al.

Karling T G (1933) Ein Beitrag zur Kenntnis der Nemertinen des Finnischen Meerbusens. Memo Soc Fauna Flora fenn 10: 76– 90

- Kirsteuer E (1967) Marine, bethonic nemerteans: how to collect and preserve them. Am Mus Novit 2290: 1–10
- Korotkevich V S (1977) Nemertinui pribrezhnuikh vod kurilskikh ostrovov. In "Fauna pribrezhnuikh zon kurilskikh ostrovov" Ed by V V Gulbin, Izdatelstvo Nauka, Moscow, pp 49–124. [In Russian]
- Moore J, Gibson R (1981) The *Geonemertes* problem (Nemertea). J Zool Lond 194: 175–201
- Norenburg J L (1986) Redescription of a brooding nemertine, *Cyanophthalma obscura* (Schultze) gen. et comb. n., with observations on its biology and discussion of the species of *Prostomatella* and related taxa. Zool Scr 15: 275–293

- Nybakken J W (2001) Marine Biology. An Ecological Approach 5th edition, Addison Wesley Longman, San Francisco
- Sánchez M, Moretto H J A (1988) A new genus of freshwater hoplonemertean from Chile. Zool J Linn Soc 92: 193–207
- Senz W (1993) Atrionemertes greenlandica gen. et sp. nov. (Nemertini: Hoplonemertini). Zool Anz 231: 99–110
- Sundberg P, Gibson R (1995) The nemerteans (Nemertea) of Rottnest Island, Western Australia. Zool Scr 24: 101–141

(Received February 1, 2002 / Accepted December 24, 2002)