

A New Genus and Five New Species of Kalyptorhynchia (Platyhelminthes: Rhabdocoela) Discovered in Northern Japan

Naoya Takeda^{1,3} and Hiroshi Kajihara²

¹ Marine Biological Research Institute of Japan, Yutaka-cho 4-3-16, Shinagawa, Tokyo 142-0042, Japan
E-mail: bobamo@gmail.com

² Faculty of Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

³ Corresponding author

(Received 7 August 2017; Accepted 27 December 2017)

<http://zoobank.org/A9B333A2-FD81-476A-A805-D280C2194964>

We have described a new genus and five new species of free-living marine flatworms in Schizorhynchia (Platyhelminthes: Rhabdocoela: Kalyptorhynchia) based on the material collected from sandy intertidal zones around Hokkaido, Northern Japan. These include *Freddius tricaudatus* gen. et sp. nov. (Cheliplanidae) and *Proschizorhynchella caudociliata* sp. nov., *P. magnoliae* sp. nov., *P. shibazakii* sp. nov., and *P. shuttlecock* sp. nov. (Schizorhynchidae). Based on this study, the number of confirmed species in the suborder Kalyptorhynchia discovered in Japanese waters has increased from one to six. *Freddius tricaudatus* gen. et sp. nov. possesses proboscis hooks that are so peculiar among Cheliplanidae that they warrant the establishment of a new genus.

Key Words: Pacific Ocean, confocal laser scanning microscopy, taxonomy, marine invertebrates.

Introduction

Flatworms belonging to the taxon Kalyptorhynchia (Platyhelminthes: Rhabditophora) possess characteristic anterior muscular proboscis for capturing prey (Smith *et al.* 2015 and references therein). The ~600 described species of such flatworms are divided into two subtaxa based on their structure of proboscis, Eukalyptorhynchia (cone-shaped proboscis) and Schizorhynchia (finger-like and dorsoventrally paired proboscis) (Tyler *et al.* 2006–2013). Previous reports on these species have mostly been from Europe, North America, and the Indo-Pacific; to date, only one species, *Proschizorhynchella pacifica* (Evdonin, 1969), has been discovered in waters around Japan (Evdonin 1969).

During a faunal survey of interstitial marine invertebrates around Hokkaido, Northern Japan, specimens representing five undescribed species belonging to the taxon Kalyptorhynchia were observed. In this paper, we describe and illustrate these species by tabulating the data of species belonging to the genus *Proschizorhynchella* Schilke, 1970.

Materials and Methods

Samples were obtained by the first author at low tide between 13 June 2011 and 1 July 2013 from five localities around Hokkaido, Japan (Fig. 1). Sandy sediments were washed with tap water, and the supernatant was sieved through a colander with a mesh size of 180 μm ; this process

was repeated 2–3 times. The colander was then placed on a petri dish filled with seawater for approximately 30 min to release the worms from the mesh. Specimens were anesthetized in 34‰ MgCl_2 aq., pipetted onto glass slides, photographed under an Olympus BX51 optical microscope, and preserved as whole mounts embedded in polyvinyl lacto-

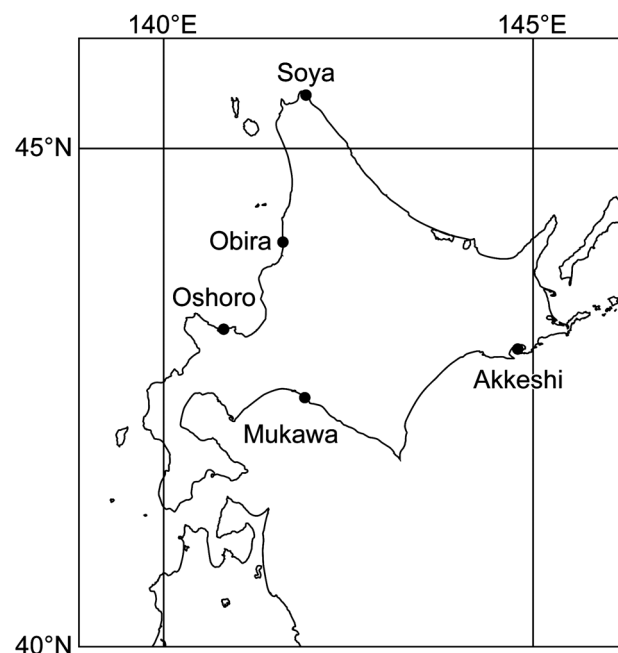


Fig. 1. Map showing sampling sites around Hokkaido, northern Japan.

phenol. The specimens were either fixed with Bouin's fluid for histological preparation or with 5% formalin in phosphate buffered saline (PBS) for fluorescent staining. Serial sections (6 µm in thickness) of the specimens were stained with Mallory's triple stain. Formalin-fixed specimens were first placed in 0.3% Triton X-100 in PBS for 15 min, in 100 nM phalloidin in PBS for 3–4 days, and in 0.3% Triton X-100 in PBS for 15 min; preserved as whole mounts embedded in Vectashield H-1000; and finally observed under a ZEISS LSM 5 DUO confocal laser scanning microscope. Type material has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

Taxonomy

Family *Cheliplanidae* Schilke, 1970

Genus *Freddius* gen. nov

Diagnosis. Cheliplanids possess hooks (*sensu* Karling 1983) articulated with movable nails; paired lateral auxiliary apparatuses are present between hooks.

Type species. *Freddius tricaudatus* sp. nov., fixed by original designation.

Etymology. The new masculine genus name is derived from the character name Freddy Krueger, a horror icon in the movie *A Nightmare on Elm Street*, alluding the resemblance between his razor-armed glove used to kill his victims and the elaborate proboscis hooks of the flatworms in the new taxon.

Freddius tricaudatus sp. nov.

(Figs 2–5)

Material examined. Holotype: ICHUM 4832, adult, whole mount, 43°01'16"N, 144°50'13"E, Akkeshi, Hokkaido, Japan, intertidal sand, 15 June 2012. Paratypes: ICHUM 4829–4831, three adults, whole mount, same data as holotype; ICHUM 4833–4835, three adults, fluorescent phalloidin stained, same data as holotype; ICHUM 4836–4838, three adults, whole mount, from type locality, 24 June 2013.

Description. Living animal body approximately 900 µm long and 130 µm wide. Pair of hooks present, asymmetrical in structure (Figs 2, 3), one 12–14 µm and the other 10–11 µm in length, each 3–4 µm wide and semi-cylindrical in shape, anteriorly articulated with three movable nails, 9–11 µm in length (Fig. 3); longer hook possesses a pair of additional lateral nails, 12–16 µm long, which are (i) anteriorly directed, (ii) articulated at both sides of the hook near its posterior end, (iii) longer than the anterior movable nails, and (iv) equipped with a pair of spines at their midpoint (Fig. 3). Paired auxiliary apparatuses present between hooks, tapered rod in shape, 4 µm long (Fig. 3C, F). Proboscis gland 55 µm long, 20 µm wide, situated posterior to proboscis (Fig. 2). Eyes absent (Fig. 2). One testis 100 µm long, 30 µm wide, not divided into follicles, situated posterior to proboscis gland (Fig. 2). Multiple sacs of unknown function, arranged posterior to testis (Fig. 2). Pharynx 100 µm long, 50 µm wide, posterior to sacs (Fig. 2). Mouth opening

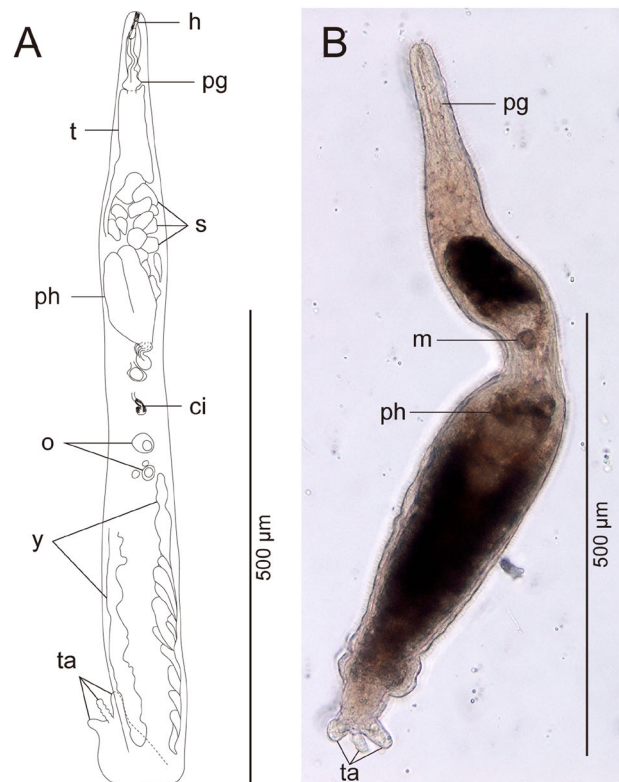


Fig. 2. *Freddius tricaudatus* gen. et sp. nov. Entire animal. A. Illustration showing the arrangement of various internal organs, ICHUM 4832 (holotype); B. Photograph taken in life, ICHUM 4836 (paratype). Abbreviations: ci, cirrus spine; h, hook; m, mouth; o, ovary; pg, proboscis gland sac; ph, pharynx; s, sac; t, testis; ta, tail; y, yolk gland.

anterior to pharynx (Fig. 4A). Male copulatory organ, posterior to pharynx (Figs 2, 4), roughly tubular; anterior part bulbous and muscular; posterior part with cone-shaped cirrus spines, 20 µm long, 10 µm wide (Figs 4B, 5). Genital pore opening on ventral side of body, posterior to cirrus spines (Fig. 4). Single ovary 50 µm long, 20 µm wide, situated posterior to genital pore (Fig. 2). Pair of yolk glands 300 µm long and 25 µm wide, situated posterior to ovary (Fig. 2). Posterior end of body trifurcated, with tail-shaped serial beads in the middle (Fig. 2).

Etymology. The specific name refers to the three tails in the new species.

Remarks. The new species is undoubtedly a member of Cheliplanidae; it possesses proboscis with side pieces and a postrostral bulb, without separate lateral gland sacs, one pair of proboscis hooks, and characteristics that are shared among all the members of the family (Karling 1983); in addition, the structure of the genital organs in this species is similar to that in other members of the family. In cheliplanids, the proboscis hooks consist of a symmetrical pair of simple, curved spines, which, however, are not elaborate enough to be articulated like in the new species. The morphology of the proboscis hook in this species is so peculiar that it cannot be classified with certainty into any of the existing four genera in the family (Tyler *et al.* 2006–2013). Therefore, we propose to establish a new genus in the Cheli-

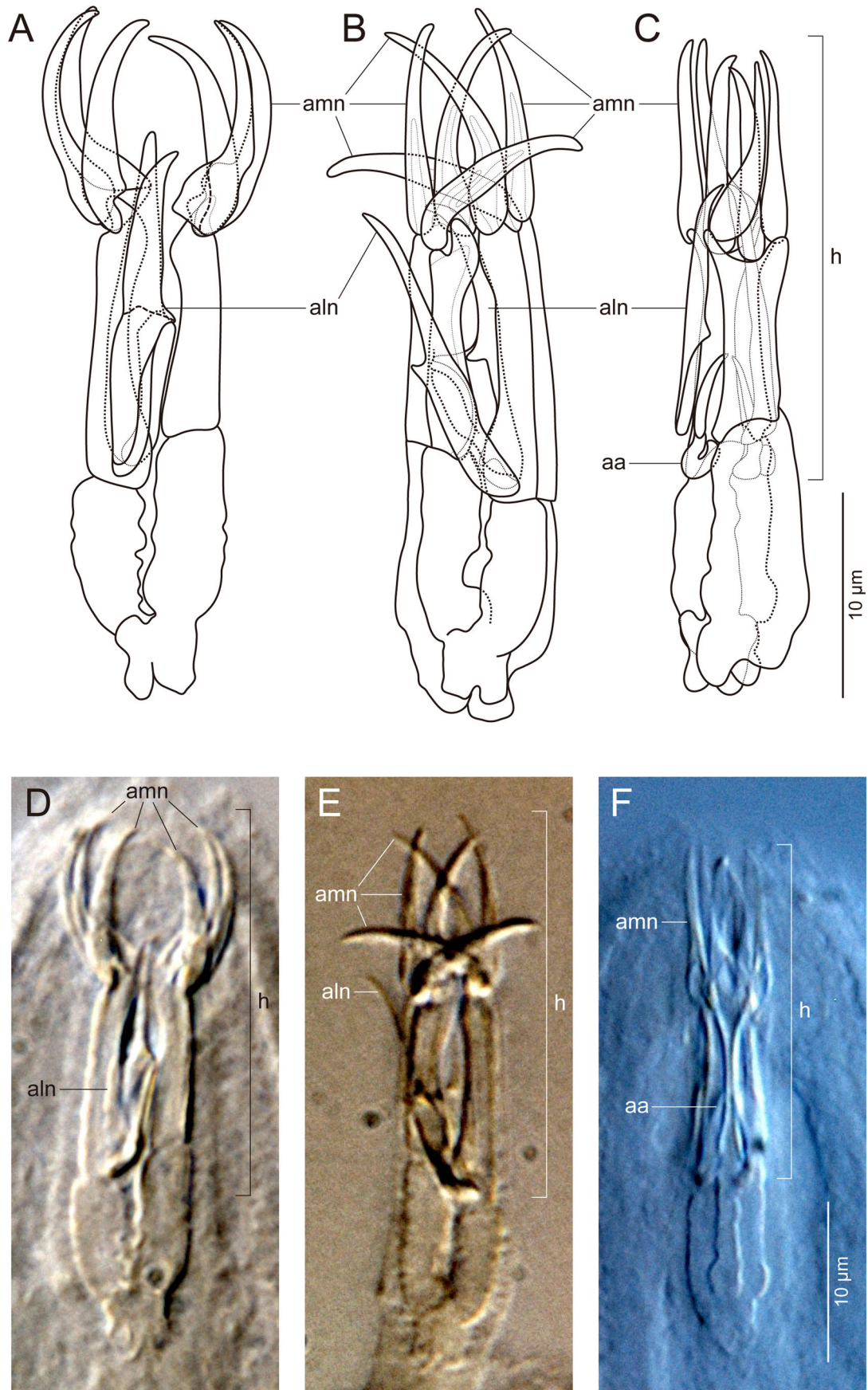


Fig. 3. *Freddius tricaudatus* gen. et sp. nov. Illustrations (A–C) and photomicrographs (D–F) of the proboscis hook in three specimens, with the anterior movable nails and auxiliary apparatuses pointing in different directions. A, D. ICHUM 4832 (holotype); B, E. ICHUM 4830 (paratype); C, F. ICHUM 4832 (holotype). Abbreviations: aa, auxiliary apparatus; aln, additional lateral nail; amn, anterior movable nail; h, hook.

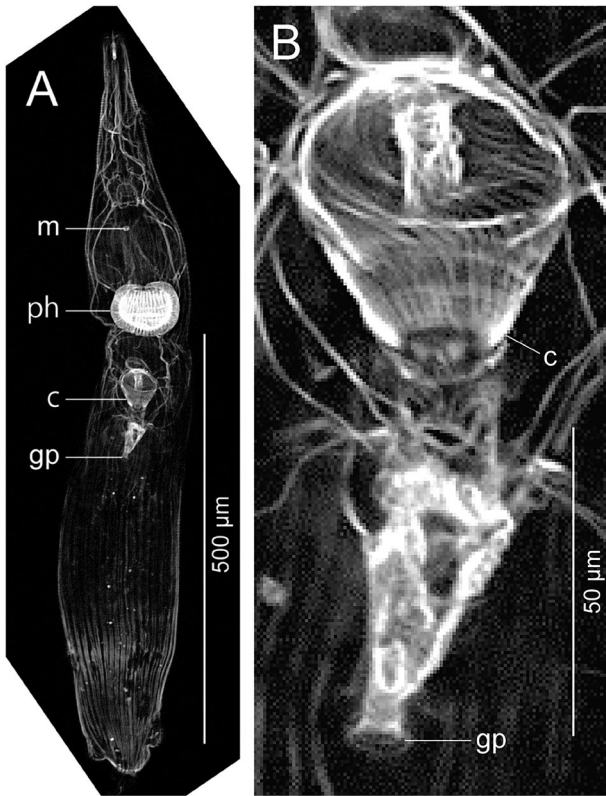


Fig. 4. *Freddius tricaudatus* gen. et sp. nov. Images obtained from confocal laser scanning microscopy, ICHUM 4833 (paratype). A. Entire animal; B. Male copulatory organ. Abbreviations: c, male copulatory organ; gp, genital pore; m, mouth; ph, pharynx.

planidae family to accommodate this new species.

Family **Schizorhynchidae** Graff, 1905
 Genus ***Proschizorhynchella*** Schilke, 1970
Proschizorhynchella caudociliata sp. nov.
 (Figs 6, 7; Table 1)

Material examined. Holotype: ICHUM 4863, adult, whole mount, 42°33'25"N, 141°55'42"E, Mukawa, Hokkaido, Japan, intertidal sand, 20 May 2012. Paratypes: ICHUM 4262, 4264, 4265, three adults, whole mounts, same data as holotype.

Description. Living animal body approximately 3.0 mm long and 0.3 mm wide (Fig. 6). Proboscis 230–270 µm long, 70–80 µm wide (Fig. 6). Pair of black eyes situated behind proboscis (Fig. 6). Two testes, each 150–280 µm in diameter (Fig. 6). Yolk gland 750 µm long, 110 µm wide (Fig. 6A). Two adhesive girdles present; anterior one located at level of posterior end of pharynx, posterior one near caudal end; each girdle composed of six adhesive papillae arranged in regular intervals (Fig. 6A). Pharynx 220 µm long, 150 µm wide (Fig. 6). Pair of seminal vesicles, each 150–310 µm long, 10–30 µm wide, located posterior to pharynx (Fig. 6A). Male copulatory organ 160 µm long, 50 µm wide (Fig. 6A). Stylet cone shaped, 45 µm long, 12 µm wide, comprised of thin sclerotic sheet rolled up four times (Figs 6A, 7). Ovary 120 µm long, 60 µm wide, located posterior to pharynx (Fig. 6A, B). Uterus, 90 µm long, 40 µm wide, located around ovary (Fig. 6A). Common genital pore opening on ventral side of body between two adhesive girdles (Fig. 6A). Bursa 190 µm long, 80 µm wide, located around common genital pore (Fig. 6A). Large oval structure (adhesive gland?)

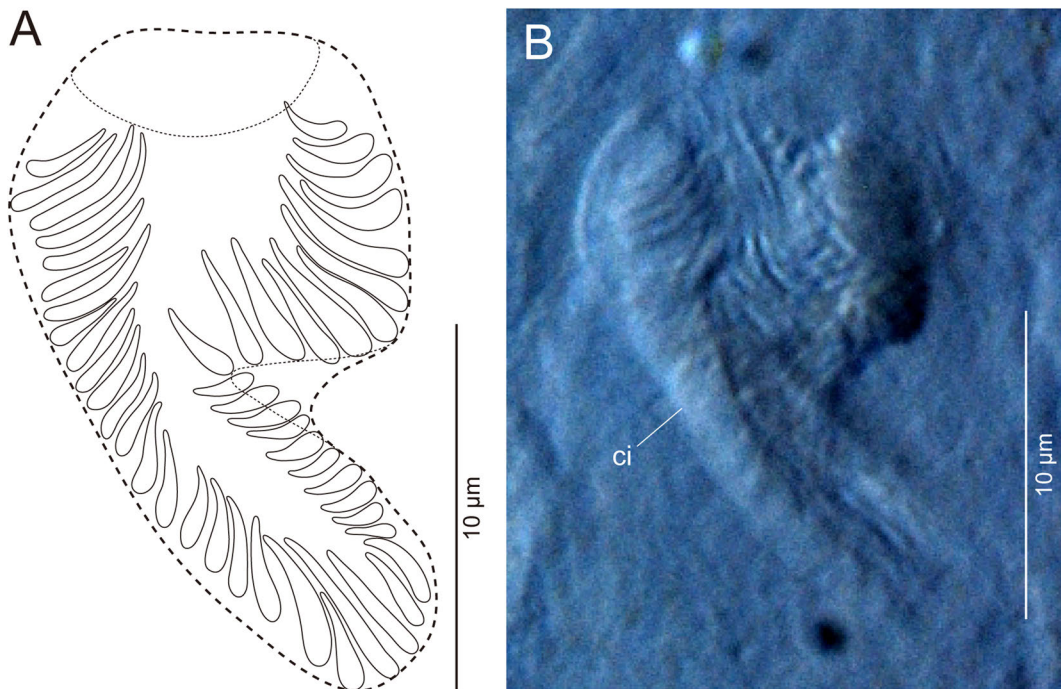


Fig. 5. *Freddius tricaudatus* gen. et sp. nov., cirrus spine, ICHUM 4832 (holotype). A. Illustration; B. Photomicrograph. Abbreviation: ci, cirrus spine.

120 µm long, 100 µm wide, located at the caudal end (Fig. 6A). Caudal end covered with long tactile cilia (Fig. 6A).

Etymology. The specific name refers to the long tactile cilia on the caudal region, which look like a brush.

Remarks. *Proschizorhynchella caudociliata* can be distinguished from all congeners, except *Proschizorhynchella*

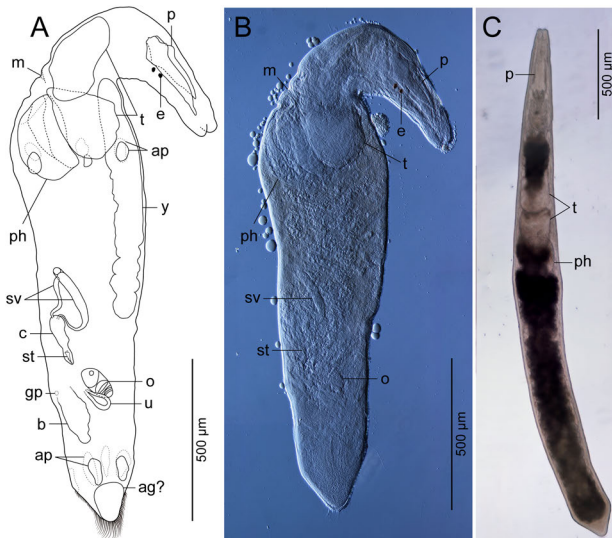


Fig. 6. *Proschizorhynchella caudociliata* sp. nov. A. Illustration showing the arrangement of various internal organs, ICHUM 4863 (holotype); B. Photomicrograph of fixed specimen, ICHUM 4863 (holotype); C. Photomicrograph taken in life, ICHUM 4862 (paratype). Abbreviations: ag, adhesive gland; ap, adhesive papilla; b, bursa; c, male copulatory organ; e, eye; gp, genital pore; m, mouth; o, ovary; p, proboscis; ph, pharynx; st, stylet; sv, seminal vesicle; t, testis; u, uterus; y, yolk gland.

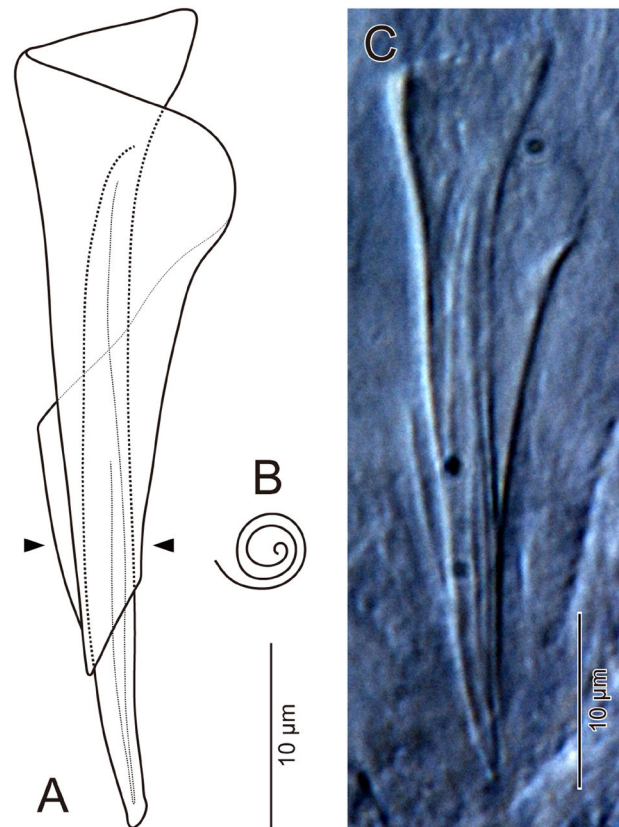


Fig. 7. *Proschizorhynchella caudociliata* sp. nov., stylet, ICHUM 4863 (holotype). A. Illustration; B. Schematic diagram of transverse section through the region indicated by the arrowheads in A; C. Photomicrograph.

Table 1. Morphological characteristics that can be used to distinguish species belonging to the genus *Proschizorhynchella*, compiled based on data reported by Steinböck (1931), Meixner (1928, 1938), Marcus (1950), L'Hardy (1965), Evdonin (1969), Schilke (1970), Doe (1974), Noldt (1986, 1989), and Karling (1989) and this study.

Species	Eye spots present (1) or absent (0)	Number of testes	Number of adhesive girdles	Number of genital pores	Cirrus	Stylet shape	Stylet structure	Bursal opening	Source
<i>P. atopus</i>	1	4	2	1	absent	conical	complete tube	absent	Marcus (1950)
<i>P. bivaginata</i>	1	2	1	1	present	cylindrical	whirl	independently behind genital pore	Schilke (1970), Noldt (1986)
<i>P. caudociliata</i> sp. nov.	1	2	2	1	absent	conical	rolled sheet	?	Present study
<i>P. echinulata</i>	1	2	2	1	present	(stylet absent)	(stylet absent)	independently behind genital pore	L'Hardy (1965)
<i>P. faroensis</i>	1	?	0	2	absent	conical	complete tube	?	Steinböck (1931)
<i>P. helgolandica</i>	1	2	3	1	absent	conical	pair of hemitubes	absent	L'Hardy (1965), Noldt (1986, 1989)
<i>P. inflata</i>	1	2	3	1	present	conical	complete tube	independently behind genital pore	Karling (1989)
<i>P. lingulata</i>	1	2×2	2	1	absent	conical	rolled sheet	to common atrium via internal vagina	Karling (1989)
<i>P. magnoliae</i> sp. nov.	1	2	3	1	absent	conical	multiple sheets	absent	Present study
<i>P. nahantensis</i>	1	2	2	1	absent	conical	complete tube	independently behind genital pore	Doe (1974)
<i>P. oculata</i>	1	2	2	1	absent	aciculate	complete tube	independently behind genital pore	Meixner (1928, 1938)
<i>P. pacifica</i>	0	2	2	1	absent	conical	complete tube	to common atrium via internal vagina	Evdonin (1969)
<i>P. papillata</i>	1	2	2	1	absent	conical	rolled sheet	to common atrium via internal vagina	Doe (1974)
<i>P. robusta</i>	1	2	1	1	absent	conical	rolled sheet	to common atrium via internal vagina	Noldt (1986, 1989)
<i>P. schilkei</i>	0	4	1	?	absent	crochet shaped	complete tube	?	Karling (1989)
<i>P. shibazakii</i> sp. nov.	1	2	2	1	absent	conical	rolled sheet	to common atrium via internal vagina	Present study
<i>P. shuttlecock</i> sp. nov.	1	2	0	1	absent	Shuttlecock shaped	one round rolled sheet	?	Present study
<i>P. spiracirro</i>	0	2	0	1	present	(stylet absent)	(stylet absent)	independently behind genital pore	Schilke (1970), Noldt (1986, 1989)

papillata (Doe, 1974) and *P. shibazakii* sp. nov., based on the characteristics listed in Table 1. These three species, however, can be distinguished on the basis of two characteristics: (i) four coils of the copulatory stylet in *P. caudociliata* and three in *P. papillata* and *P. shibazakii* and (ii) a vacuolated region and long tactile hair present near the caudal end in *P. caudociliata* but absent in *P. papillata* and *P. shibazakii*.

***Proschizorhynchella magnoliae* sp. nov.**

(Figs 8, 9; Table 1)

Material examined. Holotype: ICHUM 4859, adult, whole mount for fluorescent staining, 43°01'16"N, 144°50'13"E, Akkeshi Bay, Hokkaido, Japan, intertidal sand, 24 June 2013. Paratypes: ICHUM 4849, 4850, two adults, whole mounts, type locality, 1 July 2011; ICHUM 4851, 4852, two adults, whole mounts, type locality, 7 July 2011; ICHUM 4853–4857, five adults, whole mounts, type locality, 15 June 2012; ICHUM 4858, 4860, two adults, same data as holotype.

Description. Living animal body approximately 1.5 mm long and 160 µm wide (Fig. 8). Proboscis 240–290 µm long,

30 µm wide; pair of proboscis glands 90 µm long, 50 µm wide (Fig. 8). Pair of black eyes situated anterior to brain (Fig. 8A, B, D). Two testes, each 90–150 µm in diameter (Fig. 8A). Pharynx 120 µm long, 170 µm wide (Fig. 8A–C). Three adhesive girdles present; anterior one located at the level of posterior end of pharynx, middle one at level of seminal vesicles, posterior one near caudal end; each girdle composed of six adhesive papillae arranged in regular intervals (Fig. 8A). Pair of yolk glands 400–440 µm long, 120–170 µm wide (Fig. 8A). Pair of seminal vesicles, each 220–230 µm long, 20 µm wide, located posterior to pharynx (Figs 8A, 9A, B). Male copulatory organ tubular in shape, 120 µm long, 50 µm wide (Figs 8C, 9A), tapering toward its tip, equipped with stylet. Stylet cone shaped, 31 µm long, 13 µm wide, comprised of thin sclerotic sheets (exact number of sheets not clear but appears to be more than one), equipped with ridges and processes inside (Fig. 9C, D). Common genital pore opening on ventral side of posterior region of body, posteriorly leading to saccate common atrium, middle part of which is dorsally connected to male copulatory organ and pair of yolk glands (Fig. 9A, B). Ovary 80 µm long, 60 µm wide, connected to the end of common atrium (Fig. 9A, B).

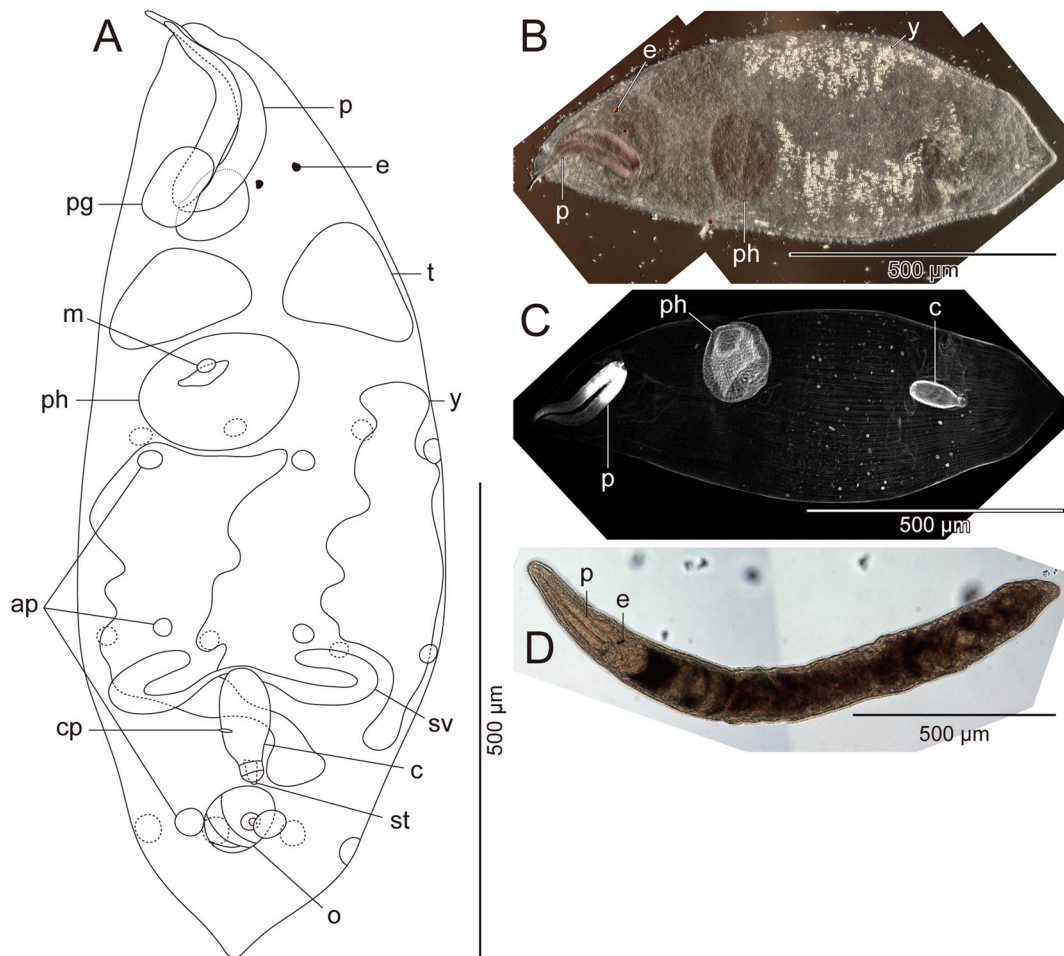


Fig. 8. *Proschizorhynchella magnoliae* sp. nov. A. Illustration of fixed specimen showing arrangement of various internal organs, ICHUM 4859 (holotype); B. Photograph of fluorescent-staining specimen of the entire animal taken under a bright field, ICHUM 4859 (holotype); C. Confocal laser scanning micrograph, ICHUM 4859 (holotype); D. Composite photomicrograph taken in life, ICHUM 4849 (paratype). Abbreviations: ap, adhesive papilla; c, male copulatory organ; cp, common genital pore; e, eye; m, mouth; o, ovary; p, proboscis; pg, proboscis gland sac; ph, pharynx; st, stylet; sv, seminal vesicle; t, testis; y, yolk gland.

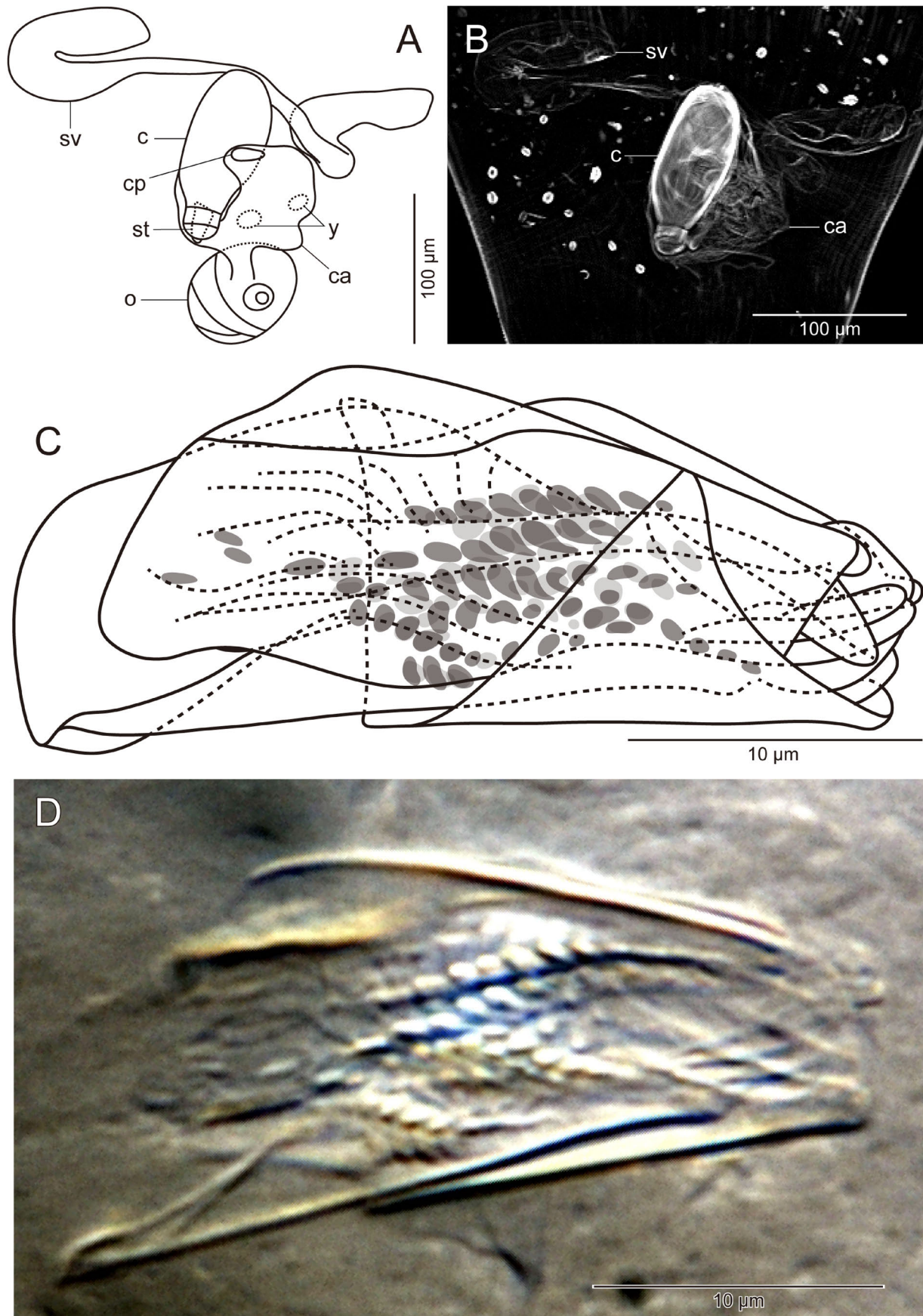


Fig. 9. *Proschizorhynchella magnoliae* sp. nov. A. Illustration showing the structure of reproductive system, ICHUM 4859 (holotype); B. Confocal laser scanning microscopy image of the reproductive system, ICHUM 4859 (holotype); C. Illustration of stylet, ICHUM 4859 (holotype); D. Photomicrograph of stylet, ICHUM 4859 (holotype). Abbreviations: c, male copulatory organ; ca, common atrium; cp, common genital pore; o, ovary; st, stylet; sv, seminal vesicle; y, yolk gland.

Etymology. The specific name is a noun in the genitive case, referring to the stylet of the new species, which resembles a flower of lily magnolia.

Remarks. *Proschizorhynchella magnoliae* sp. nov. most closely resembles *Proschizorhynchella helgolandica* (L'Hardy, 1965), which they has (i) a pair of eyes, (ii) two testes, (iii) three adhesive girdles, (iv) a single genital pore, and (v) no cirrus spine (Table 1). In *P. helgolandica*, the copulatory stylet comprises a pair of elongated cuticular sheets that face each other, with a concave confronting surface; the tip of each sheet is sharply pointed. On the other hand, in *P. magnoliae*, the copulatory stylet takes the form of spiral sheets that are complicatedly coiled with each other, with an elaborate inner surface with numerous ridges and short spines.

***Proschizorhynchella shibazakii* sp. nov.**
(Figs 10–14; Table 1)

Material examined. Holotype: ICHUM 4275, adult, whole mount, 43°12'33"N, 140°51'31"E, Oshoro, Hokkaido, Japan, intertidal sand, 13 June 2011. Paratypes: ICHUM 4276–4278, three adults, whole mounts, same data as holotype; ICHUM 4279, 4280, two adults, serial sagittal sections, same data as holotype; ICHUM 4281, 4282, two adults, serial transverse sections, same data as holotype; ICHUM 4283, one adult, whole mount, type locality, 21 May 2012; ICHUM 4861, egg, whole mount, laid by animals collected on 1 July 2013.

Description. Living animal body approximately 2.6 mm long and 0.5 mm wide (Figs 10, 11A). Four pairs of bristles located at slender anterior tip of body (Figs 10, 11C). Proboscis 350 µm long, 120 µm wide; pair of proboscis glands 140 µm long, 80 µm wide (Fig. 10). Pair of black eyes situated anterior to brain (Figs 10, 11C). Gut anteroposteriorly elongated. Two testes 300–330 µm in diameter (Figs 10, 11C). Pair of yolk glands 1 mm long, 180 µm wide (Figs 10, 11A). Pharynx 480 µm long, 330 µm wide (Figs 10, 11A). Two adhesive girdles present; anterior one located at level of posterior end of pharynx, posterior one near caudal end; each girdle comprised of six adhesive papillae arranged in regular intervals (Figs 10, 11B). Pair of seminal vesicles, each 620 µm long, 60 µm wide, located posterior to pharynx (Fig. 10). Male copulatory organ tubular in shape, 240 µm long, 30 µm wide, with ejaculatory duct surrounded by circular muscles and further surrounded by longitudinal muscles (Figs 10, 12A, B, 13); copulatory organ tapering toward its tip, equipped with stylet and situated in male genital canal. Stylet cone shaped, 29–31 µm long (31 µm in holotype), 7 µm wide, comprised of thin sclerotic sheet rolled up three times (Fig. 12C, D). Male genital canal opens to anterodorsal part of common atrium of the latter (Fig. 13). Uterus 90 µm long, 30 µm wide, anterior to common atrium (Figs 10, 13). Each yolk gland connected to each side of common atrium (Figs 10, 13). Common genital pore opening on ventral side of body between two adhesive girdles (Figs 10, 13). Ovary 110 µm long, 70 µm wide, anteriorly connected to posterodorsal portion of common atrium via a common oviduct (Figs 10, 13). Bursa oval in dorsal view,

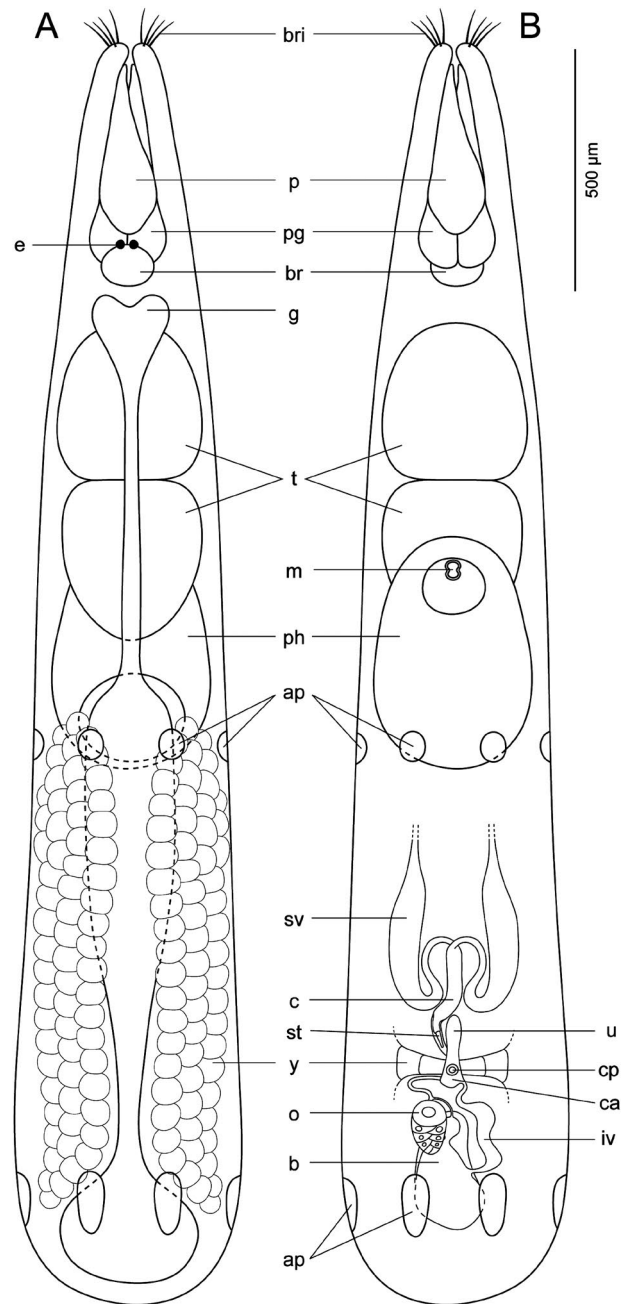


Fig. 10. *Proschizorhynchella shibazakii* sp. nov. Schematic representation of the body to show the organization of various organs. A. Dorsal view; B. Ventral view. Abbreviations: ap, adhesive papilla; b, bursa; br, brain; bri, bristle; c, male copulatory organ; ca, common atrium; cp, common genital pore; e, eye; iv, internal vagina; m, mouth; g, gut; o, ovary; p, proboscis; pg, proboscis gland sac; ph, pharynx; st, stylet; sv, seminal vesicle; t, testis; u, uterus; y, yolk gland.

250 µm long, 150 µm wide; bursal tissue divided into two (smaller anterior and larger posterior) parts by constriction; spermatids observed in posterior bursal tissue in all specimens observed; anterior bursal tissue leading forward to connect to common oviduct near ovary via narrow sperm duct (Figs 10, 13). Egg oval, 260 µm long, 200 µm wide, covered in brown shell with colorless axis (Fig. 14).

Etymology. The specific name is a noun in the genitive case, derived from the name Mr. Kouji Shibazaki, a caretaker-

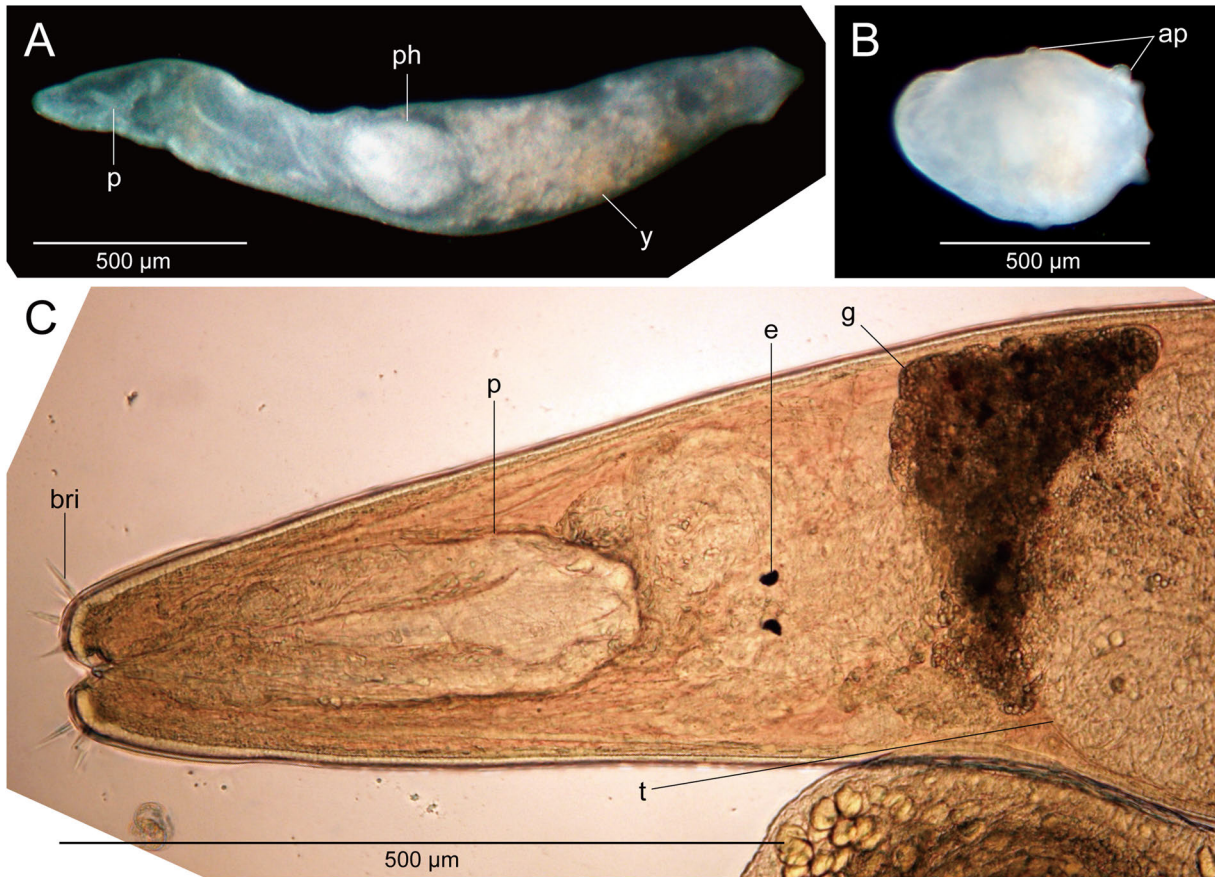


Fig. 11. *Proschizorhynchella shibazakii* sp. nov. A. Entire animal in an elongated state, ICHUM 4283 (paratype); B. Same animal in a contracted state, ICHUM 4283 (paratype); C. Photomicrograph showing the anterior part of a specimen (no voucher remains). Abbreviations: ap, adhesive papilla; bri, bristle; e, eye; g, gut; p, proboscis; ph, pharynx; t, testis; y, yolk gland.

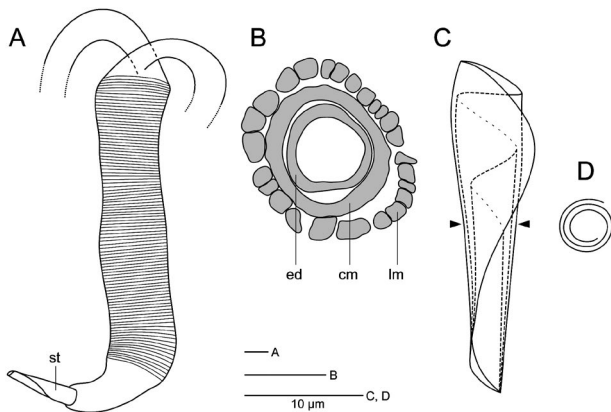


Fig. 12. *Proschizorhynchella shibazakii* sp. nov. A. Male copulatory organ, ICHUM 4275 (holotype); B. Transverse section of male copulatory organ, ICHUM 4282 (paratype); C. Stylet, ICHUM 4276 (paratype); D. Stylet, schematic diagram of transverse section through the region indicated by the arrowheads in C. Abbreviations: cm, circular muscle; ed, ejaculatory duct; lm, longitudinal muscle; st, stylet.

er of Oshoro Marine Station, Hokkaido University.

Remarks. *Proschizorhynchella shibazakii* can be distinguished from all congeners based on the characteristics listed in Table 1 except *P. papillata*. These two species, however, can

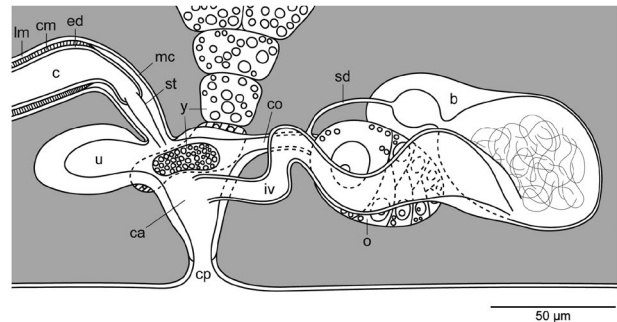


Fig. 13. *Proschizorhynchella shibazakii* sp. nov. Schematic diagram of genital organs in lateral view. Abbreviations: b, bursa; c, male copulatory organ; ca, common atrium; cm, circular muscle; co, common oviduct; cp, common genital pore; ed, ejaculatory duct; iv, internal vagina; lm, longitudinal muscle; mc, male genital canal; o, ovary; sd, sperm duct; st, stylet; u, uterus; y, yolk gland.

be distinguished based on the shape of the male copulatory organ. The differences in morphological characteristics between *P. shibazakii* sp. nov. and *P. papillata* are (i) the number of the apical sensory bristles, which is eight in *P. shibazakii* and four in *P. papillata*; (ii) the male copulatory organ, which is narrow and tubular in *P. shibazakii*, and bulb shaped in *P. papillata*; (iii) the internal part of the circular muscles, which is thin in *P. shibazakii* but thick in *P. papillata*; (iv) the border

cells which are present in *P. shibazakii* but absent in *P. papillata*; and (v) the length of the stylet, which is 29–31 μm (31 μm in holotype) in *P. shibazakii* and 55–57 μm in *P. papillata*. *Proschizorhynchella shibazakii* cannot be distinguished from *P. caudociliata* based on the characteristics listed in Table 1; however, they differ in the structure of the copulatory stylet (see Remarks for *P. caudociliata*).



Fig. 14. *Proschizorhynchella shibazakii* sp. nov. Photograph of an egg taken in life, ICHUM 4861 (paratype).

Proschizorhynchella shuttlecock sp. nov.
(Figs 15, 16; Table 1)

Material examined. Holotype: ICHUM 4846, adult, whole mount, 44°03'03"N, 141°39'46"E, Obira, Hokkaido, Japan, intertidal sand, 25 May 2012. Paratypes: ICHUM 4845, adult, whole mount, 45°29'16"N, 141°58'05"E, Soya, Hokkaido, Japan, intertidal sand, 26 May 2012; ICHUM 4847, 4848, two adults, whole mounts, same data as holotype.

Description. Living animal body approximately 2.6 mm long, 0.6 mm wide. Proboscis 600 μm long, 100 μm wide; pair of proboscis glands 170 μm long, 110 μm wide (Fig. 15A–C). Pair of black eyes situated behind proboscis (Fig. 15A–C). Two testes, each 200–290 μm in diameter (Fig. 15A, B). Pharynx typical for genus, 300 μm long, 400 μm wide (Fig. 15A, B). No adhesive girdles (Fig. 15A, B). Pair of seminal vesicles, each 410–470 μm long, 30–40 μm wide, located posterior to pharynx (Fig. 15A). Stylet badminton shuttlecock shaped, 68 μm long, comprised of incomplete tube of thin sclerotic sheet with single, narrow (~2 μm), longitudinal gap; proximal three-quarters cone shaped, 22 μm in diameter at its base, narrowing toward its tip, with many longitudinal ridges inside; distal quarter bulbous, slightly

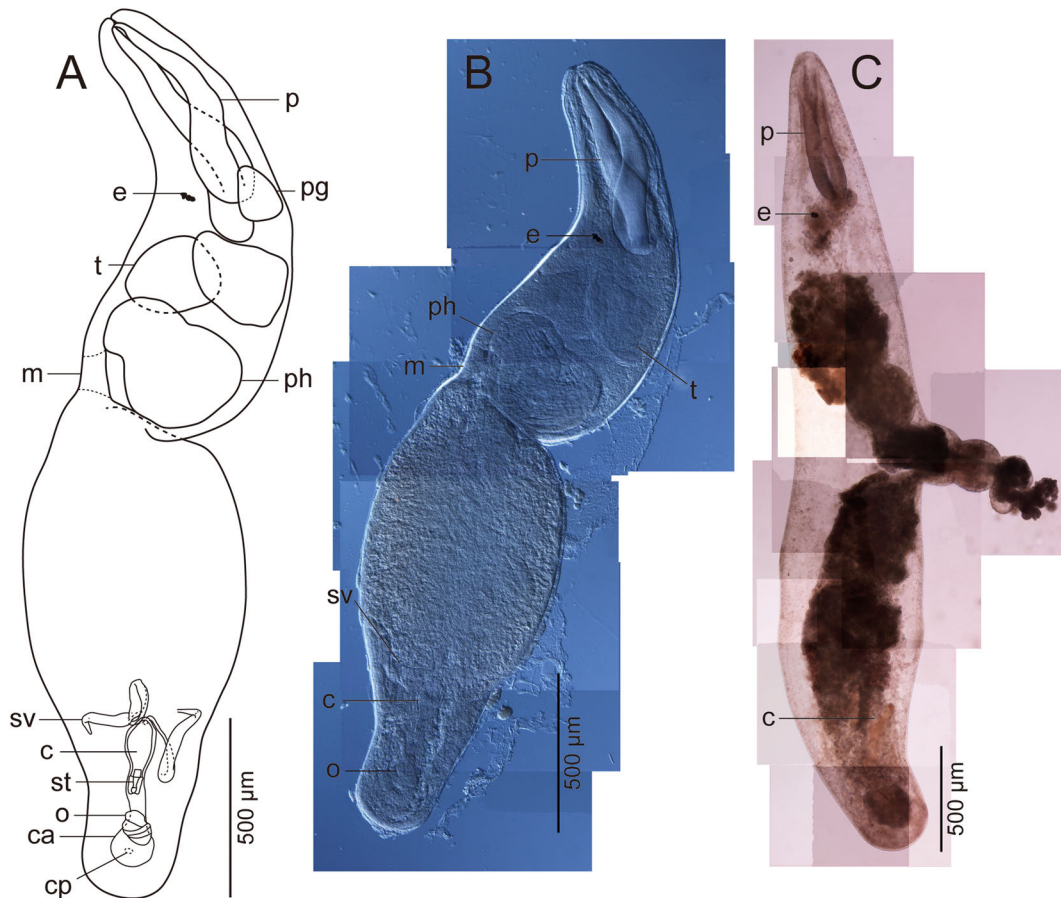


Fig. 15. *Proschizorhynchella shuttlecock* sp. nov. A. Illustration of fixed specimen, ICHUM 4846 (holotype); B. Photomicrograph of fixed specimen, ICHUM 4846 (holotype); C. Composite photomicrograph in living state, ICHUM 4845 (paratype) [the body wall is ruptured with the intestine partially protruding]. Abbreviations: c, male copulatory organ; ca, common atrium; cp, common genital pore; e, eye; m, mouth; o, ovary; p, proboscis; pg, proboscis gland sac; ph, pharynx; st, stylet; sv, seminal vesicle; t, testis.

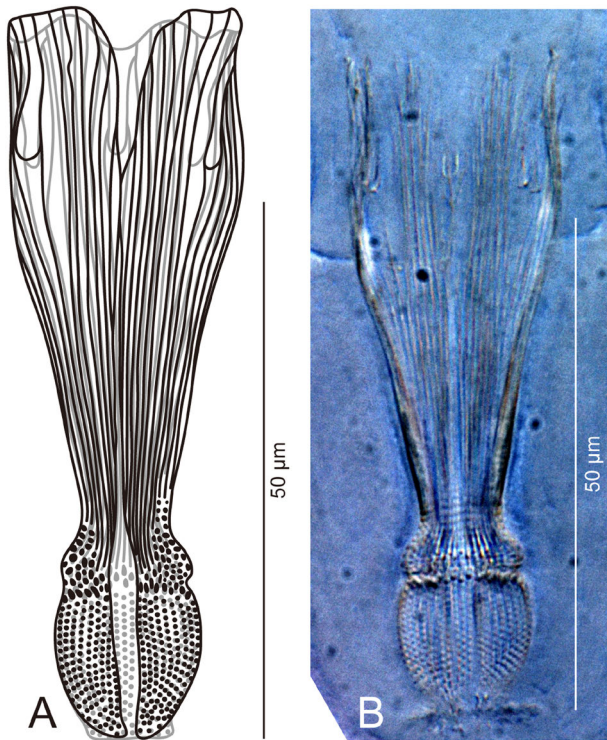


Fig. 16. *Proschizorhynchella shuttlecock* sp. nov. stylet, ICHUM 4846 (holotype). A. Illustration; B. Photomicrograph.

elongated along stylet axis, 14 µm in maximum diameter, with single, transverse constriction slightly behind its widest portion; numerous, minute, wart-like projections present inside distal bulbous region (Fig. 16A, B); cirrus spine absent. Ovary 100 µm long, 70 µm wide, located posterior to pharynx (Fig. 15A). Common atrium 110 µm in diameter, leading to genital pore opening on ventral side of body located near caudal end (Fig. 15A).

Etymology. The specific name refers to the shape of the stylet in the new species.

Remarks. The stylet in *Proschizorhynchella shuttlecock* sp. nov. is badminton shuttlecock shaped, which is unique among the congeners, which mostly show a conical stylet (Table 1). *Proschizorhynchella shuttlecock* lacks adhesive girdles and thus resembles *Proschizorhynchella faroensis* (Steinböck, 1931) and *Proschizorhynchella spiracirro* (Schilke, 1970). The latter two possess cirrus spines (Steinböck 1931; Schilke 1970) but *P. shuttlecock* does not.

Acknowledgments

We are grateful to the members of the Laboratory of Biodiversity I (Hokkaido University) for their help in sampling and their guidance.

References

- Doe, D. A. 1974. Two new *Proschizorhynchus* species from the coast of Massachusetts, USA (Turbellaria, Kalyptorhynchia). *Zoologica Scripta* 3: 101–110.
- Evdonin, L. A. 1969. Novyi predstavitel' interstitial'nykh Kaliptorinkhii (Turbellaria: Neorhabdocoela: Kalyptorhynchia) ostrova Kunashir. *Vestnik Leningradskogo Universiteta* 15: 7–14.
- Graff, L. von 1905. Marine Turbellarien Orotavas und der Küsten Europas. Teil II. Rhabdocoela. *Zeitschrift für wissenschaftliche Zoologie* 83: 68–154, pls 2–6.
- Karling, T. G. 1983. Structural and systematic studies on Turbellaria Schizorhynchia (Platyhelminthes). *Zoologica Scripta* 12: 77–89.
- Karling, T. G. 1989. New taxa of Kalyptorhynchia (Platyhelminthes) from the N. American Pacific coast. *Zoologica Scripta* 18: 19–32.
- L'Hardy, J. P. 1965. Turbellaries Schizorhynchidae des sables de Roscoff II. Le genre *Proschizorhynchus*. *Cahiers de Biologie Marine* 6: 135–161.
- Marcus, E. 1950. Turbellaria Brasileiros (8). Universidade de São Paulo, *Boletins da Faculdade de Filosofia, Ciências e Letras, Zoologia* 15: 5–192.
- Meixner, J. 1928. Aberrante Kalyptorhynchia (Turbellaria: Rhabdocoela) aus dem Sande der Kieler Bucht. *Zoologischer Anzeiger* 77: 229–253.
- Meixner, J. 1938. Turbellaria (Strudelwürmer). *Die Tierwelt der Nord- und Ostsee* 33 (IVb): 1–146.
- Noldt, U. 1986. Kalyptorhynchia (Platyhelminthes) aus dem sublittoralen Küstenbereich der Nordseeinsel Sylt. Ph.D. thesis, University of Göttingen, 222 pp.
- Noldt, U. 1989. Kalyptorhynchia (Platyhelminthes) from sublittoral coastal areas near the island of Sylt (North Sea). I. Schizorhynchia. *Microfauna Marina* 5: 7–85.
- Schilke, K. 1970. Kalyptorhynchia (Turbellaria) aus dem Eulitoral der deutschen Nordseeküste. *Helgoländer Meeresuntersuchungen* 21: 143–265.
- Smith, J. P. 3rd, Litvaitis, M. K., Gobert, S., Uyeno, T., and Artois, T. 2015. Evolution and functional morphology of the proboscis in Kalyptorhynchia (Platyhelminthes). *Integrative and Comparative Biology* 55(2): 1–12.
- Steinböck, O. 1931. Marine Turbellaria. *Zoology of the Faroes* 8: 1–26.
- Tyler, S., Schilling, S., Hooge, M., and Bush, L. F. 2006–2013. Turbellarian taxonomic database, Version 1.7. Available at <http://turbellaria.umaine.edu> (10 October 2017).