

Description of a New Genus of Candimboiidae (Polycladida: Acotylea) from the Coast of Sagami Bay

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We establish a new genus of candimboiid polyclad, *Chimaeriplana* gen. nov., based on a new species, *C. japonica* sp. nov., collected from Kanagawa, the coast of Sagami Bay, Japan. *Chimaeriplana japonica* sp. nov. is characterized by (i) an interpolated prostatic vesicle with a seminal vesicle, (ii) a penis stylet with a split tip, (iii) numerous prostatoid organs in the male atrium, (iv) a bursa copulatrix, and (v) without Lang's vesicle. In addition, we modify the definition of Candimboiidae.

Key Words: bursa copulatrix, marine flatworm, new genus, penis stylet, prostatoid organ, prostatoids.

Introduction

Candimboiidae is a family in Leptoplanoidea (Faubel 1983, 1984). Within Leptoplanoidea, Candimboiidae is notable for having a bursa copulatrix in the female copulatory apparatus. The organ is observed in another leptoplanoid family, Apidioplanidae. However, the two families are clearly distinguished by several morphological characteristics, such as the morphology of a pharynx and the presence/absence of apioids (cf. Faubel 1983; Prudhoe 1985). The family contains the only genus *Candimboides* Prudhoe, 1982; currently, two species are assigned to the genus (Oya et al. 2022: 447).

Two polyclads that could be identified with candimboiid species were collected from the coast of Sagami Bay in 1992 and 2009. The polyclads resemble flatworms in *Candimboides* in anatomical characters of copulatory apparatuses; however, the character states do not completely fit the genus diagnosis. In particular, the polyclads have numerous prostatoid organs in the male copulatory apparatus, like in flatworms in Discocelidae and Polyposthiidae (cf. Faubel 1983; Prudhoe 1985). In this study, we describe a new species based on the specimens and establish a new genus of Candimboiidae based on this species.

Materials and Methods

Sampling, observation of external characters, and specimen preparation were conducted by MoH. Information was recovered by YO from MoH's computer, field books, and lab notebooks, with consent and permission from Mieko Hagi-

ya, MoH's wife. Two polyclads were collected from intertidal zones in Kanagawa, Japan. The dorsal view of the living worms was photographed and roughly drawn. Later, the polyclads were fixed in Bouin's solution. The posterior part of the fixed specimens was dehydrated in an ethanol series, cleared in clove oil or xylene, embedded in paraffin wax, and sectioned; their thickness is unclear. The sections were stained with hematoxylin and eosin (HE) and mounted by a Canada balsam. YO tried three times to collect additional specimens at the locality of the paratype, where the detailed site can be specified from the MoH's field book, but it was unsuccessful.

A detailed observation of the preparations was carried out by YO. Histological sections were observed by an Olympus BX51 compound microscope and photographed by a Nikon D5300 digital camera under the microscope. The size of copulatory apparatuses was measured from photographs using ImageJ (Rasband 1997–2023). The morphological terminology in this study followed Hyman (1953). All graphical treatments were done with Adobe Photoshop CC, and illustrations were prepared with Adobe Illustrator CS6.

The type specimens were deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM) as a part of "Hagiya's collection," a collection of polyclad specimens collected by MoH; it was donated by MoH's wife after his death. Copies of electronic data and notebooks were also deposited in the ICHUM along with the collection for future reference.

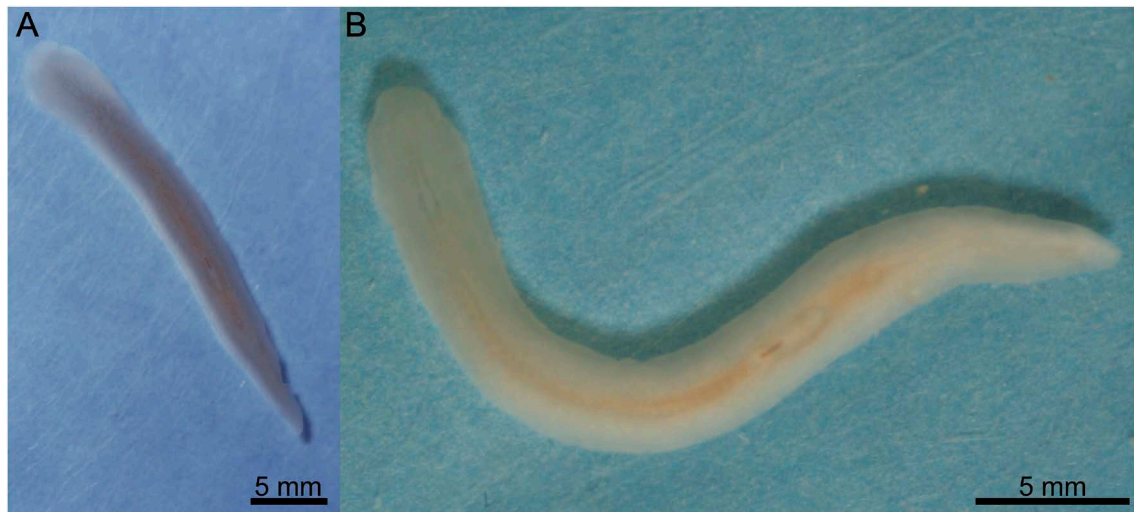


Fig. 1. *Chimaeriplana japonica* gen. et sp. nov., photographs taken in life, dorsal view. A, ICHUM 7958 (holotype); B, ICHUM 7981 (paratype). Scales estimated from MoH's lab notebooks.

Taxonomy

Family **Candimboidea** Faubel, 1983

Amended definition. Leptoplanoidea with elongate body and without tentacles. Eyespots in two elongate clusters in region of cerebral organ. Pharynx in middle third of body. Male and female gonopores well separated from posterior margin of the body. Male copulatory apparatus immediately posterior to pharynx. Prostatic vesicle muscular and lined with tall, smooth, glandular epithelium. Penis stylet lying in penis pocket. Female copulatory apparatus having bursa copulatrix [modified from Faubel (1983: 91)].

Remarks. The previous diagnosis of Candimboidea by Faubel (1983: 91) contained "Male and female genital pores approximate ..." and "Female complex includes bursa copulatrix and Lang's vesicle." To place the new genus in this family, we eliminated the references of (i) the distance between the male and female gonopores and (ii) the presence of the Lang's vesicle from Faubel's (1983) definition.

Type genus. *Candimboidea* Prudhoe, 1982.

Genus ***Chimaeriplana*** gen. nov.

[New Japanese name: Nue-hiramushi-zoku]

Type species. *Chimaeriplana japonica* sp. nov., fixed by original designation.

Definition. Candimboidea with numerous prostatic organs and penis stylet with split tip and without Lang's vesicle (Figs 1–3).

Etymology. The new generic name is feminine in gender, named after the Latin proper noun "*Chimaera*." It alludes to the type species characterized by the mixed morphology of leptoplanoid, discocelid, and polyposthiid polyclads.

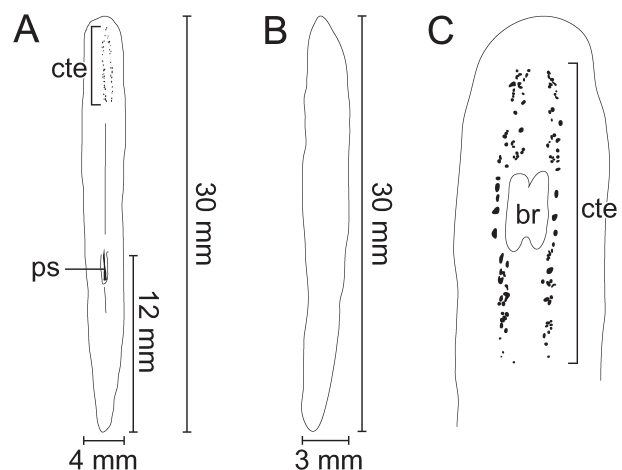


Fig. 2. *Chimaeriplana japonica* gen. et sp. nov., sketches drawn in life, fair copies from MoH's lab notebooks. A, ICHUM 7958 (holotype); B, C, ICHUM 7981 (paratype). A, B, Dorsal view; C, eyespot distribution. Abbreviations: br, brain; cte, cerebrotentacular eye cluster; ps, penis stylet.

Chimaeriplana japonica sp. nov.

[New Japanese name: Nue-hiramushi]

(Figs 1–7)

Etymology. The specific name is a Latin adjective (*japonicus*, -a, -um) derived from the occurrence of the new species in Japan. The Japanese name for the new genus and species is derived from "Nue" (a Japanese legendary monster with the face of a monkey, the body of a raccoon dog, the limbs of a tiger, and the front half of a snake for a tail) and

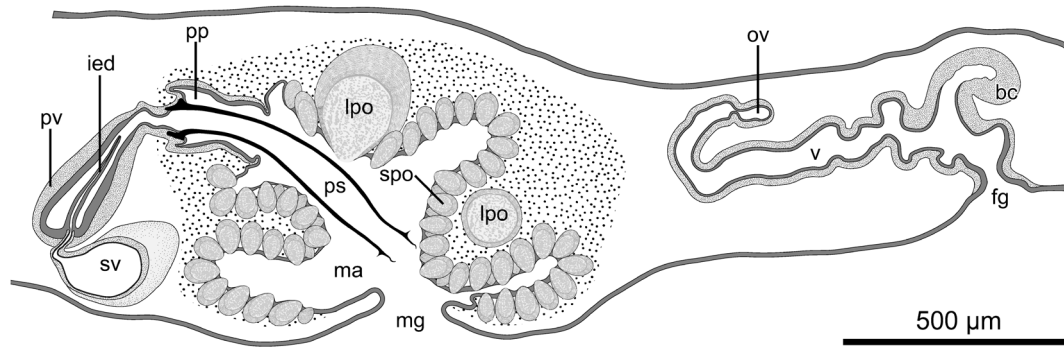


Fig. 3. Schematic diagram of copulatory apparatuses in *Chimaeriplana japonica* gen. et sp. nov., ICHUM 7958 (holotype). Abbreviations: bc, bursa copulatrix; fg, female gonopore; ied, intra-prostatic ejaculatory duct; lpo, large prostatic organ; ma, male atrium; mg, male gonopore; ov, oviduct; pp, penis pocket; ps, penis stylet; pv, prostatic vesicle; spo, small prostatic organ; sv, seminal vesicle; v, vagina.

“hiramushi” (polyclad flatworm) in the Japanese language.

Material examined. Two specimens were collected by MoH: holotype, ICHUM 7958, two slides (thickness unknown, sagittal sections of posterior half of body, stained with HE), intertidal, Moroiso (35°09'18"N, 139°36'27"E), Misaki, Kanagawa, Japan, 17 May 1992; paratype, ICHUM 7981, two slides (thickness and sectioning plane unknown, almost horizontal sections of posterior half of body, stained with HE), intertidal, Mitsuishi (35°08'16"N, 139°09'46"E), Manazuru, Kanagawa, Japan, 11 May 2009.

Description. Living specimens 30 mm long, 3–4 mm in maximum width (4 mm in holotype). Body elongated, slender, narrow toward posterior end (Figs 1, 2A, B). General appearance of body pale brownish to yellowish (Fig. 1). Tentacles lacking. Pair of cerebro-tentacular eye clusters, linear in shape, arranged near median line (Fig. 2A, C). Pharynx ruffled in shape, located slightly anterior to center of body. Penis stylet visible brownish through dorsal surface of body (Fig. 1; Supplementary Fig. 1). Gonopores well separated.

Male copulatory apparatus immediately located posterior to pharynx, consisting of seminal vesicle, interpolated prostatic vesicle, penis stylet, and numerous prostatic organs; penis papilla lacking (Fig. 3). Common sperm duct lacking; pair of sperm duct separately entering proximal end of seminal vesicle. Seminal vesicle pear-shaped, 292–321 µm long (292 µm in holotype), 165–219 µm wide (219 µm in holotype), with thick (9–20 µm) muscular wall. Distal part of seminal vesicle connecting to proximal end of prostatic vesicle. Prostatic vesicle elongated, pear-shaped, 390–404 µm long (390 µm in holotype), 169–217 µm wide (169 µm in holotype), with thick (13–43 µm) muscular wall and long (241–278 µm, 241 µm in holotype) intra-prostatic ejaculatory duct, without tubular chambers (Fig. 4A). Penis stylet 680 µm long (unclear in paratype), tubular, slightly curved, projecting penis sheath and male atrium (Figs 3, 4A, B). Surface of stylet smooth in proximal half part; that in distal half consisting of multiple ridges along with long axis (Fig. 5A–C). Each ridge independently constituting tip of stylet in distal end. Stylet with >20 tips, each tip form-

ing curved hook and possessing barb (Fig. 5D). Prostatic organs, unarmed, ovoid in shape, distributed in the inner surface of male atrium, with two types (Figs 3, 4, 6): large (212–293 µm long, 147–217 µm wide; Fig. 6B) and small (70–92 µm long, 53–64 µm wide; Fig. 6C). Five large prostatic organs present apart from each other in male atrium; numerous small prostatic organs filling gap among large ones (Fig. 6A). Each prostatic organ with glandular part opening to male atrium and muscular wall surrounding glandular part (Fig. 6). Muscular wall especially developed in large prostatic organs (up to 83 µm) (Fig. 6B, C).

Female copulatory apparatus with bursa copulatrix and without Lang's vesicle (Figs 3, 7). Vagina 1113 µm long (unclear in paratype) with ciliated epithelium, running anteriorly then turning posteriorly to exit female gonopore; about one-fifth near proximal end of vagina surrounded by cement glands (Fig. 7A). Bursa copulatrix oval, 200 µm long and 113 µm wide (unclear in paratype), with thick (36–46 µm) muscular wall, lined with epithelium differentiated from that of vagina, directing postero-dorsally (Fig. 7B, C).

Type locality. Moroiso (35°09'18"N, 139°36'27"E), Misaki, Kanagawa, Japan.

Distribution. Sagami Bay, Japan.

Habitat. Judged from MoH's lab notebooks and the environment of the sampling sites, the habitat of *C. japonica* sp. nov. is likely to be a rocky shore in intertidal zones.

Remarks. The new species should be assigned to a new genus in Candimboiidae. The presence of numerous prostatic organs is a diagnostic character of Discocelidae (Discoceloidea) and some genera in Polyposthiidae (Stylochoidea) (Faubel 1983; Prudhoe 1985). However, discocelid and polyposthiid species have an oval to elongate oval body with marginal eyespots (Faubel 1983; Prudhoe 1985), whereas the present species has a slender body without marginal eyespots (Figs 1, 2). In addition, discocelids and polyposthiids do not have a prostatic vesicle and a seminal vesicle as a set, while the new species has an interpolated prostatic vesicle with a true seminal vesicle (Figs 3–7; Table 1). Except for (i) the presence of the prostatic organs, (ii) well-

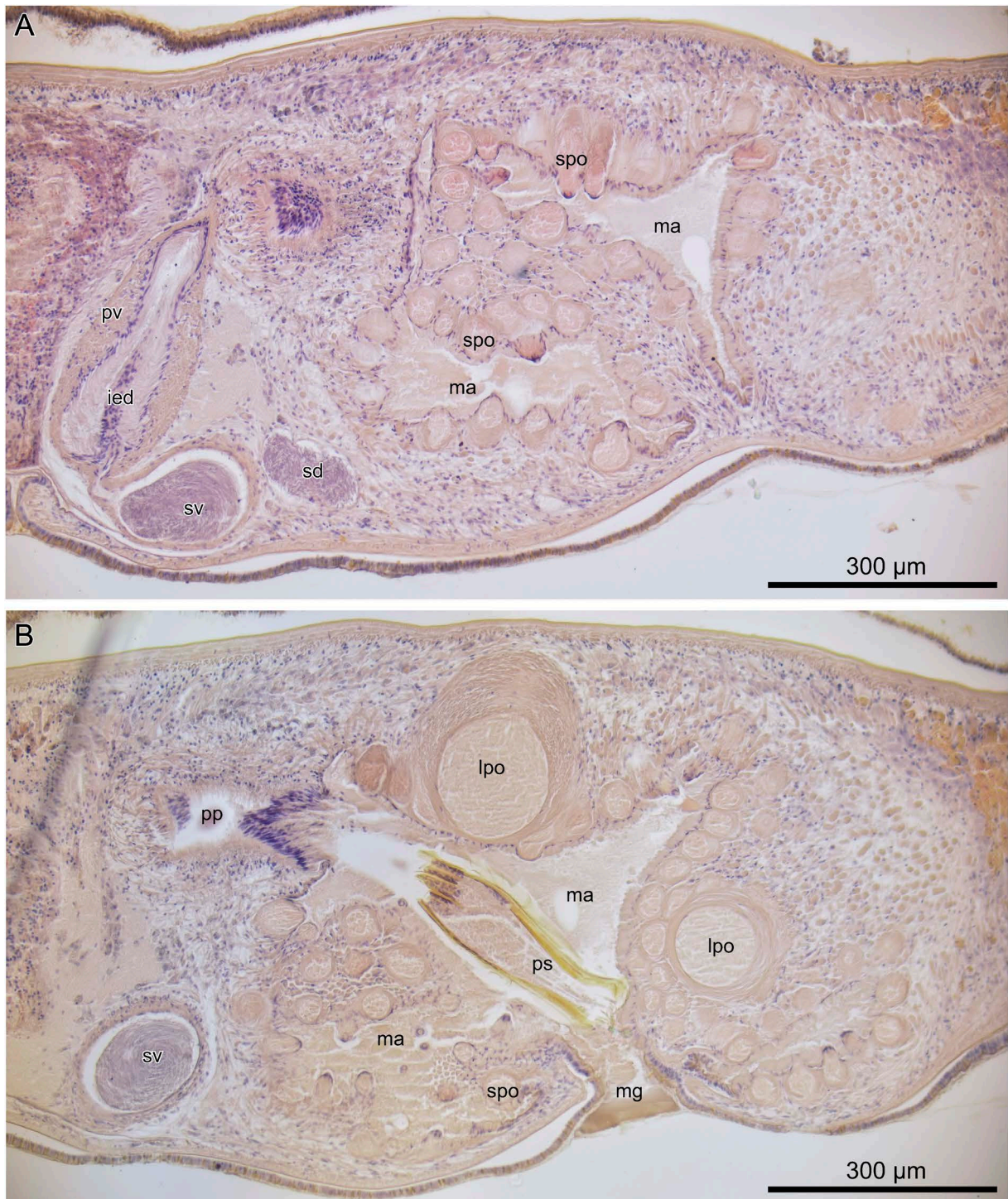


Fig. 4. Photomicrographs of sections of male copulatory apparatus in *Chimaeriplana japonica* gen. et sp. nov., ICHUM 7958 (holotype). A, Prostatic vesicle and seminal vesicle; B, penis stylet and prostatoid organs. Abbreviations: ied, intra-prostatic ejaculatory duct; lpo, large prostatoid organ; ma, male atrium; mg, male gonopore; pp, penis pocket; ps, penis stylet; pv, prostatic vesicle; spo, small prostatoid organ; sd, sperm duct; sv, seminal vesicle.

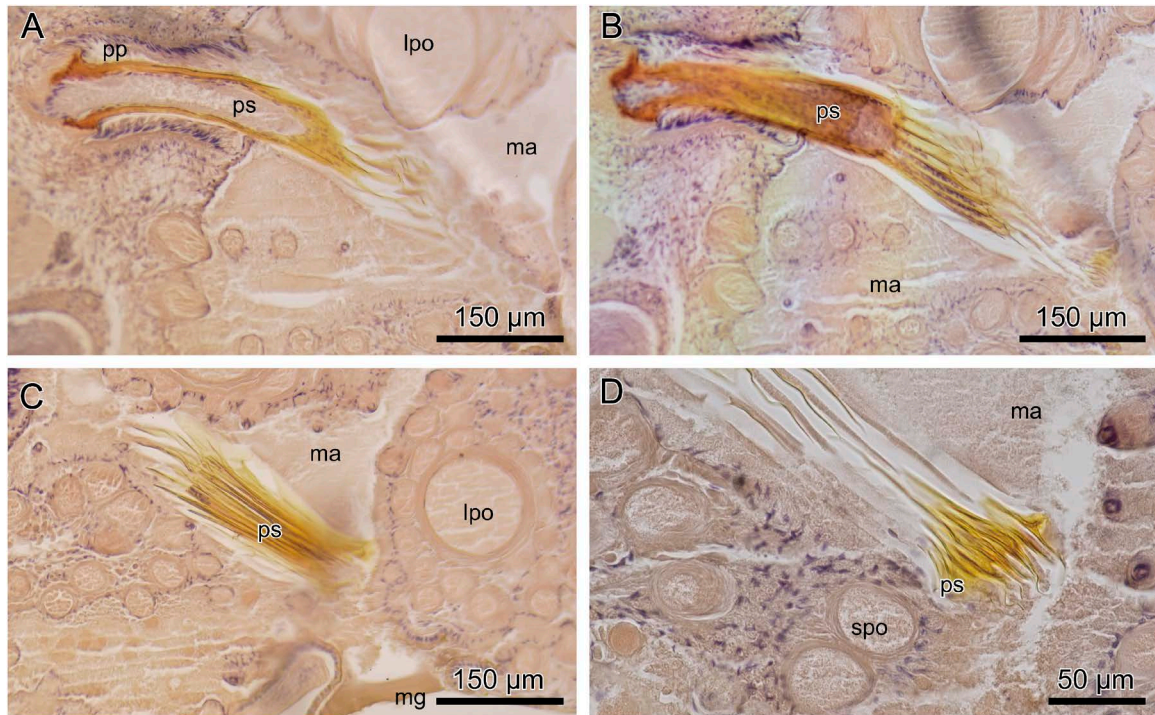


Fig. 5. Photomicrographs of penis stylet in *Chimaeriplana japonica* gen. et sp. nov., ICHUM 7958 (holotype). A, B, Proximal part; C, D, distal part. Abbreviations: lpo, large prostatoid organ; ma, male atrium; mg, male gonopore; pp, penis pocket; ps, penis stylet; spo, small prostatoid organ.

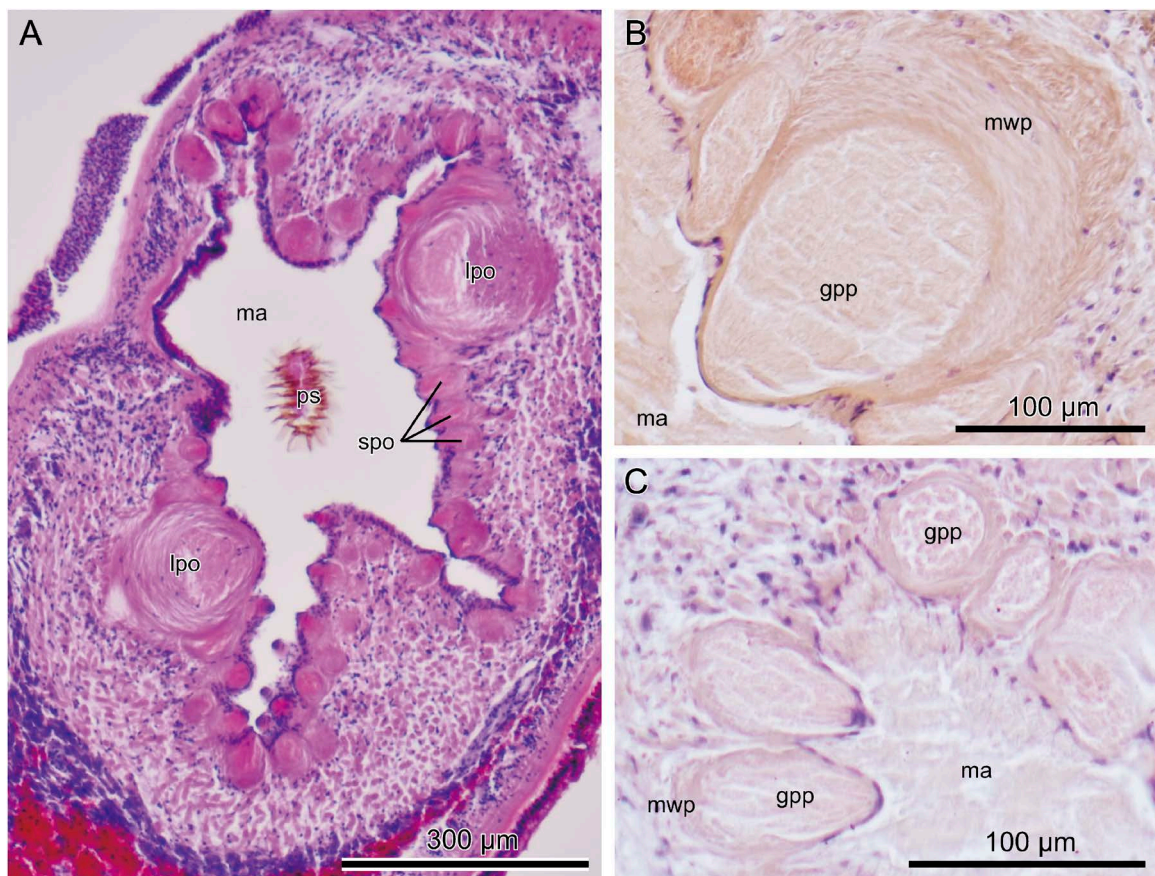


Fig. 6. Photomicrographs of prostatoid organs in *Chimaeriplana japonica* gen. et sp. nov. A, ICHUM 7981 (paratype); B, C, ICHUM 7958 (holotype). Abbreviations: gpp, glandular part of prostatoid organ; lpo, large prostatoid organ; ma, male atrium; mwp, muscular wall of prostatoid organ; ps, penis stylet; spo, small prostatoid organ.

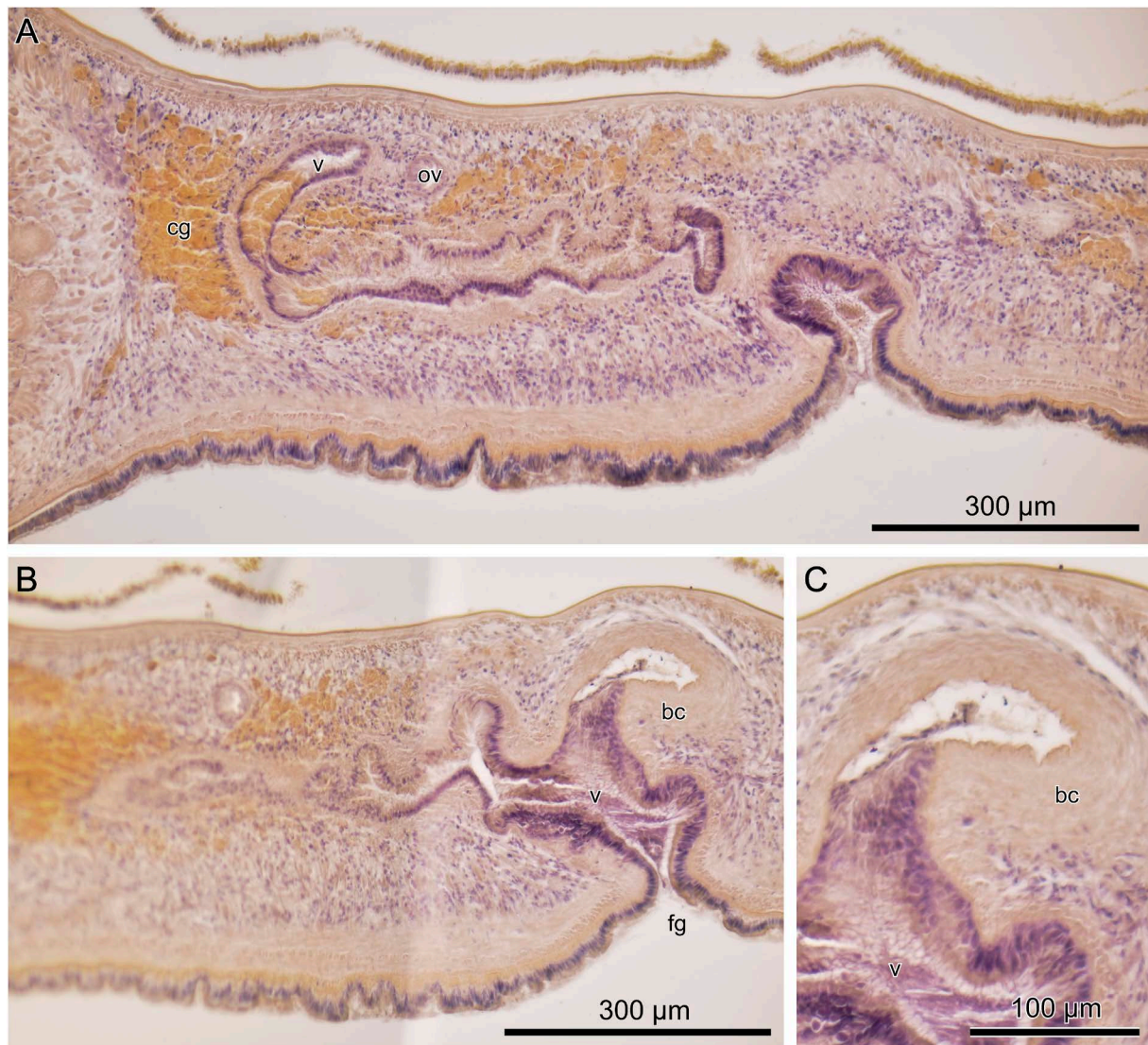


Fig. 7. Photomicrographs of sections of female copulatory apparatus in *Chimaeriplana japonica* gen. et sp. nov., ICHUM 7958 (holotype). A, Vagina; B, vagina and bursa copulatrix; C, bursa copulatrix. Abbreviations: cg; cement gland; bc, bursa copulatrix; fg, female gonopore; ov, oviduct; v, vagina.

separated gonopores, and (iii) the absence of Lang's vesicle, the new species closely resembles polyclads of *Candimboides* (Table 1; Du Bois-Reymond Marcus and Marcus 1968: figs 34, 35; Prudhoe 1982: fig. 6). Here, we establish *Chimaeriplana* gen. nov. based on *C. japonica* sp. nov.

We revise the definition of Candimboidea provided by Faubel (1983: 91), which is almost identical to the concept of *Candimboidea* by Prudhoe (1982: 371), to include *Chimaeriplana* gen. nov. in the family. The previous diagnosis of Candimboidea by Faubel (1983: 91) mentioned the proximity between the male and female gonopores and the presence of the Lang's vesicle. For the closeness of male and female gonopores, we do not believe that this character needs to be emphasized as a diagnosis at the family level because it is variable at the species level in some acotyleans (cf. Oya et al. 2022). For Lang's vesicle, the presence or absence of the

organ is often employed for distinguishing between genera in a family (e.g., Planoceridae). We eliminated these two diagnostic features from Faubel's (1983) original concept.

The prostatic organs of *C. japonica* sp. nov. are more similar to those in Discocelidae than to those in Polyposthiidae. The organs of discocelids and the present polyclad are distributed only in the wall of the male atrium and are independent of the ductal system in the male copulatory apparatus such as the sperm duct, the seminal vesicle, or the prostatic vesicle. On the other hand, prostatic organs in Polyposthiidae are observed not only in the male atrium but also in other areas on the ventral surface of the body as well as those in the male atrium have connection(s) with the sperm ducts (Bock 1913; Palombi 1924; Beauchamp 1951). As far as we have observed the histological sections of *C. japonica* sp. nov., the glandular part and the muscular wall of the

Table 1. Comparison of morphological characteristics between genera in Candimboiidae, Discocelidae, and Polypotheiidae. For the character status, we refer to Prudhoe (1982, 1985, 1989), Faubel (1983), and Bulnes (2010).

Genus	<i>Adenoplana</i> Stummer- Trautnfeld, 1933	<i>Coronadena</i> Hymen, 1940	<i>Discocelis</i> Ehrenberg, 1836	<i>Paradiscocelis</i> Prudhoe, 1989	<i>Pseudodiscocelis</i> Bulnes, 2010	<i>Thalamoplana</i> Laidlaw, 1904	<i>Tetratrema</i> Prudhoe, 1989	<i>Candimboioides</i> Prudhoe, 1982	<i>Chimaeriplana</i> gen. nov.	<i>Bergendalia</i> Laidlaw, 1903	<i>Cryptocelides</i> Bergendal, 1890	<i>Metaposthia</i> Palombi, 1923	<i>Polypthaloplana</i> Beauchamp, 1951	<i>Polypotheia</i> Bergendal, 1893	<i>Polypotheiidae</i> Palombi, 1923
Family	Discocelidae	Discocelidae	Discocelidae	Discocelidae	Discocelidae	Discocelidae	Discocelidae	Candimboiidae	Candimboiidae	Polypotheiidae*	Polypotheiidae	Polypotheiidae	Polypotheiidae	Polypotheiidae	Polypotheiidae
Body shape	elongate oval to obovate	oval or slightly ovoid	broadly oval or elongated oval	elongate oval	broadly oval	broadly oval	elongate oval	elongated, slender	elongated, slender	elongated, slender	elongate oval	oval	lanceolate (Prudhoe 1985)	oval	oval
Nuchal tentacles	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
Marginal eyespots	present	present	present	present	present	present	present	absent	absent	absent	absent	absent	absent	absent	absent
Gonopores	separated	common	common	common	common	separated	separated	separated	separated	separated	separated	separated	separated	common	separated
Seminal vesicle	absent (spermiducal bulbs present)	absent	?	absent (ejaculatory duct lined with glandular epithelium)	absent (ejaculatory duct lined with glandular epithelium)	absent (ejaculatory duct lined with glandular epithelium)	absent (ejaculatory duct lined with glandular epithelium)	present	present	absent	absent	absent	absent	absent	absent
Prostatic vesicle	absent (ejaculatory duct lined with glandular epithelium)	absent	absent	absent (ejaculatory duct lined with glandular epithelium)	absent (ejaculatory duct lined with glandular epithelium)	absent	absent (ejaculatory duct lined with glandular epithelium)	interpolated	interpolated	free	free (Faubel 1983)/absent (Prudhoe 1985)	free (Faubel 1983)/absent (Prudhoe 1985)	free	free	free
Lining of prostatic vesicle	—	—	—	—	—	—	—	smooth	smooth	smooth	smooth (Faubel 1983)	smooth (Faubel 1983)	smooth	smooth	smooth
Intra-prostatic ejaculatory duct	—	—	—	—	—	—	—	present/absent	present	—	—	—	—	—	—
Penis stylet	absent	absent	absent	absent	absent	absent	absent	present	present	absent	absent	absent	absent	absent	absent
Prostatic organ	present	present	present	present	present	present	present	absent	present	absent	present	present	present	present	present
Bursa copulatrix	absent	absent	absent	absent	absent	absent	absent	present	present	absent	absent	absent	absent	absent	absent
Lang's vesicle	present	present	present	present	absent	present	present	present	absent	absent (vaginal duct present)	present	absent	present	present	absent

* Latocestidae in Prudhoe (1985).

prostatoid organs also resemble those of the organs in discocelid flatworms (cf. Bulnes 2010: fig. 11D; Maghsoudlou and Rahimian 2013: figs 4D, 9D). Observation with other staining methods, such as Azan trichrome stain or periodic acid-Schiff stain, may reveal distinct histological or cytological differences in the prostatoid organs between *C. japonica* sp. nov. and discocelid polyclads.

The penis stylet in *C. japonica* sp. nov. is remarkable in the polyclads. The penis stylet in most polyclads is a simple, tubular structure with a smooth surface and a single tip. The penis stylet in the new species has distinct ridges on its surface and multiple tips with a barb (Fig. 5D). For now, *C. japonica* sp. nov. is probably the only polyclad that possesses such a penis stylet; however, the detailed morphology is unclear because it is broken by sectioning. In future studies, collection of new specimens and observation using a scanning electron microscope are desirable to describe the fine 3D morphology of the structure.

Supplementary Information

Supplementary Figure 1. *Chimaeriplana japonica* gen. et sp. nov., sketches drawn in life, copies from MoH's lab notebooks, annotated by YO. A, Dorsal view, ICHUM 7958 (holotype); B, dorsal view and eyespot distribution, ICHUM 7981 (paratype). Available at <https://doi.org/10.6084/m9.figshare.24217686>.

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Authors Contributions

Yuki Oya: Conceptualization; Investigation; Data curation; Visualization; Writing – original draft; Writing – review & editing. Morio Hagiya: Resources; Investigation; Data curation; Visualization.

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Declarations

Competing interests. The authors declare no conflicts of interest.

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