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Description and phylogenetic relationships of a new genus of Planoceridae (Polycladida, Acotylea) from Shimoda, Japan

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Abstract

We establish a new genus of planocerid polyclad, *Heteroplanocera* gen. nov., based on a new species, *H. katoi* sp. nov., collected at a depth of 245 m off the coast of the Izu Peninsula, West Pacific, Japan. *Heteroplanocera* gen. nov. is distinguished from other planocerid genera by the following characteristics: (*i*) a pair of nuchal tentacles, (*ii*) a muscular bulb, (*iii*) a pair of spermiducal bulbs (instead of a seminal vesicle), (*iv*) a free prostatic vesicle, (*v*) a pair of accessory organs with teeth, (*vi*) a Lang's vesicle, and *vii*) the lack of a bursa copulatrix. We provide a partial sequence of the cytochrome *c* oxidase subunit I (COI) gene (712 bp) as a DNA barcode of the new species. Furthermore, we estimate the phylogenetic position of *Heteroplanocera* gen. nov. among Planoceridae based on partial sequences of COI and 16S, 18S, and 28S ribosomal DNA. Based on our findings, we discuss the evolution and function of the (*i*) vaginal cuticularized structure and (*ii*) accessory organs among planocerid flatworms.

Keywords

eversible cirrus, flatworm, Stylochoidea, taxonomy, traumatic mating, phylogeny

INTRODUCTION

Planoceridae Stimpson, 1857 is one of the 10 families of acotylean polyclads belonging to the superfamily Stylochoidea Stimpson, 1857 (Faubel 1983, 1984; Dittmann *et al.* 2019). Members of Planoceridae are characterized by possession of eversible cirri armed with hard structures (e.g., spines, thorns, or teeth) in the male copulatory apparatus. Some planocerids also have cuticularized tissue in their female copulatory apparatus (e.g., Hyman 1953), part of which can take the form of spines in the vagina (cf. Kato 1944).

To date, seven genera have been assigned to Planoceridae (Faubel 1983; Bulnes 2010). These genera are distinguished from each other by the presence or absence of the following structures: nuchal tentacles; a muscular bulb surrounding the cirrus; a seminal vesicle and accessory organs in the male genital complex; and a Lang's vesicle and bursa copulatrix in the female reproductive organs.

In the present paper, we establish a new genus of Planoceridae based on a new species collected offshore of the Izu Peninsula, West Pacific, Japan. We provide a partial sequence of the cytochrome *c* oxidase subunit I (COI) gene as a DNA barcode for this new species. Furthermore, we estimate the phylogenetic position of the genus within Planoceridae using partial sequences of COI and 16S, 18S, and 28S ribosomal DNA (rDNA). Finally, based on our findings, we discuss the evolutionary history of the cuticularized structures in the vagina of Planoceridae as well as the function of accessory organs in the male copulatory apparatus.

MATERIALS AND METHODS

During the 18th Japanese Association for Marine Biology (JAMBIO) Coastal Organism Joint Survey, two marine worm specimens were collected from a depth of 245 m by dredging off the coast of the Izu Peninsula on the West Pacific side of Japan. The worms were anesthetized in a $MgCl_2$ solution (prepared with tap water to have the same salinity as the seawater) and then photographed following the method of Oya & Kajihara (2019). For DNA extraction, a piece of the body margin (lateral to pharynx) was cut away from each specimen and fixed in 100% ethanol. The rest of the body was fixed in Bouin's solution for 24 h, preserved in 70% ethanol, and then cut into two pieces (anterior and posterior). The anterior piece was dehydrated in an ethanol series, cleared in xylene, and observed under a Nikon SMZ 1500 stereomicroscope; this piece was preserved in 70% ethanol after observation. The posterior piece was used for histological observations, which were conducted according to the protocol of Oya & Kajihara (2019). Type specimens have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

Total DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Germany). The partial sequences of 16S (438 bp), 18S (1736 bp), and 28S rDNA (1038 bp) (hereafter referred to simply as 16S, 18S, and 28S) as well as that of COI (712 bp) were sequenced from one specimen following the methods of Oya & Kajihara (2020). The COI sequence was also determined from another specimen. In addition, the same markers from *Paraplanocera oligoglena* (Schmarda, 1859) were also determined for the purposes of phylogenetic analyses following the protocol by Oya & Kajihara (2020). Sequences were checked and edited using MEGA ver. 7.0 (Kumar *et al*. 2016), and the

genetic distances were calculated (also via MEGA ver. 7.0). All sequences determined in this study have been deposited in DDBJ/EMBL/GenBank databases with the accession numbers LC545561–LC545569.

Additional sequences of Planoceridae and outgroup species were downloaded from GenBank. The outgroup taxa comprised three acotylean species: *Discocelis* sp., *Imogine* cf. *aomori* (Kato, 1937), and *Notocomplana humilis* (Stimpson, 1857) (Table 1). The 16S, 18S, and 28S sequences were aligned using MAFFT ver. 7 (Katoh & Standley 2013), with the L-INS-i strategy selected by the "Auto" option. Ambiguous sites were removed with Gblocks ver. 0.91b (Castresana 2002) using a more stringent selection. The COI sequences were aligned manually using MEGA ver. 7.0. The concatenated dataset from the four genes was 3708 bp long and contained 12 terminal taxa.

Phylogenetic analyses were performed using (*i*) the maximum-likelihood (ML) method executed in IQtree ver. 1.6 (Nguyen *et al*. 2015) under a partition model (Chernomor *et al*. 2016) and (*ii*) Bayesian inference (BI) executed in MrBayes ver. 3.2.2 (Ronquist & Huelsenbeck 2003). The optimal substitution models for the ML analysis, which were selected with PartitionFinder ver. 2.1.1 (Lanfear *et al*. 2016) under the Akaike information criterion (Akaike 1974) using the greedy algorithm (Lanfear *et al*. 2012), were GTR + G (16S), GTR + I (18S), GTR + I + G (28S, first codon position in COI), TIM + G (third codon position in COI), and TVM + I + G (second codon position in COI); the corresponding models for BI were GTR + G (16S, third codon position in COI), GTR + I (18S, second codon position in COI), and GTR + I + G (28S, first codon positions in COI). Nodal support within the ML tree was assessed by analyses of 1000 bootstrap pseudoreplicates. For BI, the Markov chain Monte Carlo process used random

starting trees and involved four chains run for 1,000,000 generations with the first 25% of trees discarded as burn-in. We considered posterior probability (PP) values ≥0.90 and ML bootstrap (BS) values ≥70% to indicate clade support; in the text, combined nodal support is represented as PP/BS.

RESULTS

SYSTEMATICS

Family PLANOCERIDAE Stimpson, 1857

Genus *Heteroplanocera* gen. nov.

urn:lsid:zoobank.org:act:4289C802-A09E-436F-ADCB-45E9BE6DB405

TYPE SPECIES

Heteroplanocera katoi sp. nov., fixed by original designation.

DIAGNOSIS

Planoceridae with oval body, nuchal tentacles, pair of spermiducal bulbs, free prostatic vesicle with tubular folded epithelium, ejaculatory duct with prostatic-like epithelium, cirrus with numerous teeth enclosed by muscular bulb, pair of accessory organs with eosinophilic glands and teeth, rudimentary Lang's vesicle, vagina bulbosa with cuticularized epithelium, common gonopore, and without bursa copulatrix (Figures 1 and 2).

ETYMOLOGY

The new generic name is feminine in gender, formed by the combination of the prefix

hetero (meaning “other, another, different”) and the existing genus name *Planocera* Blainville, 1828, indicating the close taxonomic proximity of the new genus to the existing genus.

Heteroplanocera katoi sp. nov.

(Figures 1–4)

urn:lsid:zoobank.org:act:F26761F2-9BE9-48F2-A4AD-37914FE11872

ETYMOLOGY

The specific name is a noun in the genitive case honoring Dr. Kojiro Kato (1906–1981), who studied Japanese polyclads.

TYPE MATERIAL

Holotype: ICHUM 6081, one-third of body from anterior end preserved in 70% ethanol and sagittal sections of remaining body, 22 slides; off the coast of the Izu Peninsula, Shizuoka, Japan (34°39′11″N 138°43′12″E to 34°39′15″N 138°43′21″E; 245 m depth); coll. Y. Oya, 12 December 2018.

Paratype: ICHUM 6082, one-third of body from anterior end preserved in 70% ethanol and sagittal sections of remaining body, 20 slides; hereafter, same data as holotype.

COMPARATIVE MATERIAL EXAMINED

Paraplanocera oligoglena (Schmarda, 1859). Non-type: ICHUM 6083, sagittal sections of 20 slides; Bonomisaki, Kagoshima, Japan (31°15′15″N, 130°12′54″E; subtidal); coll.

Y. Oya, 26 July 2018.

Planocera reticulata (Stimpson, 1855). Non-type: ICHUM 6018, sagittal sections of 16 slides; hereafter, same data as ICHUM 6083.

DESCRIPTION

Live specimens 18–21 mm (18 mm in holotype) in length, 12–14 mm (12 mm in holotype) in maximum width. Body oval (Figure 1A, B). Ground body color translucent to opaque whitish (Figure 1A). Pair of nuchal tentacles located about three-tenths of body length (5.4–5.9 mm; 5.4 mm in holotype) from anterior end (Figure 1A). Tentacular eye clusters congregated in bases of each tentacle and containing 10–14 eyespots (11 in right cluster, 14 in left cluster in holotype; Figure 1C). Pair of cerebral eye clusters, each consisting of 4–6 eyespots (4 in right cluster, 5 in left cluster in holotype; Figure 1C), arranged near median line and congregated anterior to tentacular eyespots. Pharynx whitish, ruffled in shape, occupying about two-ninths of body length (4.1–4.9 mm in length; 4.1 mm in holotype), located at center of body (Figure 1B). Intestine not anastomosed, spreading throughout body except margin. Mouth opening at near center of pharynx. Ovary distributed around pharynx. Common gonopore opening at about three-tenths (5.1–6.6 mm; 5.1 mm in holotype) from posterior end of body (Figure 1D).

Male copulatory apparatus located posterior to pharynx, consisting of pair of spermiducal bulbs, free prostatic vesicle, cirrus, and pair of accessory organs; seminal vesicle lacking (Figures 2 and 3A–F). Sperm ducts running anteriorly, then turning medially at point posterior to pharynx, forming spermiducal bulbs (Figures 1D and 3C). Distal end of spermiducal bulbs connecting to distal section of sperm duct (Figure 3D), latter running anteriorly (Figure 3E), then forming common sperm duct (Figure 3F). Common sperm duct entering middle section of ejaculatory duct from ventral side. Ejaculatory duct lined with smooth, prostatic-like glandular epithelium (Figure 3A, F). Prostatic vesicle elongated, oval-shaped, possessing tubular folded epithelium, connecting proximal end of ejaculatory duct (Figures 2 and 3A, E). Prostatic vesicle and ejaculatory duct coated with thick muscular wall (Figure 3A, F). Distal end of ejaculatory duct opening to cirrus cavity. Inner epithelium of cirrus possessing three types of teeth. Teeth in proximal section of cirrus hook-shaped, large, becoming smaller and denser in distal part (Figure 4A); base of tooth grooved (Figure 4B), becoming smooth toward tip. Teeth in middle section of cirrus same shape and size as small ones in proximal section, densely distributed (Figure 4C). Teeth in distal section of cirrus curved hook-shaped, small, sparsely distributed (Figure 4D). Cirrus, prostatic vesicle, and ejaculatory duct enclosed by muscular bulb. Distal section of cirrus connecting to

male atrium, latter opening at common gonopore (Figure 3A). Ventral surface of male atrium smooth, lined with ciliated epithelium. Proximal dorsal surface of male atrium folded, lined with ciliated epithelium (Figure 3E). Distal dorsal surface of male atrium smooth, lined with cuticularized epithelium, connecting to that of vagina bulbosa (Figure 3A). Pair of accessory pouches, lined with ciliated epithelium, opening to ventral side of male atrium (Figures 3A and 4E). Accessory organs located at end of each accessory pouch, consisting of eosinophilic glands, developed muscle, and several hook-shaped teeth (Figure 4E).

Pair of oviducts forming common oviduct, latter running postero-dorsally to enter vagina. From this point, short Lang's-vesicle duct running postero-ventrally to connect to Lang's vesicle (Figure 4F). Lang's vesicle rudimentary, spherical, lined with epithelium similar to that in Lang's-vesicle duct (Figure 4F). Vagina curving antero-ventrally, running posteriorly, connecting to vagina bulbosa (Figure 3A); vagina lined with smooth, ciliated epithelium, coated with thick muscular wall. Proximal section of vagina surrounded by cement glands. Vagina bulbosa with cuticularized epithelium possessing several spines, surrounded by developed muscular wall, exiting common gonopore (Figures 3A and 4G). Bursa copulatrix absent. Mass of sperm-like structure observed in atrium of vagina bulbosa and male atrium (Figure 3A).

HABITAT

Judging from the nature of the dredged material, the species' habitat is likely to be mud-type sediment.

COI SEQUENCE

The partial COI sequences (712 bp) of the two specimens (LC545561 and LC545562) were almost identical. The uncorrected *p*-distance between the specimens was 0.004.

MOLECULAR PHYLOGENY

The BI and ML trees were identical in their topology; only the BI tree is shown (Figure 5). Within the Planoceridae clade, *Heteroplanocea katoi* gen. et sp. nov. was a sister to the *Planocera* clade with high support (0.99/91).

DISCUSSION

GENUS-LEVEL IDENTIFICATION

Among the seven existing genera in Planoceridae *sensu* Faubel (1983), *Heteroplanocera* is morphologically similar to *Paraplanocera* Laidlaw, 1903 and *Planocera* Blainville, 1828. It shares the following traits with these two genera: a (*i*) pair of nuchal tentacles, (*ii*) muscular bulb surrounding the cirrus, (*iii*) free prostatic vesicle, and (*iv*) Lang's vesicle (Figure 6A, C; Table 2). In addition, *Heteroplanocera* resembles *Paraplanocera* as it possesses (*i*) a pair of spermiducal bulbs instead of a seminal vesicle and (*ii*) a pair of accessory organs on the ventral side of the male atrium (Figure 6B). However, *Heteroplanocera* also shows resemblance to *Planocera* in that it has a rudimentary

Lang's vesicle and a developed vagina bulbosa with a cuticularized epithelium (Figure 6D).

Heteroplanocera differs from *Paraplanocera* as its accessory organ has several teeth, whereas that of *Paraplanocera* is glandular and unarmed (Figure 6B). In addition, unlike *Paraplanocera, Heteroplanocera* lacks a bursa copulatrix and developed Lang's vesicle. *Heteroplanocera* is distinguished from *Planocera* as it lacks a seminal vesicle and has the accessory organs in the male genital complex (Figure 6C, D). Moreover, among Planoceridae, a common gonopore is observed only in *Heteroplanocera* (Figure 3A). Furthermore, *Heteroplanocera* is phylogenetically separated from *Paraplanocera* and *Planocera* (Figure 5). Thus, establishing *Heteroplanocera* as a new genus is justified.

CUTICULARIZED STRUCTURE IN VAGINA BULBOSA

The cuticularized epithelium of the vagina bulbosa in *H. katoi* is likely a result of co-evolution between the male and female copulatory apparatuses. In some insects with armed male copulatory apparatuses, the inner surface of the female tract tends be thickened or sclerotized (e.g., Rönn *et al*. 2007; Kamimura 2012). This "hardening" of the copulatory apparatuses in both sexes is one of the trends observed in male-female co-evolution related to traumatic mating (Lange *et al*. 2013). Polyclad flatworms employ three methods for sperm transfer: direct copulation, dermal impregnation, and hypodermic insemination (Rawlinson *et al*. 2008). Lang (1884, p. 307) presumed that polyclads possessing hard structures (penis stylet or teeth) in the intromittent organ and a vagina bulbosa ("bursa copulatrix" in Lang (1884)) in the female copulatory apparatus employed the direct copulation. Although the copulatory behavior of *H. katoi* has not

yet been observed, we speculate that the present species also perform a direct copulation, with the cuticle on the vagina bulbosa acting as protection against the numerous teeth on the male genitalia.

Significantly, our phylogenetic analysis showed that the spines on the vagina bulbosa evolved at least twice within the *Heteroplanocera* + *Planocera* clade. Among the species we included in our analyses, only *Planocera multitentaculata* Kato, 1944, other than *H. katoi,* has distinct spines on the vagina bulbosa; whether these are also present in the *Planocera* sp. identified by Litvaitis *et al*. (2019) is unknown. We postulate that the vagina bulbosa was lined with cuticularized tissue in the last common ancestor of the *Heteroplanocera* + *Planocera* clade, and then the spines evolved independently in each lineage of *Heteroplanocera* and *P. multitentaculata* (Figure 7). Among the *Planocera* species not included in the present analysis, similar spines have also been described in *P. gilchristi* Jacubowa, 1906 and *P. uncinata* Palombi, 1939 (Jacubowa 1907; Palombi 1939). Including these species in future studies should fully elucidate the evolution of these "female spines" among planocerids.

ACCESSORY ORGANS

The accessory organs and their surrounding structure in *H. katoi* suggest that the organs are everted along with the cirrus during the course of mating. These organs possess hook-shaped teeth and a developed muscular part (Figure 4E). The muscle likely supports the eversion of the organs and the teeth likely assist in holding the mating partner during copulation; perhaps the teeth also injure the female tract. In future research, behavioral observation will improve our understanding of the function of these organs, as is also the case with the cuticularized tissue in the vagina bulbosa.

The homology of the accessory organ in Planoceridae is unclear. The components of the organ differ between *Heteroplanocera* and *Paraplanocera* (Figures 4E and 6B; see above). Planocerids in the genus *Neoplanocera* Yeri & Kaburaki, 1918 have a structure similar to the accessory organ. In *Neoplanocera*, this structure has a developed muscle similar to that in the accessory organ of *H. katoi*; however, it is papilla-like in shape and does not exist in a pair (Yeri & Kaburaki 1918, text-figure 18; Kato 1937, text-figure 14, plate 14, figure 8). Given that the phylogenetic position of *Neoplanocera* has yet to be tested by molecular analyses, it is possible that this genus is not even encompassed in Planoceridae because its external morphology (e.g., body shape and the formation of eye clusters) is more similar to that of leptoplanoids than that of planocerids (Yeri & Kaburaki 1918, text-figure 17, plate 2, figure 4; Kato 1937, text-figures 11 and 12). In order to evaluate the homology of the accessory organs among planocerids, it will be necessary in future studies to conduct not only detailed morphological and phylogenetic examinations but also developmental observations.

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REFERENCES

Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19, 716–723.

Bulnes VN (2010) Five new Polycladida (Platyhelminthes: Acotylea) species from the Aegean Sea with remarks on the prostatic structures. *Journal of Natural History* 44, 515–544.

Castresana J (2002) Gblocks, v. 0.91 b. Online version. Available from: http://molevol.cmima.csic.es/castresana/ Gblocks_server.html Accessed online 1 April 2020.

Chernomor O, von Haeseler A and Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* 65, 997–1008.

Dittmann IL, Cuadrado D, Aguado MT, Noreña C and Egger B (2019) Polyclad phylogeny persists to be problematic. *Organisms Diversity and Evolution* 19, 585–608.

Faubel A (1983) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part I. The Acotylea. *Mitteilungen des Hamburgischen Zoologischen Museums und Instituts* 80, 17–121.

Faubel A (1984) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part II. The Cotylea. *Mitteilungen des Hamburgischen Zoologischen Museums und Instituts* 81, 189–259.

Hyman LH (1953) Some polyclad flatworms from the Galapagos Islands. *Allan Hancock Pacific Expeditions* 15, 183–210.

Jacubowa L (1907) A new species of *Planocera* (*P. gilchristi*) from South Africa. *Transactions of the South African Philosophical Society* 17, 145–149.

Kamimura Y (2012) Correlated evolutionary changes in *Drosophila* female genitalia reduce the possible infection risk caused by male copulatory wounding. *Behavioral Ecology and Sociobiology* 66, 1107–1114.

Kato K (1937) Polyclads collected in Idu, Japan. *Japanese Journal of Zoology* 7, 211–232.

Kato K (1944) Polycladida of Japan. *Journal of Sigenkagaku Kenkyusyo* 1, 257–319.

Katoh K and Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and*

Evolution 30, 772–780.

Kumar S, Stecher G and Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33, 1870–1874.

Lanfear R, Calcott B, Ho SY and Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29, 1695–1701.

Lanfear R, Frandsen PB, Wright AM, Senfeld T and Calcott B (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34, 772–773.

Lang A (1884) *Die Polycladen (Seeplanarien) des Golfes von Neapel und der angrenzenden Meeresabschnitte. Eine Monographie.* Leipzig: W. Engelmann.

Lange R, Reinhardt K, Michiels NK and Anthes N (2013) Functions, diversity, and evolution of traumatic mating. *Biological Reviews* 88, 585–601.

Litvaitis MK, Bolaños DM and Quiroga SY (2019) Systematic congruence in Polycladida (Platyhelminthes, Rhabditophora): are DNA and morphology telling the same story? *Zoological Journal of the Linnean Society* 186, 865–

891.

Nguyen LT, Schmidt HA, von Haeseler A and Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32, 268–274.

Oya Y and Kajihara H (2019) A new species of *Phaenoplana* (Platyhelminthes: Polycladida) from the Ogasawara Islands. *Species Diversity* 24, 1–6.

Oya Y and Kajihara H (2020) Molecular phylogenetic analysis of Acotylea (Platyhelminthes: Polycladida). *Zoological Science* 37, 271–279.

Palombi A (1939) Turbellaria del Sud Africa. Policladi di East London. Terzo contributo. *Archivio Zoologico Italiano* 28, 123–149.

Prudhoe S (1985) *A monograph on polyclad Turbellaria*. Oxford: Oxford University Press.

Rawlinson KA, Marcela Bolaños D, Liana MK and Litvaitis MK (2008) Reproduction, development and parental care in two direct-developing flatworms (Platyhelminthes: Polycladida: Acotylea). *Journal of Natural History* 42, 2173–2192.

Ronquist F and Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.

Rönn J, Katvala M and Arnqvist G (2007) Coevolution between harmful male genitalia and female resistance in seed beetles. *Proceedings of the National Academy of Sciences* 104, 10921–10925.

Yeri M and Kaburaki T (1918) Description of some Japanese polyclad Turbellaria. *Journal of the College of Science, Tokyo Imperial University* 39, 1–54.

Figure legends and table captions

Fig. 1. *Heteroplanocera katoi* gen. et sp. nov. (ICHUM 6081, holotype), photographs taken in life (A, B, D) and in preserved state cleared with xylene (C): (A) dorsal view; (B) ventral view; (C) eyespots; (D) sperm ducts and oviducts observed from ventral view. Abbreviations: ce, cerebral eyespot; cgp, common gonopore; o, ovary; ov, oviduct; ph, pharynx; sb, spermiducal bulb; sd, sperm duct; t, tentacle; te, tentacular eyespot. Scale bars: A, B, 5 mm; C, D, 1 mm.

Fig. 2. Schematic diagram of copulatory apparatuses in *Heteroplanocera katoi* gen. et sp. nov. (ICHUM 6081, holotype): Abbreviations: ao, accessory organ; ap, accessory pouch; cc, cirrus cavity; cgp, common gonopore; cov, common oviduct; csd, common sperm duct; ed, ejaculatory duct; is, intermuscular space; ld, Lang's-vesicle duct; lv, Lang's vesicle; ma, male atrium; mb, muscular bulb; pv, prostatic vesicle; v, vagina; vb, vagina bulbosa. Scale bar: 300 μm.

Fig. 3. Photomicrographs of sagittal sections of *Heteroplanocera katoi* gen. et sp. nov. (ICHUM 6081, holotype): (A, B) Male and female copulatory apparatuses; (C–F) running of sperm duct. Abbreviations: ao, accessory organ; ap, accessory pouch; cc, cirrus cavity; cg, cement glands; cgp, common gonopore; cov, common oviduct; csd, common sperm duct; ed, ejaculatory duct; is, intermuscular space; ma, male atrium; mb, muscular bulb; pv, prostatic vesicle; sb, spermiducal bulb; sd, sperm duct; v, vagina; vb, vagina bulbosa. Scale bars: A, 500 μm; B–F, 300 μm.

Fig. 4. Photomicrographs of sagittal sections of *Heteroplanocera katoi* gen. et sp. nov. (ICHUM 6081, holotype): (A, C, D) Teeth in cirrus; (B) cross view of large tooth; (E) accessory organ; (F) Lang's vesicle; (G) spines of vagina bulbosa. Abbreviations: ao, accessory organ; ap, accessory pouch; cc, cirrus cavity; is, intermuscular space; ld, Lang's-vesicle duct; lv, Lang's vesicle; mb, muscular bulb; s, spine; vb, vagina bulbosa. Scale bars: A, C, D, F, 50 μm; B, 10 μm; E, G, 100 μm.

Fig. 5. Bayesian phylogenetic tree based on sequences from four genes (16S, 18S, and 28S ribosomal DNA; cytochrome *c* oxidase subunit I; concatenated length 3708 bp): Numbers near nodes represent posterior probability and bootstrap values, respectively.

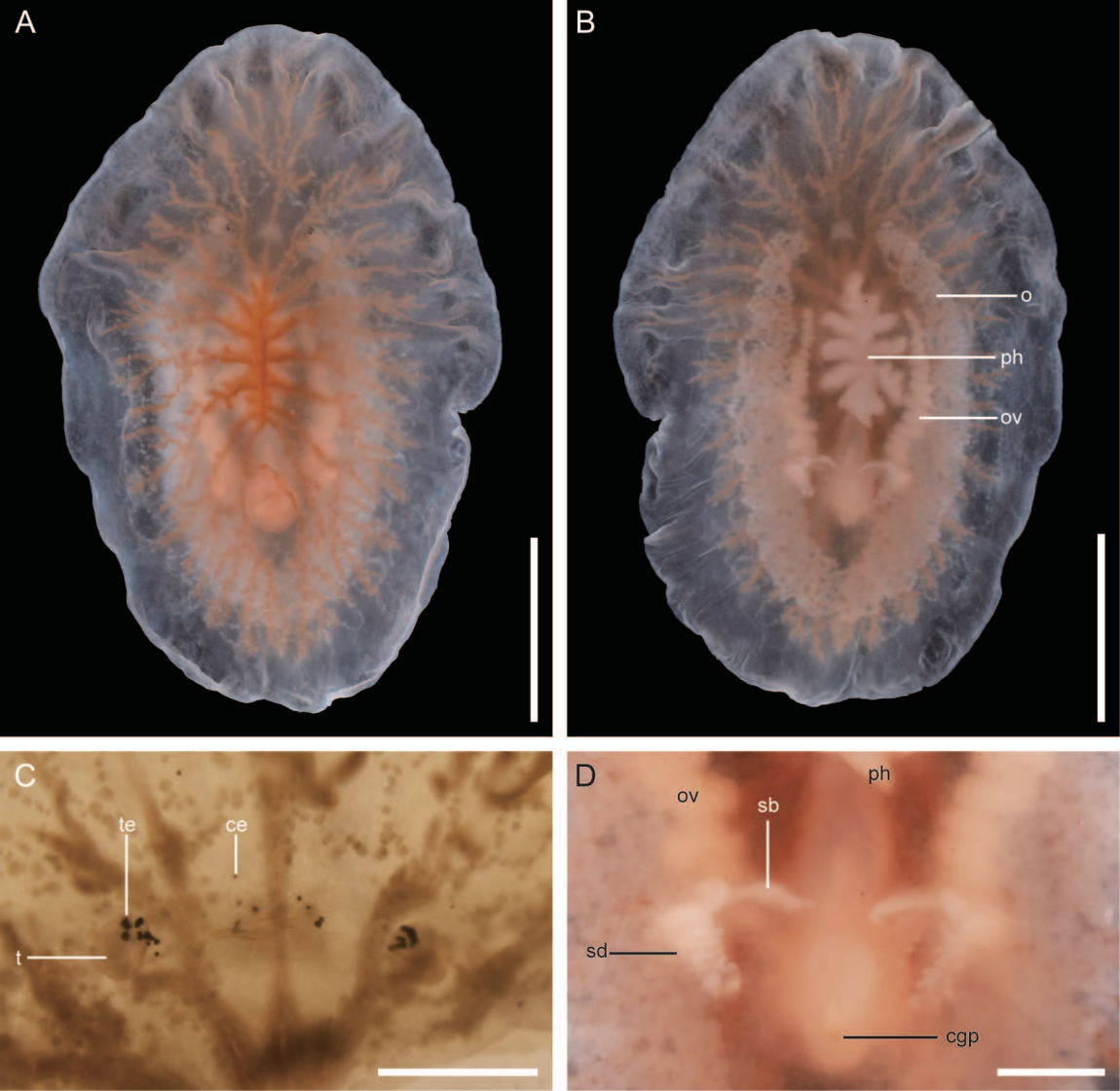
Fig. 6. Photomicrographs of sagittal sections of *Paraplanocera oligoglena* (Schmarda, 1859) (A, B; ICHUM 6083) and *Planocera reticulata* (Stimpson, 1855) (C, D; ICHUM 6018): (A, C) copulatory apparatuses; (B) accessory organ; (D) cuticle of vagina bulbosa. Abbreviations: ao, accessory organ; cc, cirrus cavity; cg, cement glands; ed,

ejaculatory duct; is, intermuscular space; lv, Lang's vesicle; mb, muscular bulb; pv, prostatic vesicle; sv, seminal vesicle; v, vagina; vb, vagina bulbosa. Scale bars: A, C, 500 μm; B, D, 100 μm.

Fig. 7. Hypothesis for the evolution of spines in the vagina bulbosa based on the tree topology obtained from the molecular phylogenetic analysis in the present study.

Table 1. List of species included in the molecular phylogenetic analysis and the respective GenBank accession numbers.

Table 2. Comparison of morphological characteristics between genera in Planoceridae *sensu* Faubel (1983).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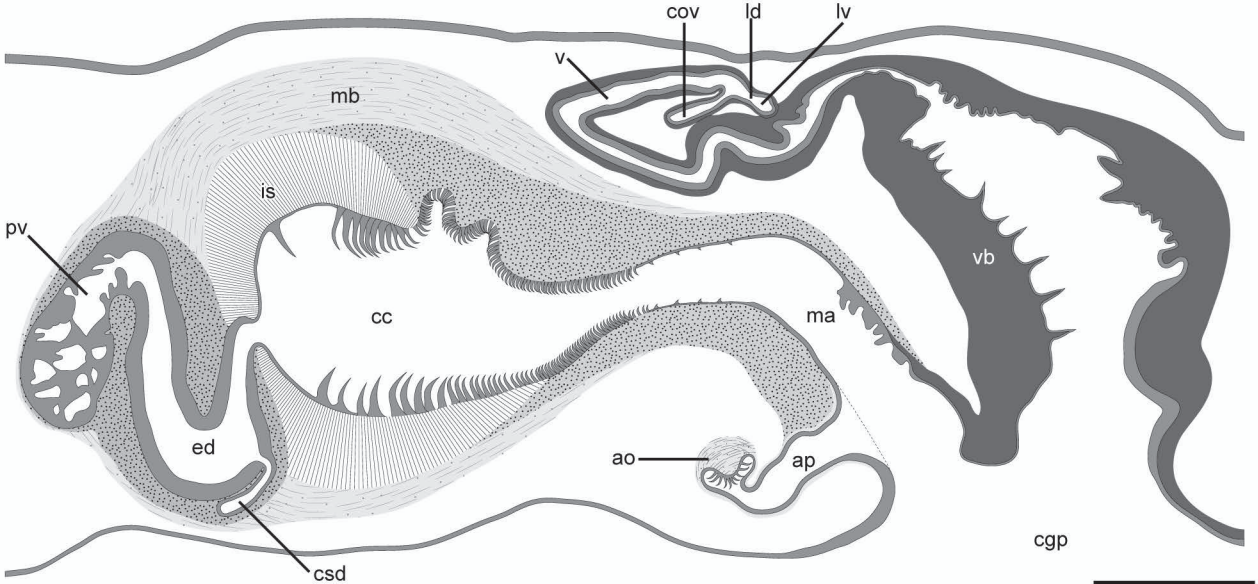
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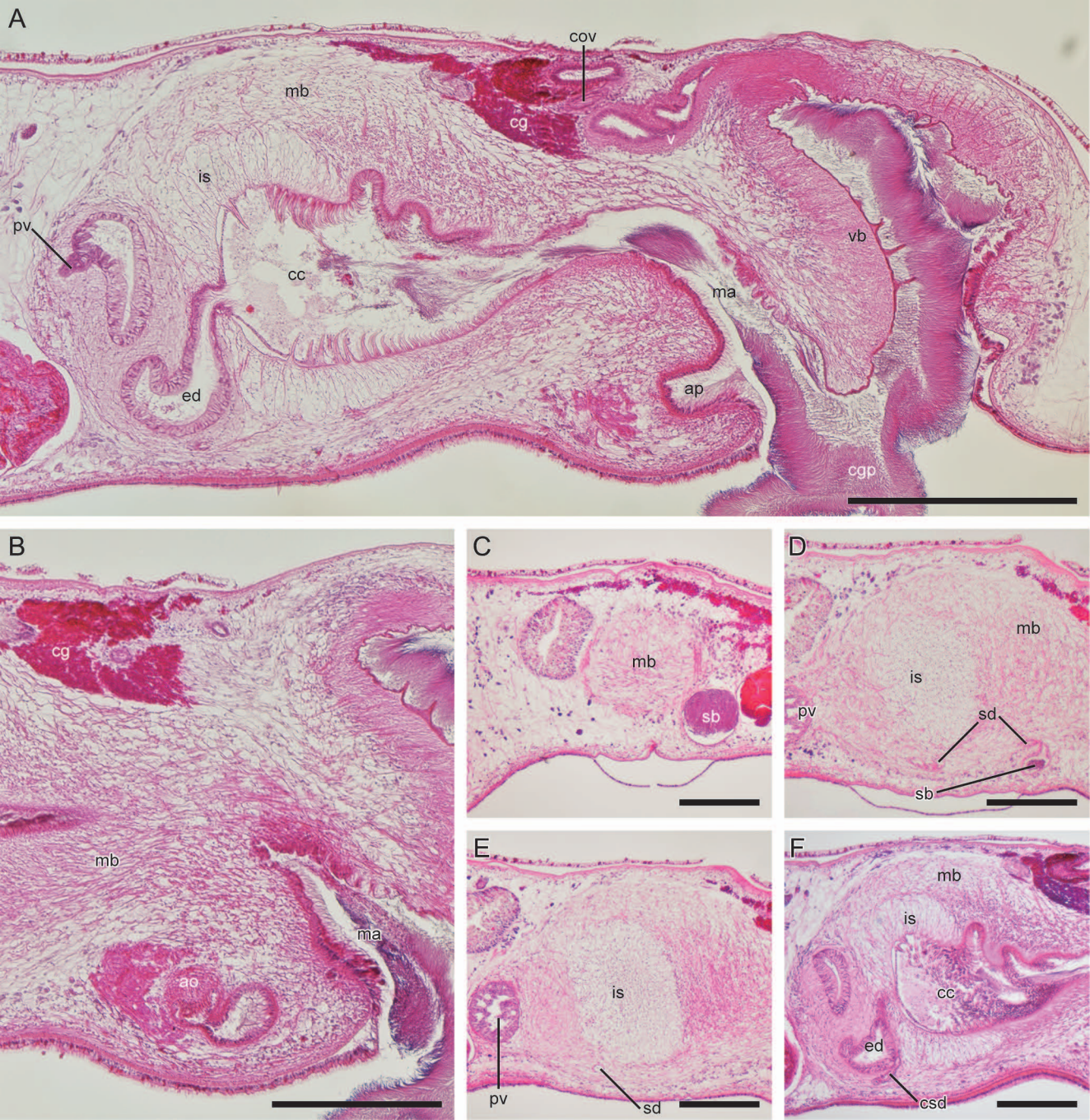
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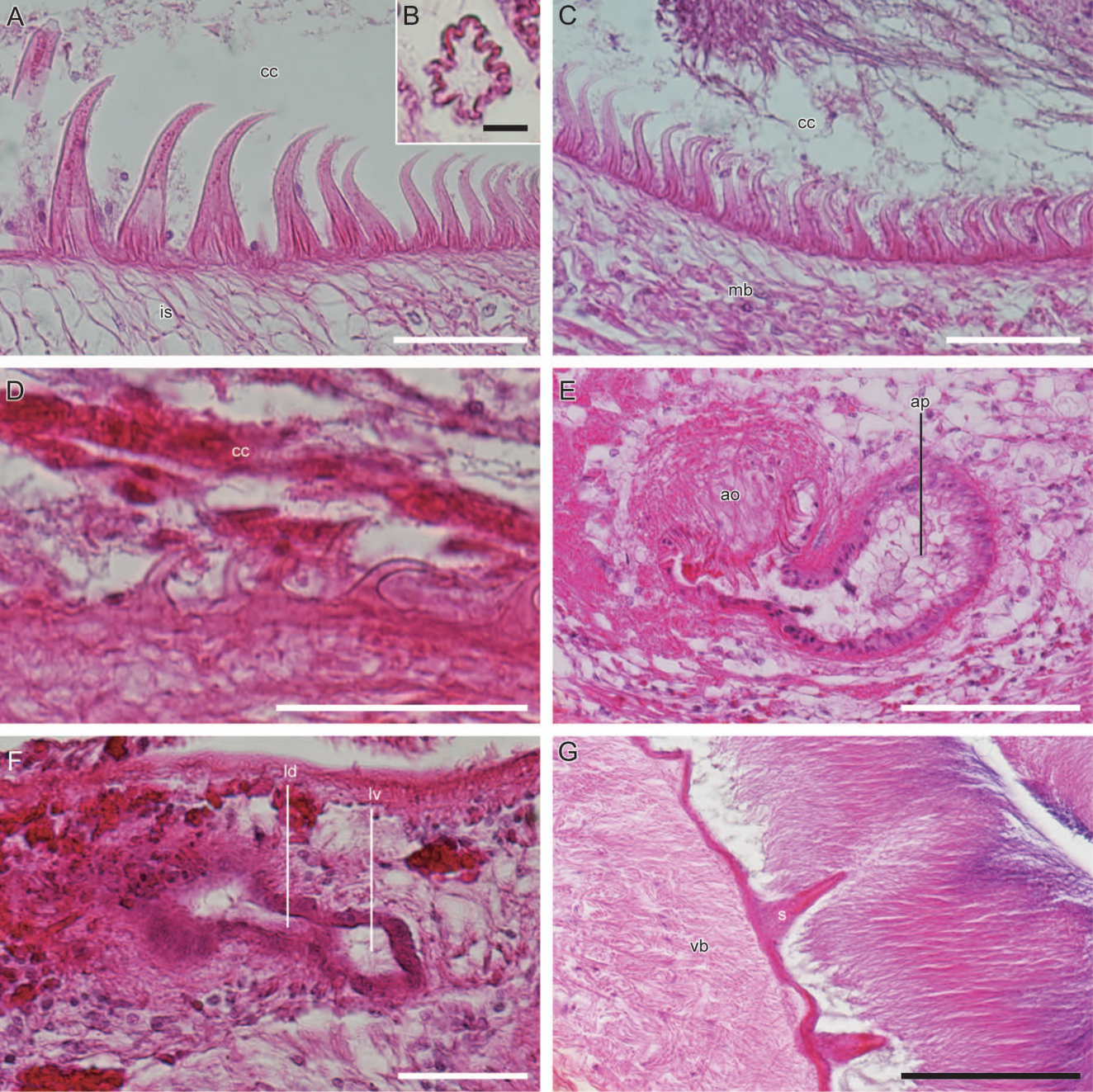
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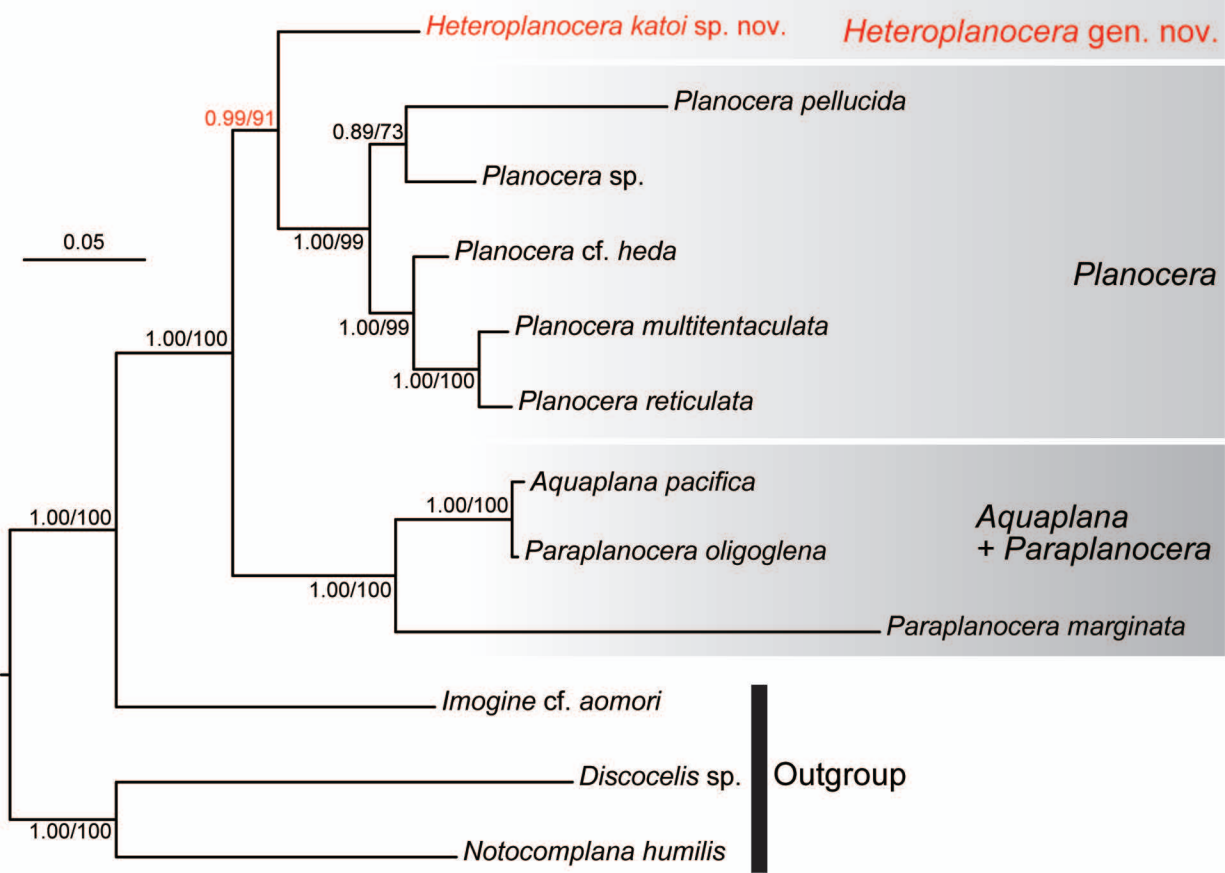
Heteroplanocera katoi sp. nov.
Heteroplanocera gen. nov.
Planocera pellucida
Planocera sp.
Planocera cf. heda
Planocera multitentaculata
Planocera reticulata
Planocera
Aquaplana pacifica
Paraplanocera oligoglena
Paraplanocera marginata
Aquaplana + Paraplanocera
Imogine cf. aomori
Discocelis sp.
Notocomplana humilis
Outgroup
0.99/91
0.89/73
1.00/99
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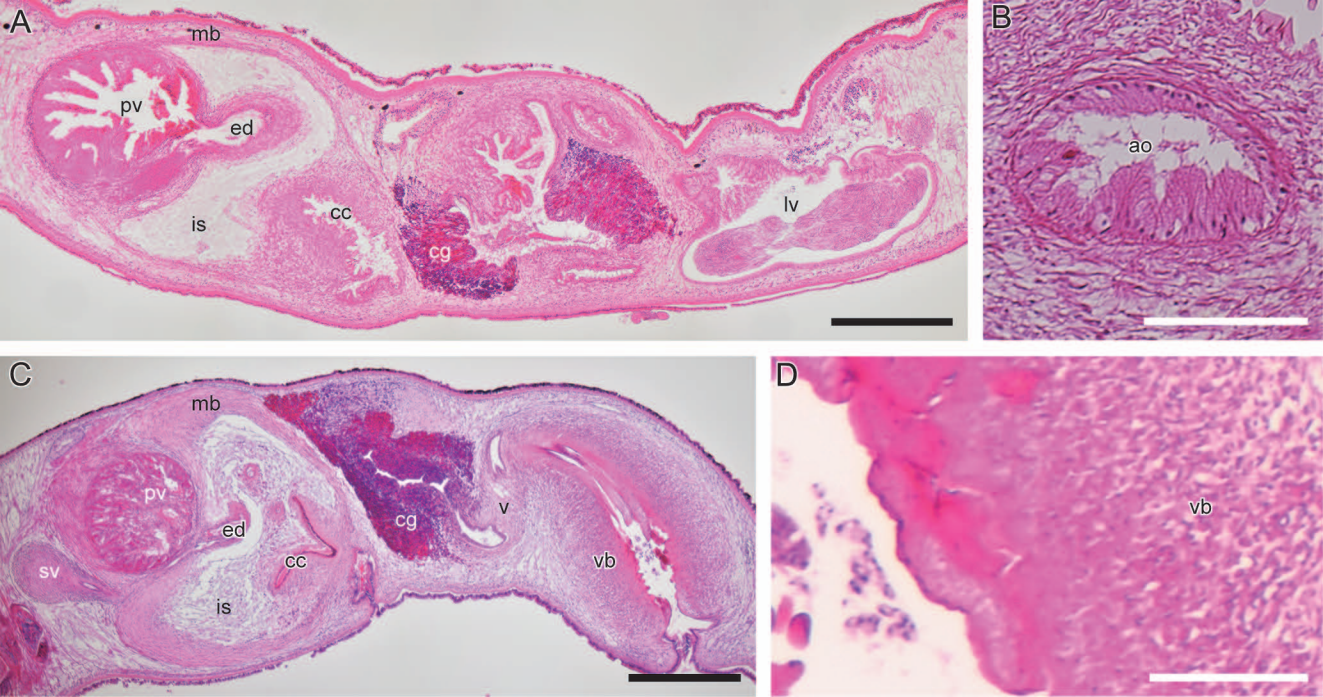
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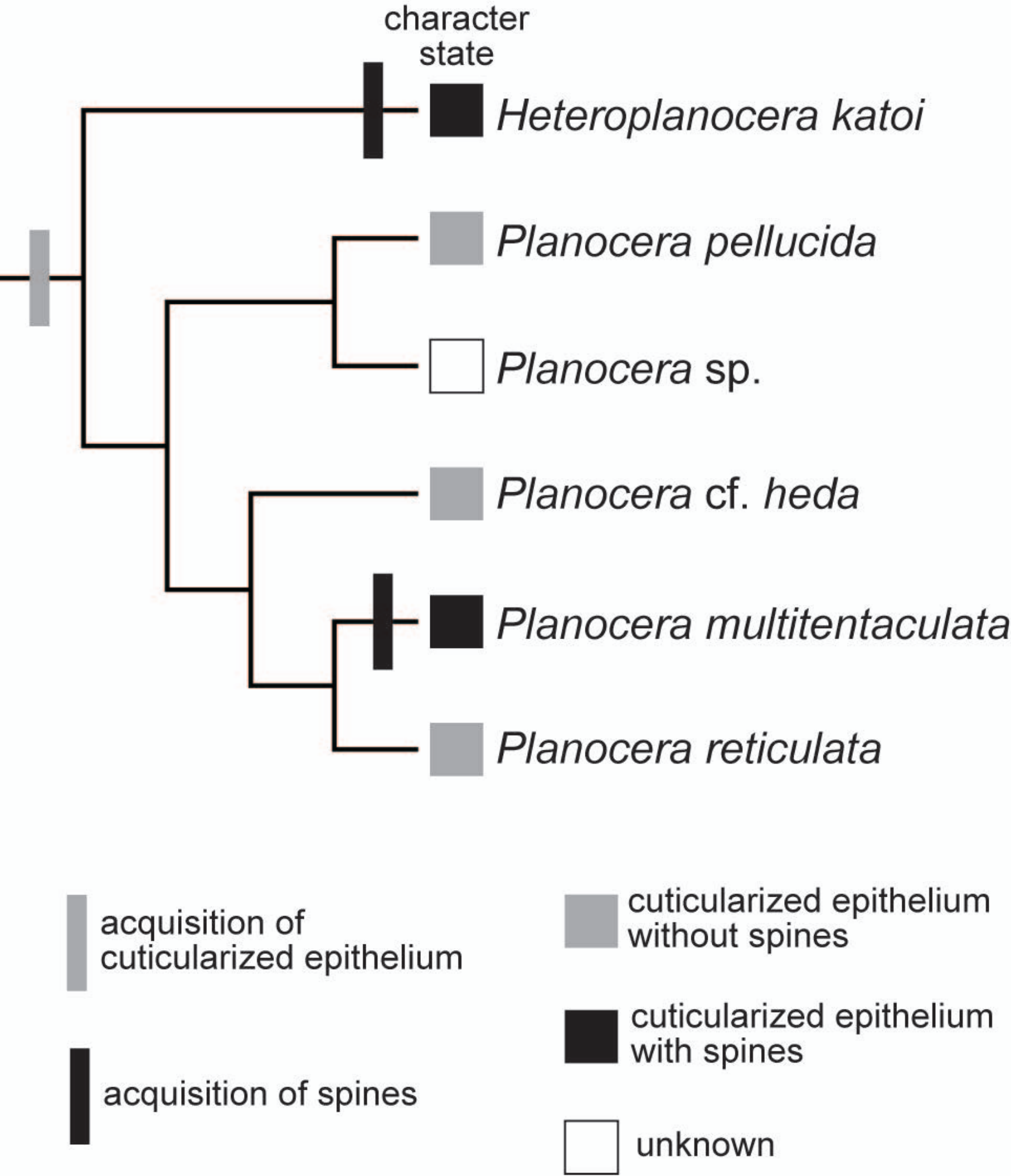
character state
Heteroplanocera katoi
Planocera pellucida
Planocera sp.
Planocera cf. heda
Planocera multitentaculata
Planocera reticulata
acquisition of cuticularized epithelium
acquisition of spines
cuticularized epithelium without spines
cuticularized epithelium with spines
unknown

Table 1. List of species included in the molecular phylogenetic analysis and the respective GenBank accession numbers.

Family	Species	16S	18S	28S	COI	Reference
Planoceridae	*Aquaplana pacifica* Hyman, 1959	—	—	MH700272	—	Litvaitis *et al*. (2019)
	Heteroplanocera katoi gen. et sp. nov.	LC545564	LC545566	LC545568	LC545562	Present study
	Paraplanocera marginata Meyer, 1922	—	—	MH700335	—	Litvaitis *et al*. (2019)
	Paraplanocera oligoglena (Schmarda, 1859)	LC545565	LC545567	LC545569	LC545563	Present study
	Planocera cf. *heda* Kato, 1944	—	—	MH700363	—	Litvaitis *et al*. (2019)
	Planocera multitentaculata Kato, 1944	LC508174	LC508150	LC508127	LC508192	Oya & Kajihara (in press)
	Planocera pellucida (Mertens, 1833)	—	MN334203	MN384696	—	Dittmann *et al*. (2019)
	Planocera reticulata (Stimpson, 1855)	LC508190	LC508172	LC508148	LC508208	Oya & Kajihara (in press)
	Planocera sp.	—	—	MH700364	—	Litvaitis *et al*. (2019)
Discocelidae	*Discocelis* sp.	LC508189	LC508170	LC508146	LC508206	Oya & Kajihara (in press)
Notocomplanidae	*Notocomplana humilis* (Stimpson, 1857)	LC508187	LC508168	LC508144	LC508204	Oya & Kajihara (in press)
Stylochidae	*Imogine* cf. *aomori* (Kato, 1937)	LC508182	LC508163	LC508140	LC508200	Oya & Kajihara (in press)

Table 2. Comparison of morphological characteristics between genera in Planoceridae *sensu* Faubel (1983).

	[1]*Aquaplana* Hyman, 1953 *sensu* Faubel (1983)	*Disparoplana* Laidlaw, 1903	*Heteroplanocera* gen. nov.	[2]*Neoplanocera* Yeri & Kaburaki, 1918	*Paraplanocera* Laidlaw, 1903	*Planocera* Blainville, 1828	*Pseudoplanocera* Bulnes, 2010	[2]*Spinicirrus* Hyman, 1953
Nuchal tentacles	present	absent	present	absent	present	present	absent	absent
Muscular bulb enclosing cirrus	present	absent	present	absent	present	present	present	present
Prostatic vesicle	present	present	present	present	present	present	present	two
Seminal vesicle/spermiducal bulbs	spermiducal bulbs	seminal vesicle	spermiducal bulbs	seminal vesicle	spermiducal bulbs	seminal vesicle	seminal vesicle	absent
Accessory organ	absent	absent	present	present	present	absent	absent	absent
Bursa copulatrix	absent	absent	absent	absent	present	absent	absent	present
Lang's vesicle	present	present	present	absent	present	present	present	absent

[1]*Aquaplana* should be treated as an invalid taxon because Faubel (1983) has transferred *Aquaplana oceanica* Hyman, 1953, the type species of *Aquaplana*, to *Paraplanocera*. It is therefore included in this table for comparison purposes only.

[2]Prudhoe (1985) classified these genera into Gnesiocerotidae Marcus & Marcus, 1966.