

A New Species of *Zygantrioides* (Platyhelminthes: Polycladida) from Amakusa, Japan

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We describe a new species of ilyplanid polyclad, *Zygantrioides serpulidicola* sp. nov., from Amakusa, Kumamoto, Japan. This is the third member of *Zygantrioides* Faubel, 1983 and the first record of the genus from the Pacific Ocean. *Zygantrioides serpulidicola* sp. nov. is characterized by *i*) the mouth opening near the common gonopore, *ii*) the sperm ducts separately entering a pear-shaped seminal vesicle, *iii*) an elongated Lang's-vesicle duct, and *iv*) the horseshoe-shaped Lang's vesicle located posterior to the common gonopore. We provide a partial sequence of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene (712 bp) as a DNA barcode for the species. Our phylogenetic analyses based on concatenated sequences of the 16S, 18S, and 28S ribosomal DNA and COI indicate that *Z. serpulidicola* sp. nov. is nested in a clade comprised of Discocolidae and Ilyplanidae; the latter does not appear to be monophyletic.

Key Words: Acotylea, Discocoloidea, DNA barcoding, marine flatworm.

Introduction

The acotylean polyclad genus *Zygantrioides* Faubel, 1983 was established as a member of Ilyplanidae and can be distinguished from other ilyplanids by possessing a penis papilla, seminal vesicle, and common genital atrium (Faubel 1983). Currently, two species are recognized in this genus, *viz.*, *Zygantrioides henriettae* (Corrêa, 1949) and *Zygantrioides plesia* (Corrêa, 1949). Both are only known in Brazilian waters and were originally placed in *Zygantriplana* Laidlaw, 1906 (Corrêa 1949). Prudhoe (1985) left the two species in *Zygantriplana*; however, recent researchers have followed Faubel's (1983) system (Bahia and Schrödl 2018; Beveridge 2018).

Faubel's (1983) familial assignment of *Zygantrioides* to Ilyplanidae has not been tested by any molecular analyses. Meanwhile, Prudhoe (1985) classified *Zygantriplana* in Leptoplanidae. So far, no members of *Zygantrioides* and *Zygantriplana* have been included in molecular phylogenetic studies (*cf.* Aguado *et al.* 2017; Bahia *et al.* 2017; Tsunashima *et al.* 2017; Dittmann *et al.* 2019; Litvaitis *et al.* 2019).

In this study, we describe a new species of *Zygantrioides* as the first representative of the genus from the Pacific Ocean, providing a partial sequence of the cytochrome *c* oxidase subunit I (COI) gene as a DNA barcode for the species. In addition, we also determine partial sequences of the 16S, 18S, and 28S ribosomal DNA (rDNA) and estimate the phylogenetic position of the new species among other acotylean polyclads by molecular analyses using the four gene markers.

Materials and Methods

Worms were collected from the surfaces of serpulid annelid tubes spread over undersurfaces of stones in the intertidal zone on Tsujishima Island, Kumamoto, Japan. Specimens were photographed with a Nikon D5300 digital camera with external strobe lighting provided by a pair of Morris Hikaru Komachi Di flash units. For DNA extraction, a piece of the body margin (lateral to ocelli) 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 and preserved in 70% ethanol; it was sagittally sectioned at 7 µm thickness and stained with hematoxylin and eosin following a protocol by Oya and Kajihara (2019). Type slides 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). As a reference for DNA barcoding, a partial sequence at the 5' end of the COI gene (712 bp) was determined from four specimens using the primer pair Acotylea_COI_F and Acotylea_COI_R (Oya and Kajihara 2017) and following a protocol by Oya and Kajihara (2017); genetic distances (uncorrected *p*-distance) were calculated using MEGA ver. 7.0 (Kumar *et al.* 2016). Two gene markers, 16S rDNA (16S) (438 bp) and 18S rDNA (18S) (1,736 bp), were sequenced from one specimen by using the primer pairs 16SarL and 16SbrH (Palumbi 1991) for 16S and hrms18S_F and hrms18S_R (Oya and Kajihara 2020) for 18S. A fragment of the 28S rDNA (28S) (*ca.* 1,300 bp) was amplified with primers LSU5 (Littlewood 1994) and rd5b

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

Superfamily/family	Species	16S	18S	28S	COI
Discoceloidea					
Cryptocelidae	<i>Amemiyaia pacifica</i> Kato, 1944	LC508185	LC508166	LC508143	LC508203
Cryptocelidae	<i>Phaenocelis purpurea</i> (Schmarda, 1859)	—	—	MH700346	—
Cryptocelidae	<i>Phaenocelis medvedica</i> Marcus, 1952	—	—	KY263706	—
Discocelidae	<i>Adenoplana evelinae</i> Marcus, 1950	—	—	MH700268	—
Discocelidae	<i>Discocelis tigrina</i> (Blanchard, 1847)	—	MN334200	MN384690	—
Discocelidae	<i>Discocelis</i> sp.	LC508189	LC508170	LC508146	LC508206
Ilyplanidae	<i>Discoplana gigas</i> (Schmarda, 1859)	—	LC508162	LC508139	LC508199
Ilyplanidae	<i>Zygantroides serpulidicola</i> sp. nov.	LC528164	LC528165	LC528166	LC528160
Leptoplanoidea					
Gnesiocerotidae	<i>Echinoplana celerrima</i> Haswell, 1907	—	MN421936	MN421930	—
Gnesiocerotidae	<i>Gnesioceros sargassicola</i> (Mertens, 1833)	—	—	MH700309	—
Gnesiocerotidae	<i>Styloplanocera fasciata</i> (Schmarda, 1859)	—	—	MH700408	—
Leptoplanidae	<i>Leptoplana tremellaris</i> (Müller, 1773)	—	MN421937	MN421931	—
Notocomplanidae	<i>Notocomplana humilis</i> (Stimpson, 1857)	LC508187	LC508168	LC508144	LC508204
Notocomplanidae	<i>Notocomplana koreana</i> (Kato, 1937)	LC176048	LC508151	LC508128	LC176014
Notoplanidae	<i>Amyris hummelincki</i> Du Bois-Reymond Marcus and Marcus, 1968	—	—	MH700269	—
Notoplanidae	<i>Notoplana australis</i> (Schmarda, 1859)	—	AJ228786	AY157153	—
Notoplanidae	<i>Notoplana atomata</i> (Müller, 1776)	—	—	MH700329	—
Notoplanidae	<i>Notoplana delicata</i> Yeri and Kaburaki, 1918	LC508188	LC508169	LC508145	LC508205
Pseudostylochidae	<i>Pseudostylochus obscurus</i> (Stimpson, 1857)	LC508180	LC508160	LC508137	LC508197
Pseudostylochidae	<i>Pseudostylochus takeshitai</i> Yeri and Kaburaki, 1918	LC508184	LC508165	LC508142	LC508202
Stylochoplanidae	<i>Armatoplana divae</i> (Marcus, 1947)	—	—	MH700273	—
Stylochoplanidae	<i>Armatoplana leptalea</i> (Marcus, 1947)	—	—	MH700275	—
Stylochoplanidae	<i>Comoplana agilis</i> (Lang, 1884)	—	MN334199	MN384685	—
Stylochoplanidae	<i>Comoplana pusilla</i> (Bock, 1924)	LC508177	LC508157	LC508134	LC508194
Stylochoplanidae	<i>Phaenoplana kopepe</i> Oya and Kajihara, 2019	LC508176	LC508156	LC508133	LC369778
Stylochoidea					
Callioplanidae	<i>Callioplana marginata</i> Stimpson, 1857	LC508179	LC508159	LC508136	LC508196
Hoploplanidae	<i>Hoploplana californica</i> Hyman, 1953	KC869753	KC869797	KC869850	—
Hoploplanidae	<i>Hoploplana ornata</i> Yeri and Kaburaki, 1918	LC508178	LC508158	LC508135	LC508195
Idioplanidae	<i>Idioplana atlantica</i> (Bock, 1913)	—	—	MH700310	—
Idioplanidae	<i>Idioplana australiensis</i> Woodworth, 1898	—	—	HQ659008	—
Latocestidae	<i>Latocestus</i> sp.	—	—	MH700317	—
Planoceridae	<i>Aquaplana pacifica</i> Hyman, 1959	—	—	MH700272	—
Planoceridae	<i>Paraplanocera marginata</i> Meyer, 1922	—	—	MH700335	—
Planoceridae	<i>Planocera pellucida</i> (Mertens, 1833)	—	MN334203	MN384696	—
Planoceridae	<i>Planocera reticulata</i> (Stimpson, 1855)	LC508190	LC508172	LC508148	LC508208

Table 1. Continued.

Superfamily/family	Species	16S	18S	28S	COI
Plehniiidae	<i>Paraplehnia pacifica</i> (Kato, 1939)	LC508175	LC508155	LC508132	LC508193
Plehniiidae	<i>Paraplehnia seisuiiae</i> Oya, Kimura, and Kajihara, 2019	LC508186	LC508167	LC467000	LC466999
Stylochidae	<i>Imogine stellae</i> Marquina, Osa, Rodríguez, Fernández-Despiau, and Noreña, 2014	—	MN334201	MN384692	—
Stylochidae	<i>Imogine</i> cf. <i>aomori</i> (Kato, 1937)	LC508182	LC508163	LC508140	LC508200
Stylochidae	<i>Leptostylochus</i> cf. <i>gracilis</i> Kato, 1934	LC508181	LC508161	LC508138	LC508198
Stylochidae	<i>Mirostylochus akkeshiensis</i> Kato, 1937	LC508191	LC508173	LC508149	LC508209
Stylochidae	<i>Stylochus ellipticus</i> (Girard, 1850)	—	Suppl. File 1 in Dittmann <i>et al.</i> (2019)	Suppl. File 1 in Dittmann <i>et al.</i> (2019)	—
Outgroup					
Cestoplanidae	<i>Cestoplana rubrocincta</i> (Grube, 1840)	—	MN334198	MN384689	—
Pericelidae	<i>Pericelis tectivorum</i> Dittmann, Dibiasi, Noreña, and Egger, 2019	—	MN334202	MK181524	—

(Schwendinger and Giribet 2005). Polymerase chain reaction (PCR) amplification conditions for 28S were 94°C for 1 min; 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1.5 min; and 72°C for 7 min. The partial sequence of 28S (905 bp) was determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 and 3730 Genetic Analyzer (Life Technologies, California, USA); internal primers, hrms28S_F (5'-GAA ACT AAC AAG GAT TCC TCT AGT AAC-3'), hrms28S_Fi1 (5'-TAC GTG AAA CCG CTG AGA GGC AAA C-3'), hrms28S_R (5'-GCT TCG ACC TGC GCA AGC ATA G-3'), and hrms28S_Ri1 (5'-TTG GTC CGT GTT TCA AGA CGG GTC A-3'), were newly designed by using PerlPrimer ver. 1.1.21 (Marshall 2003–2011). Sequences were checked and edited using MEGA ver. 7.0 (Kumar *et al.* 2016). All sequences determined in this study have been deposited in DDBJ/EMBL/GenBank databases with the accession numbers LC528160–LC528166.

Additional sequences from Acotylea were downloaded from GenBank; two cotylean species, *Cestoplana rubrocincta* (Grube, 1840) and *Pericelis tectivorum* Dittmann, Dibiasi, Noreña, and Egger, 2019, were chosen as outgroup taxa (Table 1). Alignment of 16S, 18S, and 28S sequences was done with MAFFT ver. 7.0 (Katoh and Standley 2013), using the “unalignlevel: 0.8” and “Leave gappy regions” options under the G-INS-i strategy. Ambiguous sites in the aligned 16S, 18S, and 28S dataset were removed with Gblocks ver. 0.91b (Castresana 2002) using the “Allow smaller final blocks” option. Alignment of COI was done manually with MEGA ver. 7.0 (Kumar *et al.* 2016). The concatenated dataset from the four genes was 3496 bp long and contained 46 terminal taxa.

Phylogenetic analyses were performed with the maximum-likelihood (ML) method executed in IQtree ver. 1.6 (Nguyen *et al.* 2015) under a partition model (Chernomor *et al.* 2016), and with Bayesian inference (BI) executed in MrBayes ver. 3.2.2 (Ronquist and Huelsenbeck 2003). The optimal substitution models for the ML analysis selected



Fig. 1. Photograph of a living individual of *Zygantrioides serpulidicola* sp. nov. on tubes of serpulid annelids.

with PartitionFinder ver. 2.1.1 (Lanfear *et al.* 2016) under the Akaike information criterion (AIC) (Akaike 1974) using the greedy algorithm (Lanfear *et al.* 2012) were GTR+I+G (16S, 18S, 28S, third codon position in COI), TRN+I+G (first codon position in COI), and TVM+I+G (second codon position in COI); those for BI were GTR+I+G (16S, 18S, 28S, all codon positions in COI). Nodal support within the ML tree was assessed by analyses of 1,000 bootstrap pseudoreplicates. For BI, the Markov chain Monte Carlo (MCMC) process used random starting trees and involved four chains run for 10,000,000 generations, with the first 25% of trees discarded as burn-in. We considered posterior probability (PP) values ≥ 0.90 and ML bootstrap (BS) values $\geq 70\%$ as indicating clade support; in the text, combined nodal support is indicated as PP/BS.

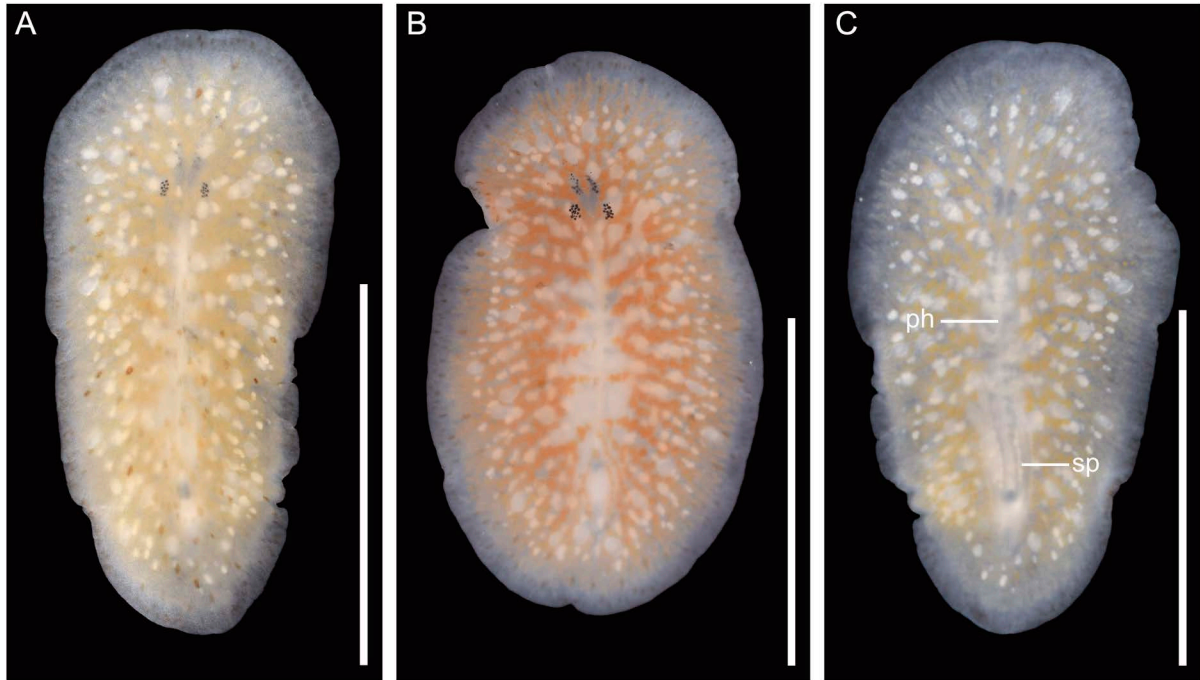


Fig. 2. *Zygantrioides serpulidicola* sp. nov., photographs taken in life. A, Dorsal view, ICHUM 6023 (holotype); B, dorsal view, ICHUM 6025 (paratype); C, ventral view, ICHUM 6023 (holotype). Abbreviations: ph, pharynx; sp, sperm duct. Scale bars: 5 mm (A–C).

Taxonomy

Genus *Zygantrioides* Faubel, 1983

Redefinition. Ilyplanidae without tentacles and marginal eyes. Pharynx somewhat oriented posteriorly. Genital tracts entering separately into common genital atrium; male complex with seminal vesicle and papillate penis. Distal part of ejaculatory duct lined with glandular ciliated epithelium. Lang's vesicle present (see Remarks).

Zygantrioides serpulidicola sp. nov.
(Figs 1–3)

Etymology. The new specific name *serpulidicola* is a noun in apposition and refers to the habit that the flatworms dwell on tubes of serpulid annelids (Fig. 1).

Material examined. Four specimens: holotype, ICHUM 6023, 6 slides, intertidal, (32°33'09"N, 130°06'32"E), Tsujishima Island, Kumamoto, Japan, 6 June 2019, Y. Oya and A. Tsuyuki leg.; paratypes (three specimens, all from the type locality, Y. Oya and A. Tsuyuki leg.); ICHUM 6024, 4 slides, 6 June 2019; ICHUM 6025, 4 slides, 13 June 2018; ICHUM 6026, 5 slides, 13 June 2018.

Description. Live specimens 6.7–8.1 mm (7.9 mm in holotype) in length, 2.7–4.8 mm (3.8 mm in holotype) in maximum width. Body oval, narrow toward posterior end when elongated (Fig. 2A, B). Ground body color translucent to whitish opaque (Fig. 2A–C). Pale brown maculae sparsely scattered on dorsal surface (Fig. 2A, B). General appearance of body whitish to orangish (Fig. 2A, B). Tentacles lacking (Fig. 3). Pair of tentacular eye clusters, each containing

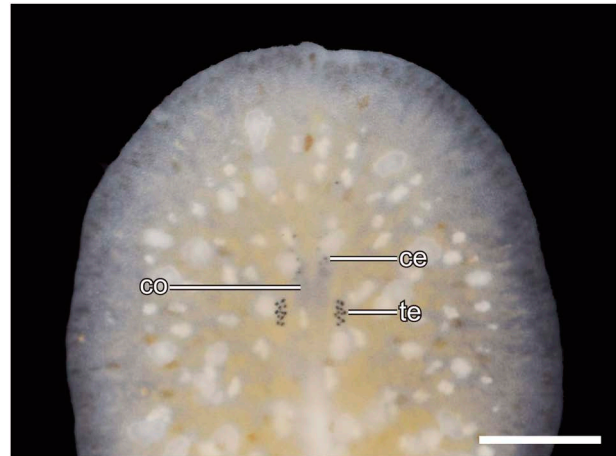


Fig. 3. *Zygantrioides serpulidicola* sp. nov., ICHUM 6023 (holotype), photograph taken in life, showing eyespot distributions. Abbreviations: ce, cerebral eyespot; co, cerebral organ; te, tentacular eyespot. Scale bar: 1 mm.

8–19 eyespots (11 in right cluster, 11 in left cluster in holotype, Fig. 3). Cerebral organ positioned slightly anteriorly to tentacular eye clusters (Fig. 3). Pair of cerebral eye clusters, each consisting of 8–22 eyespots (10 in right cluster, 8 in left cluster in holotype, Fig. 3), arranged near median line and congregated anterior to cerebral organ. Pharynx whitish, ruffled in shape, occupying about three-eighths of body length (2.6–2.9 mm in length, 2.7 mm in holotype), located at center of body (Fig. 2C); ruffles unclear and uncountable. Intestine highly branched and not anastomosed, spreading throughout body except margin and brain region. Pair of whitish sperm ducts visible through ventral body wall (Fig.

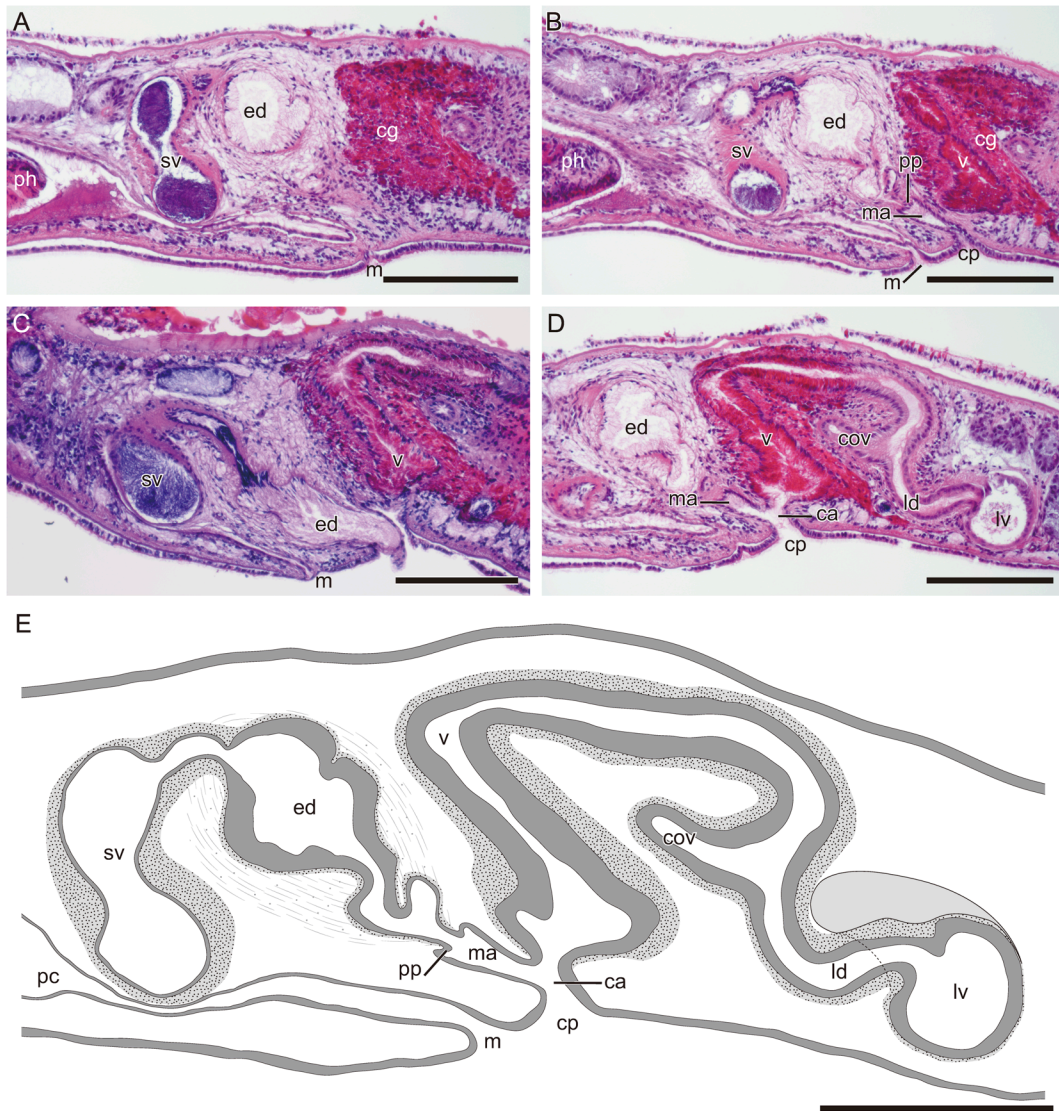


Fig. 4. *Zygantrioides serpulidicola* sp. nov., photomicrographs of sagittal sections (A–D) and schematic diagram (E). A–C, Male copulatory apparatus, ICHUM 6023 (A and B), ICHUM 6025 (C); D, female copulatory apparatus, ICHUM 6023; E, schematic diagram of copulatory complex. Abbreviations: ca, common genital atrium; cg, cement gland; cov, common oviduct; cp, common gonopore; ed, ejaculatory duct; ld, Lang's-vesicle duct; lv, Lang's vesicle; m, mouth; ma, male atrium; pc, pharyngeal cavity; ph, pharynx; pp, penis papilla; sv, seminal vesicle; v, vagina. Scale bars: 200 μ m (A–E).

2C).

Mouth opening at posterior to pharynx and near common gonopore (Fig. 4A–C). Male copulatory apparatus located posteriorly to pharynx, consisting of true seminal vesicle and penis papilla; prostatic vesicle lacking (Fig. 4A–C). Pair of sperm ducts running anteriorly, then turning medially at point of about one-fourth length of pharynx from posterior end, subsequently running posteriorly along both sides of pharynx and extending further posteriorly for short distance before turning medially (Fig. 2C) to enter separately proximal end of pear-shaped seminal vesicle with strong muscular wall (Fig. 4A, C). Distal end of seminal vesicle directly connecting to ejaculatory duct; latter having thin muscular wall lined with prostatic-like glandular epithelium, varying in shape from vesicular to almost cylindrical depending on fixation (Fig. 4B, C). Distal end of ejaculatory duct connecting to penis papilla. Penis papilla small, with-

out stylet, projecting into cylindrical male atrium. Lining epithelium of male atrium smooth.

Pair of oviducts forming common oviduct, latter running posteriorly to enter vagina. From this point, elongated Lang's-vesicle duct, lined with ciliated epithelium, running ventrally, then curving posteriorly to connect to Lang's vesicle (Fig. 4D). Lang's vesicle horseshoe-shaped, with tips orienting anteriorly and reaching to level where Lang's-vesicle duct connects to vagina (Fig. 4E). Inner epithelium of Lang's vesicle similar to that in vagina and Lang's-vesicle duct (Fig. 4D). Vagina curving postero-dorsally, running anteriorly, then recurving postero-ventrally to exit at common genital atrium (Fig. 4D, E); vagina lined with smooth, ciliated epithelium. Lang's-vesicle duct and vagina surrounded by circular muscle fibers; vagina surrounded by cement glands. Common genital atrium opening at posterior to mouth as common gonopore.

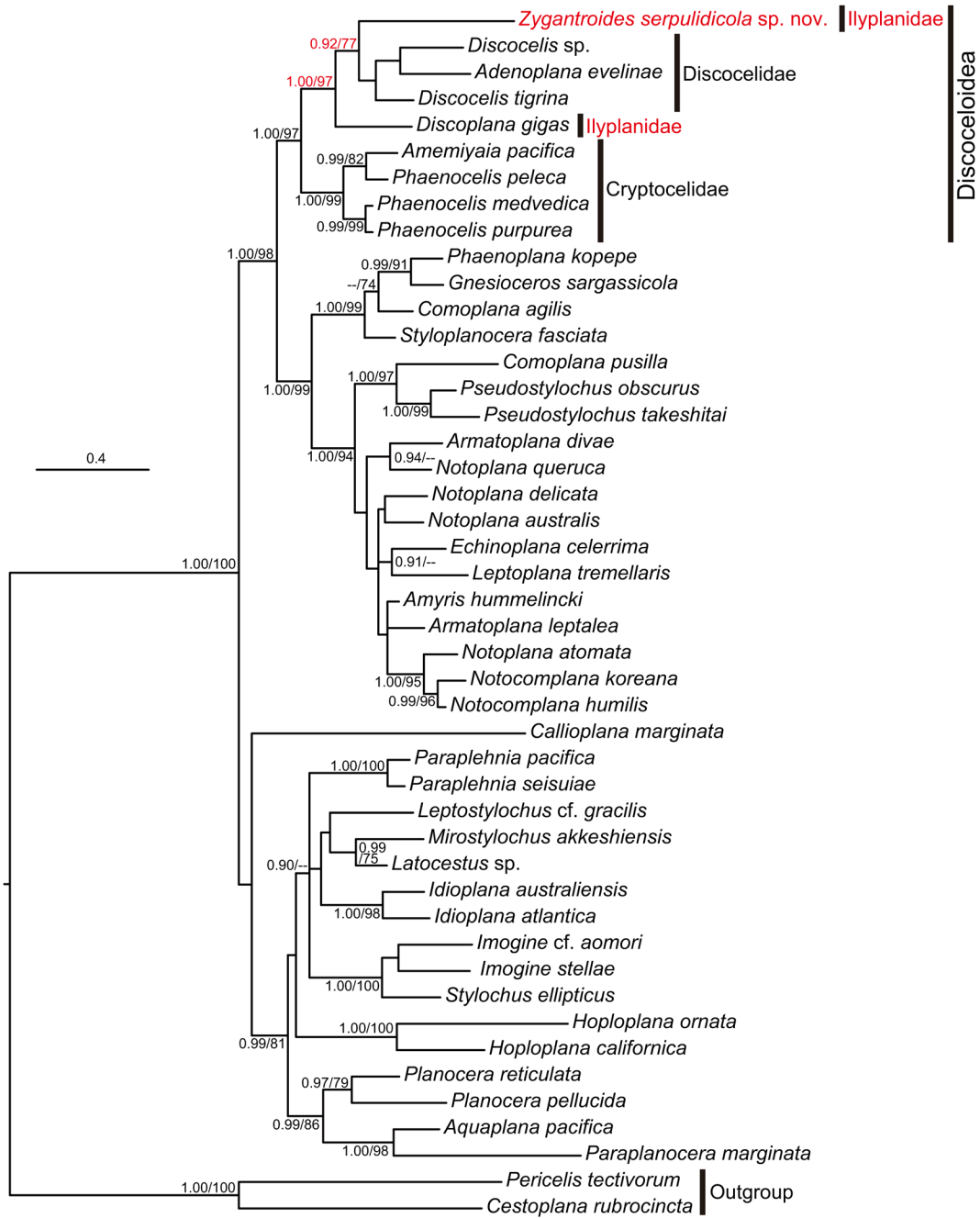


Fig. 5. Bayesian phylogenetic tree based on sequences from four genes (16S, 18S, 28S, COI) (concatenated length 3,496 bp). Numbers near nodes are posterior probability greater than 0.90 and bootstrap values greater than 70%, respectively.

Table 2. Comparison of morphological characters between species of *Zygantrioidea*.

Character	<i>Z. henriettae</i>	<i>Z. plesia</i>	<i>Z. serpulidicola</i> sp. nov.
Mouth	one-third from posterior end of pharynx	two-fifths from posterior end of the pharynx	posterior to pharynx and near common gonopore
Common sperm duct	present	present	absent
Seminal vesicle	spindle-shaped	spherical	pear-shaped
Ejaculatory duct ("granule vesicle" in Corr�ea 1949)	ovoid	approximately cordiform	varied
Lang's-vesicle duct	short	short	elongated
Lang's vesicle	positioned anteriorly to common gonopore	positioned anteriorly to common gonopore	positioned posteriorly to common gonopore
Ampulla in vagina	absent	present	absent

Habitat. Found on tubes of serpulid annelids on the undersurface of stones in the intertidal zone (Fig. 1).

Sequence of COI. The partial COI sequences (712 bp) from the four specimens (LC528160–LC528163) almost coincided with each other. The uncorrected *p*-distance among specimens showed 0.000–0.007.

Molecular phylogeny. The resulting BI and ML trees were almost identical to each other in topology; we show only the BI tree (Fig. 5). *Zygantröides serpulidicola* sp. nov. was nested in a clade comprised of discocelids and ilyplanids with high (1.00/97) nodal supports. Within this clade, *Discoplana gigas* (Schmarda, 1859) (Ilyplanidae) was sister to all the rest, and *Z. serpulidicola* sp. nov. (Ilyplanidae) was sister to Discocelidae, the latter was comprised of species in *Adenoplana* Stummer-Traunfels, 1933 and *Discocelis* Ehrenberg, 1836; Ilyplanidae was recovered non-monophyletic.

Remarks. We extend the generic diagnosis of *Zygantröides*. Faubel (1983: 40) included “Female apparatus directed backwards and oriented dorsal to the male complex” in the generic diagnosis. The female copulatory apparatus in the present species directs somewhat posteriorly but it is not arranged dorsally to the male copulatory apparatus (Fig. 4E). However, *Z. serpulidicola* satisfies other diagnostic characters of *Zygantröides*: tentacles, eyespot distribution, and structure of male and female copulatory organs (Figs 2C, 3, 4). Therefore, we judge it is more appropriate to redefine *Zygantröides* by eliminating the character of orientation of female apparatus than to establish a new genus-level taxon. The horseshoe-shaped Lang’s vesicle such as that in *Z. serpulidicola* has been often employed in genus-level definition (cf. Faubel 1983); however, we do not reflect this trait in the redefinition because the shape of Lang’s vesicle in other *Zygantröides* is not described (Corrêa 1949).

Zygantröides serpulidicola sp. nov. can be distinguished from the two congeners *Z. henriettae* and *Z. plesia* by the following characters: *i*) the position of the mouth (near the common gonopore in *Z. serpulidicola*; at about one-third from posterior end of the pharynx in *Z. henriettae*; two-fifths from posterior end of the pharynx in *Z. plesia*), *ii*) the presence/absence of the common sperm duct (absent in *Z. serpulidicola*; present in *Z. henriettae* and *Z. plesia*), *iii*) the shape of the seminal vesicle (pear-shaped in *Z. serpulidicola*; spindle-shaped in *Z. henriettae*; spherical in *Z. plesia*), *iv*) the length of the Lang’s-vesicle duct (long in *Z. serpulidicola*; short in *Z. henriettae* and *Z. plesia*), *v*) the position of the Lang’s vesicle (posterior to the common gonopore; anterior to the common gonopore in *Z. henriettae* and *Z. plesia*), and *vi*) the presence/absence of an ampulla in the vagina (absent in *Z. serpulidicola* and *Z. henriettae*; present in *Z. plesia*) (Table 2).

This is the first report of *Zygantröides* from the Pacific Ocean. Other two congeners are only reported from the Atlantic coast of southeastern Brazil (Corrêa 1949; Bahia and Schrödl 2018). The two Brazilian species have been captured from between algae, whereas our species was found on tubes of annelids.

Discussion

While the affiliation of *Zygantröides* to Ilyplanidae should be tested by future analyses including *Ilyplana aberrans* Bock, 1925 (type species of *Ilyplana* Bock, 1925, type genus of Ilyplanidae) and *Zygantröplana henriettae* (= *Zygantröides henriettae*; type species of *Zygantröides*), our tree topology suggests that Ilyplanidae is likely non-monophyletic; instead, Ilyplanidae and Discocelidae together formed a clade (Fig. 5). Five species in four genera of the two families included in our analysis, viz., *Adenoplana evelinae* (Discocelidae), *Discocelis tigrina* (Discocelidae), *Discocelis* sp. (Discocelidae), *Discoplana gigas* (Ilyplanidae), and *Z. serpulidicola* sp. nov. (Ilyplanidae), are supposed to share the following three characters: *i*) no nuchal tentacle, *ii*) no prostatic vesicle, and *iii*) horseshoe-shaped Lang’s vesicle. Therefore, these may be synapomorphic for the Discocelidae+Ilyplanidae clade. Meanwhile, *Z. serpulidicola* sp. nov. is morphologically similar to *Discoplana gigas* in that both species have an ejaculatory duct lined with prostatic-like glandular epithelium—which is one of the diagnostic characters of Ilyplanidae—and that both lack marginal eyespots. In terms of the systematic position of *Zygantröides*, if anything, our results were in favor of Faubel (1983) rather than Prudhoe (1985) in that *Z. serpulidicola* sp. nov. was more closely related to Ilyplanidae than to *Leptoplana tremellaris* (Müller, 1773) (type species of the type genus of Leptoplanidae) (Fig. 5). In any case, the family-level systematics pertaining to Discocelidae and Ilyplanidae requires further reconsiderations.

Acknowledgments

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