

Description of a new species of *Alloioiplana* (Polycladida: Stylochoplanidae) with an inference on its phylogenetic position in Leptoplanoidea

Yuki Oya,* Aoi Tsuyuki, and Hiroshi Kajihara

(YO, HK) Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan
(AT) Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

Abstract.—We herein describe a new species of stylochoplanid flatworm, *Alloioiplana yerii*, from Japan. This is the first record of the genus from the West Pacific. *Alloioiplana yerii* is characterized by *i*) a lack of nuchal tentacles, *ii*) an oval prostatic vesicle that is smaller than the seminal vesicle, *iii*) the presence of a common sperm duct, and *iv*) the presence of an intra-prostatic ejaculatory duct. 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 18S and 28S ribosomal DNA and of COI suggest that Stylochoplanidae is not monophyletic and that *Al. yerii* forms a relatively well-supported clade with *Echinoplana celerrima* Haswell, 1907 (Gnesiocerotidae) and *Notoplana* sp. (Notoplanidae).

Key words: Acotylea, DNA barcoding, marine flatworm, systematics

Polycladida is a group of marine free-living flatworms. Most polyclads are benthic and their vertical distribution ranges from the supralittoral zone (Newman & Cannon 1997) to the bathyal zone down to 3232 m (Quiroga et al. 2006). Generally, polyclad flatworms are predators feeding on other invertebrates; some polyclads are significant pests in commercial oyster cultures (Newman & Cannon 2003).

Stylochoplanidae Meixner, 1907 is a family in Leptoplanoidea Ehrenberg, 1831. Within this superfamily, Stylochoplanidae is characterized by species that possess a developed, interpolated, prostatic vesicle lined with a smooth inner epithelium; currently, the family contains 12 genera (Faubel 1983). However, a recent molecular phylogenetic study of Acotylea, which employed three stylocho-

planid genera (i.e., *Armatoplana* Faubel, 1983, *Comoplana* Faubel, 1983, and *Phaeonoplana* Faubel, 1983), revealed that Stylochoplanidae was not a monophyletic taxon (Oya & Kajihara 2020).

Alloioiplana Plehn, 1896 is a genus in Stylochoplanidae characterized by the possession of a penis stylet and the lack of a Lang's vesicle. Currently, four species are classified in this genus by Faubel (1983). To date, *Alloioiplana* has not been included in any molecular phylogenetic analyses (Aguado et al. 2017, Bahia et al. 2017, Tsunashima et al. 2017, Dittmann et al. 2019, Litvaitis et al. 2019, Oya & Kajihara 2020).

In the present study, we describe a new species of *Alloioiplana* from the coast of Japan. We also determine a partial sequence of the cytochrome *c* oxidase subunit I (COI) gene as a DNA barcode, as well as 18S and 28S ribosomal RNA genes (18S and 28S) for the inference of the

* Corresponding author.

DOI: 10.2988/0006-324X-134.1.306

phylogenetic position of the new species in Leptoplanoidea.

Materials and Methods

Flatworms were collected from Misaki (Kanagawa) and Usa (Kochi) on the Pacific coast of Japan (Table 1). Living specimens were anesthetized in MgCl₂ solution prepared with tap water until it had the same salinity as seawater. Flatworms were then photographed with a digital camera. For DNA extraction, a piece of the body margin was cut away from the 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. The specimens for histological observation were dehydrated in an ethanol series and cleared in xylene, embedded in paraffin wax, and sectioned at 7 µm thickness. The sections were stained with hematoxylin and eosin and mounted in Entellan New (Merck, Germany). Morphological terminology in this study follows Hyman (1953) and Oya & Kajihara (2017).

Total DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Germany). As a reference for DNA barcoding, a partial sequence of the COI (712 bp) gene was determined from five specimens using the primer pair Acotylea_COI_F and Acotylea_COI_R (Oya & Kajihara 2017); genetic distances were calculated using MEGA ver. 7.0 (Kumar et al. 2016). The fragments of 18S (1736 bp) and 28S (1015 bp) rDNA were also amplified from one specimen using hrms18S_F and hrms18S_R (Oya & Kajihara 2020) for 18S and fw1 and rev2 (Sonnenberg et al. 2007) for 28S. Sequences were checked and edited using MEGA ver. 7.0 (Kumar et al. 2016).

Additional sequences of leptoplanoid and outgroup species were downloaded from GenBank; two acotylean species, *Discocelis* sp. and *Stylochus* cf. *aomori*

(Kato, 1937), were chosen as outgroup taxa (Table 2). The sequences of 18S and 28S 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 the option "Do not allow many contiguous nonconserved positions." The sequences of COI were aligned and trimmed manually using MEGA ver. 7.0 (Kumar et al. 2016). The concatenated dataset from the three genes was 3163 bp long and contained 33 terminal taxa.

Phylogenetic analyses were performed with the maximum likelihood (ML) method executed in IQtree ver. 2.0 (Minh et al. 2020) under a partition model (Chernomor et al. 2016) and with Bayesian inference (BI) executed in MrBayes ver. 3.2.2 (Ronquist & Huelsenbeck 2003). The optimal substitution models for the ML analysis were selected 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) as follows: GTR+I (18S, second codon position in COI), GTR+I+G (28S), TIM+I+G (third codon position in COI), and TRN+G (first codon position in COI). For BI, the optimal substitution models were GTR+G (first codon position in COI), GTR+I (18S, second codon position in COI), and GTR+I+G (28S, second 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 (MCMC) process used random starting trees and involved four chains run for 5,000,000 generations with the first 25% of trees discarded as burn-in. We considered ML bootstrap values $\geq 70\%$ and posterior probability values ≥ 0.90 to indicate clade support.

Type slides were deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan. All sequences determined in this

Table 1.—Collection information for specimens of *Alloioplana yorii*.

Specimen number (ICHUM)	Status	Preserved state	Substrate	Locality	Date	Collector
6084	holotype	one-third of body from anterior end preserved in 70% ethanol and sagittal sections of the rest; 9 slides	subtidal, branching coralline algae	Arai-hama Beach (35°09'34"N, 139°36'42"E), Misaki, Kanagawa, Japan	26 Mar 2019	H. Kohtsuka, K. Oguchi, T. Miura
6085	paratype	same condition as holotype; 5 slides	same as holotype	same as holotype	same as holotype	same as holotype
6086	paratype	same condition as holotype; 10 slides	same as holotype	same as holotype	same as holotype	same as holotype
6087	paratype	same condition as holotype; 6 slides	at depth of 2 m, kelp holdfasts	same as holotype	19 Feb 2019	same as holotype
6088	paratype	same condition as holotype; 10 slides	same as ICHUM 6078	same as holotype	same as ICHUM 6087	same as holotype
6089	paratype	cross sections; 8 slides	same as ICHUM 6078	same as holotype	same as ICHUM 6087	same as holotype
6282	paratype	sagittal sections; 3 slides	intertidal, oyster beds on a mooring rope hung in the sea	Usa (33°26'26"N, 133°26'16"E), Kochi, Japan	25 Jul 2019	Y. Oya, K. Kakui

Table 2.—List of species included in the molecular phylogenetic analysis and respective GenBank accession numbers.

Family	Species	18S	28S	COI
Gnesiocerotidae	<i>Echinoplana celerrima</i> 1	MN421936	MN421930	—
	<i>Echinoplana celerrima</i> 2	—	HQ659020	—
	<i>Gnesioceros sargassicola</i>	—	MH700309	—
	<i>Styloplanocera fasciata</i>	—	MH700408	—
Leptoplanidae	<i>Leptoplana tremellaris</i> 1	—	KY263696	—
	<i>Leptoplana tremellaris</i> 2	MN421937	MN421931	—
Notocomplanidae	<i>Notocomplana ferruginea</i> 1	—	HQ659014	—
	<i>Notocomplana ferruginea</i> 2	—	MH700322	—
	<i>Notocomplana hagiyai</i>	LC508152	LC508129	LC176003
	<i>Notocomplana humilis</i>	LC508168	LC508144	LC508204
	<i>Notocomplana japonica</i>	LC508154	LC508131	LC176018
	<i>Notocomplana koreana</i>	LC508151	LC508128	LC176014
	<i>Notocomplana lapunda</i>	—	MH700325	—
	<i>Notocomplana septentrionalis</i>	LC508153	LC508130	LC176028
	Notoplanidae	<i>Amyris hummelincki</i>	—	MH700269
<i>Notoplana atomata</i>		—	MH700329	—
<i>Notoplana australis</i>		AJ228786	AY157153	—
<i>Notoplana delicata</i>		LC508169	LC508145	LC508205
<i>Notoplana qeshmensis</i>		—	MH700331	—
<i>Notoplana queruca</i>		—	MH700333	—
<i>Notoplana</i> sp.		—	KY263651	—
Pseudostylochidae		<i>Pseudostylochus intermedius</i>	LC508164	LC508141
	<i>Pseudostylochus obscurus</i>	LC508160	LC508137	LC508197
	<i>Pseudostylochus takeshitai</i>	LC508165	LC508142	LC508202
Stylochoplanidae	<i>Alloioiplana yerii</i> sp. nov.	LC651420	LC651421	LC582944
	<i>Armatoplana divae</i>	—	MH700273	—
	<i>Armatoplana leptalea</i> 1	—	KY263649	—
	<i>Armatoplana leptalea</i> 2	—	MH700275	—
	<i>Comoplana agilis</i>	MN334199	MN384685	—
	<i>Comoplana pusilla</i>	LC508157	LC508134	LC508194
	<i>Phaenoplana kopepe</i>	LC508156	LC508133	LC369778
Outgroup				
Discocelidae	<i>Discocelis</i> sp.	LC508170	LC508146	LC508206
Stylochidae	<i>Stylochus</i> cf. <i>aomori</i>	LC508163	LC508140	LC508200

study were deposited in DDBJ/EMBL/GenBank databases with the accession numbers LC582941–LC582944, LC651420–LC651422. This work has been registered in ZooBank with the registration number urn:lsid:zoobank.org:pub:34ADD862-F10A-43C2-B452-2B95DA01B2E2.

Systematics

Genus *Alloioiplana* Plehn, 1896

Redefinition.—Stylochoplanidae with tentacular and cerebral eye clusters, seminal

vesicle, penis armed with a slender stylet housed in a tubular atrium, and without Lang's vesicle. Gonopores or common gonopore arranged in posterior part of body (modified from Faubel (1983: p. 101)).

Remarks.—We eliminated references to character states of the nuchal tentacle and gonopores from the definition by Faubel (1983). Faubel (1983: p. 101) included the absence of the tentacle as one of the diagnostic characters of *Alloioiplana*. However, this was rather self-contradictory. As Bahia and Schrödl (2018) indicated, *Alloioiplana* sensu Faubel (1983) contained two species with nuchal tentacles, *Alloio-*

plana aulica (Marcus, 1947) and *Al. delicata* Plehn, 1896; the latter is the type species of the genus. In addition, Faubel (1983, p. 101) listed the presence of separated gonopores in the genus definition. However, *Al. aulica* has a common gonopore (Marcus 1947: p. 114, fig. 24). Other characters included by Faubel (1983: p. 101) (the eyespot distribution, the structure of male copulatory apparatus, and the position of gonopores) are applicable to all *Alloioiplana* species.

The above redefinition of *Alloioiplana* was meant to avoid confusion caused by Faubel's (1983) definition. The monophyly of this genus should be tested using molecular data, as suggested by Bahia & Schrödl (2018).

Alloioiplana yerii sp. nov.

Figs. 1–3

Etymology.—The specific name is a noun in the genitive case honoring Megumi Yeri, who studied Japanese polyclads.

Material examined.—See Table 1.

Diagnosis.—*Alloioiplana* without nuchal tentacles, with common sperm duct, oval prostatic vesicle smaller than seminal vesicle, and ejaculatory duct projecting into prostatic vesicle (Figs. 1–3).

Description.—Live specimens 7.8–17 mm in length (17 mm in holotype), 2.8–5.9 mm in maximum width (5.9 mm in holotype). Body elongate oval, narrow toward posterior end (Fig. 1A, B). Dorsal body tinged with light brown due to minute granules scattered over entire surface except around margin. Dorsal surface of body around pharynx brown. Body margin translucent. General appearance of body brownish (Fig. 1). Nuchal tentacles lacking. Cerebral and tentacular eyespots distributed continuously and forming cerebro-tentacular eye cluster. Each of cerebro-tentacular eye clusters containing 14–46 eyespots (27 in right cluster, 32 in left cluster in holotype) (Fig. 1C), arranged near median line.

Pharynx whitish, ruffled in shape, occupying about one-fourth of body length (2.0–4.6 mm in length, 4.5 mm in holotype), located at center of body (Fig. 1B). Mouth opening at slightly posterior to center of pharyngeal cavity. Intestine not anastomosed. Male and female gonopores separate; male gonopore opening at about one-fifth to one-fourth of body length (2.0–4.6 mm, 3.8 mm in holotype) from posterior end; female gonopore situated 0.1–0.5 mm (0.3 mm in holotype) posterior to male gonopore.

Male copulatory apparatus located posterior to pharynx, consisting of seminal vesicle, interpolated prostatic vesicle, and penis stylet; spermiducal bulbs lacking (Figs. 2, 3A). Pair of sperm ducts extending anteriorly, turning medially at point about one-fourth length of pharynx from posterior end, subsequently extending posteriorly along both sides of pharynx and extending further posteriorly for short distance beyond level of posterior end of pharynx, then turning anteriorly (Fig. 1B) and fusing to common sperm duct (Figs. 2, 3A). Common sperm duct entering curved-oval seminal vesicle with strong muscular wall (Figs. 2, 3A, B). Distal end of seminal vesicle slender, long, meandering, entering proximal end of prostatic vesicle and projecting as intra-prostatic ejaculatory duct (Fig. 3B). Intra-prostatic ejaculatory duct lacking muscular wall, reaching to half length of prostatic vesicle (Fig. 3B, C). Prostatic vesicle oval, lacking tubular chambers, smaller than seminal vesicle, coated with thick muscular wall and lined with smooth glandular epithelium (Fig. 3B). Post-prostatic ejaculatory duct with thin muscular wall compared to that in prostatic vesicle connecting to penis stylet. Penis stylet straight, projecting into penis pocket (Fig. 3D). Penis pocket lined with non-ciliated epithelium, opening to male atrium. Male atrium more than 10 times larger than penis pocket and lined with ciliated epithelium (Fig. 3A, D). Shape of



Fig. 1. *Alloioplana yerii*, ICHUM 6084 (holotype), photographs taken in life. A, Dorsal view; B, ventral view; C, eyespots. Abbreviations: cte, cerebro-tentacular eye; fg, female gonopore; mg, male gonopore; ov, oviduct; ph, pharynx; sd, sperm duct. Scale bars: 5 mm (A, B); 1 mm (C).

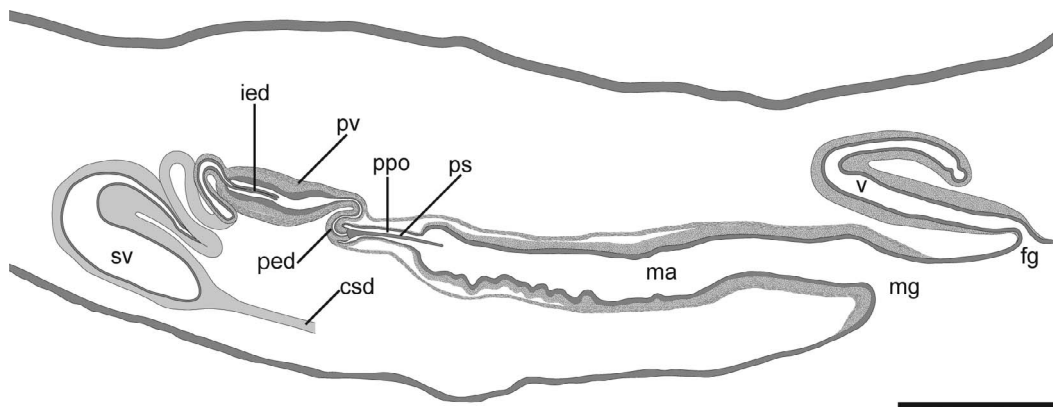


Fig. 2. *Alloioplana yerii*, schematic of male and female copulatory apparatuses, ICHUM 6084 (holotype). Abbreviations: csd, common sperm duct; fg, female gonopore; ied, intra-prostatic ejaculatory duct; ma, male atrium; mg, male gonopore; ped, post-prostatic ejaculatory duct; ppo, penis pocket; ps, penis stylet; pv, prostatic vesicle; sv, seminal vesicle; v, vagina. Scale bar: 300 μ m.

male atrium varying depending on fixation (Fig. 3A, E).

Pair of oviducts extending posteriorly and entering the proximal end of the vagina; Lang's vesicle and bursa copulatrix lacking (Figs. 2, 3F). Vagina lacking ampulla, lined with smooth, ciliated epithelium and surrounded by cement glands, curving antero-dorsally, extending anteriorly, then turning postero-ventrally to exit at female gonopore (Figs. 2, 3F).

Type locality.—Arai-hama Beach (35°09'34"N, 139°36'42"E), Misaki, Kanagawa, Japan (Table 1).

Habitat.—Intertidally to subtidally, on the surface of coralline algae and among kelp holdfasts (Kanagawa), or in oyster beds on a mooring rope hanging in the sea (Kochi) (Table 1).

Sequences of COI.—The partial COI sequences (712 bp) from the five specimens (LC582941–LC582944, LC651422) were almost identical to each other. The uncorrected p-distance among specimens was 0.001–0.010.

Molecular phylogeny.—The topology was almost identical between BI and ML trees (we show only the ML tree in Fig. 4). *Alloioplana yerii* sp. nov. was the sister to a clade comprised of *Echinoplana celerrima* Haswell, 1907 and *Notoplana* sp. identified

by Bahia et al. (2017) with relatively high bootstrap values (72%) and high posterior probability (0.99). The stylochoplanid species employed in the analyses did not form a monophyletic clade.

Remarks.—Faubel (1983) assigned four species to *Alloioplana* (Table 3). *Alloioplana yerii* sp. nov. is distinguished from these species as follows: from *Al. aulica* (Marcus, 1947) and *Al. delicata* Plehn, 1896 by the presence/absence of nuchal tentacles (absent in *Al. yerii*; present in *Al. aulica* and *Al. delicata*); from *Al. stylifera* (Hyman, 1953) by the length of the pharynx relative to the body length (about one-fourth in *Al. yerii*; about three-fifths in *Al. stylifera*) and the shape of the prostatic vesicle (oval in *Al. yerii*; long and posteriorly curved in *Al. stylifera*); and from *Al. wyona* (Du Bois-Reymond Marcus & Marcus, 1968) by the presence/absence of the common sperm duct (present in *Al. yerii*; absent in *Al. wyona*) as well as the size of the prostatic vesicle compared with that of the seminal vesicle (small in *Al. yerii*; almost the same size in *Al. wyona*). In addition, among the congeners, the intra-prostatic ejaculatory duct is only observed in *Al. yerii* sp. nov.

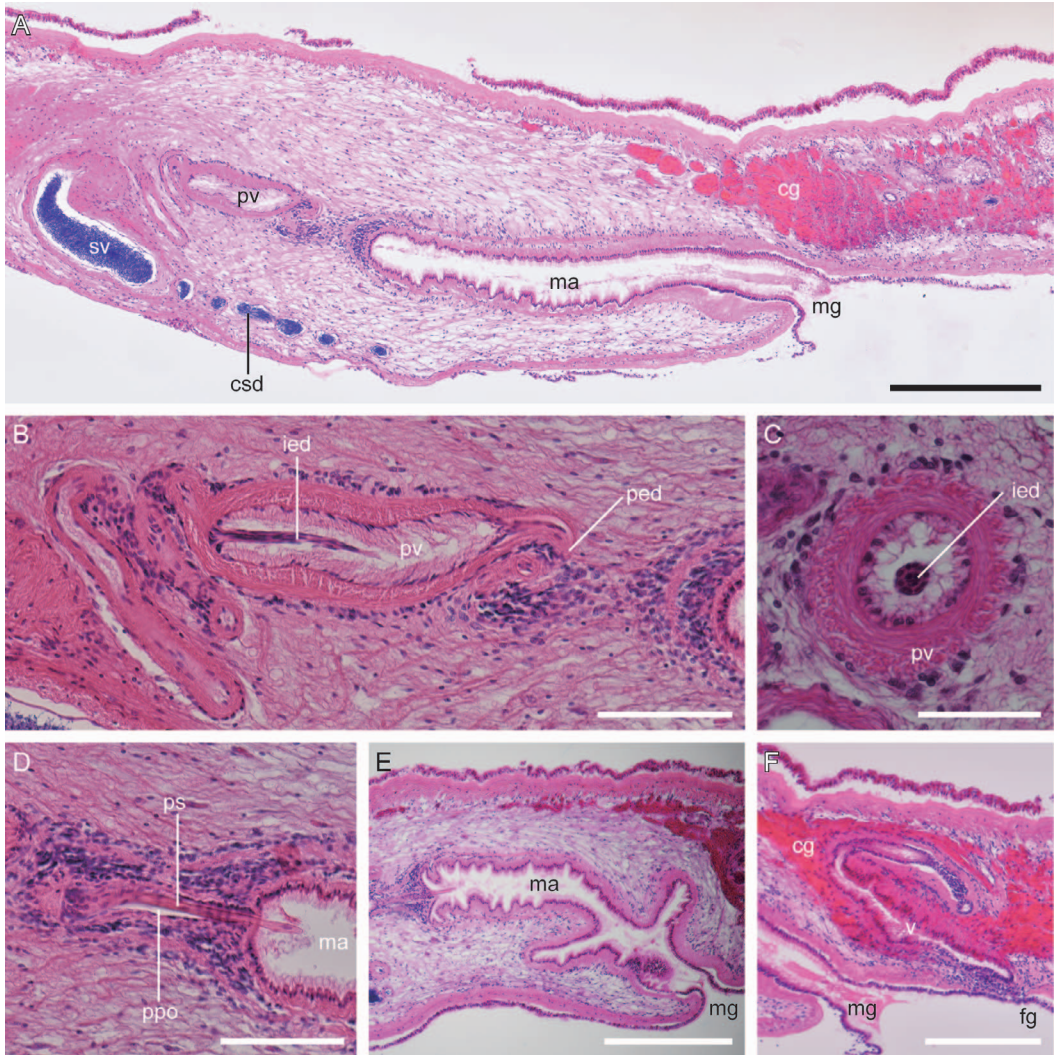


Fig. 3. *Alloioplana yerii*, photomicrographs of holotype ICHUM 6084 (sagittal sections: A, B, D, F) and paratypes ICHUM 6089 (cross section: C) and ICHUM 6082 (sagittal section: E). A, Male copulatory apparatus; B, C, prostatic vesicle and intra-prostatic ejaculatory duct; D, penis stylet; E, male atrium; F, female copulatory apparatus. Abbreviations: cg, cement glands; csd, common sperm duct; fg, female gonopore; ied, intra-prostatic ejaculatory duct; ma, male atrium; mg, male gonopore; ped, post-prostatic ejaculatory duct; ppo, penis pocket; ps, penis stylet; pv, prostatic vesicle; sv, seminal vesicle; v, vagina. Scale bars: 300 μ m (A, E); 100 μ m (B, D); 50 μ m (C); 200 μ m (F).

This is the first report of *Alloioplana* from the West Pacific. Two Atlantic species, *Al. aulica* and *Al. wyona*, have been described from Brazil (Marcus 1947; Bahia & Schrödl 2018) and the Caribbean Sea (Du Bois-Reymond Marcus & Marcus 1968), respectively. Other congeners are known from the East Pacific: *Al. delicata* from Payta, Peru

(Plehn 1896) and *Al. stylifera* from the Gulf of California (Hyman 1953).

Discussion

Our results support previous studies by Dittmann et al. (2019), Litvaitis et al.

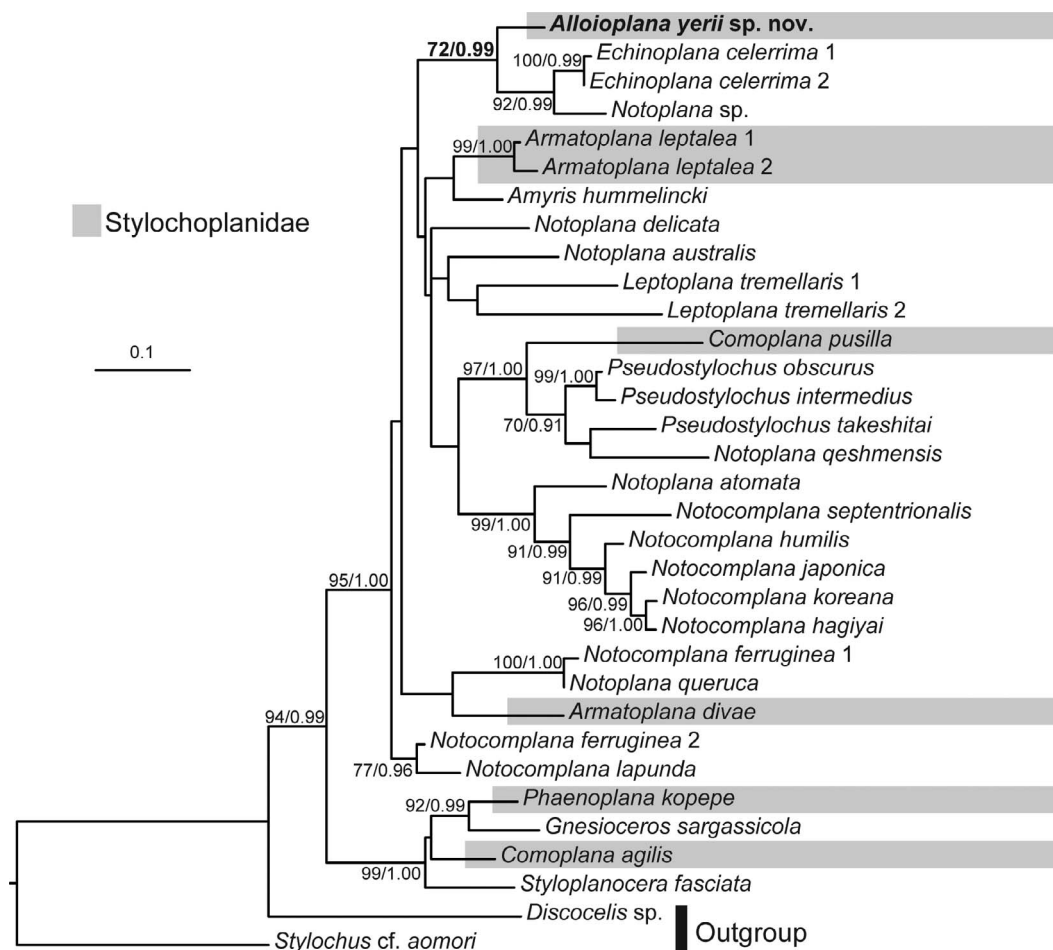


Fig. 4. Maximum likelihood tree based on sequences from three genes (18S, 28S, COI; concatenated length: 3,163 bp). Numbers near nodes are bootstrap values $\geq 70\%$ and posterior probabilities ≥ 0.90 , respectively.

(2019), and Oya & Kajihara (2020) in showing that Stylochoplanidae sensu Faubel (1983) is not monophyletic (Fig. 4). In the tree produced here, *Al. yerii* did not form a sister group relationship to any of the stylochoplanids included in our analyses. Stylochoplanidae as envisaged by Faubel (1983) should be divided into several family-level taxa as previously suggested by Dittmann et al. (2019) and Litvaitis et al. (2019).

There is a possibility that *Alloioplana* sensu Faubel (1983) is not monophyletic. Currently, *Alloioplana* contains polyclad flatworms with nuchal tentacles (*Al. aulica*

and *Al. delicata*) and without nuchal tentacles (*Al. stylifera*, *Al. wyona*, and *Al. yerii*) (Bahia and Schrödl 2018; Table 3). In other stylochoplanid polyclads, i.e., *Armatoplana*, molecular phylogenies have indicated the possibility of non-monophyly in that *Armatoplana divae* (with nuchal tentacles) and *Ar. leptalea* (without tentacles) are separately positioned in the leptoplanoid clade (Fig. 4; Litvaitis et al. 2019, Oya & Kajihara 2020). Although the presence or absence of the nuchal tentacles has hardly been applied for diagnoses, especially in species-rich genera (cf. Faubel 1983; Prudhoe 1985), the characteristic

Table 3.—Comparison of morphological characteristics between species of *Alloioiplana*. “?” means the character state is not described in the original description and drawing.

	<i>Alloioiplana aulica</i>	<i>Alloioiplana delicata</i>	<i>Alloioiplana stylifera</i>	<i>Alloioiplana wyona</i>	<i>Alloioiplana yerii</i>
Nuchal tentacles	present	present	absent	absent	absent
Length of pharynx	about one-fourth of body length	one-third of body length	three-fifths of body length	one-fifth to one-fourth of body length	about one-fourth of body length
Common sperm duct	present	?	?	absent	present
Prostatic vesicle	oval, slightly larger than seminal vesicle	elongated oval, larger than seminal vesicle	long, narrow, posteriorly curved, size unknown	oval, same size as seminal vesicle	oval, smaller than seminal vesicle
Intra-prostatic ejaculatory duct	absent	absent	absent	absent	present
Ampulla at terminal of vagina	absent	absent	absent	present	absent
Gonopores	common	separated	separated	separated	separated
Type locality	Ilha das Palmas, Santos Bay, Brazil	Payta, Peru	Angel de la Guardia Island and Tiburon Island, Gulf of California, Mexico	Simson Bay, St. Martin	Misaki, Kanagawa, Japan
Distribution	same as type locality	same as type locality	same as type locality	same as type locality	Misaki and Usa (Kochi, Japan)
Reference	Marcus (1947)	Plehn (1896)	Hyman (1953)	Du Bois-Reymond Marcus & Marcus (1968)	this study

may be useful for distinguishing higher-rank taxa (families or genera) in Leptoplanoidea, as was demonstrated by Oya & Kajihara (2020). To evaluate the suitability of this diagnostic characteristic in *Alloioiplana*, the phylogenetic positions of other *Alloioiplana* species are required.

In this study, we could not detect remarkable morphological similarities among *Al. yerii*, *E. celerrima*, and *Notoplana* sp. although these three species formed a relatively robust clade (Fig. 4). In the external morphology, *Al. yerii* and *E. celerrima* share two taxonomic characters: *i*) lacking nuchal tentacles and *ii*) having cerebro-tentacular eye clusters (Fig. 1C; Haswell 1907: p. 475, pl. 37, fig. 1); these traits are not observed in other gnesiocerotid polyclads included in the phylogeny. However, the character states in *Notoplana* sp. are unknown and these are also seen in other leptoplanoid species we employed in the analysis (e.g., *Amyris hummelincki* Du Bois-Reymond Marcus & Marcus, 1968). In the internal characters, the difference of anatomical features of the copulatory apparatuses between the three polyclads are so large that they are assigned in different families in Leptoplanoidea, respectively. Although there may be some similarities in histological characteristics of the copulatory apparatus, we could not evaluate this aspect because histological specimens of *E. celerrima* and *Notoplana* sp. were not available. Investigation of morphological synapomorphies or similarities among these species is a future subject.

Acknowledgments

We thank Mr. Hisanori Kohtsuka, Ms. Michiyo Kawabata, and Prof. Toru Miura (the University of Tokyo) and Dr. Kohei Oguchi (National Institute of Advanced Industrial Science and Technology) for providing living specimens in Misaki. We also thank Dr. Keiichi Kakui (Hokkaido University) for helping with sampling in

Kochi. YO is thankful to Dr. Hiroshi Namikawa (National Museum of Nature and Science) for providing YO an opportunity to collect flatworms in Kochi and to the staff of Usa Marine Biological Institute for support with sampling. The authors would like to thank Enago (www.enago.jp) for the English language review. This study was funded by Research Institute of Marine Invertebrates (No. 2017 IKU-3) and the Japan Society for the Promotion of Science (JSPS) under KAKENHI grant number JP20J11958 to YO.

Literature Cited

- Aguado, M. T., C. Noreña, L. Alcaraz, D. Marquina, F. Brusa, C. Damborenea, B. Almon, C. Bleidorn, & C. Grande. 2017. Phylogeny of Polycladida (Platyhelminthes) based on mtDNA data. *Organisms Diversity and Evolution* 17: 767–778.
- Akaike, H. 1974 A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.
- Bahia, J., V. Padula, & M. Schrödl. 2017. Polycladida phylogeny and evolution: integrating evidence from 28S rDNA and morphology. *Organisms Diversity and Evolution* 17: 653–678.
- Bahia, J., & M. Schrödl. 2018. Brazilian Polycladida (Rhabditophora: Platyhelminthes): rediscovery of Marcus' type material and general revision. *Zootaxa* 4490: 1–121.
- Castresana, J. 2002. Gblocks, v. 0.91 b. Online version. http://molevol.cmima.csic.es/castresana/Gblocks_server.html. (last accessed on 1 February 2021)
- Chernomor, O., A. von Haeseler, & B. Q. Minh. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* 65: 997–1008.
- Dittmann, I. L., D. Cuadrado, M. T. Aguado, C. Noreña, & B. Egger. 2019. Polyclad phylogeny persists to be problematic. *Organisms Diversity and Evolution* 19: 585–608.
- Du Bois-Reymond Marcus, E. & E. Marcus. 1968. Polycladida from Curaçao and faunistically related regions. *Studies on the Fauna of Curaçao and other Caribbean Islands* 26: 1–134.
- 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.

- Haswell, W. A. 1907. Observations on Australasian polyclads. *Transactions of the Linnaean Society of London Zoology* 9: 465–485.
- Hyman, L. H. 1953. The polyclad flatworms of the Pacific coast of North America. *Bulletin of the American Museum of Natural History* 100: 269–392.
- Katoh, K., & D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Kumar, S., G. Stecher, & K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.
- Lanfear, R., B. Calcott, S. Y. Ho, & S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.
- Lanfear, R., P. B. Frandsen, A. M. Wright, T. Senfeld, & B. Calcott. 2016. Partitionfinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773.
- Litvaitis, M. K., D. M. Bolaños, & S. Y. Quiroga. 2019. Systematic congruence in Polycladida (Platyhelminthes, Rhabditophora): are DNA and morphology telling the same story? *Zoological Journal of the Linnean Society* 186: 865–891.
- Marcus, E. 1947. Turbelários marinhos do Brasil. *Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Zoologia* 12: 99–215.
- Minh, B. Q., H. A. Schmidt, O. Chernomor, D. Schrempf, M. D. Woodhams, A. Von Haeseler, & R. Lanfear. 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530–1534.
- Newman, L. J., & L. R. G. Cannon. 1997. A new semi-terrestrial acotylean flatworm, *Myoromixa pardalota* gen. et sp. nov. (Platyhelminthes, Polycladida) from southeast Queensland. *Memoirs of the Queensland Museum* 42: 311–314.
- Newman, L. J., & L. R. G. Cannon. 2003. *Marine Flatworms: The World of Polyclads*. CSIRO Publishing, Collingwood, Australia, 97 pp.
- Oya, Y., & H. Kajihara. 2017. Description of a new *Notocomplana* species (Platyhelminthes: Acotylea), new combination and new records of Polycladida from the northeastern Sea of Japan, with a comparison of two different barcoding markers. *Zootaxa* 4282: 526–542.
- Oya, Y., & H. Kajihara. 2020. Molecular phylogenetic analysis of Acotylea (Platyhelminthes: Polycladida). *Zoological Science* 37: 271–279.
- Plehn, M. 1896. Neue Polycladen, gesammelt von Herrn Kapitän Chierchia bei der Erdumschiffung der Korvette Vettor Pisani, von Herrn Prof. Dr. Kükenthal im nördlichem Eismeer und von Herrn Prof. Dr. Semon in Java. *Jenaische Zeitschrift für Naturwissenschaft* 30: 137–181.
- Prudhoe, S. 1985. *A Monograph on Polyclad Turbellaria*. Oxford University Press, Oxford, New York, 259 pp.
- Quiroga, S. Y., D. M. Bolanos, & M. K. Litvaitis. 2006. First description of deep-sea polyclad flatworms from the North Pacific: *Anocellidus* n. gen. *profundus* n. sp. (Anocellidae, n. fam.) and *Oligocladus voightae* n. sp. (Euryleptidae). *Zootaxa* 1317: 1–19.
- Ronquist, F., & J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Sonnenberg, R., A. W. Nolte, & D. Tautz. 2007. An evaluation of LSU rDNA D1–D2 sequences for their use in species identification. *Frontiers in Zoology* 4: 1–12.
- Tsunashima, T., M. Hagiya, R. Yamada, T. Koito, N. Tsuyuki, S. Izawa, K. Kosoba, S. Itoi, & H. Sugita. 2017. A molecular framework for the taxonomy and systematics of Japanese marine turbellarian flatworms (Platyhelminthes, Polycladida). *Aquatic Biology* 26: 159–167.