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Description of a new species of *Pericelis* (Polycladida, Diposthidae) from sunken wood in the bathyal zone in Japan

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Abstract

We describe *Pericelis nivea* sp. nov. from sunken wood collected 330 m deep, off the coast of Owase, Japan. This is the first record of *Pericelis* from the bathyal zone. Unlike other congeners, *P. nivea* sp. nov. is characterized by the absence of eyespots. We provide a partial sequence of the mitochondrial cytochrome *c* oxidase subunit I gene as a DNA barcode for the new species. Phylogenetic analyses based on concatenated sequences of nuclear 18S and 28S ribosomal DNA showed that *P. nivea* sp. nov. was nested in the clade of *Pericelis* with high support; however, the relationship between *P. nivea* sp. nov. and other *Pericelis* species was unclear.

Introduction

Pericelis Laidlaw, 1902 is a genus in a cotylean polyclad family of Diposthidae Woodworth, 1898 (Litvaitis *et al*., 2019). The genus is characterized by possessing (i) an elongated oval or circular body; (ii) a pair of marginal tentacles; (iii) cerebral, tentacular, and marginal eyespots; (iv) a pharynx located at the centre of the body; (v) a seminal vesicle and an unarmed penis papilla in the male copulatory apparatus but lacking a prostatic vesicle and (vi) uterine vesicles and no Lang's vesicle in the female copulatory apparatus (Tsuyuki *et al*., 2022a). Eleven of 12 known *Pericelis* polyclads have been reported from shallow waters (intertidal to 20 m depths), mainly in tropical and subtropical areas (figure 1 in Tsuyuki *et al*., 2022a). A single species, *Pericelis tectivorum* Dittmann *et al*., 2019a, has been described from an aquarium and its habitat and distribution in nature are uncertain (Dittmann *et al*., 2019a). In recent years, new species of *Pericelis* have been successively described (Dittmann *et al*., 2019a; Ramos-Sánchez *et al*., 2020; Tsuyuki *et al*., 2020, 2022a).

We found two individuals of polyclads that could be identified as *Pericelis* on sunken wood collected from a depth of 330 m and brought to Toba Aquarium (Mie, Japan; Figure 1). One individual was successfully captured for detailed observation. The polyclad flatworm lacks eyespots; however, its copulatory apparatuses have a typical morphology of *Pericelis* polyclads. In this study, we describe a new species of eye-less *Pericelis* based on the specimen and determine cytochrome *c* oxidase subunit I (COI) sequences for DNA barcoding and 18S and 28S ribosomal RNA genes for inferring the phylogenetic positions of the new species within *Pericelis*.

Materials and methods

Sampling and fixation

Two polyclads were found on sunken wood obtained from 330 m depths by bottom trawling off the coast of Owase, Mie, Japan (Figure 1A). One individual was captured and photographed with a digital camera; another was photographed but not collected (Figure 1B, C). Fixation was performed according to the method of Tsuyuki *et al*. (2022a). The captured worm was anaesthetized in an $MgCl_2$ solution prepared with tap water to have the same salinity as seawater. The ventral view of the worm was photographed with a digital camera under an anaesthetized state. 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.

Histological observation

The whole body of the specimen was dehydrated in an ethanol series and cleared in xylene. The cleared specimen was embedded in paraffin wax and sagittally sectioned at 7 µm thickness. The sections were stained with haematoxylin and eosin and mounted in Entellan New (Merck, Germany).

Measurements of the specimens were carried out using ImageJ. The body size and pharynx length were measured from photographs of the anaesthetized specimens. The size of copulatory apparatuses was measured from photographs of the histological sections obtained by a digital camera (DP20, OLYMPUS) mounted on a microscope (Olympus BX41).

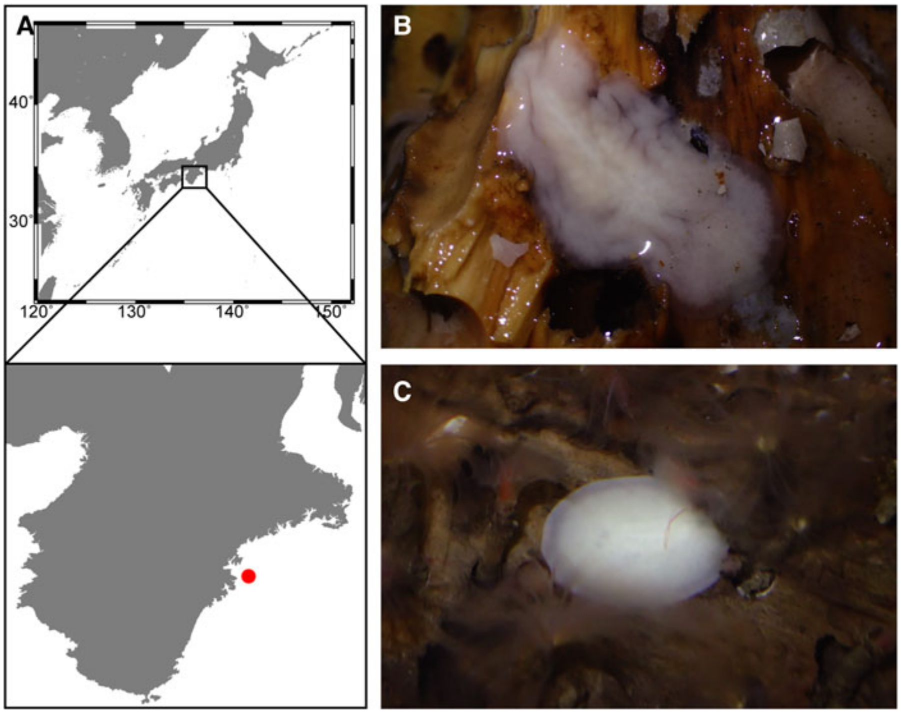

Figure 1. Collection site and photographs of living individuals of *Pericelis nivea* sp. nov. on a block of sunken wood: (A) locality of specimens, red circle indicates the collection site; (B) uncaptured individual; (C) ICHUM 8562 (holotype).

DNA extraction and sequencing

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) was determined from the specimen using the primer pair Acotylea_COI_F and Acotylea_COI_R (Oya and Kajihara, 2017). For molecular phylogenetic analyses, 18S (1736 bp) and 28S (1007 bp) fragments were sequenced using hrms18S_F and hrms18S_R (Oya and Kajihara, 2020) for 18S and fwl and rev2 (Sonnenberg *et al.*, 2007) for 28S, respectively. The procedures of PCR amplification were as follows: 94°C for 1 min; 35 cycles of 94°C for 30 s, 50°C (COI and 18S) or 52.5°C (28S) for 30 s, and 72°C for 1 min (COI), 2 min (18S), or 1.5 min (28S); and 72°C for 7 min. Sequences were checked and edited using MEGA version 7.0 (Kumar *et al.*, 2016).

Molecular phylogenetic analysis

Additional sequences of *Pericelis* and four cotylean species were downloaded from GenBank (Table 1). The 18S and 28S sequences were aligned using MAFFT version 7 (Katoh and Standley, 2013) with the L-INS-i strategy. Ambiguous sites were removed with Gblocks (Castresana, 2000) using the option 'With Half'. The concatenated dataset from the four genes was 2652 bp long and contained 16 terminal taxa.

Phylogenetic analyses were performed using the maximum likelihood (ML) method executed in IQtree version 2.0 (Minh *et al.*, 2020) under a partition model (Chernomor *et al.*, 2016) and Bayesian inference (BI) executed in MrBayes version 3.2.2 (Ronquist and Huelsenbeck, 2003). The optimal substitution models for ML analysis selected with PartitionFinder version 2.1.1 (Lanfear *et al.*, 2016) under the Akaike information criterion (Akaike, 1974) using the greedy algorithm (Lanfear *et al.*, 2012) were TRN + I (18S) and GTR + I + G (28S). For BI, optimal substitution models were GTR + I (18S) and GTR + I + G (28S). 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 10,000,000 generations, with the first 25% of trees discarded as burn-in. Convergence was confirmed using an average standard deviation of split frequencies of 0.003556, potential scale reduction factors for all parameters of 1.000–1.001, and effective sample sizes for all parameters of >5052.

Data treatment

Type slides have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan. All sequences determined in this study have been deposited in DDBJ/EMBL/GenBank databases with accession numbers LC794541–LC794543.

Results

Order **Polycladida** Lang, 1881
Suborder **Cotylea** Lang, 1884
Family **Diposthidae** Woodworth, 1898
Genus ***Pericelis*** Laidlaw, 1902
Pericelis nivea sp. nov.
[New Japanese name: *shiromuku-perikerisu*]
urn:lsid:zoobank.org:act:
8A91AC26-52FE-4BC2-A885-DEFFB910694E
(Figures 1B–4)

Material examined

Holotype, ICHUM 8562, sagittal sections (15 slides), found on sunken wood collected from 330 m deep, off the coast of Owase (34°01′N, 136°22′E), Mie, Japan, 3 February 2019, T. Moritaki leg.

Etymology

The new specific name *nivea* (*-us*, *-a*, *-um*) is a Latin adjective meaning 'snow white'. It was named after the appearance of the living worm. The new Japanese name for the new species is derived from *shiromuku* (a pure white *kimono* dress) and *perikerisu* (*Pericelis* polyclad) in the Japanese language.

Type locality

Off the coast of Owase, Mie, Japan (Figure 1A).

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

	18S	28S	Reference
Pericelis alba	–	MK299354	Cuadrado *et al.* (2021)
Pericelis byerleyana	–	MH047291	Velasquez *et al.* (2018)
	–	MK299374	Cuadrado *et al.* (2021)
Pericelis cata	–	KY263700	Bahia *et al.* (2017)
	–	MK299373	Cuadrado *et al.* (2021)
Pericelis flavomarginata	LC672041	LC568535	Tsuyuki *et al.* (2020, 2022a)
Pericelis hymanae	–	MH700339	Litvaitis *et al.* (2019)
Pericelis lactea	LC699193	LC699189	Tsuyuki *et al.* (2022a)
Pericelis maculosa	LC699194	LC699190	Tsuyuki *et al.* (2022a)
Pericelis nivea sp. nov.	LC794541	LC794542	This study
Pericelis orbicularis	–	MH700340	Litvaitis *et al.* (2019)
Pericelis tectivorum	MN334202	MK181525	Dittmann *et al.* (2019a, 2019b)
Outgroup			
Boninia yambarensis	LC699273	LC699278	Tsuyuki *et al.* (2022b)
Cestoplana rubrocincta	MN334198	MN384689	Dittmann *et al.* (2019b)
Diposthus popeae	–	MH700294	Litvaitis *et al.* (2019)
Theama mediterranea	MN384707	MN384705	Dittmann *et al.* (2019b)

Diagnosis

Pericelis without eyespots and colour pattern, with glandular epithelium in penis papilla and separated gonopores (Figures 2–4).

Description

Live specimen about 10 mm, elongated oval (Figure 2A). Anaesthetized specimen 9.3 mm long, 8.5 mm wide maximum (Figure 2C, D). Body translucent. Intestine visible whitish, highly branched, and not anastomosing, spreading throughout body, not reaching body margin. Dorsal and ventral surfaces without any colour pattern. General appearance of body white (Figures 1B, C, 2). Pair of marginal tentacles inconspicuous, not folded, slightly pointed (Figure 2B). Eyespots absent. Pharynx whitish, ruffled in shape, occupying about one-third of body length, 3.1 mm in anaesthetized state, located at almost centre of body (Figure 2A, D). Mouth opening at centre of pharyngeal cavity. Gonopores separate; female gonopore situated 281 µm posterior to male gonopore (Figures 3A, B, 4).

Male copulatory apparatus located immediately posterior to pharynx, consisting of seminal vesicle and unarmed penis papilla (Figures 3B, 4). Pair of sperm ducts entering laterally into seminal vesicle. Seminal vesicle oval, 292 µm on short axis and 446 µm on long axis, with thin (8.8–12 µm in thickness) muscular wall (Figure 3B). Distal end of seminal vesicle opening almost directly into penis papilla. Penis papilla cylindrical, 274 µm on short axis and 288 µm on long axis, with developed internal glandular epithelium, directing ventrally, occupying almost whole male atrium (Figures 3B, 4).

Female copulatory apparatus lacking Lang's vesicle (Figures 3B, 4). Pair of oviducts, each with 7–8 small uterine vesicles (Figure 3C) and single large uterine vesicle (Figure 3D) arranged from anterior to posterior, running posteriorly lateral to pharynx, leading to proximal end of vagina. Vagina 728 µm long, running posterodorsally and turning anteroventrally, opening into cement pouch. Cement glands opening cement pouch. Female atrium 166 µm long, opening to exterior through female gonopore. Sucker situated posterior to female copulatory apparatus (Figures 3A, B, 4).

Phylogenetic position

The topology was almost identical between BI and ML trees (only the ML tree is shown in Figure 5). *Pericelis nivea* sp. nov. was encompassed in the clade of *Pericelis* with high support values (87/0.99). Within the *Pericelis* species, *P. nivea* sp. nov was sister to the clade formed by other *Pericelis* except *P. lactea*; however, the nodal support was low (50/0.65).

Habitat

Sunken wood in the bathyal zone (Figure 1B, C).

Distribution

Only from the type locality.

Remarks

We assign the *P. nivea* sp. nov. to *Pericelis* although it lacks eyespots. The presence of eyespots in the body margin is a diagnostic character of the genus (cf. Tsuyuki *et al.*, 2022a). However, other morphological characteristics, such as body shape, presence of marginal tentacles, position of the pharynx, and structures of male and female reproductive organs in the present polyclad flatworm, fit the definition of the genus. The new species is also nested in the clade of *Pericelis* with high support values in phylogenetic analyses (Figure 5). Here, we avoid modifying the definition of *Pericelis* and classify the present species as an exception of the genus. The absence of eyespots in *P. nivea* sp. nov. may be related to its habitat (cf. Oya and Kajihara, 2019).

This is the first record of *Pericelis* from the bathyal zone. Among 12 species of *Pericelis*, *P. nivea* sp. nov. can be readily distinguished from other congeners by lacking eyespots and colour patterns in the dorsal surface (cf. table 3 in Tsuyuki *et al.*, 2022a). In addition, the present species differs from five species (*P. flavomarginata*, *P. hymanae*, *P. lactea*, *P. maculosa*, and *P. orbicularis*) by possessing glandular epithelium in the penis papilla. Moreover, our species is distinguished from four species (*P. alba*, *P. ernesti*, *P. nazahui*, and *P. sigmeri*) of the rest congeners by having separated gonopores. Furthermore, *P. nivea* sp. nov. is also differentiated from *P. byerleyana* by the penis-papilla

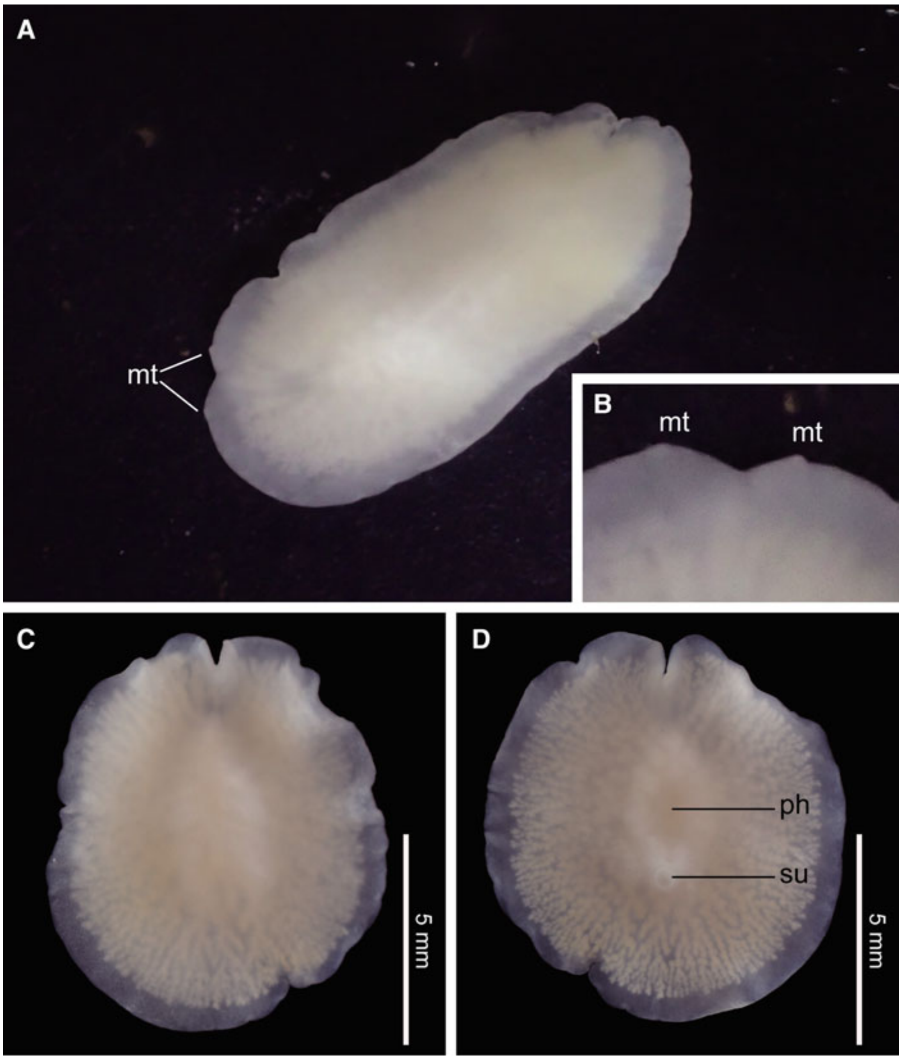

Figure 2. *Pericelis nivea* sp. nov. (ICHUM 8562, holotype), photographs taken in life: (A) dorsal view without anaesthetization, scale unknown, anterior to the left; (B) enlarged view of anterior margin (C) dorsal view with anaesthetization, anterior to the top; and (D) ventral view with anaesthetization, anterior to the top: Abbreviations: mt, marginal tentacles; ph, pharynx; su, sucker.

shape (length/width: about 1 in *P. nivea* sp. nov.; 4–5 in *P. byerleyana*). In addition to the morphology, the present polyclad is well separated from nine *Pericelis* species by the molecular information (Figure 5). Here, we judged the worm to be a new species of *Pericelis*.

Discussion

This polyclad is the fourth polyclad species described from the bathyal zone around Japan (Oya and Kajihara, 2019, 2021; Oya *et al.*, 2019, this study). In Japan, approximately 150 species of Polycladida have been reported from the coast of Japan (Kato, 1944), representing 15% of the described polyclads in the world. In addition, despite easily accessible sites such as the intertidal zone, new polyclad flatworms have been successively described from Japan (e.g. Oya *et al.*, 2021, 2022); this fact suggests that Japanese waters have a rich polyclad fauna. Although knowledge of the polyclad fauna in deep areas is scarce, it is natural that many species will be discovered on the deep sea bottom around Japan as the faunal survey progresses.

Unintentionally captured specimens are important for investigating the diversity of deep-sea polyclads. Deep-sea polyclads are rarely collected; for example, *Paraplehnia seisuiae* Oya *et al.*, 2019, which was described from the bathyal zone of the Kumano Sea, has not been collected except for a single specimen of the holotype although the area has been continuously surveyed since 2017 (Kimura *et al.*, 2018, 2019a, 2019b; Jimi *et al.*, 2020). As Quiroga *et al.* (2006) pointed out, polyclads in deep waters may be broken or wafted away during dredging in many cases even though many species inhabit the seafloor; Quiroga *et al.* (2006) stated that sampling by research submersibles or remotely operated vehicles is the only way to collect intact polyclads in the deep sea. These machines are indeed effective; however, it is not considered suitable for surveying large areas of the seafloor. In terms of covering the limitations of the methods, bycatch in other research and commercial fisheries would be an effective way to collect bathyal polyclads.

Pericelis nivea sp. nov. is expected to be a predator on wood falls. In *Pericelis*, several observations about feeding habits have been reported (Bahia *et al.*, 2014; Dittmann *et al.*, 2019a; Tsuyuki *et al.*, 2020). Bahia *et al.* (2014) described that *P. cata* fed on a sea slug, *Felimare lajensis* (Troncoso *et al.*, 1998) when they were placed in the same container and Dittmann *et al.* (2019a) observed that *P. tectivorum* preyed on a marine snail, *Tectus fenestratus* (Gmelin, 1791). In another study, Tsuyuki *et al.* (2020) reported that *P. flavomarginata* fed on a scaleworm, *Iphione muricata* (Lamarck, 1818). Like these congeners, *P. nivea* sp. nov. may feed on other invertebrates, such as annelids and molluscs, on sunken wood. As Quiroga *et al.* (2008) pointed out, taxonomic studies of polyclads on wood falls would be important not only for revealing polyclad fauna but also for understanding a community in deep-sea environments.

Polyclad flatworms may have independently colonized deep-sea wood falls in several lineages. Four species of polyclads

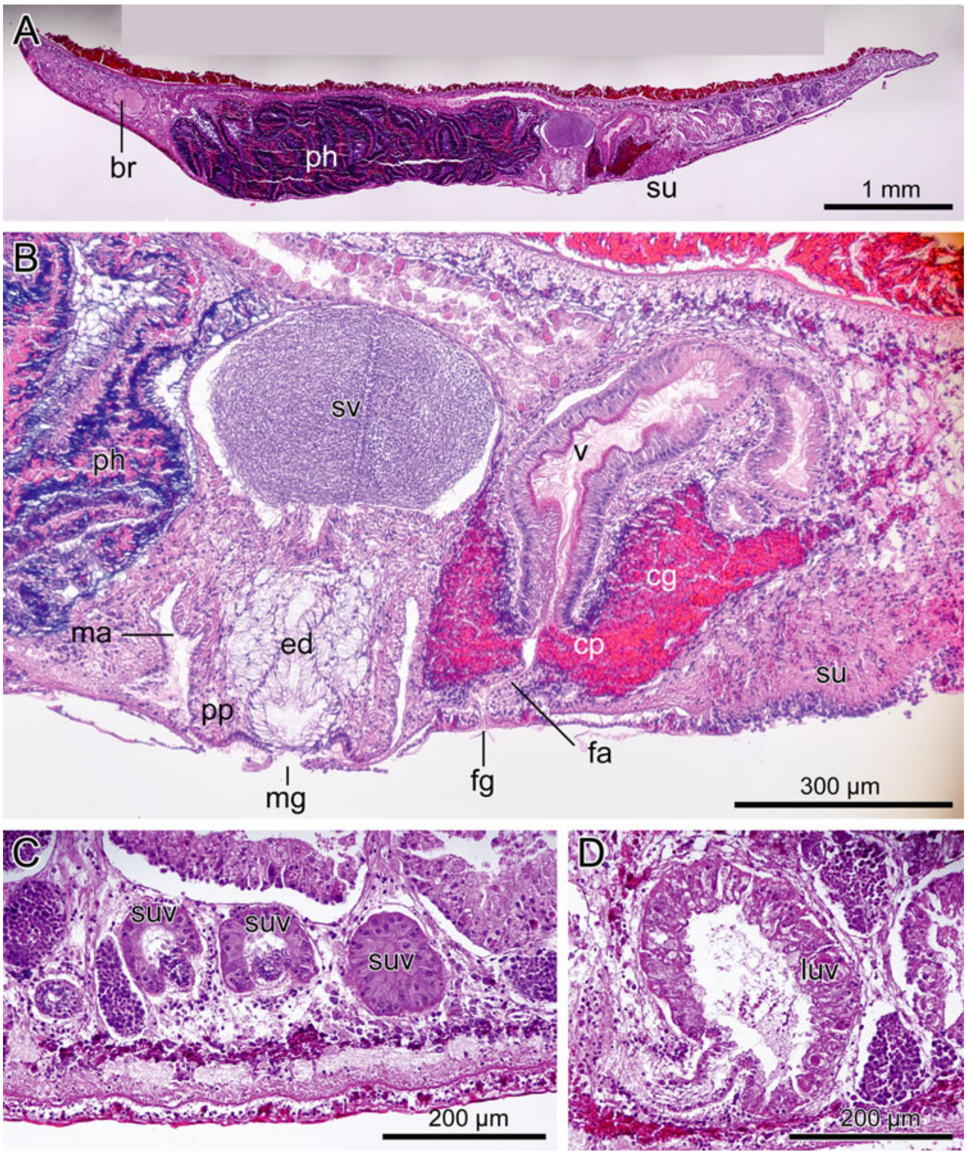

Figure 3. Photomicrographs of sagittal sections of *Pericelis nivea* sp. nov. (ICHUM 8562, holotype), anterior to the left: (A) whole body; (B) male and female copulatory apparatuses; (C and D) uterine vesicle. Abbreviations: br, brain; cg, cement gland; cp, cement pouch; ed, ejaculatory duct; fa, female atrium; fg, female gonopore; luv, large uterine vesicle; ma, male atrium; mg, male gonopore; ph, pharynx; pp, penis papilla; sv, seminal vesicle; su, sucker; suv, small uterine vesicle; v, vagina.

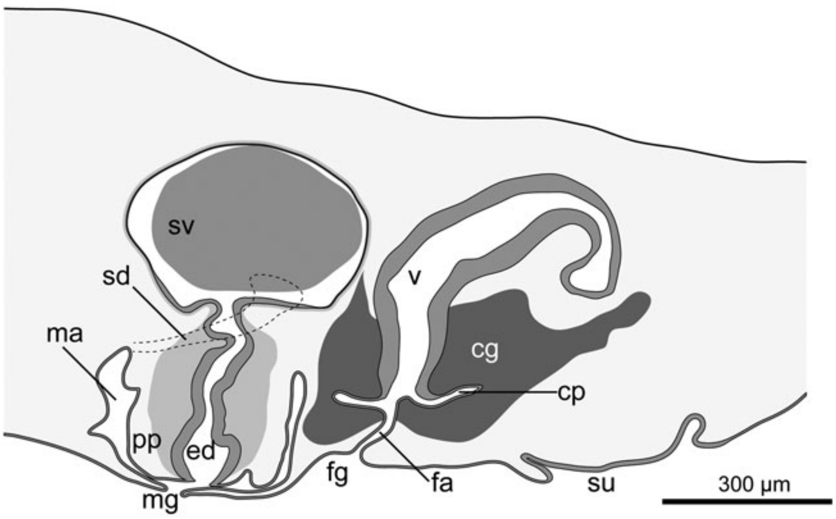

Figure 4. Schematic diagram of copulatory apparatuses in *Pericelis nivea* sp. nov, anterior to the left. Abbreviations: cg, cement gland; cp, cement pouch; ed, ejaculatory duct; fa, female atrium; fg, female gonopore; ma, male atrium; mg, male gonopore; pp, penis papilla; sd, sperm duct; sv, seminal vesicle; su, sucker; v, vagina.

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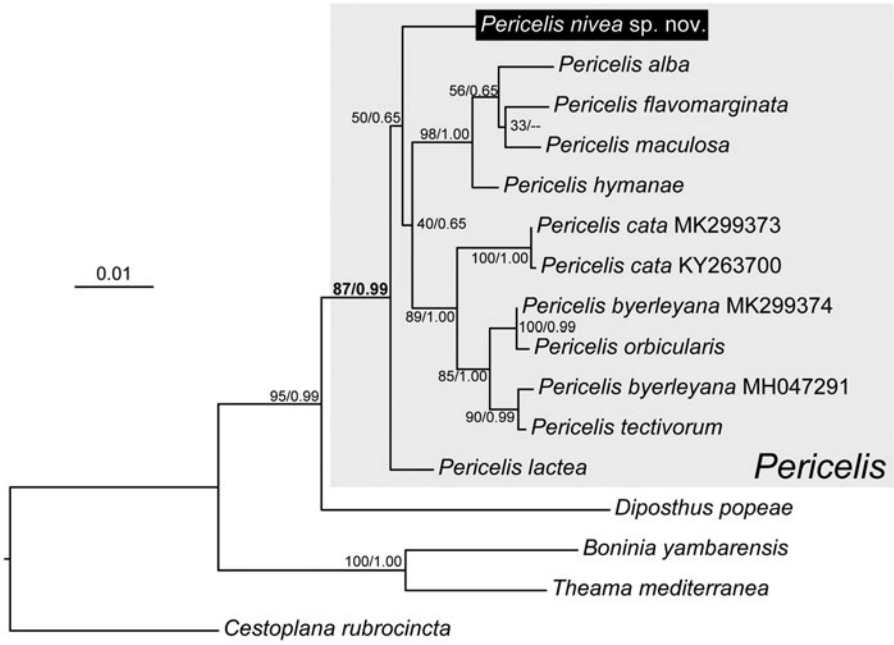

Figure 5. Maximum likelihood (ML) phylogenetic tree based on sequences from two genes (18S and 28S; concatenated length: 2652 bp). The numbers near nodes are ML bootstrap values/posterior probability.

from two acotylean (*Anocellidus profundus* Quiroga *et al.*, 2006 in Anocellidae Quiroga *et al.*, 2006 and *Didangia carneyi* Quiroga *et al.*, 2008 in Didangiidae Faubel, 1983) and one cotylean families (*Oligocladus bathymodiensis* Quiroga *et al.*, 2008 and *O. voightae* Quiroga *et al.*, 2006 in Euryleptidae Stimpson, 1857) have been described from sunken wood in the deep sea (Quiroga *et al.*, 2006, 2008). In the group known in the wood falls, *Oligocladus* Lang, 1884 is expected to provide some insights into the colonization of deep-sea substrates because it contains species inhabiting shallow waters (e.g. Noreña *et al.*, 2014) as well as bathyal zones (Quiroga *et al.*, 2008) to abyssal zones (Quiroga *et al.*, 2006). *Pericelis* may be another candidate of polyclad flatworms to study the colonization process from shallow waters to bathyal wood falls.

Data availability. The data that support the findings of this study are available from the corresponding author, Y. O., upon reasonable request.

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Author contributions. Y. O. prepared the histological sections, conducted morphological observations, performed molecular analyses, and wrote the manuscript. T. M. collected the specimens and photographed the living polyclads. A. T. improved the description and the figures. All authors read and approved the manuscript.

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Competing interest. None.

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