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Description of the snail-eating flatworm in marine aquaria, *Pericelis tectivorum* sp. nov. (Polycladida, Platyhelminthes)

ISABEL L. DITTMANN[1], WOLFGANG DIBIASI[1], CAROLINA NOREÑA[2] & BERNHARD EGGER[1,3]

[1]*University of Innsbruck, Institute of Zoology, Technikerstr. 25, 6020 Innsbruck, Austria.*
E-mail: Isabel L Dittmann: isi.dittmann@gmail.com; Wolfgang Dibiasi: wolfgang@dibiasi.com
[2]*Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal 2, 28006 Madrid, Spain. E-mail: mcnnj92@mncn.csic.es*
[3]*Corresponding author. E-mail: bernhard.egger@uibk.ac.at*

Abstract

We describe a new marine snail-eating flatworm, *Pericelis tectivorum* **sp. nov.**, found in coral-bearing marine aquaria. *Pericelis tectivorum* **sp. nov.** is characterised by several differential characters of the external and internal morphology like 1) a long line of frontal eyes extending anteriorly; 2) the length of the penis papilla; 3) the spherical seminal vesicle; 4) the lack of the enlargements of the ejaculatory duct; 5) the uterine vesicles, which start posterior of the female genital at the level of the sucker and 6) the distinct sucker. The combination of these characters in one species is unique and therefore the studied specimens are recognised by us as a new species. We additionally present a phylogenetic reconstruction using partial 28S rDNA sequences including three congeners. Our analysis demonstrates that *P. tectivorum* **sp. nov.** differs also genetically from other *Pericelis* species included in this analysis.

Key words: Leopardenstrudelwurm (German), aquarium predator, integrative taxonomy, Cotylea, morphology

Introduction

The Polycladida is a large taxon of free-living flatworms, which is found almost exclusively in marine habitats. Most of these species are known from tropical seas (Newman & Cannon 1995, Bahia *et al*. 2017). Sometimes, these animals can also be found in marine aquaria, where they are often noted due to their preying behaviour, especially if their prey is of interest to humans (Rawlinson *et al*. 2011). Most polyclads prey on a variety of marine invertebrates, like molluscs, urochordates, crustaceans or cnidarians, whereas some polyclads show prey preference (Rawlinson *et al*. 2011). Worldwide about 1000 species have been scientifically described so far (Bahia *et al*. 2017), including four species of the genus *Pericelis* Laidlaw, 1902.

Pericelis belongs to the suborder Cotylea (Lang 1884), although some features like a large encapsulated brain with well-defined globuli cell masses, a long, ruffled pharynx, the anteriorly directed uteri or the location of the copulatory complex in the posterior body quarter are more representative for the suborder Acotylea (Poulter 1974, Quiroga *et al*. 2015). Living *Pericelis* can be classified as cotyleans primarily because of their tiny marginal tentacles and their sucker posterior to the female genital opening (Poulter 1974). The dorsal marginal eyes, which can be found in a continuous series all around the body, characterise the genus *Pericelis* and many other polyclad genera, such as *Aprostatum*, *Ilyplana*, *Nonatoma*, or *Parastylochus* (Prudhoe 1985). Besides the type species *P. byerleyana* (Collingwood, 1876), three additional species are recognised, *P. hymanae* Poulter 1974, *P. cata* Marcus & Marcus, 1968 and *P. orbicularis* (Schmarda, 1859).

In this study, we describe a new representative of this genus, *Pericelis tectivorum* **sp. nov.** Our description is based on live observations and pictures, serial histological sections and comparison of partial 28S rDNA sequences with other representatives of the genus *Pericelis*.

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Material and methods

Animals. The animals were obtained from a commercial coral-bearing marine aquarium shop in Innsbruck and a private coral-bearing marine aquarium in Landeck (Austria). Of eleven individuals in total, four animals were used; three specimens (numbered #5, #7 and #10; all from Innsbruck) for histological and molecular investigation, and another just for molecular investigations (#2; from Landeck). Only data of two sectioned animals are shown (#5, #10). All eleven collected individuals were used for live observations, and were kept in darkness in plastic boxes (ca. 15 x 20 cm) at room temperature. Feeding behaviour was observed by adding snails to the plastic boxes containing worms, and periodically checking if the food was accepted.

Histology. Animals were fixed using the techniques described in Lee *et al*. (2006). Specimens were placed on filter paper, which was transferred on frozen fixative, dropping cold fixative on the animal's dorsal surface. The fixative solution was 3.5% formaldehyde (FA) in phosphate buffered saline (PBS). The copulatory region lies just posterior to the pharynx, hence for two specimens (#5, #7), we fixed only the posterior fourth of the body of sexually mature specimens, which was cut off the living animal with a razor blade. Only one specimen (#10) was used entirely. A soft brush was used for positioning and to ensure the specimens remained flat under the fixative (Lee *et al*. 2006). The specimens were left for at least twelve days in fixation solution at 4°C (fridge). Subsequently, the specimens were washed (PBS; aqua dest.), dehydrated in an ethanol series, cleared with intermedium (methyl benzoate over night; benzene 30 min), submerged in a 1:3 benzene:paraplast solution overnight and then embedded in paraplast. Specimens were serially sectioned at about 25–30 µm (#5) and at 10 µm (#7, #10) and stained with Azan trichromic stain after Romeis (1989).

Documentation. Live animals (#2, #5, #7) were photographed with a Canon EOS 5D Mark II camera equipped with a Canon Lens EF 100 mm 2.8L Macro IS USM objective and a Canon Macro Twin Lite MT-26EX-RT flash. Sections were documented with a Leica DM 5000B compound microscope equipped with a Leica DFC 490 digital camera for photographing. Further image processing was performed with Adobe Photoshop CS2 and 7. Drawings were produced in Adobe Illustrator CS2 and CS6.

DNA extraction, PCR amplification and sequencing. DNA was extracted from a small piece of marginal tissue and stored in absolute ethanol. DNA extraction was performed following a phenol-chloroform protocol (Chen *et al*. 2010). Concentration and quality of extracted DNA was checked by NanoDrop (NanoDrop Fluorospectrometer Thermo Fisher Scientific, USA). Partial 28S rDNA markers were amplified using published 28S primers (see Table 1 for primer sequences and references). Polymerase chain reaction (PCR) was performed using Taq DNA polymerase (New England BioLabs, USA), 1 µl of forward and reverse primers and between 8.6 ng and 107 ng of DNA template. #2, #7 and #10 were amplified with a standard PCR protocol, while for #5 a touchdown protocol was employed. The standard PCR protocol was: 5 min of initial denaturation at 94°C; and then 35 cycles of: 30 s of denaturation at 94°C; annealing at 53°C for 30 s; extension at 72°C for 2 min. Final extension at 72°C for 10 min. The touchdown PCR conditions were: 5 min of initial denaturation at 94°C; 12 cycles of 30 s of denaturation at 94°C; annealing at 68–45°C for 30 s; extension at 72°C for 2 min. 23 cycles of 30 s of denaturation at 94°C; annealing at 45°C for 30 s; extension at 72°C for 2 min. Final extension at 72°C for 10 min. Successful amplicons were purified with the Wizard® SV gel and PCR clean-up system (Promega, USA) according to the manufacturer's quick protocol. The purified PCR products were sequenced by Microsynth Austria GmbH, using premixed primers listed in Table 1. Sequences were assembled and edited using the software CLC Main Workbench 7 (https://www.qiagenbioinformatics.com/).

TABLE 1. Primers.

28S_LSU5_fw	TAGGTCGACCCGCTGAAYTTAAGCA	Larsson *et al*. 2008	Specimens: #2; #5; #7; #10
L1642R	CCAGCGCCATCCATTTTCA	Larsson *et al*. 2008	#2; #7
28S_6R	GGAACCCTTCTCCACTTCAGT	Charbagi-Barbirou *et al*. 2011	#5; #10

Dataset for phylogenetic analyses. Additional partial 28S sequences were downloaded from NCBI (*Pericelis orbicularis* EU679116.1; *Pericelis cata* EU679115.1; *Pericelis cata* EU679114.1; *Pericelis byerleyana* MH047291.1. As outgroup, *Theama* sp. KC869845.1 was selected and downloaded. Accession numbers of the newly generated sequences of *Pericelis tectivorum* **sp. nov.** are MK181524 (#2, Landeck), MK181525 (#5, Innsbruck), MK181526 (#7, Innsbruck) and MK181527 (#10, Innsbruck). The dataset was aligned using the MAFFT Q-INS-i algorithm (Katoh & Standley 2013) and sequences were manually trimmed to a length of 941 nucleotides. Phylogenetic reconstruction using Bayesian inference was done with MrBayes 3.2.6 (Ronquist *et al*. 2012) with the settings 'lset nst=6 rates=invgamma' (as suggested by jModelTest 2.1.10, Posada 2008) for 10

million generations (samplefreq=100, diagnfreq=1000). Standard deviation of split frequencies was well below 0.001, and the first 25% of trees were discarded as burn-in.

Results

Rhabditophora Ehlers, 1985

Order Polycladida Lang, 1881

Suborder Cotylea Lang, 1884

Superfamily Periceloidea Laidlaw, 1902

Family Pericelidae Laidlaw, 1902

Genus *Pericelis* Laidlaw, 1902

Pericelis tectivorum sp. nov.

(Figs. 1–6)

Material examined. *Pericelis tectivorum* **sp. nov.** specimens #5; #10, sagittally sectioned.

Type material. Sagittal serial sections of the holotype (NHMW_EvMicro 5771/1-45) and paratype (NHMW EvMicro 5772/1-250), and marginal tissue sample in 100% ethanol of the paratype (NHMW EvWet 21221) submitted to the Natural History Museum Vienna, Austria).

Holotype. One sagittally sectioned specimen (#5) stained with Azan.

Paratype. One sagittally sectioned specimen (#10) stained with Azan.

Type locality. Commercial seawater aquarium 'Alpenriff' in Innsbruck (Tirol; Austria).

Other material observed. Live observations of four live specimens from Landeck (including specimen #2), seven from Innsbruck (including specimens #5, #7 and #10). Partial 28S sequences of one specimen from Landeck (#2) and three specimens from Innsbruck (#5, #7, #10). **Histological remarks.** One sample, #7, was found to be not fixed well and the tissue appeared broken and disjointed in the sections. Therefore, it was not used for further analyses. The Azan trichrome staining looks markedly different in colour and intensity between holotype (#5) and paratype (#10), although the same recipe was used. The thickness of the sections (25–30 µm in #5 and 10 µm in #10) and the age of the prepared solutions may have influenced the appearance.

Etymology. After the observed prey of the species, *Tectus fenestratus* (Gmelin, 1791), a snail of the family Tegulidae and after the Latin word 'vorare' which means 'devour' in English.

Synonym. In German, this or a related species are referred to as the 'Leopardenstrudelwurm' (the 'leopard turbellarian').

Appearance. Elongated oval body, holotype 7 cm long and 4 cm wide (paratype: 5 cm long and 3 cm wide). The pharynx length is about 50% of the body length. Two thin marginal tentacles at anterior body edge (Fig 1A, B). Margin slightly ruffled. Cerebral eyes clusters posterior to a well-marked V-shaped notch between the tentacles, well separated, elongated, oval in form, directly merging to a line of frontal eyes extending in a fan-like shape towards the tentacles. From anterior to posterior, the line of frontal eyes is about the same length as the cerebral eye cluster (Fig. 2). Tentacle eyes are especially dense at the tips (Figs. 1B, 2). Dorsal colouration with white spots on dark brown background, highest density of spots along the margins, darkest in colour along the median line (Fig. 1A). Ventral colouration: whitish, nearly bluish grey, dendritic markings in the shade of pearl white (Fig. 1D). Genital pores in the posterior third. Male and female genital pores very close (ca. 50 µm apart) to each other, but no common gonopore. Sucker lies just posterior to the female gonopore (Figs. 1E, 3A, 4B).

Reproductive system. In both, the holotype and the paratype, the male and female copulatory organs are well developed (Fig. 3). Male copulatory complex shows a spherical seminal vesicle (Fig. 5E–K); two paired, heavily muscularised spermiducal bulbs (Fig. 5A–B, T–W), laterally orientated. Without prostatic vesicle or prostatic glands; ejaculatory duct narrow and long. Penis papilla cylindrical (0.5–0.6 mm long), U-shaped (holotype, Figs.

5G–P, 6A) or pointing ventro-posteriorly (paratype, Fig. 4A, C). Tapered in the last distal section. The whole penis papilla projects into the male atrium.Female genital complex with about six uterine vesicles per side, starting posteriorly at the level of the sucker and proceeding anteriorly (Figs. 3A, 4B). Female atrium or vagina externa of *P. tectivorum* **sp. nov.** runs upwards from the female gonopore and expands at the level of the cement pouch (Figs. 3B, 4A). Vagina interna narrows afterwards, turns downwards and opens into the oviduct.

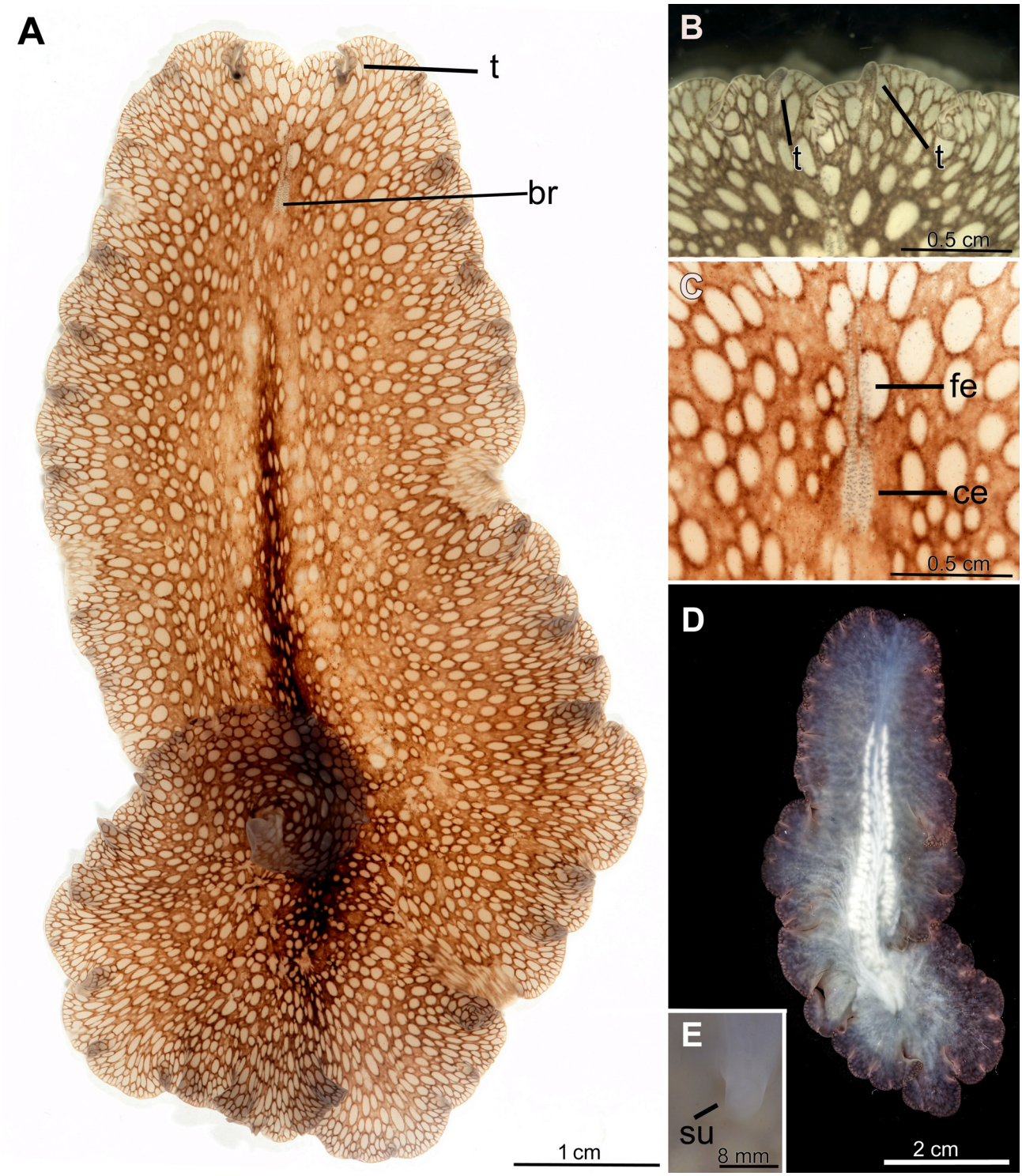

FIGURE 1. Live images of *P. tectivorum* **sp. nov. A.** Dorsal view. **B.** Detailed view of the tentacles. **C.** Detailed view of the cerebral and anteriorly extending frontal eyes. **D.** Dorsal view. **E.** Detailed view of the sucker. br = brain; ce = cerebral eyes; fe = anteriorly extending frontal eyes; su = sucker; t = tentacle. Orientation: anterior directed upwards.

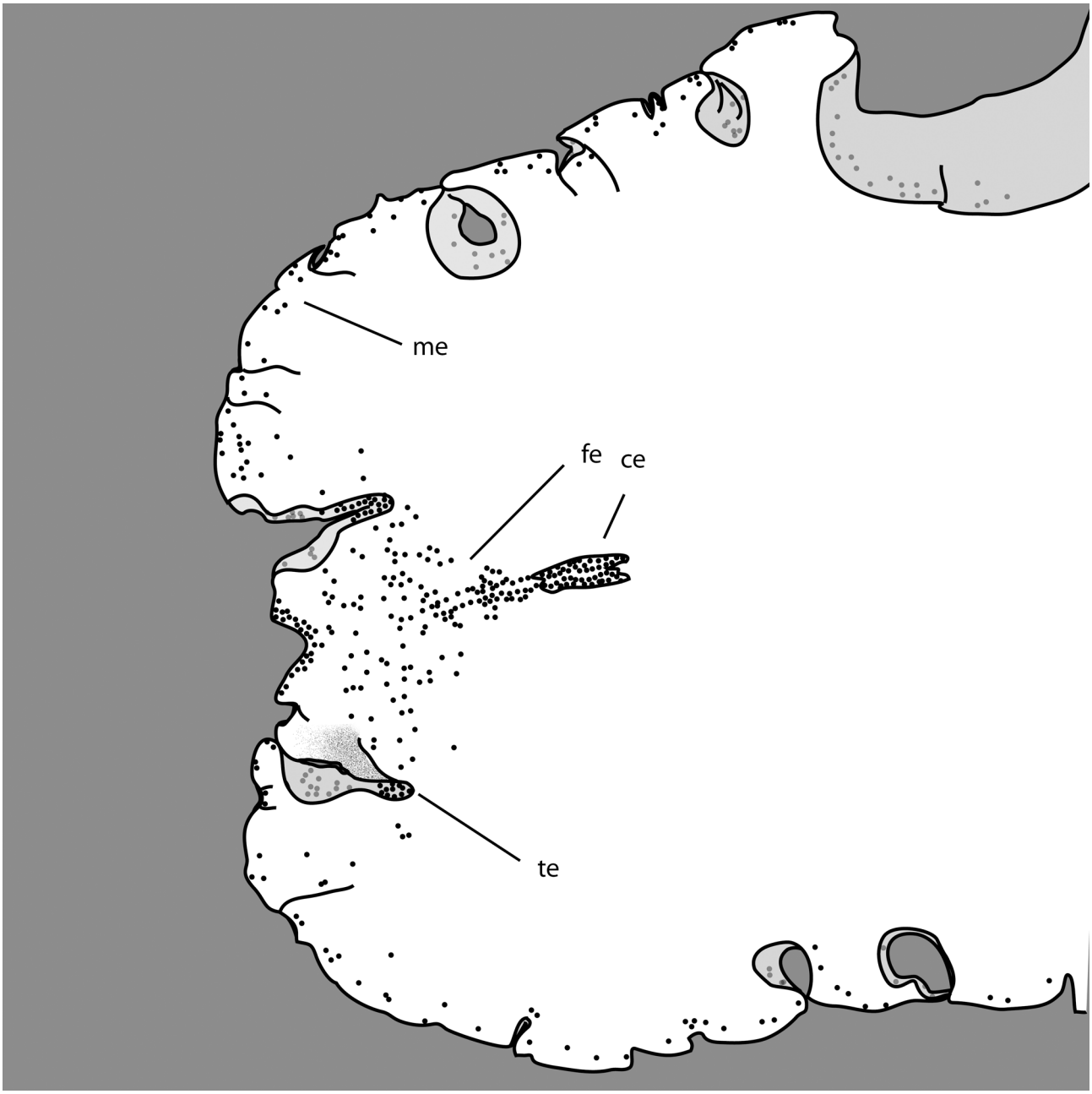

FIGURE 2. Drawing of the eye configuration of *P. tectivorum* **sp. nov.** ce = cerebral eyes; fe = frontal eyes; me = marginal eyes; te = tentacle eyes. Orientation: anterior to the left.

Lab cultures and feeding. *Pericelis tectivorum* **sp. nov.** was observed to prey on *Tectus fenestratus* mainly at night. During daytime, worms were hidden under stones or other objects in the commercial aquarium (pers. com. Christian Hepperger). In lab cultures, the worms were observed to slide over the snail shell and stay in this position for several minutes. In some cases, the snail started strongly to turn back and forth. However, we were not able to recognise if this was active movement of the snail or if it was moved by *P. tectivorum* **sp. nov.** The feeding act could not be observed, but devoured snails were recognised by the presence of empty snail shells and associated opercula. Both the snail shell and the operculum were intact and not damaged by the worm. Dark food particles were clearly noticeable in the gut of *Pericelis*.

Molecular analyses based on partial 28S rDNA sequences. In our phylogenetic reconstruction of four different species of the genus *Pericelis* (Fig. 7), the specimens of *P. tectivorum* **sp. nov.** are recovered with maximal support as sister group of *P. byerleyana*, and these two are sister group of *P. orbicularis*. *P. cata* is sister group of all other available *Pericelis* species.

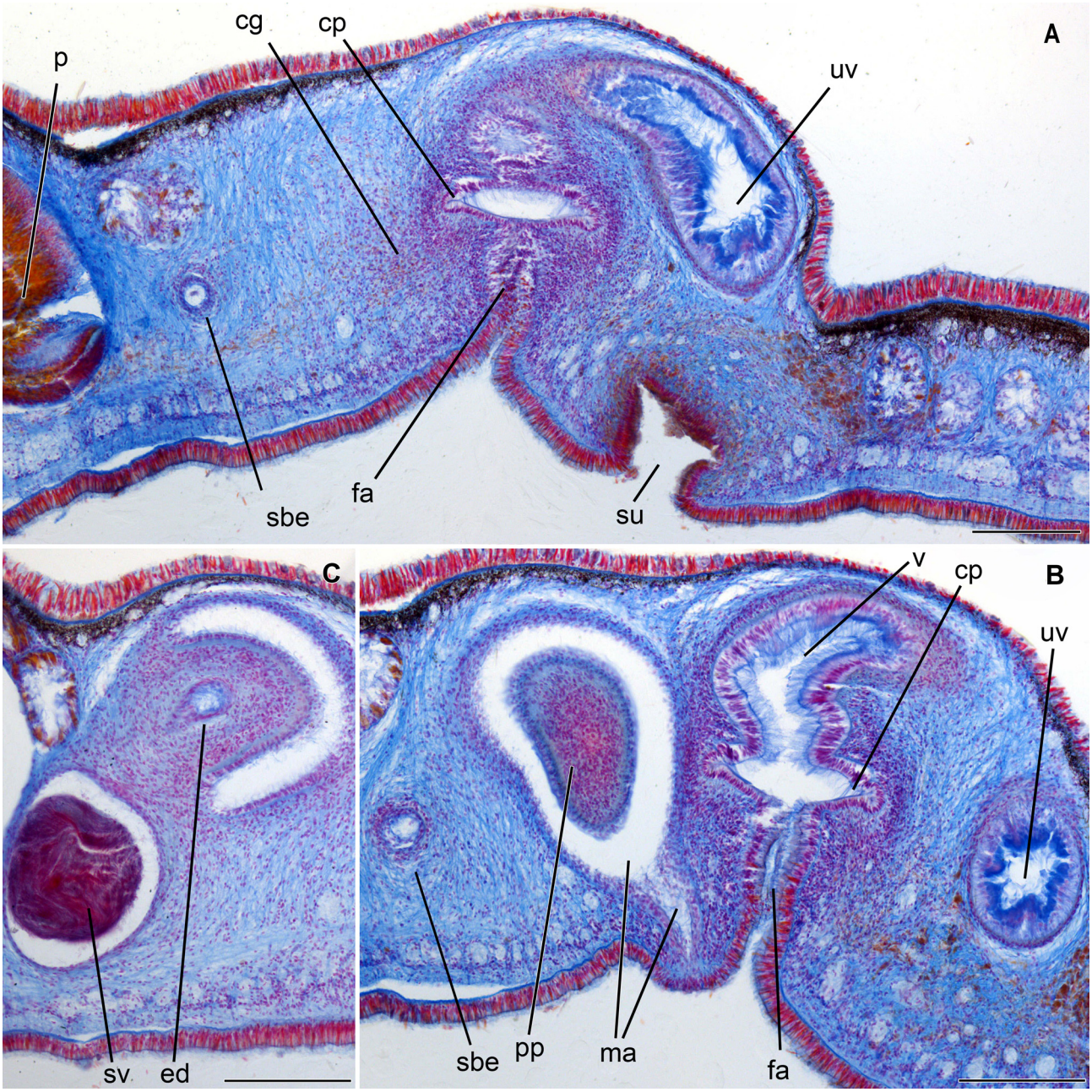

FIGURE 3. Sagittal sections of male and female genitals, as well as of the sucker of *P. tectivorum* **sp. nov.** (holotype). **A.** Female genitals and sucker. **B.** Penis papilla and female genitals. **C.** Spherical seminal vesicle and penis papilla. cg = cement glands; cp = cement pouch, ed = ejaculatory duct; fa = female atrium; ma = male atrium; p = pharynx; pp = penis papilla; sbe = spermiducal bulb entrance; su = sucker; sv = seminal vesicle; uv = uterine vesicle; v = vagina. Orientation: anterior to the left. Scale 200 µm.

Sequence identity was found to be 100% between all sequences of *Pericelis tectivorum* **sp. nov.** (one specimen from Landeck, three from Innsbruck) in the 941-nucleotide partial 28S alignment. Between *P. tectivorum* **sp. nov.** and *P. byerleyana*, 99.35% identity (6 nucleotides difference), and between *P. tectivorum* **sp. nov.** and *P. orbicularis*, 98.18% identity (17 nucleotides difference) was observed.

In a longer alignment with a length of 1362 nucleotides of only *P. tectivorum* **sp. nov.** sequences, three sequences from Landeck and Innsbruck were 100% identical (#2, #5, #10), while #7 was in two nucleotide positions (99.85% identity).

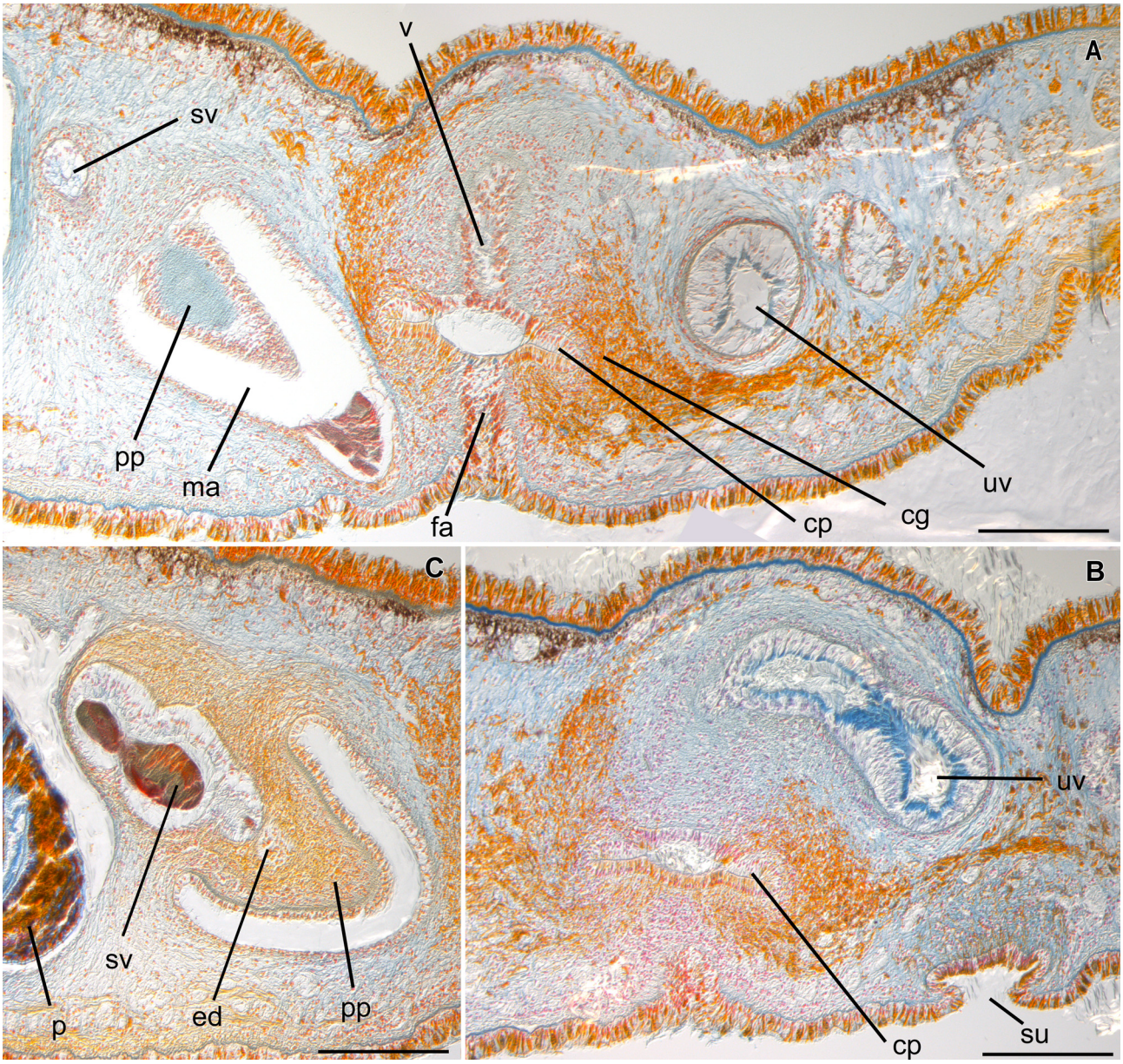

FIGURE 4. Sagittal sections of male and female genitals, as well as of the sucker of *P. tectivorum* **sp. nov.** (paratype). A. Penis papilla and female genitals. B. Female genitals and sucker. C. Spherical seminal vesicle and penis papilla. cg = cement glands; cp = cement pouch, ed = ejaculatory duct; fa = female atrium; ma = male atrium; p = pharynx; pp = penis papilla; su = sucker; sv = seminal vesicle; uv = uterine vesicle; v = vagina. Orientation: anterior to the left. Scale 200 µm.

Discussion

The family Pericelidae is riddled by a variety of divergent descriptions (see Table 2) of *Pericelis byerleyana* (Collingwood, 1876), the type of the genus (Laidlaw 1902). Descriptions by Collingwood (1876), Laidlaw (1902), Meixner (1907), Kato (1943) or Velasquez *et al*. (2018) about morphology and anatomy of different specimens of the nominally same *Pericelis* species vary widely (see Table 2) and make the determination more difficult. Additionally, some *Pericelis* species resemble each other in some points of the external morphology, like colour and eye patterns. In total, four species of the genus *Pericelis* have been described so far: *P. orbicularis* (Schmarda, 1859), *P. byerleyana* (Collingwood, 1876), *P. cata* Marcus & Marcus, 1968, and *P. hymanae* Poulter, 1974. Therefore, it is important to compare all morphological characters of the new species, *P. tectivorum* **sp. nov.**, with the four other well-known species (Table 2) and distinguish between the available molecular sequences.

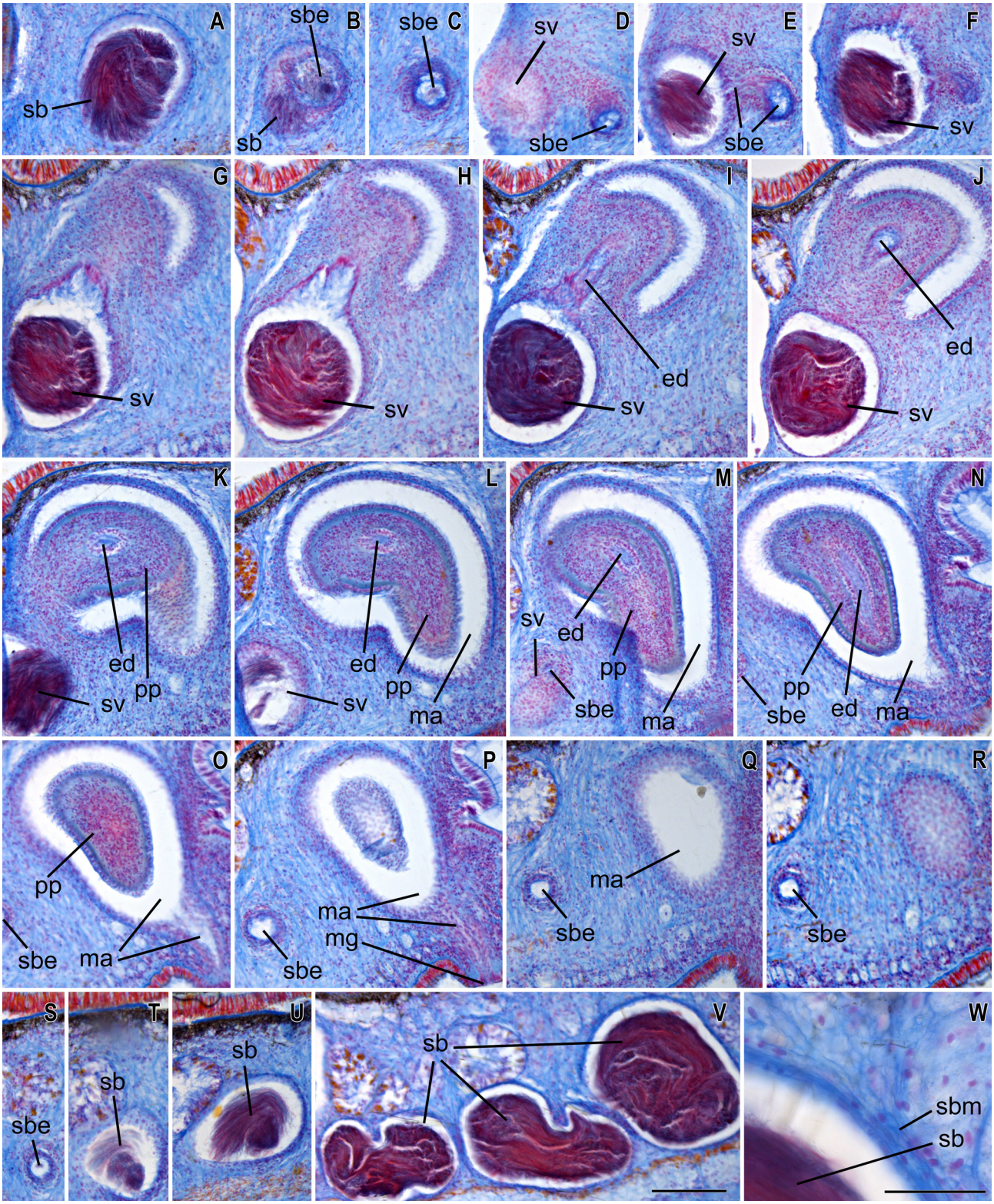

FIGURE 5. Sagittal serial sections of the male genital of *P. tectivorum* **sp. nov.** (holotype). A–D. First spermiducal bulbs with entrance. E. Junction between spermiducal bulbs to seminal vesicle. F–J. Seminal vesicle. K–N. Penis papilla. O–R. Male atrium. S–W. Second spermiducal bulbs with entrance. ed = ejaculatory duct; ma = male atrium; mg = male gonopore; pp = penis papilla; sb = spermiducal bulbs; sbm = sperm bulb muscles; sbe = spermiducal bulbs entrance; sv = seminal vesicle. Orientation: anterior to the left. Scale A-V 200 µm, W 50 µm.

External morphology. *Colour and pattern.* The colouration of *P. tectivorum* **sp. nov.**, which consists of white spots on dark brown background, is almost identical to the colouration of *P. byerleyana*, which is described as

"beautifully marbled, with light brown rings" by Collingwood (1876). However, except for *P. hymanae*, which is coloured off-white (Poulter 1974), all species of the genus *Pericelis* resemble each other in the combination of colour pattern, cream or beige with a reticulated brown pattern. But even if the combination of colour pattern is similar, they are not identical. The dorsal side of *P. cata* shows a dark pattern interrupted by round white areas, which sometimes coalesce to larger blotches. Some of the dark patches contain scattered black spots (Marcus & Marcus 1968). *Pericelis orbicularis* is coloured by a reddish brown, fine-lined network on a paler ground (Schmarda 1859; Hyman 1955; Marcus & Marcus 1968). However, the colouration, which often differs intra-specifically, is not sufficient for species identification, but gives us a first indication that the new species belongs neither to *P. hymanae,* nor to *P. cata*. Only looking at the colouration, the distinction between *P. tectivorum* **sp. nov.***, P. byerleyana* and *P. orbicularis* is problematic.

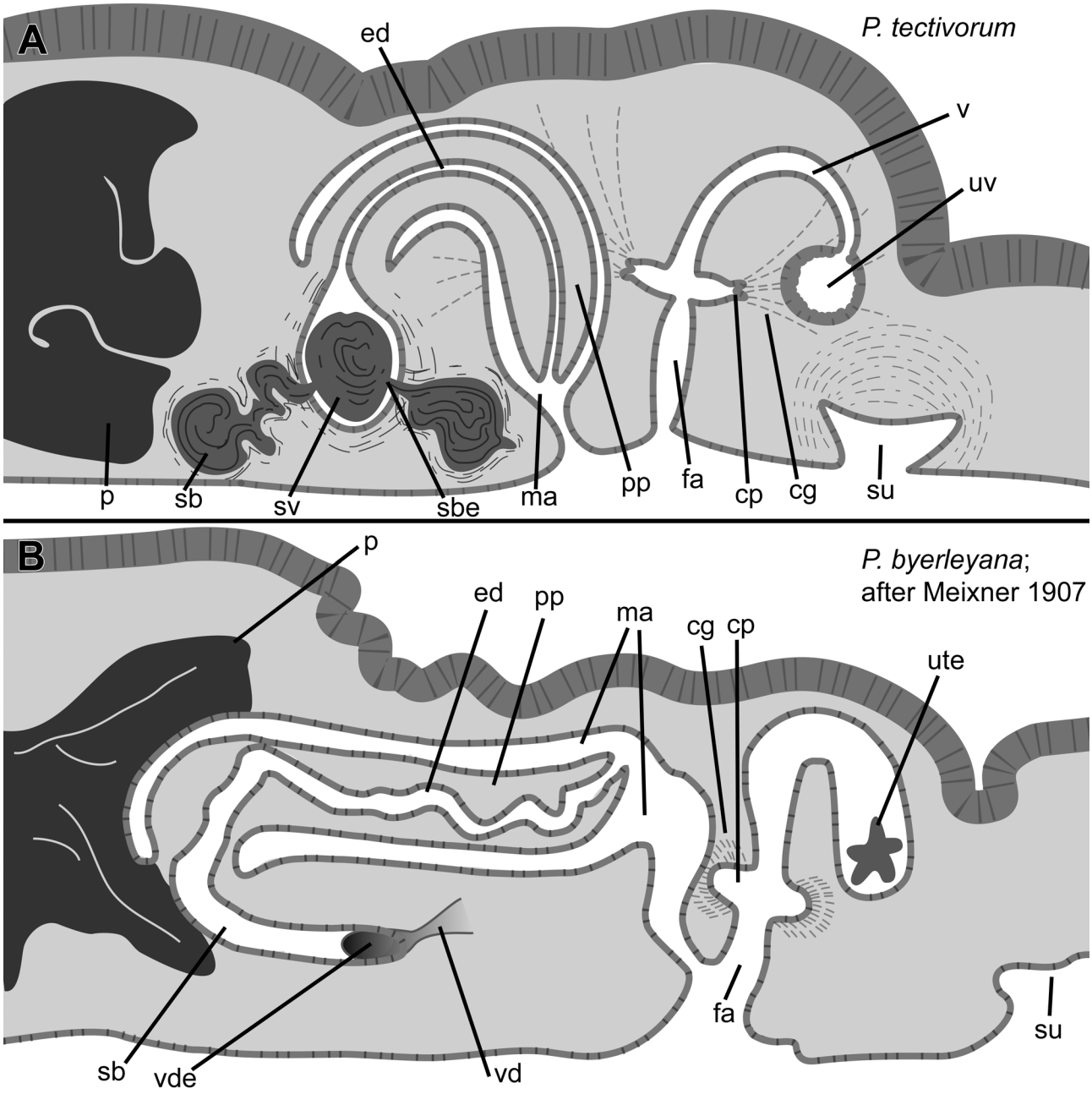

FIGURE 6. Comparison of the genitals of *P. tectivorum* **sp. nov.** and *P. byerleyana*. A. Reconstruction of the genital of *P. tectivorum* **sp. nov.** B. Reconstruction of the genital of *P. byerleyana* after Meixner 1907. cg = cement glands; cp = cement pouch ed = ejaculatory duct; fa = female atrium; ma = male atrium; p = pharynx; pp = penis papilla; sb = spermiducal bulbs; sbe = spermiducal bulbs entrance; su = sucker; sv = seminal vesicle; uv = uterine vesicle; ute = uterine entrance; v = vagina; vd = vasa deferentia; vde = vasa deferentia entrance. Orientation: anterior to the left.

Eye spot cluster. Another informative character is the configuration and form of eye spot clusters, in particular those of cerebral and frontal eyes. A clear delimitation of cerebral and frontal eyes is often difficult. The two clusters of cerebral eyes of *P. tectivorum* **sp. nov.** are separated, elongated, oval-shaped and directly merging to a line of frontal eyes extending in a fan-like shape anteriorly (Fig. 2). This configuration resembles the description of the cerebral eye cluster of *P. byerleyana* (Laidlaw 1902; Meixner 1907), however, the line of frontal eyes (excluding the more anterior fan-shaped part) in *P. tectivorum* **sp. nov.** is about double in length than in *P. byerleyana* (Fig. 8A, B) compared to the length of the cerebral eye cluster.

The cerebral eyes of *P. hymanae* are described and drawn as paired elongated oval groups located behind the anterior margin, with frontal eyes fanning out towards the tentacles and few frontal eyes in the region of the midline (Poulter 1974). The description of the cerebral eye clusters of *P. hymanae* is distinctly different from the eye clusters in *P. tectivorum* **sp. nov.** (Fig. 8A, F).

Like *P. hymanae,* the cerebral eye cluster of *P. cata* forms an elongated oval cluster (e.g. Marcus & Marcus 1968, Bahia & Padula 2009, Queiroz *et al*. 2013) different, therefore, from *P. tectivorum* **sp. nov.** (Fig. 8A, C).

The cerebral eyes of *P. orbicularis* are described in different ways. Schmarda (1859) and Stummer-Traunfels (1933) draw the cerebral eyes as two well separated stripes forming a wedge (Schmarda 1859; Stummer-Traunfels 1933) (Fig. 8E). On the other hand, Hyman (1955) describes "a group of eyes, scarcely paired, overlies the brain region and from these cerebral eyes cerebro-frontal eyes spread to the anterior margin in a fanlike manner". In contrast, and similar to Schmarda (1859) and Stummer-Traunfels (1933), Marcus & Marcus (1968) describe the cerebral eye clusters as two broad stripes running close to each other, not as a loose cluster as drawn by Hyman (1955) (Marcus & Marcus 1968) (Fig. 8D). The description of Marcus & Marcus (1968) for *P. orbicularis* is less clear, as no related figure is available. We cannot clarify this controversy, but we may say that neither of these descriptions of the eye cluster of *P. orbicularis* resemble the eye clusters of *P. tectivorum* **sp. nov.** (Fig. 8A, D, E). In conclusion, the eye spot cluster of *P. tectivorum* **sp. nov.** is closest to *P. byerleyana*, but differs from all descriptions of the genus *Pericelis.*

Size. The largest specimen of *P. tectivorum* **sp. nov.** was up to 7 cm long and up to 4 cm wide, while *P. byerleyana* was found with a body length of up to 6 cm (Kato 1943), but most often with a significantly shorter body of 1.8 to 3.5 cm (see Table 2). The largest animal found of *P. hymanae* has a reported length of about 4.8 cm (Poulter 1974), and that of *P. cata* of about 6 cm (Marcus & Marcus 1968). The smallest species described is *P. orbicularis* with a length of up to 2.5 cm (Marcus & Marcus 1968).

Pharynx. In *P. tectivorum* **sp. nov.** the pharynx length as a percentage of body length is about 50%, similar to all other described *Pericelis* species except *P. orbicularis*, where the relative pharynx length is noticeably smaller (see Table 2).

Internal morphology. For species determination of polyclads, the internal morphology is often decisive. The most informative character for identification of flatworm species in general and indeed for animals belonging to the genus of *Pericelis* are the copulatory organs (Marcus & Marcus 1968).

Male copulatory complex. The penis papilla of the holotype of *P. tectivorum* **sp. nov.** is strongly bent in a U-shape (Figs. 5, 6), whereas the penis papilla of the paratype is straight (Fig. 4). The description of *P. byerleyana* by Laidlaw (1902) defines the penis as muscular, directed backwards, conical in shape and tapering to a fine point, which projects into a long and extremely narrow male atrium. Meixner (1907) describes the penis of *P. byerleyana* as long, cylindrical in form, just tapering before the distal end and located parallel to the ventral side of the body (Fig. 6B). These inconsistencies in the descriptions of the shape and bending of the male genitals within the same species are likely to result from their position at the moment of fixation. The bending of the penis papilla might therefore be a rather weak character for species determination. However, the length of the penis papilla of *P. tectivorum* **sp. nov.** measures between 0.5 and 0.6 mm, whereas the penis papilla of *P. byerleyana* is 1.1 mm long (Meixner 1907) even though the overall body length of *P. tectivorum* **sp. nov.** is twice as large as *P. byerleyana*. The bending of the penis papilla is absent in *P. hymanae, P. cata* and *P. orbicularis*, and their respective lengths are 0.7 mm, 0.5 mm and unknown (see Table 2).

Also interesting to note is the very prominent, spherical seminal vesicle of *P. tectivorum* **sp. nov.** All species of *Pericelis,* except *P. byerleyana,* have a very prominent seminal vesicle. Meixner (1907) characterises the seminal vesicle of *P. byerleyana* as not very prominent. He assumes that an extremely muscular, elongated section of the ejaculatory duct without enlargement of the lumen is able to function as seminal vesicle (Meixner 1907). The seminal vesicle of *P. tectivorum* **sp. nov.** is in contrast strongly enlarged and spherical in form, similar to *P. hymanae, P. cata* and *P. orbicularis.*

Following the argument of Poulter (1974), another distinctive character is the nature of the ejaculatory duct. *Pericelis tectivorum* **sp. nov.** together with *P. hymanae, P. byerleyana* and *P. cata* lacks the enlargement of the ejaculatory duct shown in *P. orbicularis* (Poulter 1974). The luminal enlargement of the ejaculatory duct of *P. orbicularis,* drawn by Hyman (1955, fig 4), is interpreted by Hyman (1955) as a prostatic vesicle, however no evidence of prostatic secretion was found (Hyman 1955). In *P. orbicularis*, this muscular widening of the ejaculatory duct is lined with a granular secretory epithelium (Stummer-Traunfels 1933). This granular secretory epithelium, but without an enlargement of the ejaculatory duct, can also be found in *P. byerleyana (*Meixner 1907) and *P. cata* (Hyman 1955; Marcus & Marcus 1968), but not in *P. hymanae* (Poulter 1974) or in *P. tectivorum* **sp. nov.**

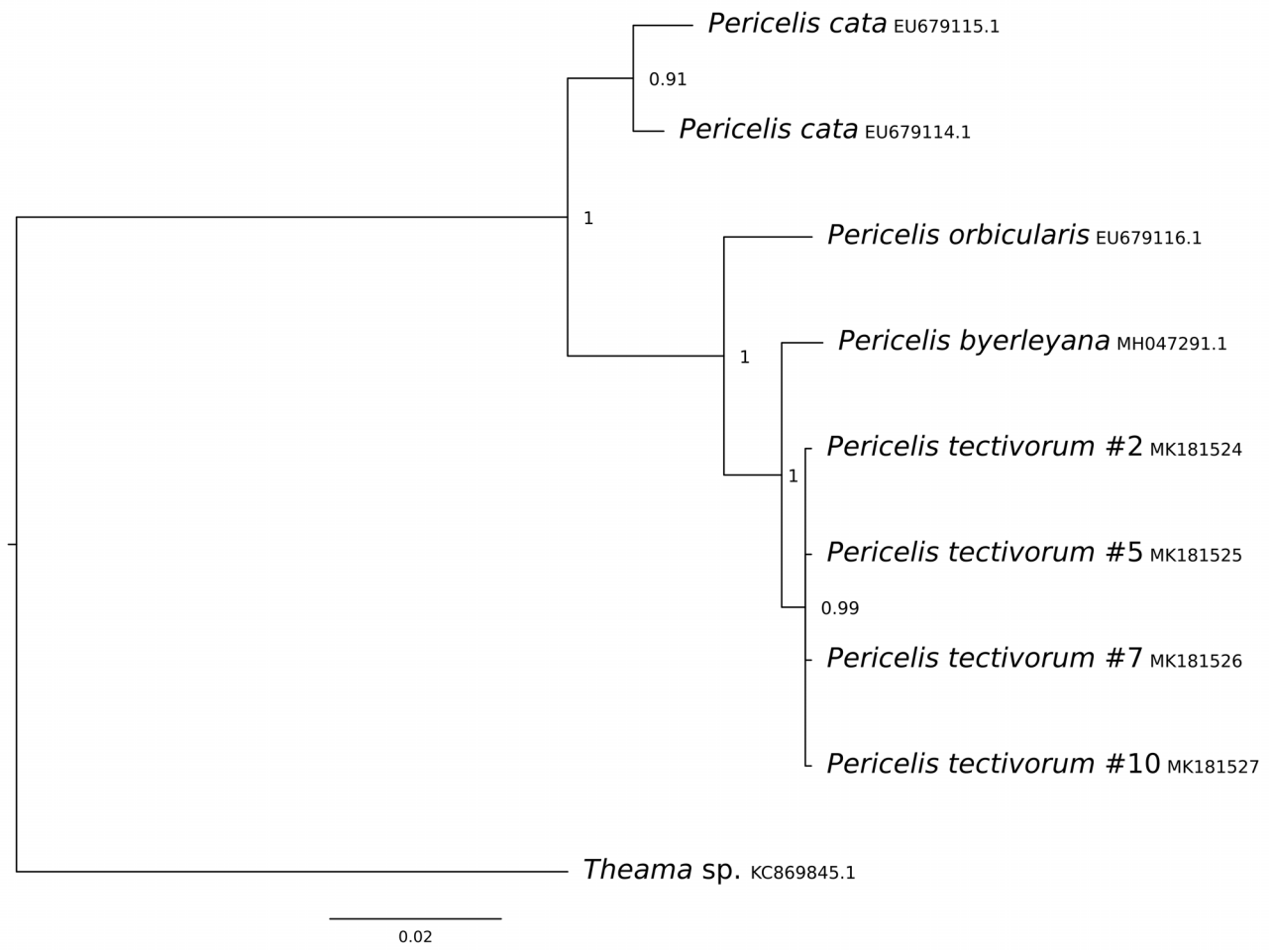

FIGURE 7. *Pericelis* genus-level partial 28S rDNA phylogeny using Bayesian inference, rooted with *Theama* sp. Accession numbers shown after species names.

Another factor to be taken into account is the size of the male atrium, which is the cavity that extends between the gonopore and the base of the penis papilla (Faubel 1984). Similar to *P. byerleyana* and *P. hymanae,* the male atrium of *P. tectivorum* **sp. nov.** rises almost up to the seminal vesicle (Meixner 1907; Poulter 1974). The male atrium of *P. orbicularis* and *P. cata,* in contrast, is much shorter and does not extend to the seminal vesicle (Hyman 1955, Marcus & Marcus 1968). Like in all congeners, the male atrium of *P. tectivorum* **sp. nov.** merges ventrally into the male gonopore. The male and the closely situated female gonopore of *P. tectivorum* **sp. nov.** are separated. This separation can also be found in *P. cata* and in *P. hymanae,* whereas the separation of the male and female gonopore in *P. byerleyana* and in *P. orbicularis* is not resolved, as the interpretation of the separation of the male and female gonopore is hypothesised to be strongly influenced by fixation artefacts and therefore not a useful differential character (Meixner 1907, Marcus & Marcus 1968), possibly similar to the bending of the penis papilla.

Female copulatory organ. Due to the presence of the uterine vesicles, Laidlaw (1902) suggests that the uteri

themselves are a very remarkable feature. In contrast to the uterine vesicles of *P. byerleyana* (Laidlaw 1902), *P. hymanae* (Poulter 1974) and *P. orbicularis* (Hyman 1955), which begin anterior to the female genital pore, the uterine vesicles of *P. tectivorum* **sp. nov.** begin behind the female genital pore, and run anteriorly. The latter feature can also be observed in *P. cata* (Marcus & Marcus 1968) and in *P. orbicularis* as depicted by Marcus & Marcus (1968) (see Table 2).

Sucker. The suckers of *P. tectivorum* **sp. nov.** (Fig. 5A), *P. cata* (Marcus & Marcus 1968, fig. 59) and *P. orbicularis* (Hyman 1955, fig. 4) are more developed than the suckers of *P. byerleyana* and *P. hymanae* (see Table 2). The latter are clearly smaller and less muscularised ('rudimentary sucker' according to Poulter (1974)).

Type locality. Both holotype and paratype of *Pericelis tectivorum* **sp. nov.** were obtained from the same aquarium. Unfortunately, after talking with the aquarium's owner it turned out that the different organisms and live rocks contained in the aquarium came from locations all over the world, and additionally some parts were obtained from other aquaria, whose contents were also derived from many different locations. The same problem exists for the animals derived from an aquarium in Landeck. Essentially, it is impossible to deduce the natural habitat of *P. tectivorum* **sp. nov.** at this point, but it seems likely that long term cultures are (involuntarily) maintained in these aquaria, as the worms were found for years without adding new material.

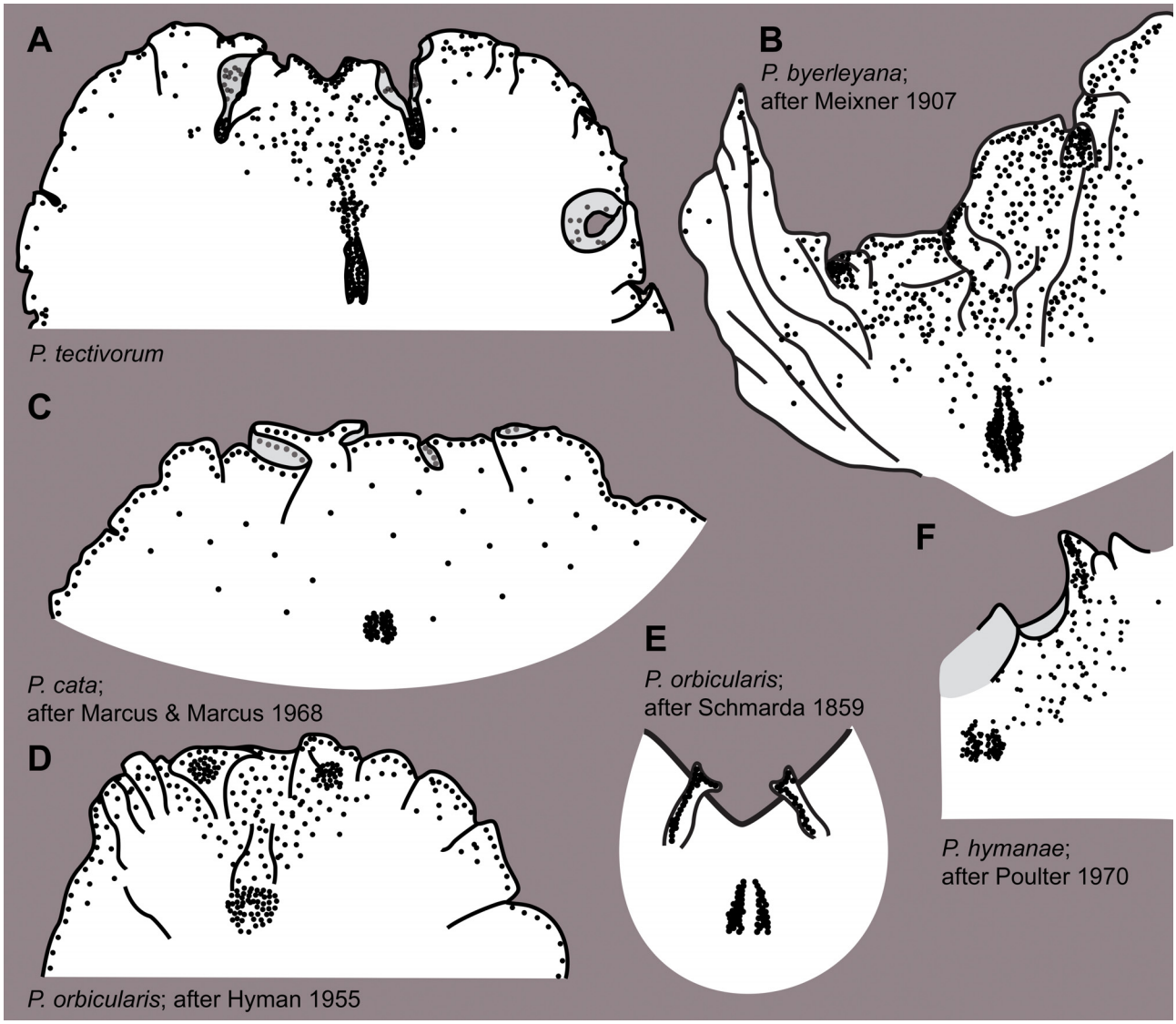

FIGURE 8. Schematic drawings of eye spot clusters in comparison. Anterior directed upwards. Not drawn to scale.

Molecular phylogeny and distribution. Based on the molecular phylogenetic tree (Fig. 7) and 100% sequence identity we find that *Pericelis* from both sampling localities (Landeck and Innsbruck) belong to the same species, *P. tectivorum* **sp. nov.**, which are distinctly different from all other available *Pericelis* sequences. The

sequence identity between *P. byerleyana* and *P. tectivorum* **sp. nov.** is quite high; either they are very closely related species, also reflected by some morphological similarities (see Table 2), or the specimen provided by Velasquez *et al*. (2018), which was not histologically examined and the colour and eye patterns of which most closely resemble *P. tectivorum* **sp. nov.**, could possibly be a representative of *P. tectivorum* **sp. nov.** instead of *P. byerleyana*. At this point, there are not enough sequences available to clarify this conjecture, but it is conspicuous that the sampling localities given for *P. byerleyana* span from the Eastern Arabian Sea to the Western Pacific Ocean, except for the specimen provided by Velasquez *et al*. (2018), which was found in the northern tip of the Red Sea (see Table 2).

Currently, we are not able to make a statement about the phylogenetic position of *P. hymanae* within the genus *Pericelis* and its relation to *P. tectivorum* **sp. nov.**, as no molecular data are available. The molecular data support our morphological findings very well, as *P. byerleyana* and *P. orbicularis*, in this order, are most similar to *P. tectivorum* **sp. nov.**, both in morphology and sequence identity.

TABLE 2. Comparison of colouration, pharynx length as a percentage of body length, line of frontal eyes, body size and genital organs of the genus *Pericelis.* **1** line of frontal eyes extending anterior; **2** body length [mm]; **3** bending of the penis papilla; **4** the length of the penis-papilla [mm]; **5** spherical seminal vesicle; **6** enlargement of the ejaculatory duct (prostatic vesicle); **7** length of the male atrium; **8** position of the first uteri vesicles; **9** sucker. + present, - absent, ? unclear, l long, s short, p posterior to the female genital, a anterior of the female genital, d distinct, r rudimentary.

Pericelis	author(s)/distribution	colouration	pharynx	1	2	3	4	5	6	7	8	9
***tectivorum* sp. nov.** (holotype)	this work; marine aquaria in Innsbruck	white ovals on brown background	ca. 50%	+	70	+	0.6	+	-	l	p	d
***tectivorum* sp. nov.** (paratype)	this work; marine aquaria in Innsbruck	white ovals on brown background	ca. 50%	+	50	-	0.5	+	-	l	p	d
byerleyana	Collingwood 1876; west coast of Borneo	marbled with brown rings, including roundish spaces of whitish colour	?	-	19	?	?	?	?	?	?	?
	Laidlaw 1902; Minicoy (Eastern Arabian Sea)	marbled with brown rings, including roundish spaces of whitish colour	?	-	35	-	?	-	-	s	a	r
	Meixner 1907; Moucha Island (Gulf of Tadjoura) and Pulau Jaga Utara (Java Sea)	marbled with brown rings, including roundish spaces of whitish colour	> 50%	-	18	+	1.1	-	-	l	?	r
	Kato 1943; Palau (Western Pacific Ocean)	marbled with brown rings, including roundish spaces of whitish colour	> 50%	-	60	+	?	-	-	l	?	r
	Velasquez *et al*. 2018; Eilat (Red Sea)	marbled with brown rings, including roundish spaces of whitish colour	> 50%	+	27	?	?	?	?	?	?	r
hymanae	Poulter 1974; Hawaii (Pacific Ocean)	white	33–53%	-	48	-	0.7	+	-	l	a	r
cata	Marcus & Marcus 1968; Curaçao (Caribbean Sea)	freckled	46–55%	-	60	-	0.5	+	-	s	p	d
orbicularis	Schmarda 1859; south coast of Jamaica (Caribbean Sea)	brown network on white ground	?	-	21	-	?	?	?	?	?	?
	Stummer-Traunfels 1933; Jamaica (Caribbean Sea)	brown network on white ground	20–25%	-	16	-	?	+	+	l	?	d
	Hyman 1955; Texas (Gulf of Mexico)	brown network on white ground	22–38%	-	35	-	?	+	+	l	a	d
	Marcus & Marcus 1968; Key Biscayne (Florida)	brown network on white ground	37%	-	25	-	?	?	?	?	p	d

Conclusion

In conclusion, *P. tectivorum* **sp. nov.** is most similar to *P. byerleyana* (Collingwood 1876) both morphologically and molecularly, but differs from *P. byerleyana* as well as from *P. hymanae* (Poulter 1974), *P. cata* (Marcus & Marcus 1968) and *P. orbicularis* (Schmarda 1859) in the following features: 1) a long line of frontal eyes extending anteriorly; 2) the length of the penis papilla in relation to the body length; 3) the spherical seminal vesicle; 4) the lack of the widening of the ejaculatory duct; 5) the uterine vesicles, which are starting posterior of the female genital at the level of the sucker and 6) the distinct sucker. The molecular data support *P. tectivorum* **sp. nov.** as a distinct species neither belonging to *P. byerleyana*, *P. cata,* nor to *P. orbicularis*.

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References

Bahia, J. & Padula, V. (2009) First record of *Pseudoceros bicolor* and *Pericelis cata* (Platyhelminthes: Polycladida) from Brazil. *Marine Biodiversity Records*, 2, 1–5. https://doi.org/10.1017/S1755267209000918

Bahia, J., Padula, V. & Schrödl, M. (2017) Polycladida phylogeny and evolution: integrating evidence from 28S rDNA and morphology. *Organisms Diversity & Evolution*, 17, 653–678. https://doi.org/10.1007/s13127-017-0327-5

Charbagi-Barbirou, K., Álvarez-Presas, M., Vila, M., Gammoudi, M., Tekaya, S. & Riutort, M. (2011) Marine triclads (Platyhelminthes: Tricladida: Maricola), a preliminary molecular approach to their phylogeny. *CBM-Cahiers de Biologie Marine*, 52 (3), 303.

Chen, H., Rangasamy, M., Tan, S.Y., Wang, H. & Siegfried, B.D. (2010). Evaluation of five methods for total DNA extraction from western corn rootworm beetles. *PLoS ONE*, 5 (8), e11963. https://doi.org/10.1371/journal.pone.0011963

Collingwood, D. (1876) VI. On thirty-one Species of Marine Planarians, collected partly by the late Dr. Kelaart, FLS, at Trincomalee, and partly by Dr. Collingwood, FLS, in the Eastern Seas. *Transactions of the Linnean Society of London.* 2nd Series: *Zoology*, 1 (3), 83–98.

Ehlers, U. (1985) *Das phylogenetische System der Plathelminthes*. G Fischer, Stuttgart, New York, pp. 317.

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

Gmelin, J.F. (1791) Vermes. In: Gmelin, J.F. (Ed.), *Caroli a Linnaei Systema Naturae per Regna Tria Naturae*, Ed. 13. Tome 1(6). G.E. Beer, Lipsiae [Leipzig]. pp. 3021–3910.

Hyman, L.H. (1955) A further study of the polyclad flatworms of the West Indian region. *Bulletin of Marine Science,* 5 (4), 259–268.

Kato, K. (1943). Polyclads from Palao. *Bulletin of the Biogeographical Society of Japan,* 13 (12), 79–90.

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution*, 30 (4), 772–780. https://doi.org/10.1093/molbev/mst010

Laidlaw, F.F. (1902) The marine Turbellaria, with an account of the anatomy of some of the species. *The Fauna and Geography of the Maldive and Laccadive Archipelagoes,* 1, 282–312.

Lang, A. (1881) *Der Bau von Gunda segmentata und die Verwandtschaft der Plathelminthen mit Coelenteraten und Hirudineen*. 3, 187–251.

Lang, A. (1884) *Die Polycladen (Seeplanarien) des Golfes von Neapel und der angrenzenden Meeres-Abschnitte: eine Monographie*. Wilhelm Engelmann, Leipzig, 688 pp.
Larsson, K. & Jondelius, U. (2008) Phylogeny of Catenulida and support for Platyhelminthes. *Organisms Diversity & Evolution.* 8 (5), 378–387.
https://doi.org/10.1016/j.ode.2008.09.002
Lee, K.-M., Beal, M. & Johnston, E.H. (2006) A new predatory flatworm (Platyhelminthes, Polycladida) from Botany Bay, New South Wales, Australia. *Journal of Natural History*, 39 (47), 3987–3995.
https://doi.org/10.1080/00222930500485263
Marcus, E. & Marcus, E. (1968) Polycladida from Curaç ao and faunistically related regions. *Studies on the Fauna of Curaçao and other Caribbean Islands,* 101, 1–133.
Meixner, A. (1907) Polycladen von der Somaliküste, nebst einer Revision der Stylochinen. *Zeitschrift für wissenschaftliche Zoologie,* 88, 368–498.
Newman, L.J. & Cannon, L.R.G. (1995) The importance of the fixation of colour, pattern and form in tropical Pseudocerotidae (Platyhelminthes, Polycladida). *Hydrobiologia,* 305 (1), 141–143.
https://doi.org/10.1007/BF00036376
Posada, D. (2008). jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, 25 (7), 1253–1256.
https://doi.org/10.1093/molbev/msn083
Prudhoe, S. (1985) *A monograph on polyclad Turbellaria.* London/Oxford: British Museum of Natural History and Oxford University Press.
Poulter, J.L. (1974) A new species of the genus *Pericelis*, a polyclad flatworm from Hawaii. *In*: Riser, N.W. & Morse, M.P. (Eds.), *Biology of the Turbellaria* McGraw-Hill, New York, pp. 93–107.
Queiroz, V., Sales, L., Neves, E.G. & Johnsson, R. (2013) *Pericelis cata* Marcus and Marcus, 1968 (Platyhelminthes: Polycladida): first record from northeast of Brazil. *Check List,* 9 (3), 628–630.
https://doi.org/10.15560/9.3.628
Rawlinson, K.A., Gillis, R.E., Billingings, Jr. & Borneman, E.H. (2011) Taxonomy and life history of the *Acropora*-eating flatworm *Amakusaplana acroporae* nov. sp. (Polycladida: Prosthiostomidae). *Coral Reefs,* 30 (3), 693–705.
https://doi.org/10.1007/s00338-011-0745-3
Romeis, B. (1989) *Mikroskopische Technik (17 Auflage)*. Verlag Oldenbourg, München, 697 pp.
Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology,* 61 (3), 539–542.
https://doi.org/10.1093/sysbio/sys029
Schmarda, L.K. (1859) *Neue wirbellose Thiere beobachtet und gesammelt auf einer Reise um die Erde 1853 bis 1857 (Vol. 1)*. Wilhelm Engelmann, Leipzig.
Stummer-Traunfels, R. (1933) Polycladida (continued). *In*: Bronn, H.G. (ed.), *Klassen und Ordnungen des Tier-Reichs IV. (Vermes)*. Akademic Verlagges, Leipzig, pp. 3485–3596.
Velasquez, X., Bolaños, D.M. & Benayahu, Y. (2018) New records of cotylean flatworms (Platyhelminthes: Polycladida: Rhabditophora) from coastal habitats of Israel. *Zootaxa,* 4438 (2), 237–260.
https://doi.org/10.11646/zootaxa.4438.2.2