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A new species of *Cycloporus* from the Adriatic Sea, with an updated phylogeny of the families Euryleptidae and Stylostomidae (Polycladida, Platyhelminthes)

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Abstract

We describe *Cycloporus pinkipus* sp. n., a new polyclad flatworm species from the Adriatic coast of Croatia using live images, histological sections, and a molecular marker. It is the fifteenth described species of *Cycloporus* Lang, 1884 and the second described congener in the Mediterranean. The genus *Cycloporus* is characterised by a small oval body, tentacular bumps and the name-giving marginal pores. *Cycloporus pinkipus* sp. n. has a smooth dorsal surface, which is transparent creamy white with light brown to yellow spots, covered with prominent serial pink spots on the inner rim of the body margin. There is little variation of the genital organs between different species of the genus, therefore we recognise *C. pinkipus* sp. n. as a new species in particular due to its unique coloration, and a unique partial large nuclear ribosomal subunit (28S) sequence. In recent years the family Euryleptidae Stimpson, 1857, which also contains the genus *Cycloporus*, was discussed and revised in several molecular studies. In an updated molecular phylogeny of the Polycladida based on partial 18S and 28S rDNA marker genes, *C. pinkipus* sp. n. was recovered in a clade of many other *Cycloporus* species within Euryleptidae.

Key words: Turbellaria, Cotylea, Taxonomy, Systematics, Histology

Introduction

The genus *Cycloporus* was established by Lang (1884) and currently comprises fourteen species (Newman & Cannon 2002; Pitale & Apte 2021). Because of its small size, *Cycloporus* is rather difficult to sample and can easily be overlooked (Newman & Cannon 2002). However, species of *Cycloporus* were found in marine waters all over the world, predominantly in Australian waters (Newman & Cannon 2002). Just one species, the type species *Cycloporus papillosus* (Sars, 1878), was found in the Mediterranean so far (Norenã *et al.* 2014). The genus *Cycloporus* is characterised by its small oval body, the bulb-like tentacles and the marginal pores. Most representatives of the genus *Cycloporus* are distinguished mainly through the presence or absence of dorsal papillae and their dorsal colouration (Newman & Cannon 2002), as is the case for many cotylean genera. Following the traditional classification, the genus *Cycloporus* belongs to the family Euryleptidae (Faubel 1984; Prudhoe 1985). Faubel (1984) characterises the family mainly by the presence of tentacles, a forward-directed cylindrical pharynx reaching up to the level of the brain, separated gonopores, and a forward-directed male copulatory organ with a true prostatic vesicle. In recent years both, Euryleptidae and *Cycloporus*, were found to be non-monophyletic in molecular studies (Bahia *et al.* 2017; Dittmann *et al.* 2019; Litvaitis *et al.* 2019). Besides the species description of *Cycloporus pinkipus* sp. n., we therefore generated a phylogenetic reconstruction using the partial 18S and 28S rDNA marker genes including *Cycloporus pinkipus* sp. n. The combination of previously published datasets (Bahia *et al.* 2017; Tsunashima *et al.* 2017; Dittmann *et al.* 2019; Litvaitis *et al.* 2019; Tsuyuki *et al.* 2020; Cuadrado *et al.* 2021; McNab *et al.* 2022) provides a new and updated phylogeny of the Polycladida, with special attention to the families Euryleptidae and Stylostomidae Dittmann, Cuadrado, Aguado, Noreña & Egger, 2019.

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Material and methods

Animals

Six specimens were collected in the port of Punat, Krk, Croatia (45°01’23”N14°37’41”E) in October 2018 and May 2019. The animals were found in brown algae and separated by using 1:1 7.14% $MgCl_2 \times 6H_2O$ and artificial sea water (ASW) (see Grosbusch *et al.* 2019).

Histology

Three specimens of *Cycloporus pinkipus* sp. n. were fixed after the technique described in Lee *et al.* (2006). Briefly, worms were placed on filter paper, which was transferred to frozen 3.5% formaldehyde (FA) in phosphate buffered saline (PBS), dripping cold fixative on the specimen. The animals were stored at 4°C in fixative for several months. For histological examination, the specimens were washed three times for 10 min in PBS and distilled water, dehydrated in an ethanol series, cleared with intermedium (methyl benzoate overnight, benzene 30 min), submerged in a 1:3 benzene:Paraplast solution overnight and then embedded in Paraplast (Leica Biosystems, St. Louis, USA). Specimens were serially sectioned with a Reichert-Jung Autocut 2040 Microtome (Leica Biosystems, Vienna, Austria) at 5 µm and stained with AZAN trichrome stain after Romeis (1989).

Documentation

Live animals were documented with a Leica MZ 16F stereomicroscope equipped with a Leica DFC450 C digital camera for incident light microscopy (Leica Microsystems Heerbrugg, Switzerland) and a KOZO ZOOM stereomicroscope (KOZO, Nanjing, China) equipped with a Moticam 1080 digital camera for transmitted light microscopy (Motic Asia, Hong Kong, China). Sections were photographed with a Leica DM 5000B compound microscope equipped with a Leica DFC 490 digital camera (Leica Microsystems Heerbrugg, Switzerland). Further image processing was performed with Adobe Photoshop 7. Drawings were produced in Adobe Illustrator CS6.

DNA extraction, PCR amplification and sequencing

A complete, starved adult of *Cycloporus pinkipus* sp. n., was extracted following the protocol in Dittmann *et al.* (2019). For polymerase chain reaction (PCR) of the partial nuclear small and large ribosomal subunits (18S and 28S), the primers 4fb and 1806R, 5fk and S30 (all 18S), and 4LSU5 and L1642R (28S) were used with Taq polymerase (Larsson *et al.* 2008; Dittmann *et al.* 2019) with the following PCR conditions: 94°C 2 min, 35 cycles of (94°C 30 sec, 50°C 30 sec, 72°C 60 sec), 72°C 7 min, 12 °C storage. Successful amplicons were sequenced with the respective PCR primers by Microsynth (Austria).

Phylogenetic analyses

For this study, all available polyclad 28S sequences on GenBank (accessed on 2022-02-22) were downloaded. The macrostomid flatworm *Macrostomum lignano* was used as an outgroup (see Suppl. Mat. 1). Including our new *C. pinkipus* sp. n. partial 28S sequence, a total of 614 polyclad terminals were aligned using MAFFT E-INS-i v7.310 (Katoh & Standley 2013), and the alignment was curated with the least stringent options available in Gblocks v0.91b (Talavera and Castresana 2007). The final matrix length had 1084 nucleotide positions. We also made an alignment using only cotylean sequences, with a total of 400 polyclad terminals and a final matrix length of 1021 positions, and a concatenated alignment of cotylean 18S and 28S sequences with 32 terminals and a matrix length of 2702 positions. Phylogenetic reconstructions were performed with IQ-TREE 2.1.3 (Minh *et al.* 2020) including ModelFinder (Kalyaanamoorthy *et al.* 2017). The best fitting model according to the Bayesian information criterion was GTR+F+R5 for the all-polyclad 28S matrix, GTR+F+R4 for the only-cotylean 28S matrix, and TNe+R2 for the 18S and GTR+F+R4 for the 28S partition of the concatenated 18S+28S matrix. 200 non-parametric bootstrap replicates were calculated, displayed in FigTree 1.4.3 (http://tree.bio.ed.ac.uk) and further edited in Adobe Illustrator CS6.

Results

Order Polycladida Lang, 1881

Suborder Cotylea Lang, 1884

Superfamily Pseudocerotoidea Lang, 1884

Family Euryleptidae Stimpson, 1857

Genus *Cycloporus* Lang, 1884

Cycloporus pinkipus Egger & Dittmann sp. n.

(Figs. 1–6)

Material examined. *Cycloporus pinkipus* sp. n. specimens #1 and #2 used for molecular analysis. Specimens #3 and #5 sagittally sectioned. Specimens #1, #2, #3, #4 and #5 used for live observations. Specimen #6 cross-sectioned.

Type material. Serial sections of holotype and paratypes submitted to the Natural History Museum Vienna, Austria. GenBank accession numbers of partial nuclear ribosomal subunits of specimen #2 are OQ676574 (18S) and OQ676575 (28S). The ZooBank registration number is urn:lsid:zoobank.org:act:6765D50D-C5C8-44D3-A032-29FDFAC6165F.

Holotype. One sagittally sectioned specimen (#3) stained with AZAN (NHMW-ZOO-EV-M-5880).

Paratype 1. One sagittally sectioned specimen (#5) stained with AZAN (NHMW-ZOO-EV-M-5881).

Paratype 2. One cross-sectioned specimen (#6) stained with AZAN (NHMW-ZOO-EV-M-5882).

Type locality. Port of Punat, Krk, Croatia (45°01’23”N14°37’41”E).

Habitat. Animals were found in brown algae.

Etymology. The species epithet ‘pinkipus’ refers to the typical pink spots which characterises the dorsal colouration, and rhymes with ‘Cycloporus’.

Description

Appearance. Small, dorsoventrally flattened worm (between 2.1–4 mm long and 0.8–3 mm wide, holotype about 2.6 mm long and 1.1 mm wide) with elongated oval body, two small bump-like tentacles at the anterior end (Fig. 1). Margin sometimes slightly ruffled (Fig. 1F–G). Two separated, elongated cerebral eye clusters with about 37 eyes in each cluster (37.08±4.96, n = 3) (Figs. 1A; 2E–F). These clusters are arranged in an elongated shape (Figs. 1A; 2E–F). Each tentacle is bearing a cluster of about 36 tentacular eyes (35.91±3.74, n=3) (Figs. 1A; 2E–F). Dorsal surface smooth, transparent cream white with light brown to yellow spots (Fig. 1A, C–E,G). Several orange-brown spots posterior to the cerebral eyes (Fig. 1A, C–E,G). Pink spots in a broad stripe along the inner rim of the margin (Figs. 1A–E, G; 2F). If the gut diverticula are filled with food, a dark, reticulate, anastomosing pattern with six lateral intestinal branches emerges (Fig. 1F–G). Pharynx shaped cylindrically (Fig. 1B), mouth opening just posterior to the brain (Fig. 3B). Male and female genital pores well separated, posterior to the mouth opening (Figs. 3; 4A, M; 5). Sucker in the third quarter of the body (Fig. 1B). Intestinal branches are anastomosing. Six lateral intestinal branches each side, forking from the median intestinal branch, which is frontally passing between cerebral eye clusters. Colourless marginal pores of the intestinal branches open laterally, visible in cross sections (Fig. 6).

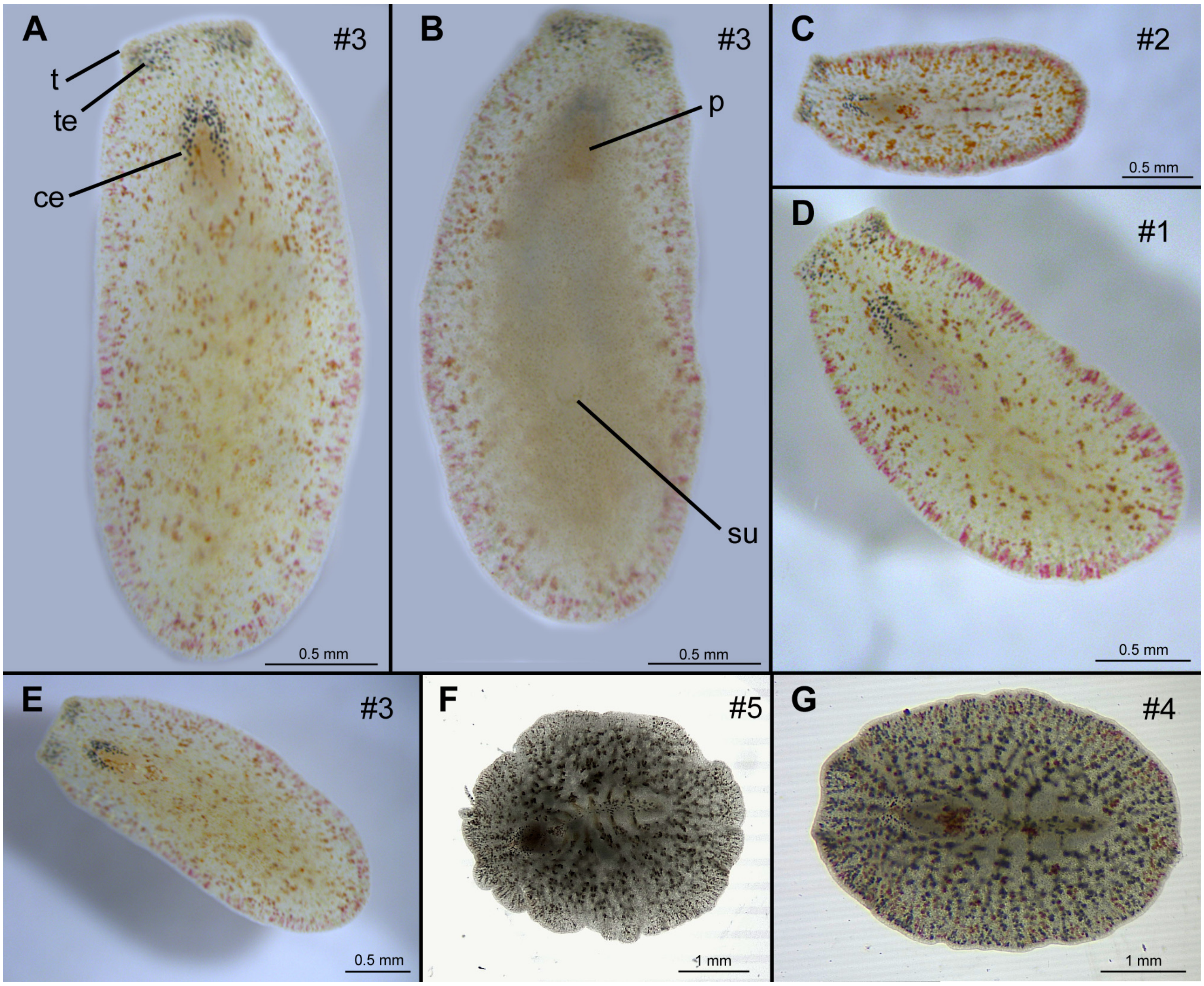

FIGURE 1. Live images of *Cycloporus pinkipus* sp. n. **A–E.** Incident light, **F–G.** Transmitted light. **A., E.** Dorsal view of the holotype (specimen #3). **B.** Ventral view of the holotype (specimen #3). **C**. Dorsal view of specimen #2. **D.** Dorsal view of specimen #1. **F.** Dorsal view of paratype 1 (specimen #5). **G.** Dorsal view of specimen #4. ce = cerebral eyes, p = pharynx, su = sucker, t = tentacle, te = tentacle eyes. Orientation: A–B anterior directed upwards, C–G anterior directed to the left.

Reproductive system. Male copulatory complex consists of a short stylet-bearing penis papilla with a length of about 140 µm (paratype), a spherical prostatic and seminal vesicle, and vasa deferentia (Figs. 3A–C; 4A–L; 5). Penis papilla in a penis sheath, prostatic and seminal vesicle posterior to the male gonopore and directed anteriorly (Figs. 3A–C; 4A–E; 5). Prostatic vesicle and seminal vesicle open separately into penis papilla (Figs. 3C; 5). Prostatic vesicle right anterior to seminal vesicle (Figs. 3A–C; 4A–L), which is connected to two well-developed vasa deferentia (Figs. 3A; 4B–E, K–M; 5). Female gonopore posterior to male gonopore (Figs. 3D–E; 4M, O; 5). Female genital complex comprises of short female atrium and female duct (vagina) which expands at the level of the cement pouch (Figs. 3E; 4M, O–P; 5). Large number of cement glands around cement pouch (Figs. 3E; 4M; 5). Female genital complex characterised by uteri connected with three uterine vesicles (Figs. 4P; 5).

Molecular analyses based on partial 18S and 28S rDNA sequences

Our phylogenetic 28S trees (Fig. 7, Suppl. Mat. 2) show that *C. pinkipus* sp. n. clusters in the main clade of *Cycloporus* consisting of *C. gabriellae Marcus, 1950, C. variegatus Kato, 1934, C. papillosus and C. japonicus Kato, 1944.* This clade clusters within Euryleptidae as sister group to *Eurylepta* Ehrenberg, 1831*, Prostheceraeus*

Schmarda, 1859 and *Maritigrella Newman & Cannon, 2000*. *Cycloporus pinkipus* sp. n. appears as sister group to a clade consisting of *C. papillosus* and a *C. variegatus* sequence (KY263659.2). According to our trees, published *Cycloporus* and *Eurylepta* sequences do not appear to be monophyletic (red and green sequence names in Fig. 7). Also *C. variegatus* and *C. gabriellae* are not monophyletic. In a combined 18S and 28S tree, *Cycloporus pinkipus* **sp. n.** is the only representative of its genus and sister group to Euryleptidae (Suppl. Mat. 2).

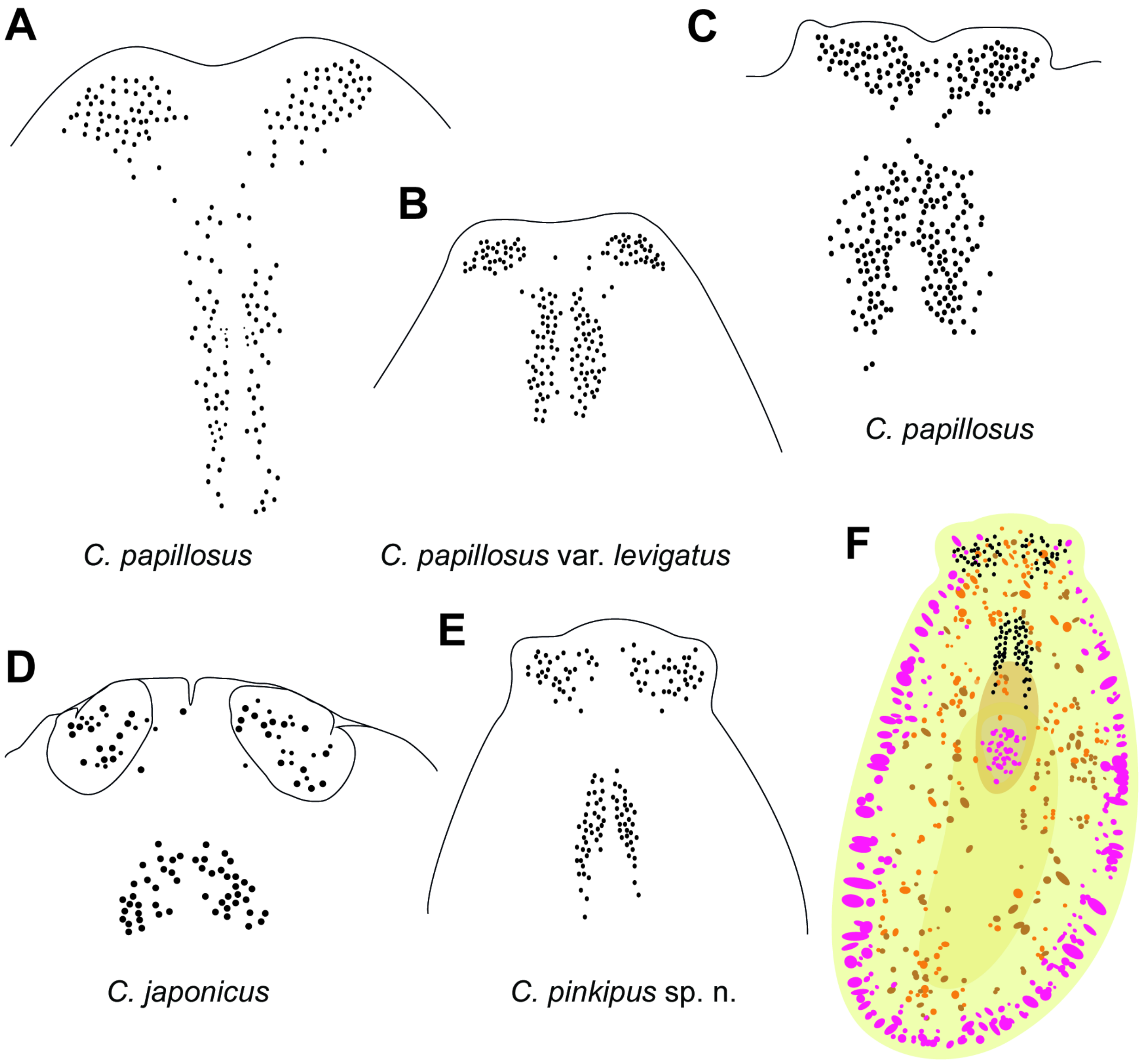

FIGURE 2. Schematic drawings of eye spot arrangement in similar *Cycloporus* species. **A–E.** Schematic drawings of eye spot clusters of different species and specimens after Lang (1884) (**A–B.**), after Marquina *et al.* (2015) (**C.**), and Kato (1937) (**D.**). **F.** Schematic drawing of the dorsal colouration of *Cycloporus pinkipus* sp. n. Anterior directed upwards. Not drawn to scale.

Discussion

Taxonomic remarks

The herein described new species, *Cycloporus pinkipus* sp. n., was diagnosed as member of the genus *Cycloporus* by the combination of the following features: 1) small in size (few mm) with elongated oval body; 2) bump-like tentacles with several tentacular eyes; 3) two separated and elongated cerebral eye clusters; 4) anastomosing intestinal branches; 5) frontal median intestinal branch passing between cerebral eye clusters; 6) sucker posterior to genital pores; 7) separation of mouth opening, male and female pore; 8) mouth opening right posterior to the brain; 9) presence of stylet; 10) male copulatory complex directed forward; 11) prostatic vesicle located dorsal to the penis papilla; 12) several uterine vesicles present and 13) marginal pores of the intestinal branches, eponymous for the genus *Cycloporus*. Our observations of these pores (Fig. 6) resemble the drawings and description of *Cycloporus papillosus* (Lang 1884, plate 27, fig 2), the type species of the genus *Cycloporus*.

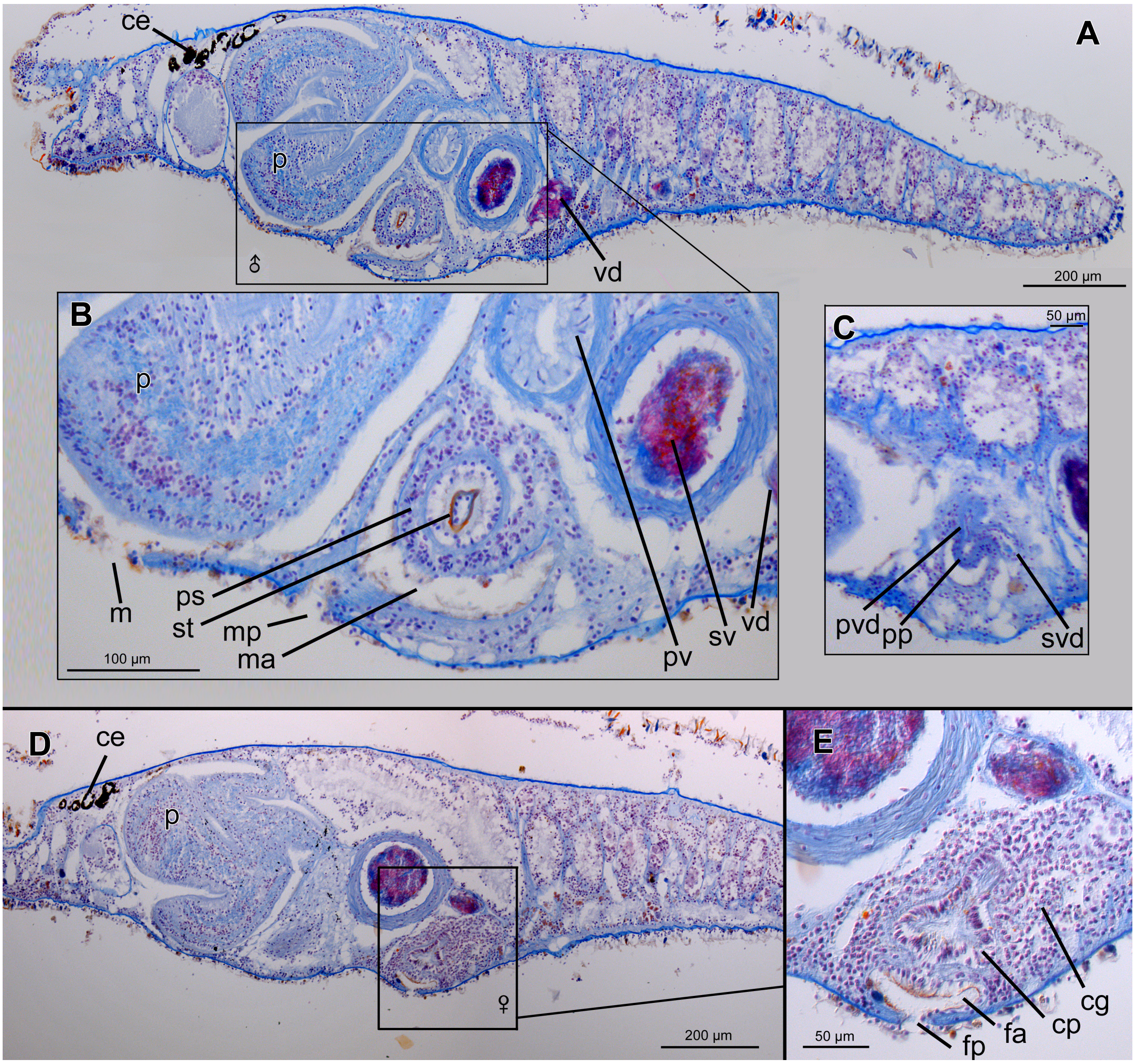

FIGURE 3. Sagittal sections of genital apparatus of *Cycloporus pinkipus* sp. n. (holotype; specimen #3). **A.** Male genital. **B.** Detailed view of male genital and mouth opening **C.** Detailed view of the ducts of prostatic vesicle and seminal vesicle. **D.** Female genital overview. **E.** Detailed view of female genital. ce = cerebral eyes, cg = cement glands, cp = cement pouch, fa = female atrium, fp = female pore, m = mouth opening, ma = male atrium, mp = male pore, p = pharynx, ps = penis sheath, pp = penis papilla, pv = prostatic vesicle, pvd = prostatic vesicle duct, st = stylet, sv = seminal vesicle, svd = seminal vesicle duct, vd = vas deferens. Orientation: anterior to the left.

So far, fourteen species of *Cycloporus* have been described (Newman & Cannon 2002; Pitale & Apte 2021), which were mainly determined as new species based on their dorsal colouration (Newman & Cannon 2002). The colouration of *C. pinkipus* sp. n. does not resemble any previously known *Cycloporus* species. However, the feeding status affects the optical appearance, as in fed animals the dark, reticulate, anastomosing pattern of the gut diverticula is visible in transmitted light microscopy (Fig. 1F–G). Besides the colouration, also the presence of dorsal papillae characterises some *Cycloporus* species (see Newman & Cannon 2002). In *C. pinkipus* sp. n., no dorsal papillae are observable. Other characters used for species determination within the genus *Cycloporus* are the relative number of

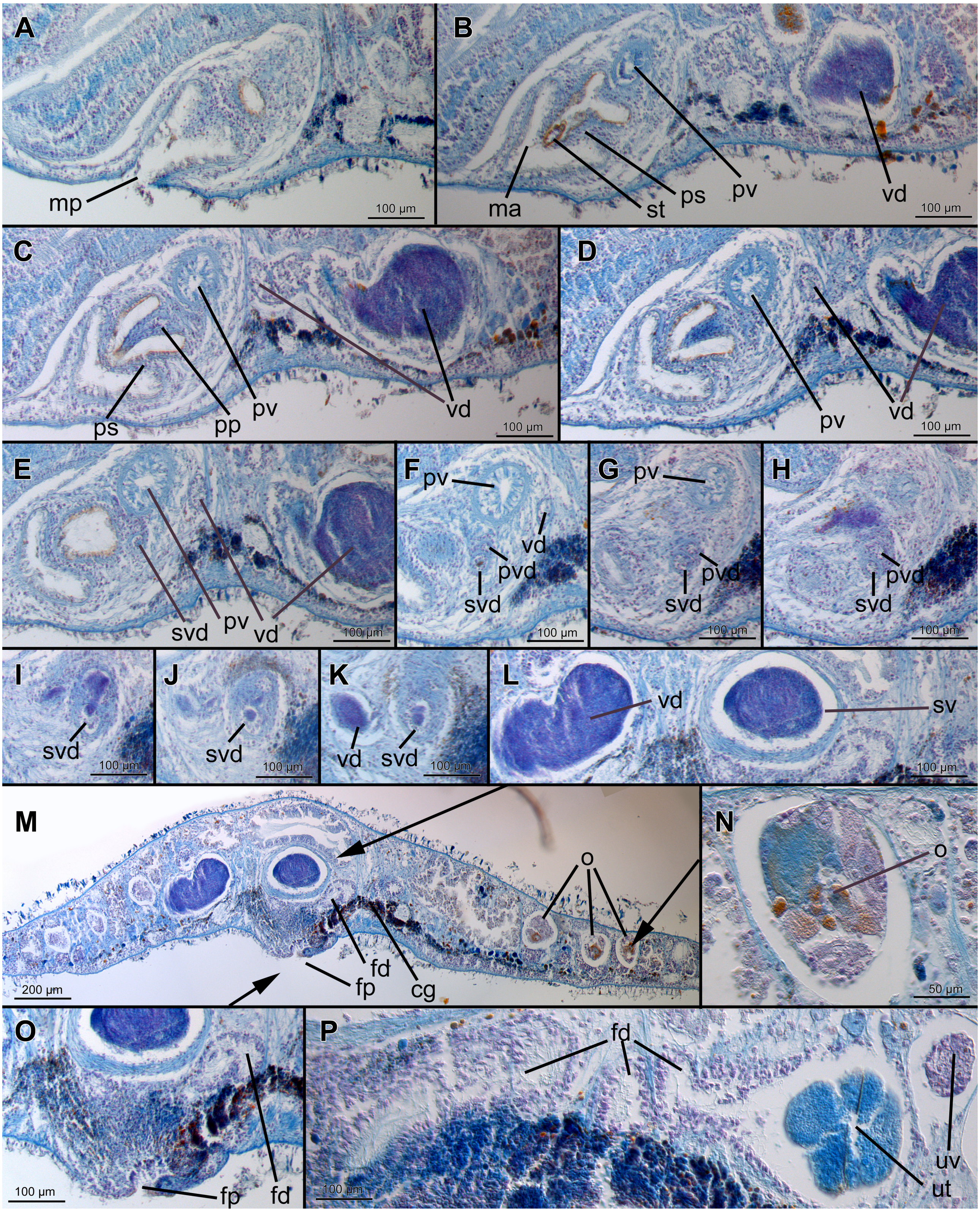

FIGURE 4. Sagittal sections of genital apparatus of *Cycloporus pinkipus* sp. n. (paratype 1; specimen #5). **A–D.** Penis papilla, stylet, prostatic vesicle and vas deferens. **E–L.** Junction between seminal vesical and vas deferens. **M.** Female genital. Arrows indicate the region of detailed views. **N.** Detailed view of ovary. **O.** Detailed view of female pore. **P.** Female duct, uterus and uterine vesicle. cg = cement glands, fd =female duct, fp = female pore, ma = male atrium, mp = male pore, o = ovary, pp = penis papilla, ps = penis sheath, pv = prostatic vesicle, pvd = prostatic vesicle duct, st = stylet, sv = seminal vesicle, svd = seminal vesicle duct, ut = uterus, uv = uterine vesicle, vd = vas deferens. Orientation: anterior to the left. Same scale bars in **A–L.**, **O–P.**

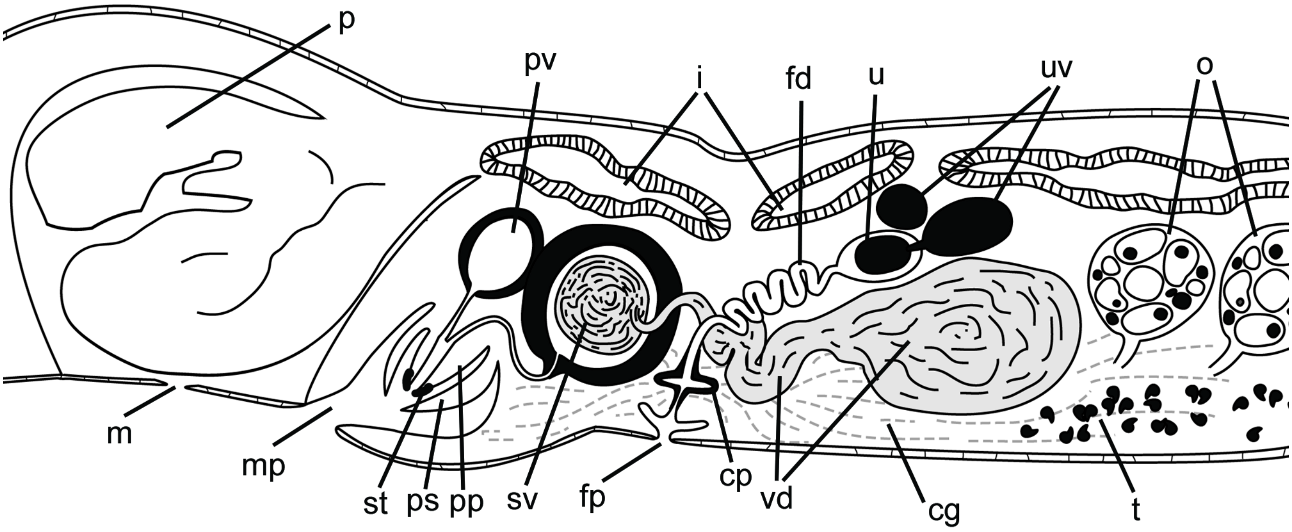

FIGURE 5. Sagittal reconstruction of the genital of *Cycloporus pinkipus* sp. n. cg = cement glands, cp = cement pouch, fd =female duct, fp = female pore, i = intestine, m = mouth opening, mp = male pore, o = ovary, p = pharynx, ps = penis sheath, pp = penis papilla, pv = prostatic vesicle, st = stylet, sv = seminal vesicle, t = testis, u = uterus, uv = uterine vesicle, vd = vas deferens. Orientation: anterior to the left.

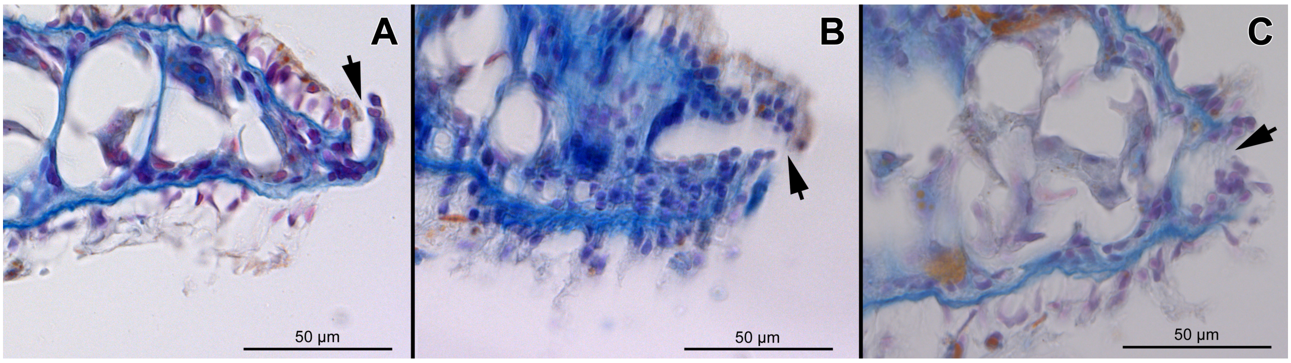

FIGURE 6. A–C. Cross sections of marginal pores of *Cycloporus pinkipus* sp. n. Arrows point to marginal pores.

cerebral and tentacular eyes (10–70 per side) as well as the number of lateral intestinal branches (4–9) (Newman & Cannon 2002). However, both characters are questionable as they vary intra-specifically (cerebral and tentacular eyes) and are furthermore often hard to observe or to count (Newman & Cannon 2002). Only one species, *Cycloporus japonicus* is distinguished as a separate species mainly due to the arrangement of eyes (Kato 1944) (Fig. 2). In most *Cycloporus* species, no details of the reproductive anatomy are given, and there is just little variation between studied species. Therefore, the reproductive organs cannot be used for species determination in *Cycloporus* (Newman & Cannon 2002).

History of *Cycloporus papillosus*/Differential diagnosis

So far, only one *Cycloporus* species, *C. papillosus*, was described from the Mediterranean, featuring the eponymous papillae (Lang 1884). A variety without papillae was named *C. papillosus* var. *levigatus* Lang (1884). Lang (1884) argued that based on the external appearance, *C. papillosus* could be classified into five to six different species, but decided against it due to the very similar internal morphology. In the meanwhile, the external morphology turned out to be an important character for the species determination of *Cycloporus* (Newman & Cannon 2002).

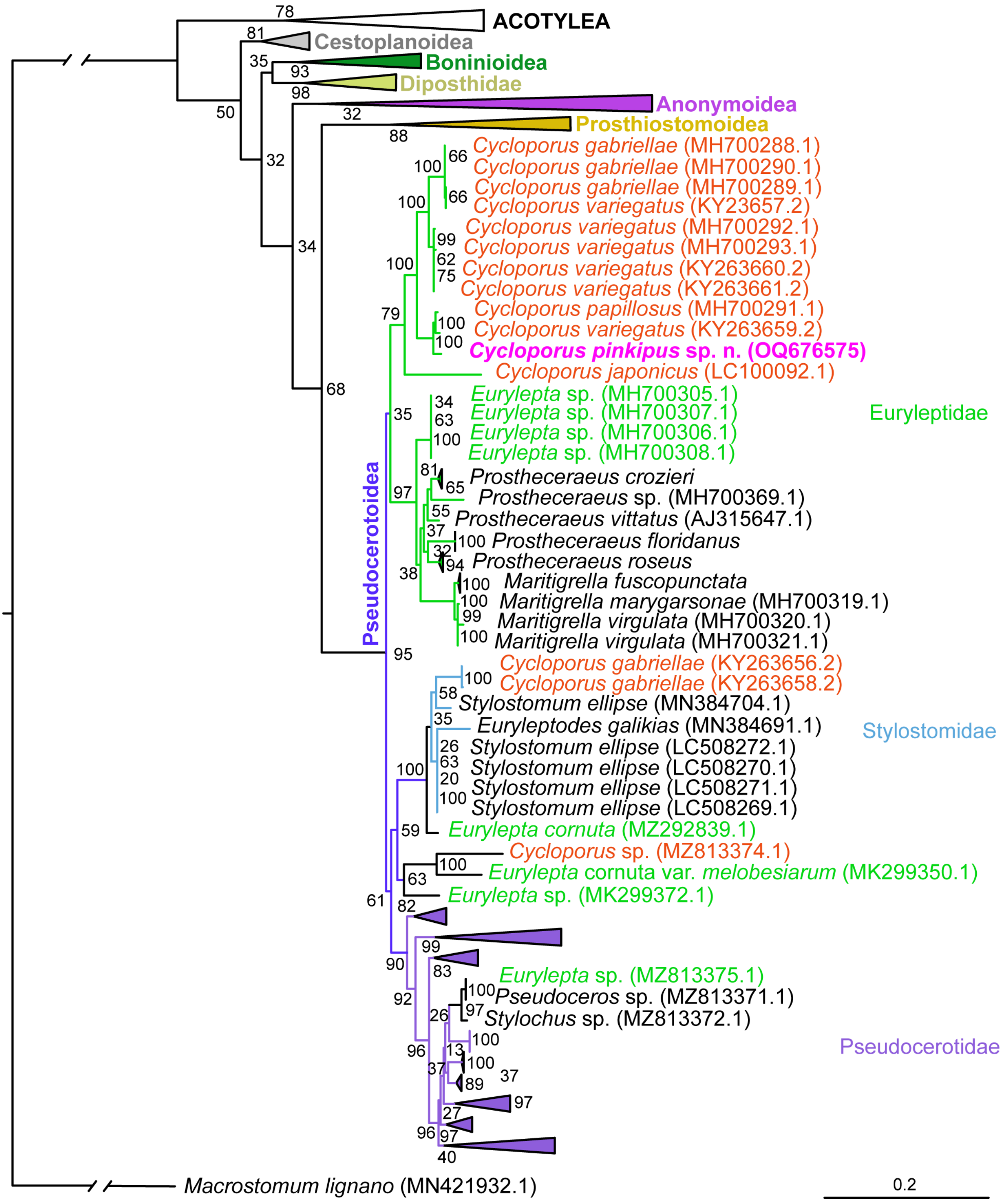

FIGURE 7. Phylogenetic maximum likelihood reconstruction using partial 28S sequences (accession numbers in brackets) of polyclads, rooted with *Macrostomum lignano*; branches other than Euryleptidae and Stylostomidae collapsed. Bootstrap nodal support of 200 non-parametric bootstrap replicates. Full tree in Suppl. Mat. 2. *Cycloporus pinkipus* sp. n. marked in pink. Additional representatives of *Cycloporus* written in red. Representatives of *Eurylepta* written in light green. Branches of Euryleptidae in light green. Branches of Stylostomidae in light blue. Branches of Pseudocerotidae in purple. Scale bar indicates the number of substitutions per site.

Lang described *C. papillosus* in 1884 and synonymised it with *Proceros tuberculatus* Schmidtlein, 1880. As Schmidtlein did not provide a description of *P. tuberculatus*, the material of which he had received from Lang (Schmidtlein 1880), *P. tuberculatus* is a *nomen nudum* and unavailable according to Article 12.1 of the International Code of Zoological Nomenclature (ICZN 1999). The formal species description under the name *C. papillosus* was published in Lang (1884). Some years later, Francotte (1897) found that Lang's description of *C. papillosus* also fits the description of *Planaria schlosseri* Giard, 1873, and also observed variants without dorsal papillae. Bock (1913) synonymised *Thysanozoon papillosus* Sars, 1878 with *C. papillosus* of Lang (1884) as *C. papillosus* (Sars, 1878) and noted colour variants and specimens without dorsal papillae. He provided a photograph of a sagittal section through the genital region of *C. papillosus* but remained sceptical that Giard's *Planaria schlosseri* was the same species as *C. papillosus* (Bock 1913). We therefore regard *P. schlosseri* as a *nomen dubium.* The latest study dealing with *C. papillosus* was conducted by Noreña *et al.* (2014), giving a detailed sagittal reconstruction of the genital region, and noting several colour variations, all with dorsal papillae (Noreña *et al.* 2014). All studied specimens of *C. papillosus* are from Atlantic coasts, with the exception of Lang (1884), who worked with material from the Mediterranean. Lang (1884) also introduced a variation without dorsal papillae under the name *C. papillosus* var. *levigatus.*

The size and colouration of *C. pinkipus* sp. n. differ from *C. papillosus* or *C. papillosus* var. *levigatus*. The latter have a length between 10 and 16 mm (except 5 mm in Bock 1913), while *C. pinkipus* sp. n. measures only between 2 and 4 mm. Lang (1884) describes the colouration of *C. papillosus* var. *levigatus* as transparent and slightly reddish, with yellow spots at the positions of the missing papillae, and yellow marginal pores. The carmine red intestinal branches dominate the colour pattern (Lang 1884, plate 8, fig. 5, Fig. 8). The observed colour patterns in *C. papillosus* are according to Lang (1884) white to yellow/orange, with either white or carmine red dorsal papillae; the marginal pores are conspicuously red-brown. The dorsal colour pattern in *C. pinkipus* sp. n. is similar to the written descriptions of *C. papillosus*, but the given pictures are dissimilar (Fig. 8), and there are no dorsal papillae in *C. pinkipus* sp. n. In addition, the marginal pores are colourless and inconspicuous in *C. pinkipus* sp. n., in contrast to *C. papillosus*. The colour patterns given for *C. papillosus* var. *levigatus* and *C. pinkipus* sp. n. are substantially different (Figs. 1–2; 8). Our molecular data also confirm the separation of the two species (Fig. 7).

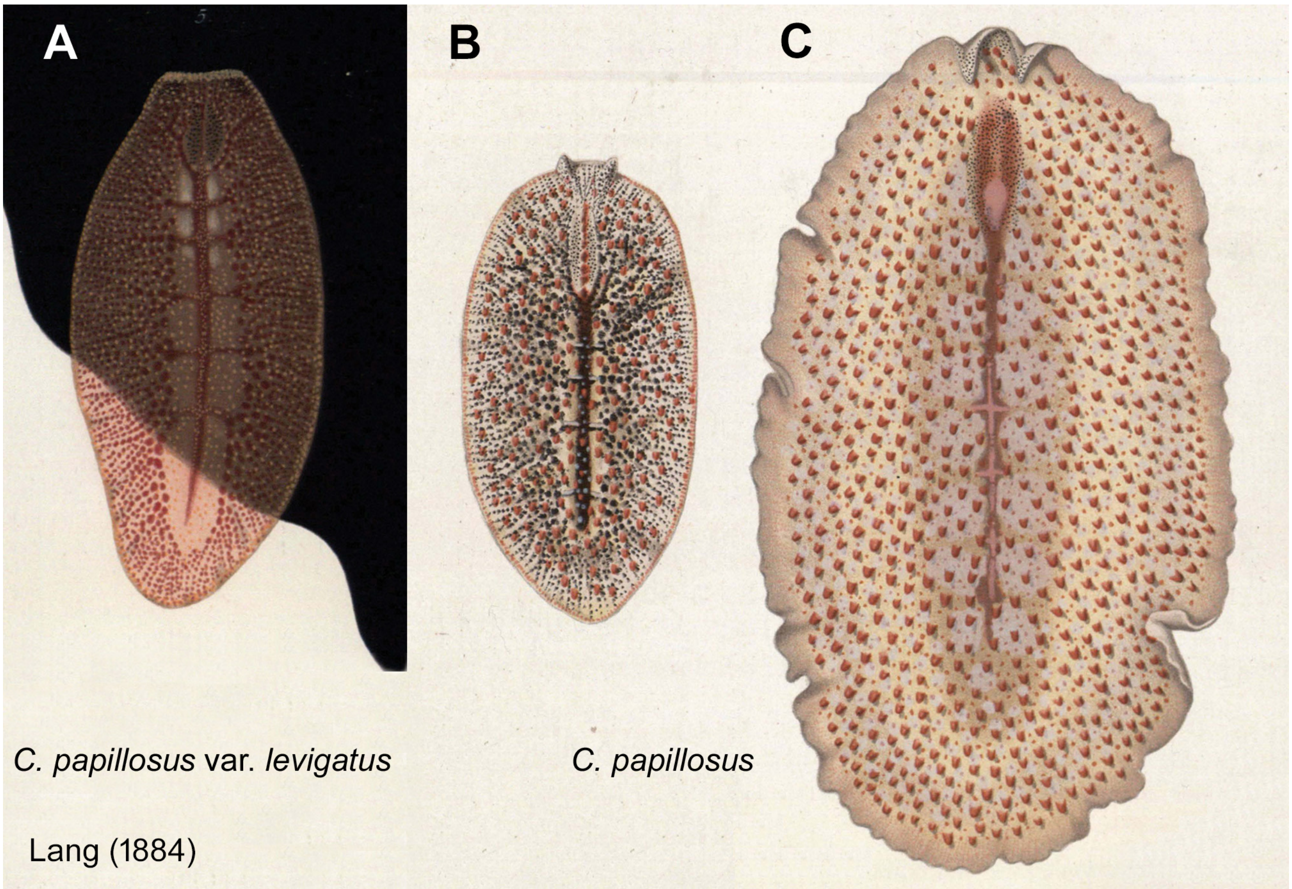

FIGURE 8. Drawings of **A.** *Cycloporus papillosus* var. *levigatus* and **B–C.** *Cycloporus papillosus* as shown in Lang (1884). Original scale.

Is the genus *Cycloporus* monophyletic?

The family Euryleptidae appears in several recent molecular studies to be non-monophyletic (Bahia *et al.* 2017; Dittmann *et al.* 2019; Litvaitis *et al.* 2019, fig 4). *Sensu* Faubel (1984), this family contains five genera represented with sequences on GenBank (*Cycloporus*, *Eurylepta, Euryleptodes* Heath & McGregor, 1912, *Prostheceraeus*, *Stylostomum* Lang, 1884, and the following genera without sequences so far: *Acerotisa* Strand, 1928, *Anciliplana* Heath & McGregor, 1912, *Katheurylepta* Faubel, 1984, *Leptoteredra* Hallez, 1913, *Oligoclado* Pearse, 1938, *Oligocladus* Lang, 1884, *Parastylostomum* Faubel, 1984, *Pareurylepta* Faubel, 1984, *Praestheceraeus* Faubel, 1984, and *Stygolepta* Faubel, 1984). In particular, the phylogenetic positions of some representatives of the genus *Cycloporus,* among them *C. gabriellae*, seem to be problematic (Bahia *et al.* 2017; Dittmann *et al.* 2019). In our phylogentic reconstruction using all available partial 28S sequences of members of Cotylea (Fig. 7), *C. pinkipus* sp. n. is within the main clade of *Cycloporus*, as sister group to a clade consisting of *C. papillosus* (from Honduras) and *C. variegatus* (from Spain). Another sequence of *C. variegatus* (from Brazil) clusters within *C. gabriellae* (from Jamaica and from Florida) and is thus likely a misdetermined *C. gabriellae* (see Table 1), while all remaining sequences of *C. variegatus* (from Brazil and from Colombia, from different authors) form a monophylum. It seems to be possible that the specimen determined as *C. variegatus* (from Spain) is a morphotype of *C. papillosus*, like the unpapillated *C. papillosus* var. *levigatus*, or a new species of *Cycloporus* (see Table 1).

TABLE 1. Suggested determinations for published sequences.

Accession number	Published species name	Suggested determination
KY263656.2	*Cycloporus gabriellae*	*Stylostomum* sp.
KY263658.2	*Cycloporus gabriellae*	*Stylostomum* sp.
KY263657.2	*Cycloporus variegatus*	*Cycloporus gabriellae*
KY263659.2	*Cycloporus variegatus*	*Cycloporus* sp.
MZ292839.1	*Eurylepta cornuta*	Stylostomidae sp.
MZ813374.1	*Cycloporus* sp.	Stylostomidae sp.
MK299350.1	*Eurylepta cornuata* var. *melobesiarum*	Stylostomidae sp.
MK299372.1	*Eurylepta* sp.	Stylostomidae sp.
MZ831375.1	*Eurylepta* sp.	Pseudocerotidae sp.
MZ813371.1	*Pseudoceros* sp.	Pseudocerotidae sp.
MZ813372.1	*Stylochus* sp.	Pseudocerotidae sp.

Two sequences labelled as *C. gabriellae* (from Brazil) appear within Stylostomidae as sister group to *Stylostomum ellipse (Dalyell, 1853)* (from the Mediterranean) (Fig. 7). We hypothesise that these sequences belong to *Stylostomum* (see Table 1), as the external morphology of *Cycloporus* and *Stylostomum* shows several similarities (small in size, oval body shape, often with small, rudimentary tentacles) and can easily be confused (Lang 1884). We revise the definition of the family Stylostomidae given in Dittmann *et al.* (2019) by removing *Cycloporus gabriellae* from the family and currently only leaving the genera *Stylostomum* and *Euryleptodes.* A sequence labelled as *Cycloporus* sp. is most closely related to two sequences labelled as *Eurylepta*, which are discussed below. In conclusion, we think that *Cycloporus* is a monophyletic group well characterised by marginal pores.

Different *Eurylepta* sequences appear in five different pseudocerotoid clades in our phylogenetic reconstruction (Fig. 7, green names). A sequence of the type species, *Eurylepta cornuta (Müller OF, 1776),* is included, but, unfortunately, there is no histological material available to ascertain determination (McNab *et al.* 2022). An indication for a possible misdetermination is that two *Eurylepta* sequences (including *E. cornuta*), determined by the same lab, are appearing in very different parts of the tree (*Eurylepta cornuta* within Stylostomidae, *Eurylepta* sp. within Pseudocerotidae, see Table 1). Two other *Eurylepta* sequences (MK299350.1, MK299372) published by Cuadrado *et al.* (2021) are paraphyletic with a *Cycloporus* sp. sequence (Fig. 7); all three sequences together form the sister group of Stylostomidae. Only *Eurylepta* sp. sequences published by Litvaitis *et al.* (2019) form the expected clade with *Prostheceraeus* and *Maritigrella* and were also histologically determined.

One of the species leading to an *Eurylepta* sequence was morphologically and histologically determined as

Eurylepta cornuta var. *melobesiarum* Lang, 1884 (Cuadrado *et al.* 2021). Schmidtlein (1880) mentions a *Proceros melobesiarum*, but as for *Proceros tubercatulatus*, no species description is given and the name is therefore unavailable (see above). Lang (1884) gave *P. melobesiarum* as a synonym of *E. cornuta* var. *melobesiarum* and noted that *Eurylepta cornuta* var. *melobesiarum* shows a morphological discrepancy to *Eurylepta cornuta* (the type species of *Eurylepta*, which is the type genus of Euryleptidae) concerning the shape and length of the tentacles, the furcation of the gut, as well as the depression located closely to the female genital opening (Lang 1884, also see fig 6 in Cuadrado *et al.* 2021). We therefore consider that *Eurylepta cornuta* var. *melobesiarum* is possibly a new species, and regarding its position in the phylogenetic tree, most likely belonging to a different genus or even family than *Eurylepta cornuta* (Fig. 7).

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper.

Supplementary Material 1. Sequences used for phylogenetic reconstructions in Fig. 7 and Suppl. Mat. 2, including species names, accession numbers and locations of species of interest.

Supplementary Material 2. Phylogenetic maximum likelihood reconstructions using partial 28S and 18S sequences (with accession numbers in front of species names) of polyclads, rooted with *Macrostomum lignano* or *Cestoplana rubrocincta*. Bootstrap nodal support of 200 non-parametric bootstrap replicates. Scale bar indicates the number of substitutions per site.