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A New Species of *Cestoplanoida* (Platyhelminthes: Polycladida) from Southern Taiwan, with the First Molecular Data for the Genus

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Cestoplanoida Faubel, 1983, a poorly known monotypic genus within Cestoplanidae Lang, 1884, characterized by multiple female copulatory apparatuses, was previously known only from *C. polypora* (Meyer, 1921). We describe a second species, *C. wanlitongensis* sp. nov., from the intertidal zone of Pingtung County, southern Taiwan. It resembles *C. polypora* in external morphology and copulatory apparatuses but differs in having the pharynx in the posterior quarter rather than the posterior third of the body. Histological examination reveals protandrous sequential hermaphroditism and the absence of Lang's vesicle, which is also absent in *C. polypora* based on a reassessment of its original description. This observation prompts the exclusion of Lang's vesicle from the genus diagnosis. Phylogenetic analyses of partial 28S rDNA sequences tentatively place *C. wanlitongensis* sp. nov. as sister to *Eucestoplana* Faubel, 1983, within a strongly supported monophyletic clade representing Cestoplanidae. This study expands the taxonomic and molecular knowledge of *Cestoplanoida*, revises the generic diagnosis, and contributes to understanding the phylogenetic relationships within Cestoplanidae, providing a framework for future systematic and evolutionary studies.

Keywords: Cotylea, Cestoplanidae, Multiple female apparatuses, Molecular phylogeny, Maximum likelihood

BACKGROUND

The family Cestoplanidae Lang, 1884 is characterized by an elongated or slender body, a feature reflected in the ribbon-like meaning of its name. Members lack tentacles. Numerous eyespots are typically distributed between the cerebral region and the anterior submarginal area (except for eyeless deep-sea species). A ventral adhesive structure may be present near the posterior extremity. The pharynx is ruffled and situated in the posterior half of the body. The male copulatory apparatus is directed anteriorly, and the true prostatic vesicle is lined with smooth glandular epithelium (Faubel 1983; Prudhoe 1985; Oya and Kajihara 2018). Historically, Lang (1884) established Cestoplanidae as a monogeneric taxon to accommodate the genus *Cestoplana* Lang, 1884. Within this systematic framework, the family was assigned to the tribe Acotylea Lang, 1884 (currently recognized as a suborder), as its morphological characters conformed to the established diagnosis of the group. This assignment relied on the absence of a ventral sucker, a definitive diagnostic criterion at the time, while Lang (1884) simultaneously emphasized the family's highly specialized and isolated status within the suborder due to its aberrant posterior anatomy. The family remained monogeneric for nearly

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a century. Throughout this interval, the progressive description of new species revealed substantial intrageneric morphological heterogeneity, which eventually necessitated a taxonomic reassessment. By evaluating refined characters of the reproductive system and pharyngeal position, Faubel (1983) restructured the family and erected five additional genera: *Acestoplana* Faubel, 1983, *Cestoplanella* Faubel, 1983, *Cestoplanides* Faubel, 1983, *Cestoplanoida* Faubel, 1983, and *Eucestoplana* Faubel, 1983. This six-genus classification framework has persisted to the present day.

Since the establishment of Cestoplanidae, many authors (*e.g.*, Laidlaw 1903; Kato 1937 1938; Hyman 1939; Marcus 1949; Prudhoe 1982) have reported several internal features in the family that are typically associated with the suborder Cotylea Lang, 1884 (as summarized by Rawlinson and Litvaitis 2008; Bahia et al. 2017), such as cement glands, the male copulatory apparatus located posteriorly to the male gonopore, a penis sheath formed by the atrial wall, and the absence of Lang's vesicle. Despite these cotylean-like features having been noted by early authors, the family was conservatively retained within Acotylea. Bahia et al. (2017) attributed this to an over-reliance on limited morphological characters and clarified the systematic position of Cestoplanidae as an early-diverging lineage within Cotylea by integrating 28S rDNA sequences with multiple morphological traits. This finding was further substantiated by Litvaitis et al. (2019), who utilized an expanded dataset to confirm the family's basal placement within the suborder. Subsequently, Goodheart et al. (2023) constructed a transcriptome-based phylogeny of selected polyclad taxa, in which Cotylea was resolved into two major clades. One of these, referred to as clade 1, comprises Cestoplanidae, Theamatidae Marcus, 1949, Boniniidae Bock, 1923, and Pericelidae Laidlaw, 1902, with Cestoplanidae recovered as one of the earliest diverging members of the group. Although the inferred relationships among cotylean families vary among studies, Cestoplanidae has been consistently recovered as an early-diverging lineage within Cotylea.

Following Faubel's (1983) taxonomic restructuring, taxonomic research at the species level on Cestoplanidae has remained limited, with only two additional species described in the subsequent four decades. These are *Cestoplana nopperabo* Oya and Kajihara, 2018, a bathyal species from Sagami Bay, Japan, which deviates from the typical morphology of Cestoplanidae by lacking a ribbon-shaped body and eyespots; and *Eucestoplana ittanmomen* Tsuyuki, Oya and Kajihara, 2023, a relatively small and slender species from the intertidal zone of the Okinawa Islands, Japan. These descriptions bring the total number of recognized species within the family to 13 (Tyler et al. 2006–2025). Integration of earlier taxonomic studies (Kato 1937 1938) indicates that research on Cestoplanidae in the Indo-West Pacific has been primarily concentrated in Japanese waters. While occasional regional surveys of Polycladida recording the family have been conducted outside of Japan, such as reports from Micronesia (Newman et al. 2003) and the Indian Ocean (Baroliya et al. 2025), research on Cestoplanidae within the Indo-West Pacific remains remarkably scarce and geographically restricted.

The first report of a cestoplanid flatworm species from Taiwan appeared in a field guide by Jie and Kuo (2014), in which the specimens were initially identified as *Cestoplana* sp. Subsequent histological examination of these specimens and additional material revealed the presence of multiple female copulatory apparatuses positioned posterior to a single male copulatory apparatus, a key diagnostic feature of *Cestoplanoida*, previously a monotypic genus represented solely by *C. polypora* (Meyer, 1921), originally described from the Red Sea and not reported since. In this study, we describe a second species of *Cestoplanoida* based on specimens collected from southern Taiwan and provide an updated diagnosis for the genus. To elucidate the phylogenetic relationships of the new species and determine its genetic distances from related taxa, we analyzed partial 28S ribosomal DNA (28S rDNA) sequences of the new species, alongside those of other cestoplanid flatworms and representative taxa of related families within the Cotylea available in public databases. Additionally, cytochrome *c* oxidase subunit I (*COI*) barcodes were generated to facilitate future taxonomic identification and molecular phylogenetic studies.

MATERIALS AND METHODS

Specimen collection and processing

Specimens were collected from intertidal zones with flat coral reef substrates at three locations in Pingtung County, Taiwan: Houbihu (21°56'8"N, 120°44'49"E) and Wanlitong (21°59'49"N, 120°42'7"E) in Hengchun Township, and Haikou (22°4'54"N, 120°42'3"E) in Checheng Township (Fig. 1). Sampling was conducted at night during low tide, within one hour of the tidal minimum. Exposed reef surfaces and shallow tide pools were searched, and flatworms were guided into seawater-filled bottles with a fine watercolor brush. In the laboratory, living specimens were photographed using a Nikon D800 camera equipped

with a macro lens to record their external morphology and coloration. Morphometric data were obtained from photographs of living specimens via ImageJ (ver. 1.54 g, Schneider et al. 2012) and from fixed specimens using a caliper.

Tissues for DNA analysis were excised from the posterior margins of specimens and preserved in 95% ethanol. The remaining bodies were fixed in frozen 4% formaldehyde (diluted from formalin with seawater), following a simplified method adapted from Newman and Cannon (1995). Specimens were stored at 4°C for at least 24 hours and subsequently transferred to 70% ethanol for histological examination and long-term preservation.

Reproductive regions were dissected from fixed specimens, embedded in paraffin, and sagittally sectioned at 5–7 µm thickness. Sections were stained with hematoxylin and eosin. Reproductive structures in histological sections were examined and measured using an Olympus BX51 compound microscope, with images captured by a Jenoptik ProgRes CF cooled digital camera. All voucher specimens and histological slides were deposited in the Platyhelminthes collection of the National Taiwan Museum, Taipei, Taiwan (TMPL).

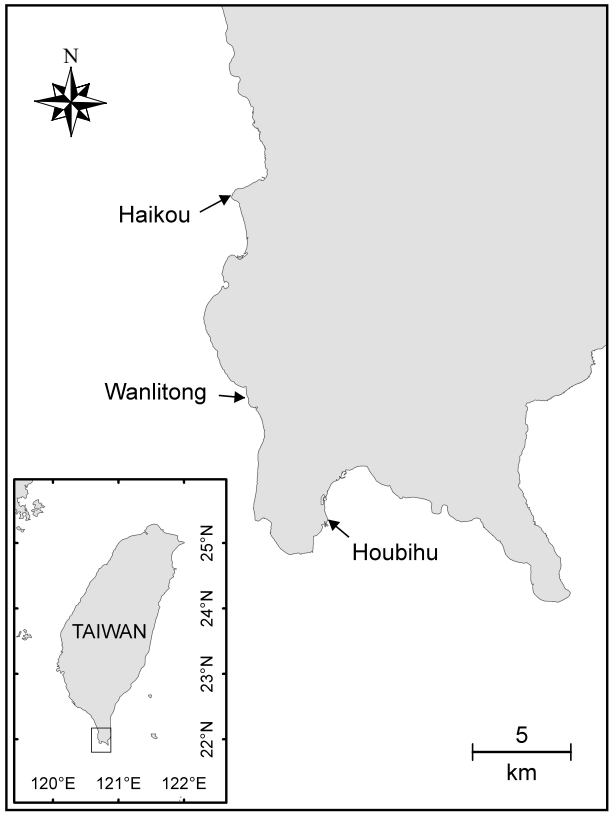

Fig. 1. Collection localities of *Cestoplanoida wanlitongensis* sp. nov. in southern Taiwan. Arrows indicate the sampling sites.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from prepared tissue samples using the Gene-Spin Genomic DNA Isolation Kit (Protech Technology Enterprise Co., Ltd., Taiwan) following the manufacturer's instructions. Partial 28S rDNA was amplified in two segments with the primer pairs D1D2fw1-CU28iR and CC28iF-D1D2rev (Table 1). The barcode region of the *COI* gene was amplified using the primer pair Cesto391F-COIR391 (Table 1), which was developed based on the complete mitochondrial genome sequences of four polyclad flatworms available in GenBank: *Enchiridium* sp. (KT363734), *Hoploplana elisabelloi* (KT363735), *Prosthiostomum siphunculus* (KT363736), and *Stylochoplana maculata* (KP965863), as reported by Aguado et al. (2016).

Polymerase chain reaction (PCR) was performed using an Eppendorf 5531 Gradient Mastercycler. Each 20 µl reaction mixture contained 1 µl of DNA template, 1 µl of each primer (10 µM), 1 µl of $MgCl_2$ (25 mM), 6 µl of DNase/RNase-free distilled water, and 10 µl of Fast-Run™ 2× Taq Master Mix (Protech Technology Enterprise Co., Ltd., Taiwan) for 28S rDNA amplification. For *COI* amplification, Fast-Run™ 2× Taq Master Mix was replaced with GoTaq® Green Master Mix (Promega Corp., USA), while all other reaction components remained unchanged. The PCR protocol consisted of an initial denaturation at 94°C

Table 1. Primer sequences used in this study for DNA amplification

Primer	Sequence (5'-3')	Reference
D1D2fw1	AGCGGAGGAAAAGAAACTA	Sonnenberg et al. 2007
CU28iR	CTGCGCCTTTAGGTTTCGTA	This study
CC28iF	CTGAGAGGCAAACTGGTGGAGCT	This study
D1D2rev2	ACGATCGATTTGCACGTCAG	Sonnenberg et al. 2007
Cesto391F	ATTATATTCTACAAATCATAAGGATATAGG	This study
COIR391	CTTTCCTCTATAAAATGTTACTATTTGAG	This study

for 4 minutes, followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing for 40 seconds, and extension at 72°C for 1.5 minutes. Annealing temperatures were 52°C for *COI*, 55°C for primer pair D1D2fw1–CU28iR, and 52°C for CC28iF–D1D2rev. A final extension was performed at 72°C for 5 minutes. PCR products were submitted to Tri-I Biotech Inc. (Taipei, Taiwan) for sequencing. Trace files were assembled, merged, and manually checked using CLC Main Workbench (ver. 7.9, QIAGEN Aarhus, Denmark). All validated sequences were deposited in GenBank.

Molecular phylogenetic analyses

Partial 28S rDNA sequences for phylogenetic analyses were obtained from GenBank and generated in this study (Table 2). Sequence alignment was conducted using MAFFT (ver. 7, Katoh et al. 2019), with the L-INS-i strategy as selected by the AUTO option. The best-fit substitution model (GTR+G+I) was determined based on the corrected Akaike Information Criterion (AICc) in MEGA (ver. 11.0.13, Tamura et al. 2021). *Alloioplana yerii* Oya, Tsuyuki and Kajihara, 2021 was employed as the outgroup for phylogenetic analyses of the target group. Maximum likelihood (ML) analysis was conducted in MEGA 11 with 1,000 bootstrap replicates. Bayesian inference (BI) analysis was performed using MrBayes (ver. 3.2.7, Ronquist and Huelsenbeck 2003). The Markov chain Monte Carlo (MCMC) analysis was run for 10,000,000 generations, with sampling every 1,000 generations. The first 25% of generations were discarded as burn-in, and the remaining samples were used to construct a majority-rule consensus tree and estimate posterior probabilities. Bootstrap values (≥ 70%) and posterior probability (≥ 0.8) were indicated at the nodes of the ML tree. Uncorrected *p*-distances were calculated in MEGA 11 using default settings.

RESULTS

TAXONOMY

Suborder Cotylea Lang, 1884
Family Cestoplanidae Lang, 1884
Genus *Cestoplanoida* Faubel, 1983

Emended diagnosis: Body highly elongated and ribbon-shaped. Pharynx situated in posterior third of body. Eyes numerous, arranged in fan-shaped pattern anteriorly, with base beginning posterior to cerebral region and extending forward toward submargin. Adhesive structure absent. Single male copulatory apparatus in immediate postpharyngeal position, comprising true seminal vesicle, elongated interpolated prostatic vesicle, and penis papilla that may bear stylet. Multiple female copulatory apparatuses present, each comprising cement gland, cement duct, vagina, atrium, and gonopore; gonopores arranged in single longitudinal row posterior to male gonopore Lang's vesicle absent.

Type species: *Cestoplanoida polypora* (Meyer, 1921).

Table 2. Taxa and GenBank accession numbers used in the 28S rDNA phylogenetic analyses

Species	Accession numbers	Reference
Acotylea Lang, 1884		
Stylochoplanidae Faubel, 1983		
Alloioplana yerii Oya, Tsuyuki and Kajihara, 2021	LC651421	Oya et al. (2021)
Cotylea Lang, 1884		
Boniniidae Bock, 1923		
Boninia yambarensis Tsuyuki, Oya and Kajihara, 2022	LC699282	Tsuyuki et al. (2022)
Cestoplanidae Lang, 1884		
Cestoplanoida wanlitongensis sp. nov.	PP515897	This study
Cestoplana nopperabo Oya and Kajihara, 2018	LC322284	Oya and Kajihara (2018)
Cestoplana rubrocincta (Grube, 1840)	MN384689	Dittmann et al. (2019)
Cestoplana salar Marcus, 1949	KY263653	Bahia et al. (2017)
Cestoplana techa Du Bois-Reymond Marcus, 1957	KY263655	Bahia et al. (2017)
Eucestoplana cf. *cuneata* (Sopott-Ehlers and Schmidt, 1975)	LC740493	Tsuyuki et al. (2023a)
Pericelidae Laidlaw, 1902		
Pericelis flavomarginata Tsuyuki, Oya, Jimi and Kajihara, 2020	LC568535	Tsuyuki et al. (2020)
Theamatidae Marcus, 1949		
Theama japonica Tsuyuki, Oya and Kajihara, 2023	LC740210	Tsuyuki et al. (2023b)

Cestoplanoida wanlitongensis **sp. nov. Kuo, Li and Jie**

(Figs. 2–5)

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Material examined: Holotype: TMPL000053, live measurements unavailable, fixed 89 × 15 mm, Wanlitong; coll. W.-B. Jie and S.-C. Kuo, 6 Apr. 2013; reproductive region dissected into anterior and posterior sections, sagittally sectioned (39 and 40 slides, respectively), plus 12 transverse sections anterior to pharynx.

Paratypes (5 specimens): TMPL000054, live 140 × 22 mm, fixed 144 × 23 mm (dead upon fixation), Haikou; coll. S.-C. Wu, 15 Apr. 2020; GenBank: PP515895 (28S), PP524912 (*COI*). TMPL000055, live 157 × 28 mm, fixed 108 × 21 mm, Haikou; coll. S.-C. Wu, 15 Apr. 2020; GenBank: PP515896 (28S), PP524913 (*COI*). TMPL000056, live measurements unavailable, fixed 73 × 15 mm, Haikou; coll. S.-C. Wu, 4 May 2020, sagittally sectioned (20 slides); GenBank: PQ770702 (28S). TMPL000057, live 150 × 13 mm, fixed 64 × 13 mm, posterior body end damaged, Houbihu; coll. S.-C. Wu, 14 Apr. 2023; sagittally sectioned (39 slides), plus 12 transverse sections anterior to pharynx; GenBank: PP515897 (28S). TMPL000058, live 124 × 19 mm, fixed 72 × 16 mm, posterior body end damaged, Houbihu; coll. S.-C. Wu, 14 May 2023; sagittally sectioned (38 slides); GenBank: PP515898 (28S).

Other material: TMPL000052, live and fixed measurements unavailable, specimen twisted, all other data identical to the holotype.

Etymology: The specific epithet is derived from Wanlitong, the locality where the holotype was collected.

Diagnosis: Body ribbon-shaped. Eyes numerous, in broad fan-shaped frontal arrangement; base posterior to cerebral region, extending anterolaterally; eyes absent from cerebral region and body margin. Pharynx ruffled, in posterior quarter of body. Adhesive structure absent. Single male copulatory apparatus immediately postpharyngeal; penis papilla with sclerotized stylet. Multiple female copulatory apparatuses; gonopores in single longitudinal row posterior to male gonopore; Lang's vesicle absent.

Description: Body highly elongate, ribbon-shaped; anterior margin bluntly rounded, posterior end tapering. Tentacles absent. Dorsal surface smooth, uniformly grayish brown with grayish white margin (Fig. 2A); cerebral region slightly lighter. Ventral surface grayish white, grading to grayish brown toward submargin (Fig. 2B). Preserved coloration grayish brown on both surfaces after one year; grayish brown with deep brown marginal rims after four years; uniformly brown with deep brown rims after ten years. Eyes numerous (426 in TMPL000057; 689 in TMPL000058), in broad fan-shaped arrangement in anterior dorsal region; eye cluster base immediately posterior to cerebral region; eyes absent from cerebral region and body margin. Dense eye distribution along midline, with forward extension from base and decreasing density toward anterior submargin (Fig. 2C).

Pharynx ruffled, within posterior quarter of body length (Fig. 2A, 2B); position 0.71–0.85 (0.77 in holotype) relative to body length from anterior margin, based on fixed specimens. Mouth ventral, near pharyngeal center (Fig. 2B). Intestinal trunk extending anteriorly from pharynx to posterior cerebral region, with numerous pairs of robust lateral branches (Fig. 2A); trunk absent posterior to pharynx, replaced by fine branches forming sparse reticulate network (Fig. 2D).

Male gonopore in immediate postpharyngeal position. Female gonopores 7–23 (13 in holotype), in single longitudinal row posterior to male gonopore, extending toward posterior extremity (Fig. 2E). Rhabdites distinctly long near dorsal posterior extremity. Ventral posterior end slightly concave along margin, with prominent rhabdites (Fig. 2F). No specialized adhesive structure present.

Male copulatory apparatus composed of true seminal vesicle, elongated interpolated prostatic vesicle, penis papilla and gonopore. Sperm ducts expanding into numerous densely packed spermiducal vesicles forming broad longitudinal bands on either side of midline, extending from region posterior to seminal vesicle to body end (Fig. 3A). Seminal vesicle posterior to male gonopore, near dorsal side; 489–733 µm long, 186–219 µm wide (733 × 204 µm in holotype); wall thick, muscular; highly convoluted. Proximal end anteroventrally directed, receiving single duct from merged anterior sperm ducts ventral to vesicle; distal end anteriorly directed, slightly leftward, joined to elongated prostatic vesicle by short, curved ejaculatory duct (Figs. 3B, 4A). Prostatic vesicle 334–607 µm long, 111–138 µm wide (561 × 138 µm in holotype); wall thick, muscular; epithelium densely glandular, extending anteriorly, then bending ventrally to join base of penis papilla (Figs. 3B, 4A). Penis papilla elongated conical, vertically directed downward, 53–137 µm long (81 µm in holotype), enclosed within blunt-shaped penis sheath, and bearing sclerotized stylet at its distal end (Fig. 3C). Male antrum deeply invaginated, reaching nearly half body thickness (Figs. 3B, 4A).

Multiple female copulatory apparatuses arranged along body midline, originating ventral to seminal vesicle and extending toward posterior extremity

(Figs. 3B, 4B). Each apparatus composed of cement gland, cement duct, vagina, atrium, and gonopore (Fig. 3D). Paired uteri in two longitudinal rows on either side of midline, positioned at mid-body height (Fig. 4C), extending from approximately one-quarter of body length to near posterior end (Fig. 2B); irregularly expanded into series of closely spaced rounded or oval egg-filled enlargements, some interconnected by additional ducts. Vagina ciliated (Fig. 3E), U-shaped with ventrally directed opening; proximal end lacking

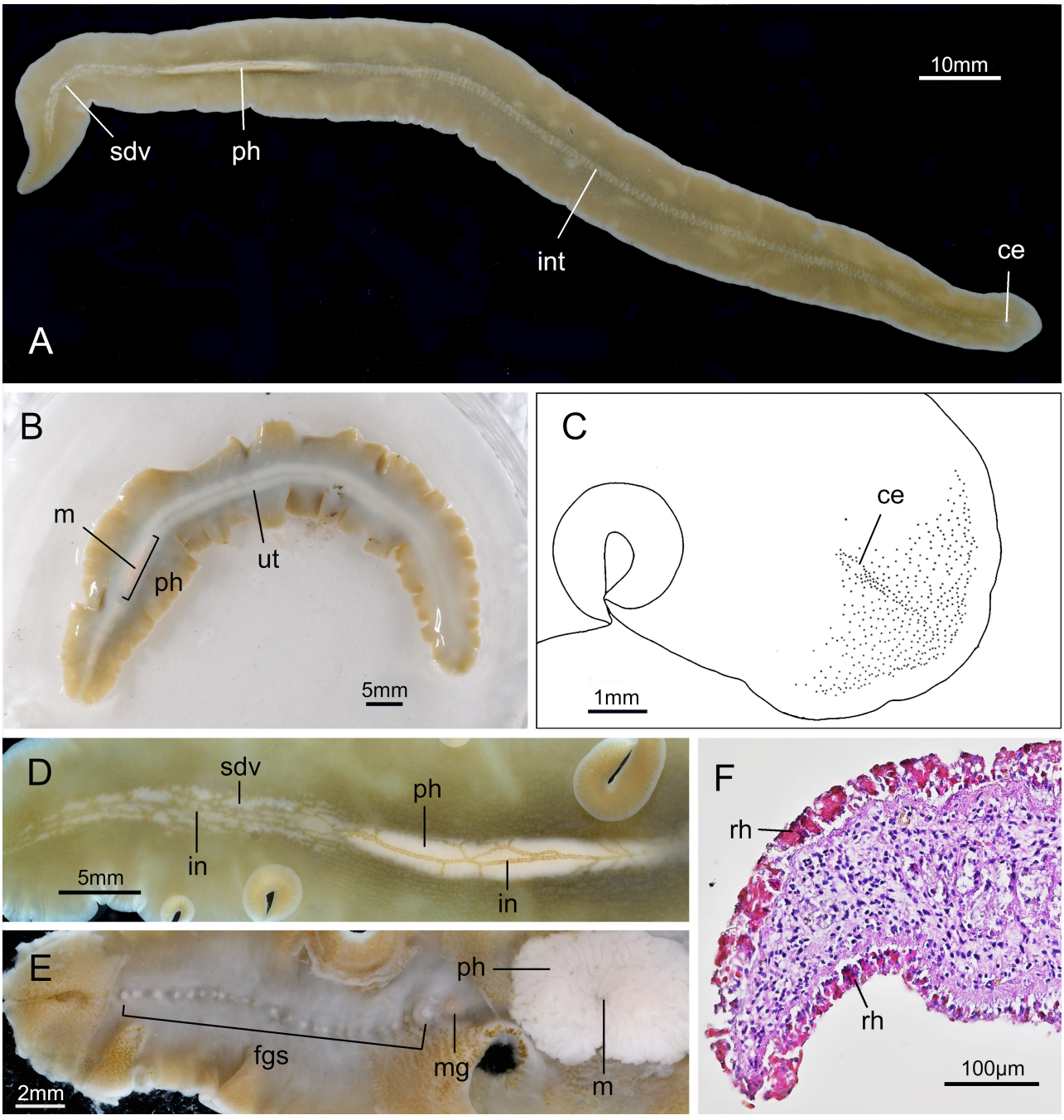

Fig. 2. *Cestoplanoida wanlitongensis* sp. nov. (A, C, D: TMPL000057; B, F: TMPL000053; E: TMPL000054), anterior end to the right. A, dorsal view of living specimen; B, ventral view of fixed specimen, showing a pair of expanded uteri; C, illustration of the distribution of eyes, represented by dots; D, dorsal view of posterior region of living specimen, showing reticulated intestine and a white band corresponding to the aggregated spermiducal vesicles; E, ventral view of fixed specimen showing arrangement of gonopores; F, histological sagittal section of posterior end, showing conspicuous rhabdites. Abbreviations: ce, cerebral region; fgs, female gonopores; in, intestine; int, intestinal trunk; m, mouth; mg, male gonopore; ph, pharynx; rh, rhabdites; sdv, spermiducal vesicles; ut, uterus.

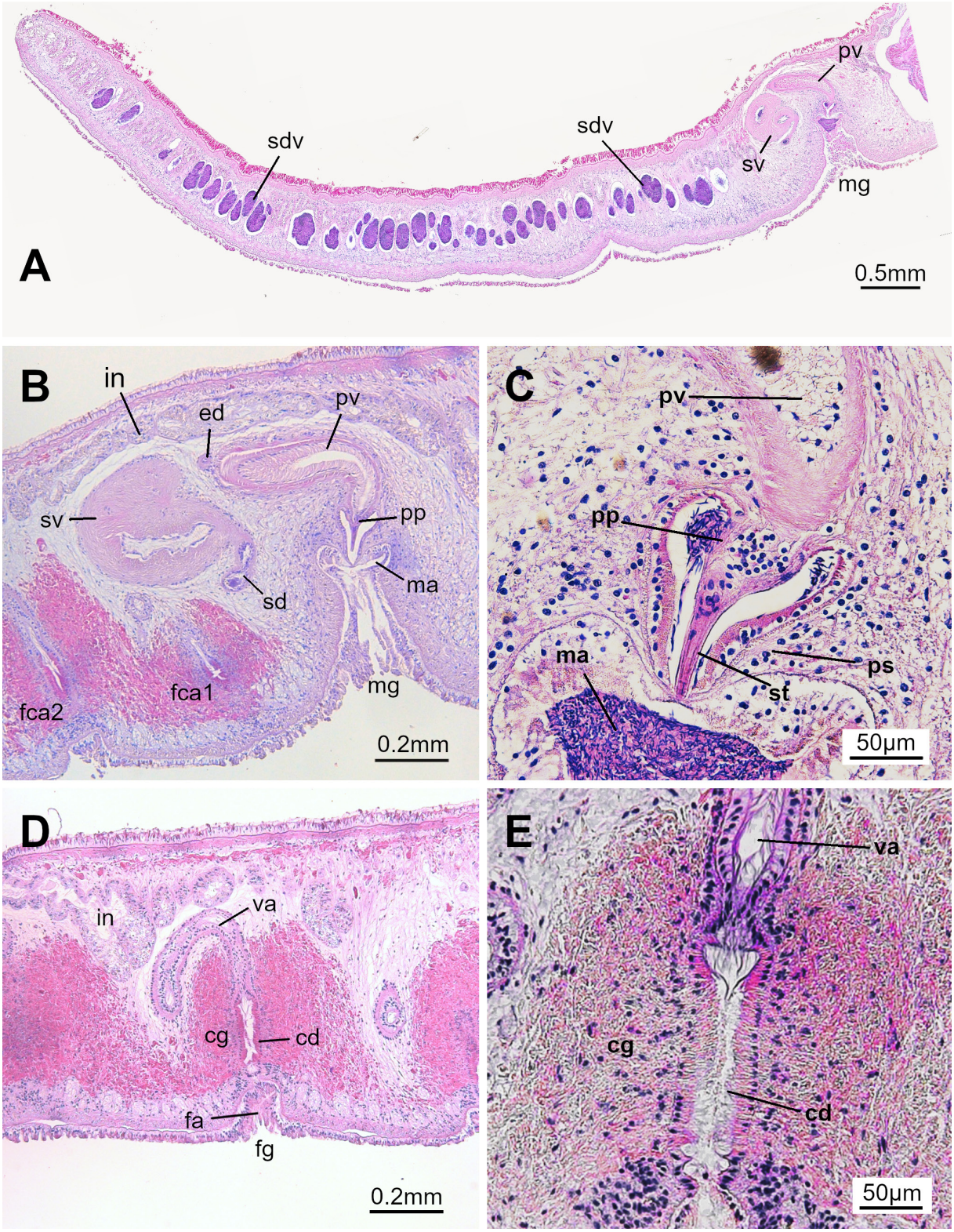

Fig. 3. Histological sections of reproductive structures of *Cestoplanoida wanlitongensis* sp. nov. (A, C: TMPL000057; B, D, E: TMPL000053), anterior end to the right. A, male structures; B, male and female copulatory apparatuses of mature specimen; C, penis papilla showing stylet; D, third female apparatus; E, cement complex of the tenth female apparatus. Abbreviations: cd, cement duct; cg, cement gland; ed, ejaculatory duct; fa, female atrium; fca, female copulatory apparatus; fg, female gonopore; in, intestine; ma, male atrium; mg, male gonopore; pp, penis papilla; ps, penis sheath; pv, prostatic vesicle; st, stylet; sd, sperm duct; sdv, spermiducal vesicles; sv, seminal vesicle; va, vagina.

Lang's vesicle, connected to paired uterine ducts extending to uteri on either side (Figs. 3D, 4C). Uterine ducts occasionally expanded and filled with eggs, resembling uterine enlargements. Distal end of vagina connecting to cement duct, both enclosed by cement gland (Fig. 3D, 3E). Cement duct extending ventrally to gonopore. All female copulatory apparatuses exhibiting similar morphology, except for first two, where vagina and cement duct may slant posteriorly, possibly due to restricted dorsal development from overlying seminal vesicle. Ovaries dorsally positioned above uteri, generally vertically ovoid with germinative zone ventral and growing zone dorsal (Fig. 5).

Distribution: Only known from the southwestern Pingtung County, Taiwan.

Ecology: *Cestoplanoida wanlitongensis* sp. nov. is nocturnal, inhabiting coral reef flats within the coastal intertidal zone. It exhibits pronounced photophobic behavior, rapidly retreating into crevices when exposed to light. Individuals have been observed preying on polychaetes; notably, during *COI* sequence amplification, one primary amplicon was assigned to Polychaeta, providing direct molecular evidence of predation.

Remarks: *Cestoplanoida wanlitongensis* sp. nov. shares with *C. polypora* a ribbon-shaped, uniformly grayish brown body, numerous anterior dorsal eyespots in fan-shaped arrangement, and similar organization of copulatory apparatuses. The primary distinguishing feature of *C. wanlitongensis* sp. nov. is the more posterior position of the pharynx, at approximately three quarters of body length from the anterior end, compared to approximately two thirds in *C. polypora* (Meyer 1921: 150, pl. 3(15)). Morphometric analysis

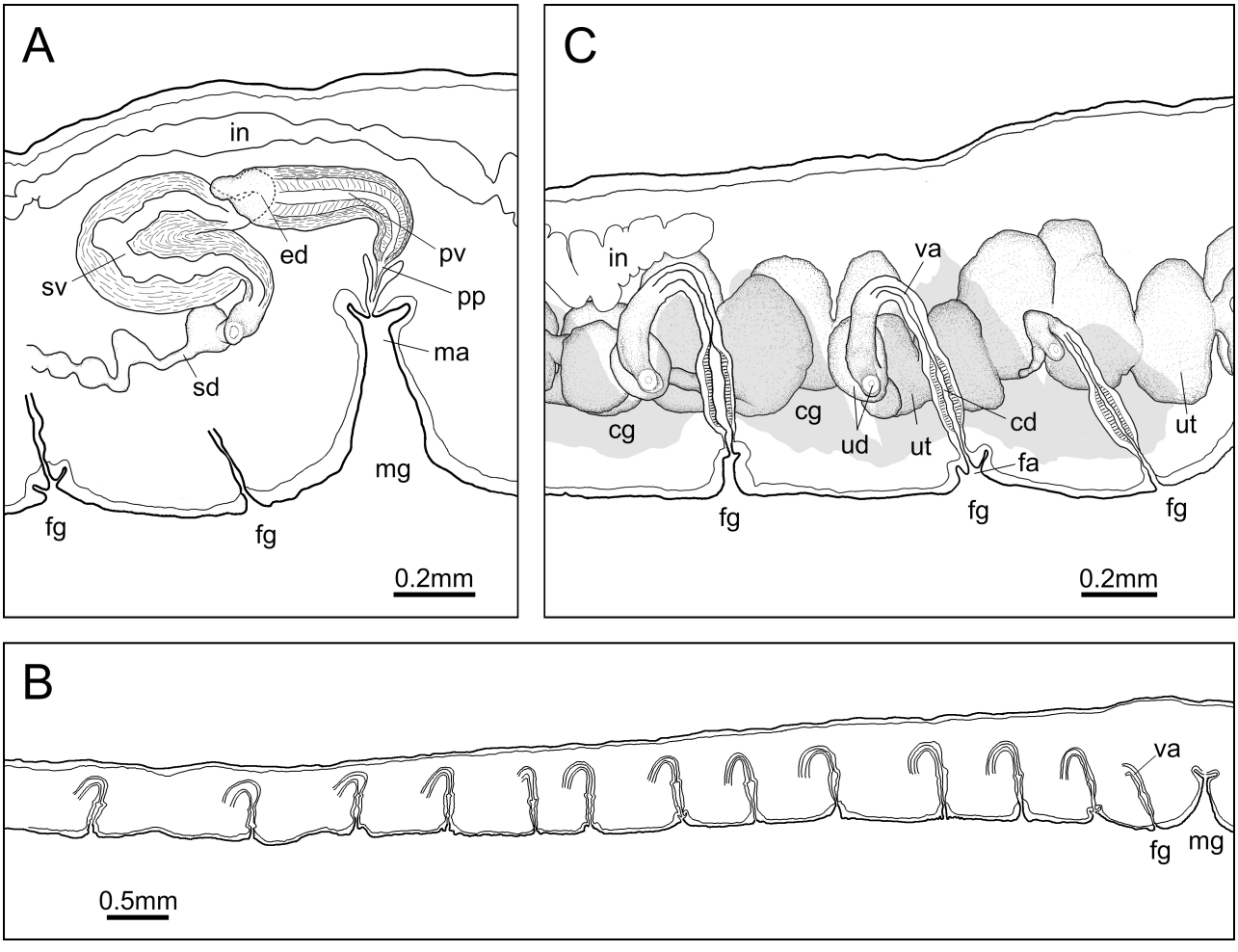

Fig. 4. Reconstruction of copulatory apparatuses of *Cestoplanoida wanlitongensis* sp. nov. (TMPL000053), anterior end to the right. A, male apparatus, showing only left sperm duct; B, arrangement of female apparatuses, cement glands not shown; C, first to third female apparatuses, with left uterus also shown. Abbreviations: cd, cement duct; cg, cement gland (gray shading); ed, ejaculatory duct; fa, female atrium; fg, female gonopore; in, intestine; ma, male atrium; mg, male gonopore; pp, penis papilla; pv, prostatic vesicle; sd, sperm duct; sv, seminal vesicle; ud, uterine duct; ut, uterus; va, vagina.

of the original illustration of *C. polypora* yields a ratio of the distance from the pharynx center to the anterior end relative to total body length of approximately 0.68, significantly lower than the mean ratio of 0.77 in *C. wanlitongensis* sp. nov. (one sample *t*-test, $p < 0.01$). Additionally, Meyer (1921: 149, pl. 3(15)) noted that most preserved specimens of *C. polypora* exhibit dark marginal stripes or parallel longitudinal stripes on the dorsal surface; in contrast, specimens of *C. wanlitongensis* sp. nov. lack these color patterns across all preservation durations. These morphological and preservation related coloration differences support the distinction of *C. wanlitongensis* sp. nov. from *C. polypora*.

Based on histological sections, Meyer (1921: 152, pl. 3(18–19)) observed that each female gonopore in *C. polypora* is associated with a duct (interpreted herein as the vagina) terminating proximally in a roundish sac. She speculated that this sac-like structure might represent either the terminal portion of the duct or a turning point along its course. Possibly due to limitations in the resolution of the histological sections, Meyer regrettably noted her inability to identify any connections among these individual female reproductive apparatuses or with the afferent ducts of the reproductive organs. Faubel (1983) seemingly interpreted the structure at the proximal end of the vagina described by Meyer as a blind sac, identifying it as a Lang's vesicle, and subsequently included the presence of Lang's vesicle as one of the diagnostic characters in his establishment of the genus *Cestoplanoida*. Histological examination of *C. wanlitongensis* sp. nov. revealed that the proximal end of the vagina corresponds to the confluence of uterine ducts, which originate from the uteri on both sides, and that this region does not form a blind sac. Accordingly, the generic diagnosis of *Cestoplanoida* should be revised to exclude the presence of Lang's vesicles.

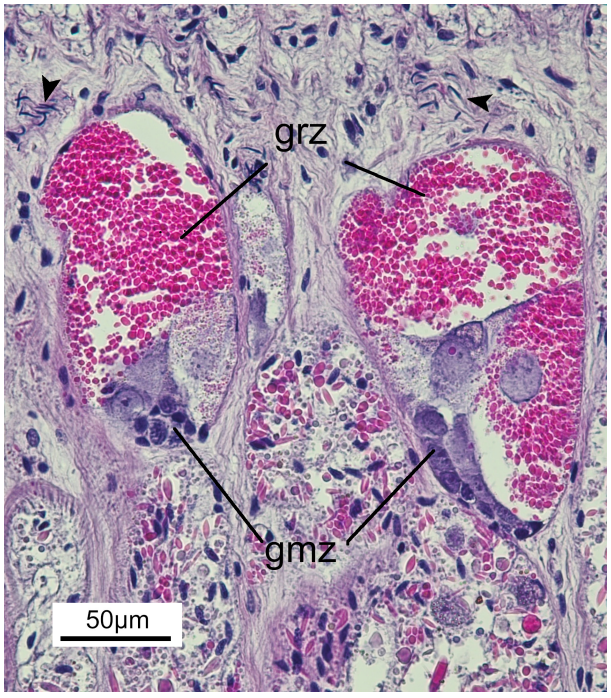

Fig. 5. Cross section of ovary of *Cestoplanoida wanlitongensis* sp. nov. (TMPL000053), showing ventral germinative zone (gmz), dorsal growing zone (grz), and sperm (arrowhead).

All the type specimens possessed a male gonopore. Female gonopores were observed in TMPL000053, TMPL000054, TMPL000055, and TMPL000056, with counts of 13, 23, 18, and 9, respectively; conversely, no female gonopores were externally visible in TMPL000057 and TMPL000058. Histological examination of TMPL000053 and TMPL000056 revealed multiple fully developed female copulatory apparatuses, with uteri containing numerous ovoid to spherical expansions filled with eggs. In both specimens, the male copulatory apparatus and spermiducal vesicles were simultaneously present. In TMPL000053, the spermiducal vesicles were markedly reduced and degenerated, dispersed among the expanded uterine structures. Similarly, in TMPL000056, degenerated spermiducal vesicles were interposed between the expanded uterine structures; however, intact, sperm filled vesicles remained in the posterior region where no female copulatory apparatuses had developed. Correspondingly, Meyer (1921: pl. 3(18)) illustrated an anatomical state in *C. polypora* depicting the concurrent presence of male and female reproductive structures, a condition consistent with the developmental stage observed in TMPL000056. In contrast, TMPL000057 showed no female copulatory apparatuses, while TMPL000058 exhibited seven in early stages of development. Uteri were not observed in either specimen. These observations are consistent with the absence of externally visible female gonopores. Both specimens possessed well developed male reproductive systems, characterized by numerous prominent sperm filled spermiducal vesicles. Notably, these two specimens had the shortest post fixation body lengths among the examined material, suggesting that the development of female copulatory apparatuses occurs after the maturation of the male system.

Molecular phylogeny and genetic distance

Partial 28S rDNA sequences of *Cestoplanoida wanlitongensis* sp. nov. were successfully obtained from five individuals, and these sequences were identical. Partial *COI* sequences were generated from two individuals of the new species and were also identical.

Phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI) methods based on partial 28S rDNA sequences recovered nearly identical topologies; only the ML tree is presented herein (Fig. 6). The resulting tree shows that *C. wanlitongensis* sp. nov. is nested within a monophyletic family Cestoplanidae, with strong nodal support (bootstrap value, BS = 99; posterior probability, PP = 0.83). Within this clade, the new species is recovered as sister to *Eucestoplana* cf. *cuneata*, although with weak support (BS = 59, PP = 0.50). Pairwise uncorrected *p*-distances for the 28S rDNA sequences show that *C. wanlitongensis* sp. nov. exhibits 6.002–7.181% divergence from species of *Cestoplana*, whereas 4.930% to *E.* cf. *cuneata* (Table 3). These results suggest that, despite the weak nodal support, *C. wanlitongensis* sp. nov. is phylogenetically closer to *E.* cf. *cuneata* than to members of *Cestoplana*.

DISCUSSION

Cestoplanoida wanlitongensis sp. nov. represents the second species described in the genus. Direct morphological comparisons with *C. polypora* could not be conducted due to the unavailability of type specimens. Although the histologically observed reproductive structures of *C. wanlitongensis* sp. nov. closely resemble those described by Meyer (1921) for *C. polypora*, the two species differ primarily in the position of the pharynx, which is located more posteriorly in *C. wanlitongensis* sp. nov. Differences in preserved coloration also provide additional but less definitive evidence supporting the separation of the two species.

Histological examination of the type specimens of *C. wanlitongensis* sp. nov. indicates a progressive series of morphological transitions in the reproductive system, supporting a pattern of protandrous sequential hermaphroditism. The male reproductive system matures prior to the female system. Subsequently, female copulatory apparatuses develop sequentially from anterior to posterior. This development is associated with the degeneration of spermiducal vesicles in the same direction. In posterior regions where female structures have not yet formed, spermiducal vesicles remain present and filled with sperm; this indicates a transitional phase during which both male and female reproductive systems are likely functional as the individual progresses toward maturity. Finally, while the spermiducal vesicles degenerate, the male copulatory apparatus nonetheless remains intact. This observed pattern indicates a shift toward predominantly female reproductive function as the individual matures. Furthermore, each female copulatory apparatus connects to a shared pair of uteri. This structural arrangement suggests that the multiple female copulatory apparatuses do not involve the addition of uteri for increased egg storage capacity, but likely serve to enhance the efficiency of egg release. Given the intense wave action

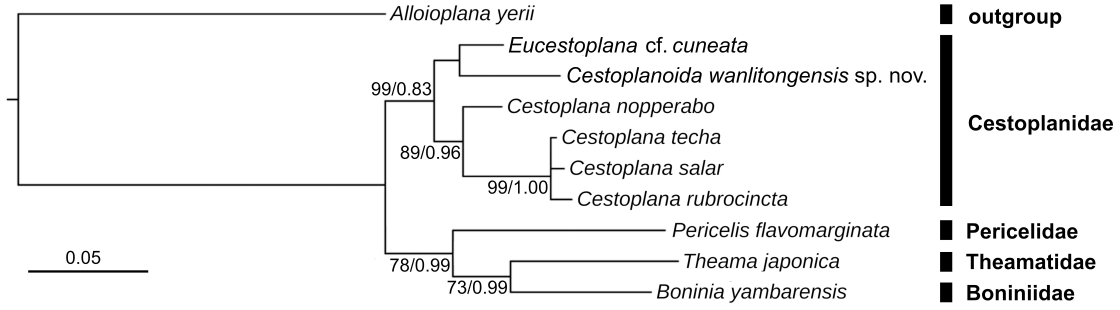

Fig. 6. Maximum likelihood (ML) phylogenetic tree of selected Polycladida based on partial 28S rDNA sequences. Numbers at nodes indicate ML bootstrap values and Bayesian posterior probabilities.

Table 3. Pairwise uncorrected *p*-distance (%) matrix of Cestoplanidae species for 28S rDNA

Species	1	2	3	4	5
1. *Cestoplana nopperabo*	-				
2. *Cestoplana rubrocincta*	4.930	-			
3. *Cestoplana salar*	5.038	1.285	-		
4. *Cestoplana techa*	4.721	1.072	0.750	-	
5. *Cestoplanoida wanlitongensis* sp. nov.	6.002	7.181	6.860	6.760	-
6. *Eucestoplana* cf. *cuneata*	4.609	6.538	6.324	5.901	4.930

and fluctuating water levels of the lower intertidal zone, such a configuration may be advantageous for maintaining reproductive performance under these unstable conditions.

Differences in the positions of the germinative and growing zones within the ovary between Acotylea and Cotylea have long been noted (Prudhoe 1985). Acotylean species exhibit a dorsal germinative zone and a ventral growing zone, whereas cotyleans display the reverse arrangement. This pattern has been reaffirmed in several recent studies (*e.g.*, Liana and Litvaitis 2009; Gammoudi et al. 2016a b; Gammoudi and Tekaya 2017). *Cestoplanoida wanlitongensis* sp. nov. exhibits a ventral germinative zone and dorsal growing zone, further corroborating the consistency of this pattern among studied cotyleans. The present study expands the taxonomic coverage of ovarian configuration observations to include the genus *Cestoplanoida*. While ovarian configurations appear to differ between the two suborders, current histological sampling remains insufficient to draw definitive conclusions. Future investigations across broader taxonomic groups are required to determine if these traits represent reliable synapomorphies for these two suborders.

This study provides the first molecular data for the genus *Cestoplanoida*. Currently, partial 28S rDNA and *COI* sequences are available for only three of the six recognized genera within Cestoplanidae. The three represented genera were recovered as a monophyletic group. *Cestoplanoida wanlitongensis* sp. nov. clusters with *Eucestoplana*, albeit with weak statistical support. As the monophyly of Cestoplanidae remains unresolved, additional sequence data from the remaining genera, including *Acestoplana*, *Cestoplanella* and *Cestoplanides*, are needed to clarify familial relationships and better resolve the evolutionary history of the group.

CONCLUSIONS

Cestoplanoida wanlitongensis sp. nov. is an elongate and ribbon-shaped cotylean flatworm inhabiting coral reef flats in the intertidal zone of southern Taiwan. It represents the second described species of the genus. The new species differs from the type species, *C. polypora*, by the more posterior position of the pharynx, which is located in the posterior quarter of the body. Based on a detailed re-examination of the original description of the type species and histological observations of the new species, the presence of a Lang's vesicle is excluded from the generic diagnosis. Histological evidence further reveals a pattern of protandrous sequential hermaphroditism and identifies a ventral germinative zone within the ovary, consistent with the pattern observed in other cotyleans. Molecular phylogenetic analyses based on partial 28S rDNA sequences support the placement of *Cestoplanoida* within the family Cestoplanidae as sister to *Eucestoplana*.

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Availability of data and materials: Voucher specimens are deposited in the National Taiwan Museum, Taipei, Taiwan. DNA sequences generated in the study have been deposited in GenBank database.

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