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Diversity and phylogenetic relationships of 'tetraphyllidean' Clade 3 (Cestoda) based on new material from orectolobiform sharks in Australia and Taiwan

Janine N. Caira[1] and **K. Jensen**[2]

[1] Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA;

[2] Department of Ecology & Evolutionary Biology and the Biodiversity Institute, University of Kansas, Lawrence, Kansas, USA

Abstract: In an effort to expand knowledge of Clade 3—one of the ten clades that compose the non-monophyletic order 'Tetraphyllidea' all current members of which parasitise orectolobiform sharks—we targeted species of orectolobiform sharks that had not previously been examined for 'tetraphyllidean' cestodes. That work led to the discovery of three new species off Australia and Taiwan. *Ambitalveolus* gen. n. was erected to accommodate these species. *Ambitalveolus costelloae* gen. n. et sp. n., *Ambitalveolus kempi* sp. n., and *Ambitalveolus penghuensis* sp. n. differ from one another in scolex size, genital pore position, and number of marginal loculi, proglottids, and testes. Among 'tetraphyllideans', the new genus most closely resembles the two other genera in Clade 3. It differs from *Carpobothrium* Shipley et Hornell, 1906 in lacking anterior and posterior flap-like extensions of its bothridia; instead, its bothridia are essentially circular. It differs from *Caulopatera* Cutmore, Bennett et Cribb, 2010 in that its vitelline follicles are in two lateral bands, rather than circum-medullary, and in that its bothridia bear, rather than lack, conspicuous marginal loculi. A key to the three genera in Clade 3 is provided. A phylogenetic analysis including new sequence data for one of the three new species of *Ambitalveolus* gen. n., the only species of *Caulopatera*, and all four described species and one undescribed species of *Carpobothrium* supports previously hypothesised close affinities between *Caulopatera* and *Carpobothrium*, with the new genus as their sister group. This is the first report of 'tetraphyllidean' cestodes from the orectolobiform shark family Brachaeluridae Applegate. The association of the new species with orectolobiform sharks is consistent with those of the other members of Clade 3. However, whereas species of *Carpobothrium* and *Caulopatera* parasitise members of the hemiscylliid genus *Chiloscyllium* Müller et Henle, species of *Amitalveolus* gen. n. parasitise members of the Brachaeluridae and Orectolobidae Gill.

Keywords: Orectolobiformes, tapeworms, new species, new genus, 28S rDNA, phylogeny

This paper aims to help advance the process of unravelling the non-monophyly of the order of cestodes traditionally referred to as the 'Tetraphyllidea' by expanding our understanding of the subgroup of 'tetraphyllideans' referred to by Caira et al. (2017) as Clade 3. These authors considered this subgroup to consist of the genus *Carpobothrium* Shipley et Hornell, 1906 and the monotypic *Caulopatera* Cutmore, Bennett et Cribb, 2010, all five described species of which parasitise orectolobiform sharks of the genus *Chiloscyllium* Müller et Henle. The strategy we employed here was to investigate the existence of additional members of Clade 3 in orectolobiform taxa whose cestode faunas are poorly known. Of the 11 genera of sharks in the order Orectolobiformes, cestodes have been reported from one or more species in all but the monotypic genera *Eucrossorhinus* Regan and *Rhincodon* Smith, and the ditypic *Brachaelurus* Ogilby. Examination of *Brachaelurus waddi* (Bloch et Schneider) from Australia yielded two new cestode species

that appear to belong to Clade 3. The cestode faunas of most of the ten valid species of *Orectolobus* Bonaparte have also not previously been examined; existing records consist solely of species in the orders Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014 (see Williams 1962, Campbell and Beveridge 2002, Fyler 2011, Cutmore et al. 2018) and Phyllobothriidea Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014 (see Butler 1987, Cutmore et al. 2017). Examination of cestodes from *Orectolobus japonicus* Regan from Taiwan led to the discovery of a third new species that also appears to belong to Clade 3.

To explore the phylogenetic relationships among these taxa we generated sequence data for the D1–D3 region of the 28S rRNA gene *de novo* for one to three specimens of each of the four described and one undescribed species of *Carpobothrium*, as well as for one specimen of *Caulopatera pagei* Cutmore, Bennett et Cribb, 2010 and two speci-

mens of the new species from *O. japonicus*. The result was the first phylogenetic tree of the relationships among members of Clade 3. Morphological differences in combination with the results of our phylogenetic analysis suggest that the new species represent a distinct genus within Clade 3. That genus and its three new species are described below. This brings the total number of genera in Clade 3 to three.

MATERIALS AND METHODS

Specimen collection

Each shark specimen from which cestodes were collected was assigned a unique combination of collection code and collection number. Additional information on each host specimen is available in the Global Cestode Database (Caira et al. 2021a) using this unique collection code and number. Shark specimens examined were as follows: six females ranging in total length (TL) from 51 to 61 cm and one male 60 cm in TL of *Brachaelurus waddi* collected in the Pacific Ocean off Tuncurry (32.173192 S, 152.510617 E), New South Wales, Australia, between September 24th and 30th, 1991 (SA-17, SA-23, SA-29, SA-35, SA-36, SA-38, and SA-46); one female of *Orectolobus japonicus* 74 cm in TL collected in the Taiwan Strait off Magong (23.563611 N, 119.575278 E), Penghu Island, Taiwan, on May 13th, 2005 (TW-19) and one female of *Chiloscyllium plagiosum* (Anonymous [Bennett]) 69.6 cm in TL collected in the Philippine Sea off Dasi (24.869722 N, 121.836667 E), Taiwan, on May 11th, 2005 (TW-7); one female of *Chiloscyllium griseum* Müller et Henle 63.2 cm in TL collected from the Bay of Bengal off Digha (21.630389 N, 87.544139 E), India, on June 13th, 2013 (IN-64); one male of *Chiloscyllium* sp. 1 (*sensu* Fernando et al. 2019) 41.5 cm in TL from the Gulf of Mannar off Vankalai (8.894167 N, 79.928333 E), Northern Province, Sri Lanka, on March 13th, 2018 (SL-71); two specimens of *Chiloscyllium indicum* (Gmelin), one male 50.2 cm in TL collected from the South China Sea off Mukah (2.897822 N, 112.095589 E), Sarawak, Malaysia, on April 28th, 2004 (BO-443) and one female 52.1 cm in TL collected from the South China Sea off Sepuk Laut (0.214333 S, 109.083417 E), West Kalimantan, Indonesia, on July 13th, 2008 (KA-365); two females of *Chiloscyllium hasseltii* Bleeker 65 and 69 cm in TL collected from the South China Sea off Sematan (1.804292 N 109.779769 E), Sarawak, Malaysia, on June 2nd, 2002 (BO-17, BO-18). In addition, material from a female of *Chiloscyllium* cf. *punctatum* Müller et Henle 117 cm in TL collected from Moreton Bay off St. Helena Island (27.383333 S, 153.233333 E), Queensland, Australia, on May 21st, 2007 (SC-1) was sent to us by Scott Cutmore.

In each case, the spiral intestine was removed through a ventral incision in the body cavity and fixed in seawater-buffered formalin or 95% ethanol. Formalin-fixed spiral intestines were subsequently transferred to 70% ethanol for storage. A subset of cestodes was removed from each spiral intestine prior to fixation, sorted under a dissecting microscope, and preserved in seawater-buffered formalin or 95% ethanol.

Morphological methods

Methods for preparing and examining cestodes for descriptive work with light and scanning electron microscopy (SEM) for most specimens followed Caira et al. (2021b), as did the methods for generating illustrations and taking measurements. Measure-

ments are given in the text as the range (in micrometres unless stated otherwise); in instances in which more than four measurements were taken, the range is following in parentheses by the mean, standard deviation, number of specimens measured, and total number of measurements if more than one measurement was made per worm. Owing to the limited available material of *Ambitalveolus penghuensis* sp. n., the previously mounted hologenophore (see below) specimen JW166 was unmounted from Canada balsam by immersion in methyl salicylate for approximately 48 hrs. The unmounted scolex was processed as described in Caira et al. (2021b) and the posterior portion of the worm was remounted in Canada balsam. Unfortunately, the time in methyl salicylate was insufficient to remove all of the Canada balsam and thus most of the surfaces of the scolex are obscured by remnants of the mounting medium. Nonetheless, we present scanning electron micrographs of the scolex and of the proximal surface of a bothridium.

Re-examination of whole mounts and proglottid cross sections of cestodes from *B. waddi*, originally identified as *Carpobothrium* n. sp. by Caira et al. (1999, 2001) (LRP 2380–2396), informed by recent advances in our understanding of the morphology of *Carpobothrium* (see Koontz and Caira 2016, Caira et al. 2021b), revealed this identification to be incorrect. These specimens are actually *Ambitalveolus costelloae* sp. n. A subset of this material was included in the type series of this species.

Microthrix terminology follows Chervy (2009). Museum abbreviations are as follows: LRP, Lawrence R. Penner Parasitology Collection, University of Connecticut, Storrs, Connecticut, USA; NMNS, Natural Museum of Natural Science, Department of Zoology, Taichung, Taiwan; QM, Queensland Museum, Brisbane, Queensland, Australia; USNM, Natural Museum of Natural History, Smithsonian Institution, Department of Invertebrate Zoology, Washington, D.C., USA.

Molecular methods and phylogenetic analysis

Sequence data were generated *de novo* for the D1–D3 region of the 28S rRNA gene for 11 specimens. In each case, genomic DNA was extracted from the middle portion of the strobila and the remainder of each specimen (i.e., hologenophore *sensu* Pleijel et al. 2008) was prepared as a whole mount and deposited in the LRP. These specimens and their LRP and GenBank accession numbers and unique specimen numbers are as follows: two specimens of *A. penghuensis* sp. n. (JW166, LRP 10790, OM213001 and JW167, LRP 10791, OM213002) ex *O. japonicus* (TW-19); one specimen of *Caulopatera pagei* (JW1393, LRP 10792, OM2130013) ex *C.* cf. *punctatum* (SC-1); one specimen of *Carpobothrium megaphallum* Subhapradha, 1955 (JW143, LRP 10784, OM213004) ex *C. griseum* (IN-64); one specimen of *Carpobothrium marjorieae* Caira, Otto et Jensen, 2021 (JW328, LRP 10788, OM213005) ex *Chiloscyllium* sp. 1 *sensu* Fernando et al. (2019) (SL-71); one specimen of *Carpobothrium* sp. n. 2 (KW221, LRP 10789, OM213006) ex *C. plagiosum* (TW-7); two specimens of *Carpobothrium chiloscyllii* Shipley et Hornell, 1906 (JW117, LRP 10787, OM213007 and AW7a, LRP 10793, OM213008) ex *C. indicum* (KA-365, BO-443); and three specimens of *Carpobothrium eleanorae* Koontz et Caira, 2016 (JW138, LRP 10785, OM213009; AW8, LRP No.10794, OM213010; JW114, LRP 10786, OM213011) ex *C. hasseltii* (BO-17, BO-18). Comparable sequence data were downloaded from GenBank for a fourth specimen of *C. eleanorae* (KF685755;
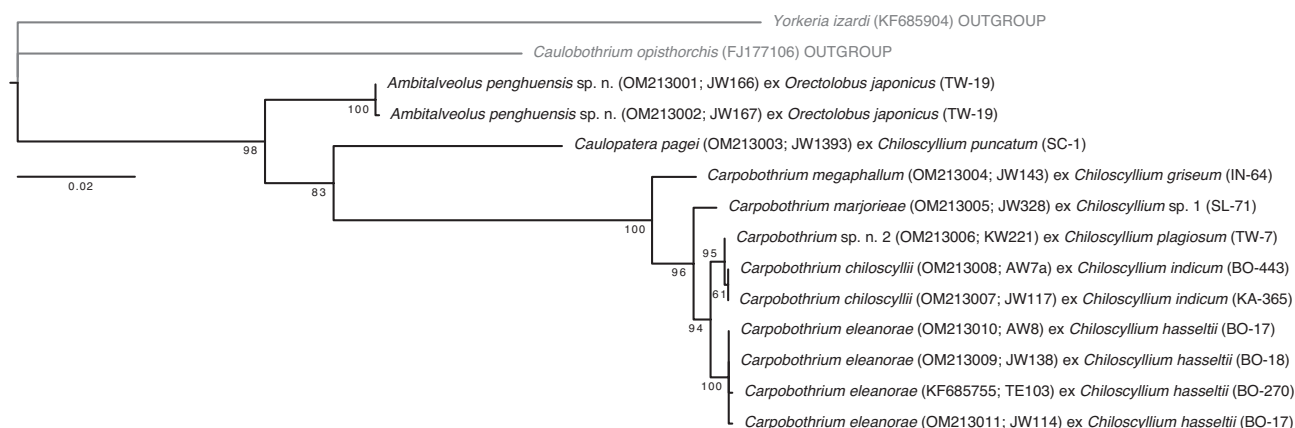
Fig. 1. Phylogenetic tree resulting from maximum likelihood analysis of the D1–D3 region of the 28S rDNA gene for species of *Ambitalveolus*, *Caulopatera*, and *Carpobothrium*. Scale bar indicates number of nucleotide substitutions per site. Numbers associated with nodes are bootstrap support values. Scale bar indicates number of nucleotide substitutions per site. Taxon labels are presented as cestode species name with GenBank accession number and cestode specimen number in parentheses, followed by host species name and host specimen number in parentheses.

Caira et al. 2014) as well as for the outgroup taxa *Yorkeria izardi* Caira, Jensen et Rajan, 2007 (KF685904; Caira et al. 2014) and *Caulobothrium opisthorchis* Riser, 1955 (FJ177106; Healy et al. 2009). The latter two outgroups were chosen based on the topology of the tree resulting from the more extensive analysis of acetabulate cestodes of Caira et al. (2014). Extraction, amplification, and Sanger sequencing of DNA followed Caira et al. (2020) and Jensen et al. (2021).

Sequences were initially aligned and trimmed in Geneious Prime 2019.1.3 or 2019.2.3 (Biomatters Inc., Newark, New Jersey). They were then realigned using webPRANK (Löytynoja and Goldman 2010; https://www.ebi.ac.uk/goldman-srv/webprank/) using the default settings, but with the '+F flag' removed. The best-fitting model of evolution was determined using jModelTest 2.1.7 v20150220 (Darriba et al. 2012) based on evaluation of 88 models. Sample-size corrected Akaike Information Criterion (AICc) values were used to evaluate goodness of fit. Based on the results of the jModelTest analysis, GTR + G was employed as the model of evolution. A maximum likelihood (ML) analysis and a ML bootstrap (BS) analysis were conducted using Garli v. 2.01.1067 (Zwickl 2006) on the University of Kansas (KU) Center for Research Computing Shared Community Cluster. Default Garli configuration settings were used for both ML and BS analyses with the following exceptions: "streefname = random", "attachmentspertaxon = 28" (i.e., twice the number of terminals in the matrix), and "outputphyliptree = 1". In addition, "genthreshfortopoterm = 100000" and "significanttopochange = 0.00001" for 1,000 search replicates were specified for the ML analysis, and "genthreshfortopoterm = 10000" and "significanttopochange = 0.01" for 1,000 bootstrap replicates were specified for the BS analysis. Bootstrap values were displayed on the best ML tree using SumTrees v. 4.0.0 (Sukumaran and Holder 2015) implemented in DendroPy v. 4.0.3 (Sukumaran and Holder 2010).

RESULTS

Phylogenetic analysis

The tree resulting from our ML phylogenetic analysis is shown in Fig. 1. The topology confirms both the pre-

vious hypothesis of close affinities between *Caulopatera* and *Carpobothrium*, and that *Caulopatera* belongs in 'tetraphyllidean' Clade 3. The topology also indicates that the new species described here belong to a lineage within Clade 3 that is distinct from both *Caulopatera* and *Carpobothrium*. This result, in combination with the morphological evidence provided below, supports erection of a new genus to house these species. The topology suggests that this new genus is the earliest diverging lineage in Clade 3.

Ambitalveolus gen. n.

ZooBank number for genus:
urn:lsid:zoobank.org:act:D15D07EC-5837-49D2-A21B-819222B6D550

Diagnosis. Worms euapolytic. Scolex with 4 stalked bothridia; cephalic peduncle present; myzorhynchus absent. Bothridia circular, with distinct rim of marginal loculi. Spinitriches on proximal bothridial surfaces gladiate, scolopate, or absent; spinitriches on distal bothridial surfaces palmate. Proglottids acraspedote, wider than long becoming longer than wide with maturity. Testes numerous, restricted to region anterior to genital pore on poral and aporal sides of proglottid, 1 layer deep. Vas deferens minimal, extending anterior, lateral, and posterior to cirrus sac. Cirrus sac pyriform, narrowly oblong, or J-shaped, containing coiled cirrus; cirrus armed with spinitriches. Genital pores lateral, irregularly alternating; genital atrium shallow. Ovary near posterior end of proglottid, inverted U-shaped in frontal view, tetralobed in cross section. Vagina sinuous, extending from ootype along midline of proglottid, crossing cirrus sac to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Vitellarium follicular; follicles in 2 lateral bands; each band consisting of multiple columns of follicles, extending length of proglottid, uninterrupted by terminal genitalia or ovary. Uterus medial, ventral, sacciform, extending from anterior margin of ovary to posterior margin of cirrus sac. Uterine duct present, entering uterus near its anterior margin. Excretory vessels 4, arranged in 1 dorsal

and 1 ventral pair on each lateral margin of proglottid. Parasites of orectolobiform sharks.

Type species: *Ambitalveolus costelloae* gen. n. et sp. n.

Additional species: *Ambitalveolus kempi* sp. n., *Ambitalveolus penghuensis* sp. n.

Etymology: The generic name is from the Latin *ambitus*, encircling, and *alveolus*, the diminutive of the Latin, cavity, referring to the marginal loculi that occur throughout the circumference of the bothridia.

Remarks. The new genus differs from the 21 genera in the nine other families and clades of 'tetraphyllideans' beyond Clade 3 recognised by Caira et al. (2017) as follows. It differs from genera in the Calliobothriidae Perrier, 1897 (i.e., *Biloculuncus* Nasin, Caira et Euzet, 1997, *Calliobothrium* van Beneden, 1850, *Erudituncus* Healy, Scholz et Caira, 2001, and *Symcallio* Bernot, Caira et Pickering, 2015) and the Balanobothriidae Pintner, 1928 (i.e., *Balanobothrium* Hornell, 1912, *Pachybothrium* Baer et Euzet, 1962, *Pedibothrium* Linton, 1908, S*piniloculus* Southwell, 1925, and *Yorkeria* Southwell, 1927) in lacking, rather than possessing, bothridial hooks. It differs from genera in the Dioecotaeniidae Schmidt, 1969 (i.e., *Dioecotaenia* Schmidt, 1969), the Gastrolecithidae Euzet, 1955 (i.e., *Ceratobothrium* Monticelli, 1892 and *Dinobothrium* van Beneden, 1889), the Rhoptrobothriidae Caira, Jensen et Ruhnke, 2017 (since considered a family within the Phyllobothriidea by Jensen et al. 2021); i.e., *Myzophyllobothrium* Shipley et Hornell, 1906), the Serendipeidae Brooks et Evenhuis, 1995 (i.e., *Duplicibothrium* Williams et Campbell, 1978, *Glyphobothrium* Williams et Campbell, 1977, and *Serendip* Brooks et Barriga, 1995), Clade 1 (i.e., *Pentaloculum* Alexander, 1963 and *Zyxibothrium* Hayden et Campbell, 1981; *sensu* Eudy et al. 2019), and both described genera in Clade 4 (i.e., *Caulobothrium* Baer, 1948 and *Pithophorus* Southwell, 1925) in that its bothridia lack, rather than bear, a distinct anterior/posterior axis. In the latter respect, the new genus resembles the only genus in Clade 2 (i.e., *Anthobothrium* van Beneden, 1850). However, it conspicuously differs from the latter genus in that it lacks, rather than possesses, laciniations on the posterior margins of its proglottids and in that its testes are restricted to the region anterior to the genital pore on the poral and aporal sides of the progottid, rather than extending throughout the length of the proglottid.

With respect to members of Clade 3, although its proglottid anatomy is very reminiscent of that of *Carpobothrium*, the new genus is easily distinguished from the latter genus in that its bothridia lack anterior and posterior bothridial flaps and instead are essentially circular. Furthermore, although the margins of the bothridia of members of the new genus have the ability to fold inward when contracted (e.g., Figs. 2B, 4B), this configuration is very different from the construction of the bothridia of species of *Carpobothrium*, which bear conspicuous bundles of horizontal musculature at their anterior and posterior margins giving the bothridia a permanently pouch-like configuration. Although in bearing circular bothridia the new genus resembles

Caulopatera, it conspicuously differs from *Caulopatera* in that its vitelline follicles are arranged in two lateral bands, rather than being circum-medullary, and its genital pore is in the posterior, rather than anterior, half of the proglottid. Moreover, the bothridia of *Caulopatera* were described as lacking marginal loculi by Cutmore et al. (2010), but the new genus bears conspicuous marginal loculi throughout the entire circumference of its bothridia.

Ambitalveolus costelloae gen. n. et sp. n.   Figs. 2, 3

ZooBank number for species:

urn:lsid:zoobank.org:act:DDEBA94C-EC11-4E45-B219-352CD4421A94

Description (based on 3 incomplete immature, 3 complete mature, and 4 incomplete mature worms; cross sections of posterior proglottids of 1 strobila and its voucher): Worms euapolytic, 22.2–26.5 mm (n = 3) long; maximum width 823–1,251 (1,042 ± 155; 10) at level of scolex. Scolex consisting of 4 stalked bothridia and inconspicuous cephalic peduncle. Scolex proper 773–991 (904 ± 72; 7) long. Bothridia circular, 368–849 (563 ± 122; 8; 19) long, 417–732 (547 ± 85; 9; 29) wide, with 52–59 (55 ± 2; 8; 19) conspicuous marginal loculi, lacking apical sucker. Cephalic peduncle 158–249 (200 ± 28; 7) long, 227–372 (326 ± 54; 7) wide. Proximal bothridial surface covered with acicular filitriches (Fig. 3D); distal surface of bothridial rim covered with acicular filitriches (Fig. 3G); remainder of distal bothridial surface densely covered with palmate spinitriches (Fig. 3E, F), filitriches not observed; microtriches on stalks and cephalic peduncle not observed.

Proglottids acraspedote, 74–89 (n = 3) in total number, initially wider than long, becoming longer than wide with maturity; immature proglottids 70–85 (n = 3) in number; mature proglottids 4 (n = 3) in number. Terminal proglottid 1,131–2,535 (n = 3) long, 366–692 (n = 3) wide, length to width ratio 1.6–6.9:1 (n = 3). Testes oval in frontal view, 27–44 (35 ± 4; 5; 20) long, 26–61 (48 ± 8; 5; 20) wide, >200 in number, 1 layer deep, restricted to region anterior to genital pore on poral and aporal sides of proglottid, arranged in 8–9 columns. Cirrus sac pyriform to weakly J-shaped, 218–321 (n = 4) long, 63–112 (n = 4) wide, length to width ratio 2.9–3.5:1 (n = 4), containing coiled cirrus; cirrus armed with spinitriches. Vas deferens minimal, in relatively tight coils extending slightly anterior, medial, and slightly posterior to cirrus sac. Genital atrium shallow; genital pores lateral, alternating irregularly, 35–39% (n = 3) of proglottid length from posterior end of proglottid.

Ovary near posterior end of proglottid, with slightly lobulated margins, inverted U-shaped in frontal view, tetralobed in cross section, 112–155 (n = 3) long, 145–213 (n = 3) wide. Ovicapt at posterior margin of ovarian isthmus. Mehlis' gland posterior to ovary. Vagina sinuous, extending along mid-line of proglottid from ovarian bridge to level of cirrus sac, then crossing cirrus sac ventrally and extending along anterior margin of cirrus sac to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Vitellarium follicular; follicles arranged in 2 lateral bands; each band
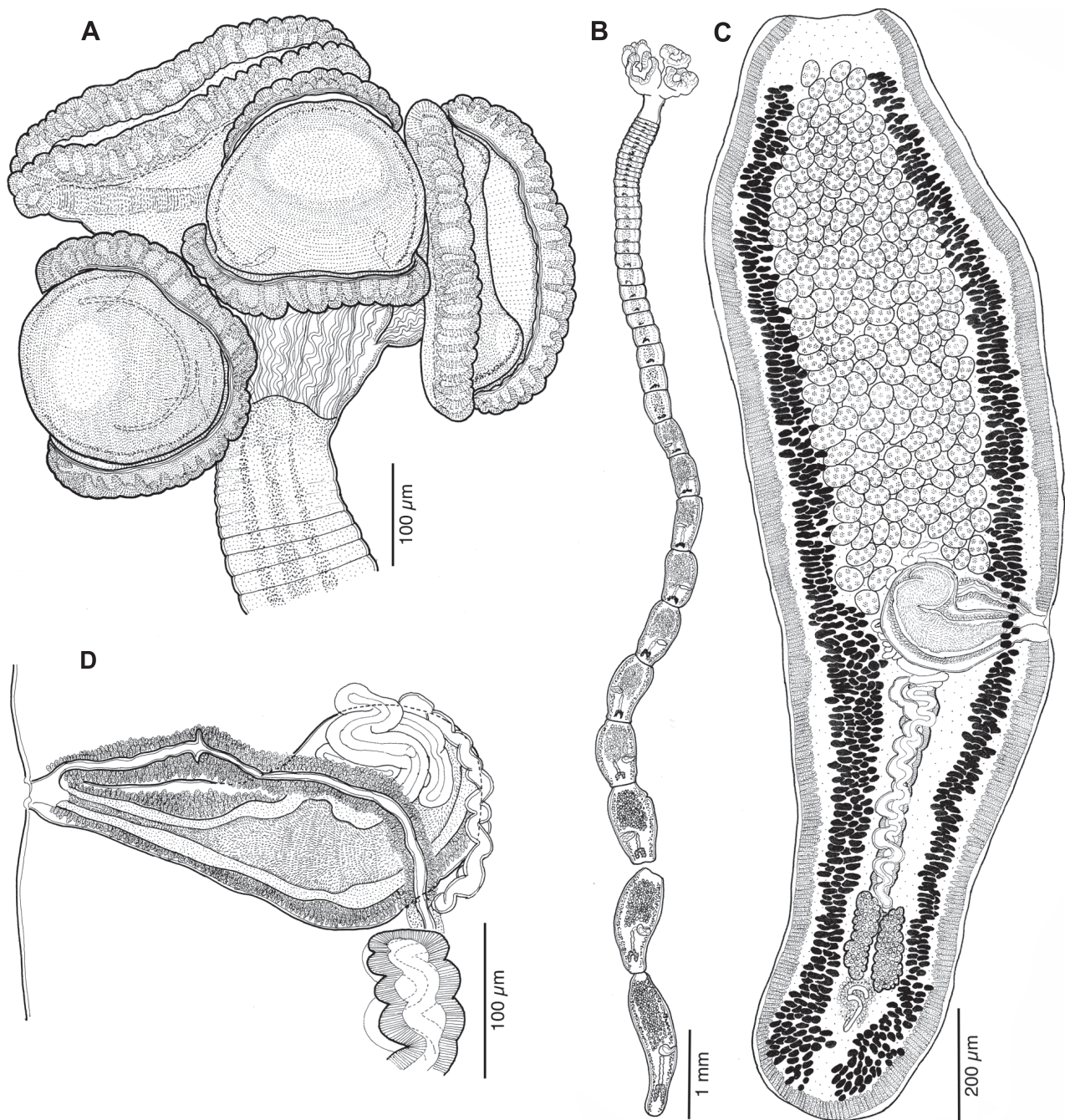
Fig. 2. Line drawings of *Ambitalveolus costelloae* gen. n. et sp. n. from *Brachaelurus waddi* (Bloch et Schneider). **A** – scolex (paratype; USNM 1661753); **B** – whole worm (holotype; QM G239502); **C** – mature proglottid, dorsal view (holotype; QM G239502); **D** – detail of terminal genitalia (paratype; USNM 1661752).

consisting of multiple columns of follicles, extending from near anterior margin of proglottid to posterior margin of proglottid, uninterrupted by terminal genitalia or ovary. Uterus relatively thick-walled, saccate, ventral to vagina, medial, extending anteriorly from ovarian isthmus to posterior margin of cirrus sac; uterine duct sinuous, dorsal to uterus, entering uterus near its anterior margin. Excretory vessels 4, arranged in 1 dorsal and 1 ventral pair on each lateral margin of proglottid. Eggs not observed.

Type host: *Brachaelurus waddi* (Bloch et Schneider) (Orectolobiformes: Brachaeluridae Applegate).

Type locality: Pacific Ocean off Tuncurry (32.173192 S, 152.510617 E), New South Wales, Australia.

Additional locality: None.

Site of infection: Spiral intestine.

Type material: Holotype (QM G239502) and 2 paratypes (QM G239503, G239504); cross sections and paratype voucher (LRP 2389–2396), 4 paratypes (LRP 2380, 2381, 2388, and 10428); 3 paratypes (USNM 1661751–1661753).

Sequence data: None.

Etymology: This species is named for Alison Costello who conducted some of the early work on this species including preparation of whole mounts of a subset of specimens in the type series.
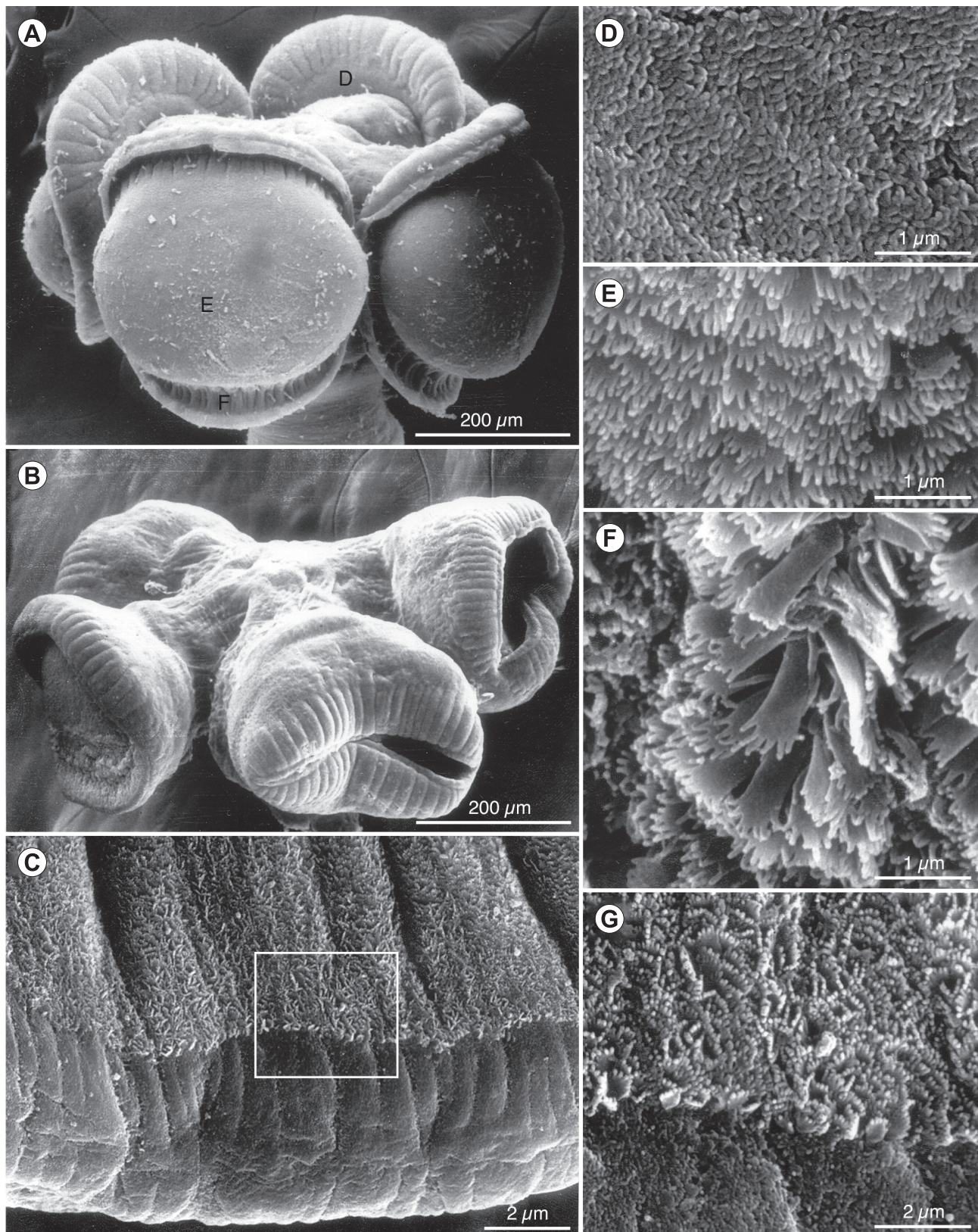
Fig. 3. Scanning electron micrographs of *Ambitalveolus costelloae* gen. n. et sp. n. from *Brachaelurus waddi* (Bloch et Schneider). **A** – relaxed scolex, small letters indicate location of details shown in Fig. 3D–F; **B** – contracted scolex; **C** – distal surface of margin of bothridium, rectangle illustrates location of detail in Fig. 3G; **D** – acicular filitriches on proximal surface of bothridium; **E** – palmate spinitriches on distal surface of bothridium away from margin; **F** – palmate spinitriches on distal surface of marginal loculi; **G** – distal surface of margin of bothridium showing boundary between filitriches and palmate spinitriches.

Ambitalveolus kempi sp. n.   Figs. 4, 5

ZooBank number for species:

urn:lsid:zoobank.org:act:3BF7B8AB-4B4F-43ED-9AB9-287BCFB59C3B

Description (based on 2 immature, 4 mature, and 2 gravid worms; measurements for mature and gravid worms combined): Worms euapolytic, 13.8–36.3 mm (21.5 ± 8.1; 6) long; maximum width 1,899–2,638 (2,181 ± 256; 6) at level of scolex. Scolex consisting of 4 stalked bothridia and cephalic peduncle. Scolex proper 1,232–1,852 (1,471 ± 267; 6) long. Bothridia circular, 755–1,061 (914 ± 101; 7; 12) long by 930–1,293 (1,096 ± 116; 5; 9) wide, with 41–50 (45 ± 3; 5; 8) conspicuous marginal loculi, lacking apical sucker. Cephalic peduncle 178–328 (276 ± 51; 6) long, 366–477 (424 ± 44; 6) wide. Proximal bothridial surface densely covered with acicular filitriches (Fig. 5C) near rim, with scolopate spinitriches and acicular filitriches away from rim (Fig. 5D); distal bothridial surface covered with palmate spinitriches and capilliform filitriches (Fig. 5E); microtriches on stalks and cephalic peduncle not observed.

Proglottids acraspedote, 56–65 (60 ± 3; 6) in total number, initially wider than long, becoming longer than wide with maturity; immature proglottids 54–63 (58 ± 4; 5) in number; mature proglottids 1–3 (2 ± 0.8; 5) in number; gravid proglottids 0–1 (n = 2) in number. Terminal proglottid 2,774–5,214 (4,066 ± 960; 6) long, 629–977 (835 ± 127; 6) wide, length to width ratio 3.4–8.3:1 (5.1 ± 1.9; 6). Testes irregularly oval in frontal view, 40–105 (69 ± 20; 6; 24) long, 55–95 (74 ± 10; 6; 24) wide, 200–258 (223 ± 24; 5) in number, 1 layer deep, restricted to region anterior to genital pore on poral and aporal sides of proglottid, arranged in 5–7 columns. Cirrus sac narrowly oblong or weakly pyriform, 414–472 (443 ± 24; 6) long, 133–219 (172 ± 34; 6) wide, length to width ratio 2.0–3.4:1 (2.7 ± 0.5; 7), containing coiled cirrus; cirrus armed with spinitriches. Vas deferens minimal, in relatively tight coils extending slightly anterior, medial, and slightly posterior to cirrus sac. Genital atrium shallow; genital pores lateral, alternating irregularly, 40–52% (45 ± 4.2; 6) of proglottid length from posterior end of proglottid.

Ovary near posterior end of proglottid, with lobulated margins, inverted U-shaped in frontal view, tetralobed in cross section, 210–358 (292 ± 61; 6) long, 187–375 (271 ± 61; 5) wide. Ovicapt at posterior margin of ovarian isthmus. Mehlis' gland posterior to ovary. Vagina sinuous, extending along mid-line of proglottid from ovarian bridge to level of cirrus sac, then crossing cirrus sac ventrally and extending along anterior margin of cirrus sac to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Vitellarium follicular; follicles arranged in 2 lateral bands; each band consisting of multiple columns of follicles, extending from near anterior margin of proglottid to posterior margin of proglottid, uninterrupted by terminal genitalia or ovary. Uterus relatively thick-walled, saccate, ventral to vagina, medial, extending anteriorly from ovarian isthmus to posterior margin of cirrus sac; uterine duct sinuous, dorsal to uterus, entering uterus near its anterior margin. Excretory vessels 4, arranged in 1 dorsal and 1

ventral pair on each lateral margin of proglottid. Eggs not observed.

T y p e   h o s t : *Brachaelurus waddi* (Bloch et Schneider) (Orectolobiformes: Brachaeluridae Applegate).

T y p e   l o c a l i t y : Pacific Ocean off Tuncurry (32.173192 S, 152.510617 E), New South Wales, Australia.

A d d i t i o n a l   l o c a l i t y : None.

S i t e   o f   i n f e c t i o n : Spiral intestine.

T y p e   m a t e r i a l : Holotype (QM G239505) and 2 paratypes (QM G239506, G239507); 3 paratypes (LRP 10429–10431); 2 paratypes (USNM 1661754, 1661755).

S e q u e n c e   d a t a : None.

E t y m o l o g y : This species is named for Denis Kemp, captain of the Sea Tang, who was essential in helping us connect with fishers in the Tuncurry Fishing Cooperative who encountered *B. waddi* as by-catch in their trapping endeavours.

Remarks. This new species differs conspicuously from *A. costelloae* in its possession of a scolex proper that is both longer (1,232–1,852 μm *vs*. 773–992 μm) and wider (1,899–2,638 μm *vs*. 823–1,251 μm), a smaller number of marginal loculi (41–50 μm *vs*. 52–59 μm), fewer proglottids (56–65 μm *vs*. 74–89 μm), and a more anterior genital pore (40–52 μm *vs*. 35–39% from the posterior end of the proglottid).

Ambitalveolus penghuensis sp. n.   Figs. 6, 7

ZooBank number for species:

urn:lsid:zoobank.org:act:6A12D4DC-1483-445E-BA52-C46EE69C4748

Description (based on 1 mature worm and 1 scolex): Worm euapolytic, 19.4 mm long; maximum width 1,042 at level of scolex. Scolex consisting of 4 stalked bothridia. Scolex proper 745 long. Bothridia circular (Fig. 7A), 309–637 (n = 2) long, 276–439 (n = 2) wide, with 38–42 (n = 1) conspicuous marginal loculi, lacking apical sucker. Cephalic peduncle unclear. Proximal bothridial surface densely covered with gladiate spinitriches, filitriches not observed (Fig. 7B); microtriches on distal bothridal surfaces, stalks, and cephalic peduncle not observed.

Proglottids acraspedote, 38 in total number, initially wider than long, becoming longer than wide with maturity; immature proglottids 37 in number; mature proglottids 1 in number. Terminal proglottid 3,308 long, 493 wide, length to width ratio 6.7 : 1. Testes irregularly oval in frontal view, 34–41 long, 61–66 wide, 182 in number, in 1 layer deep, restricted to region anterior to genital pore on poral and aporal sides of proglottid, arranged in 3–5 columns. Cirrus sac J-shaped, 375 long, 125 wide, length to width ratio 3 : 1, containing coiled cirrus; cirrus armed with spinitriches. Vas deferens minimal, in relatively tight coils extending slightly anterior, medial, and slightly posterior to cirrus sac. Genital atrium shallow; genital pores lateral, alternating irregularly, 42% of proglottid length from posterior end of proglottid.

Ovary near posterior end of proglottid, with lobulated margins, inverted U-shaped in frontal view, tetralobed in cross section, 118 long, 113 wide. Ovicapt at posterior margin of ovarian isthmus. Mehlis' gland posterior to ova-
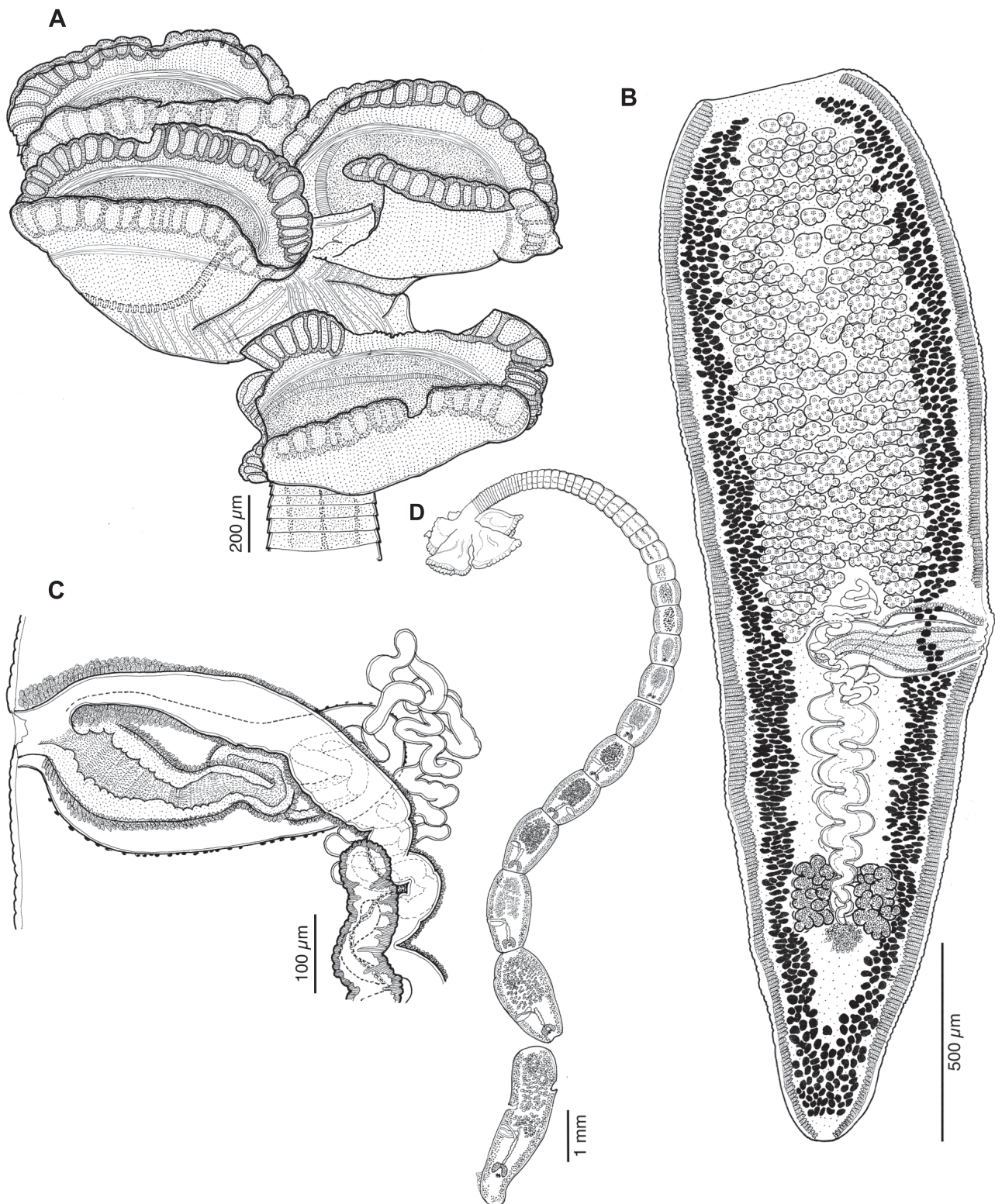
Fig. 4. Line drawings of *Ambitalveolus kempi* sp. n. from *Brachaelurus waddi* (Bloch et Schneider). **A** – scolex (paratype; QM G239507); **B** – mature proglottid, dorsal view (paratype; LRP 10429); **C** – detail of terminal genitalia (holotype; QM G239505); **D** – whole worm (holotype; QM G239505).
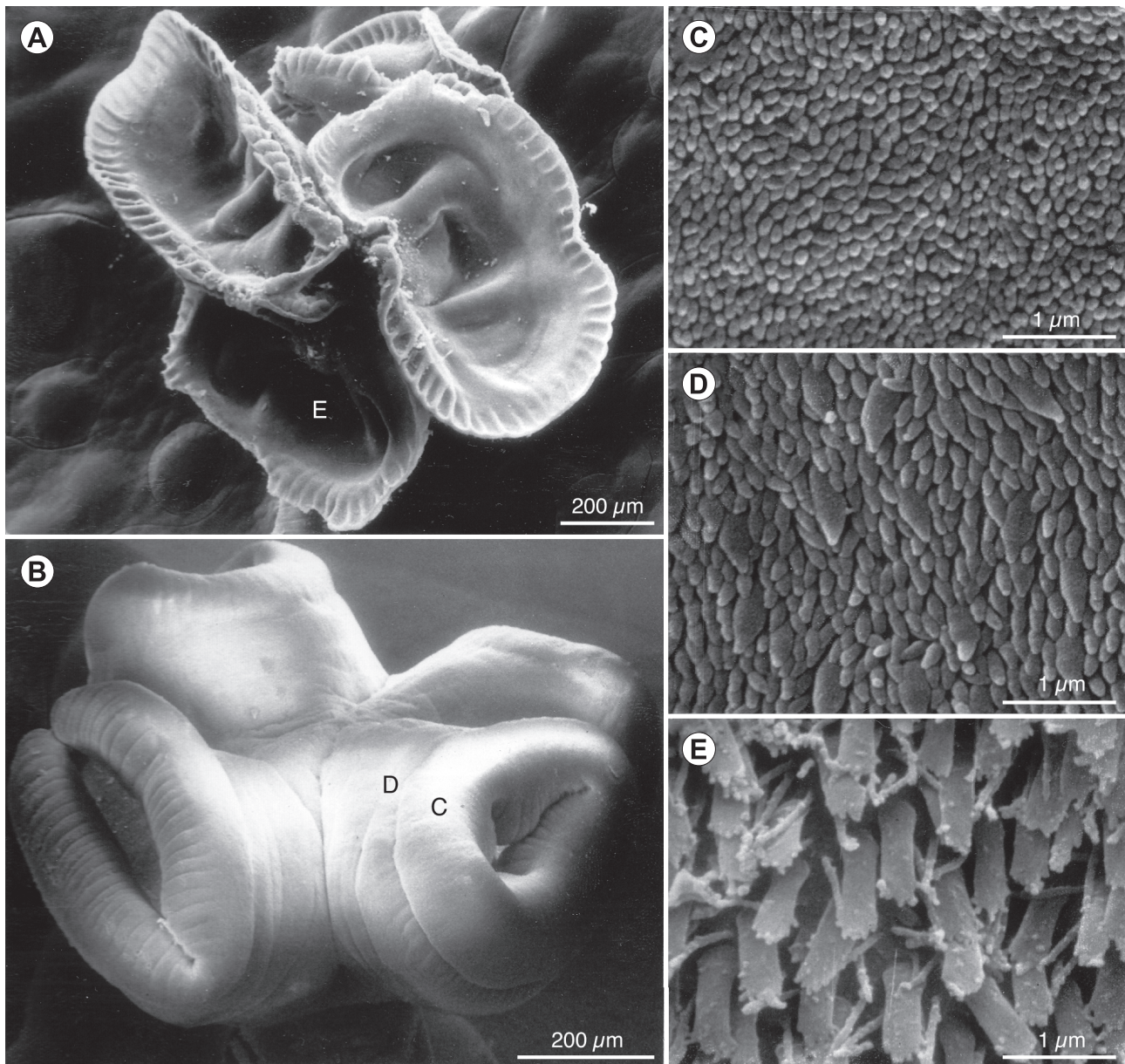
Fig. 5. Scanning electron micrographs of *Ambitalveolus kempi* sp. n. from *Brachaelurus waddi* (Bloch et Schneider). **A** – relaxed scolex; small letter indicates location of detail shown in Fig. 5E; **B** – contracted scolex; small letters indicate location of details shown in Fig. 5C, D; **C** – acicular filitriches on proximal surface of bothridium near rim; **D** – scolopate spinitriches and acicular filitriches on proximal surface of bothridium away from rim; **E** – palmate spinitriches and capilliform filitriches on distal surface of bothridium.

ry. Vagina sinuous, extending along mid-line of proglottid from ovarian bridge to level of cirrus sac, then crossing cirrus sac ventrally and extending along anterior margin of cirrus sac to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Vitellarium follicular; follicles arranged in 2 lateral bands; each band consisting of multiple columns of follicles, extending from near anterior margin of proglottid to posterior margin of proglottid, uninterrupted by terminal genitalia or ovary. Uterus relatively thick-walled, saccate, ventral to vagina, medial, extending anteriorly from ovarian isthmus to posterior margin of cirrus sac; uterine duct sinuous, dorsal to uterus, entering uterus near its anterior margin. Excretory vessels 4, ar-ranged in 1 dorsal and 1 ventral pair on each lateral margin of proglottid. Eggs not observed.

Type host: *Orectolobus japonicus* Regan (Orectolobiformes: Orectolobidae Gill).

Type locality: Taiwan Strait off Magong (23.563611 N, 119.575278 E), Penghu Island, Taiwan.

Additional locality: None.

Site of infection: Spiral intestine.

Type material: Holotype (NMNS 8506-001) and 1 para-type (USNM 1661756).

Sequence data: GenBank accession OM213001 (hologenophore, TW-19 [JW166], LRP 10790); GenBank accession OM213002 (hologenophore, TW-19 [JW167], LRP 10791).
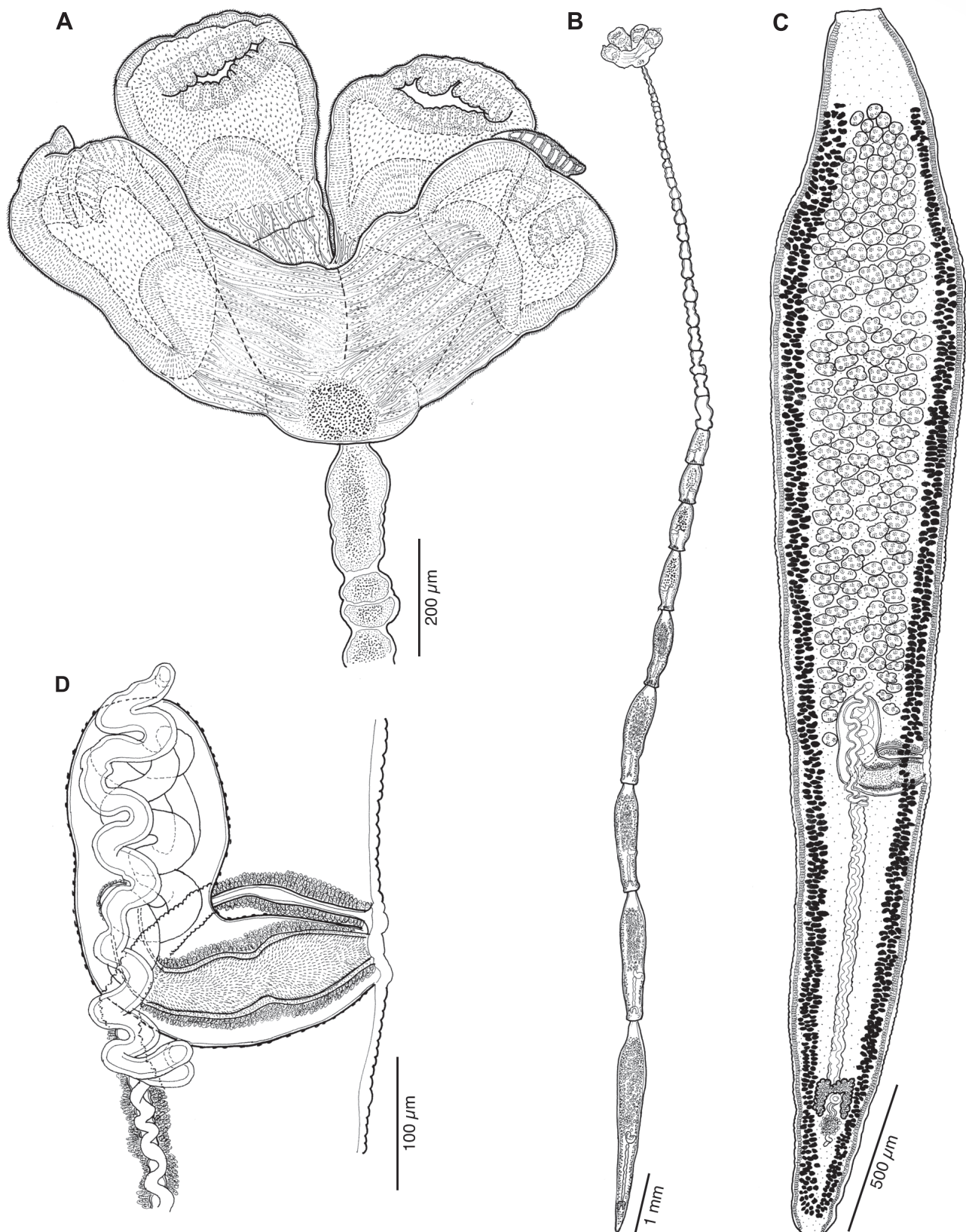
Fig. 6. Line drawings of *Ambitalveolus penghuensis* sp. n. from *Orectolobus japonicus* Regan. **A** – scolex (holotype; NMNS 8506-001); **B** – whole worm (holotype; NMNS 8506-001); **C** – mature proglottid, dorsal view (paratype; NMNS 8506-001); **D** – detail of terminal genitalia (holotype; NMNS 8506-001).
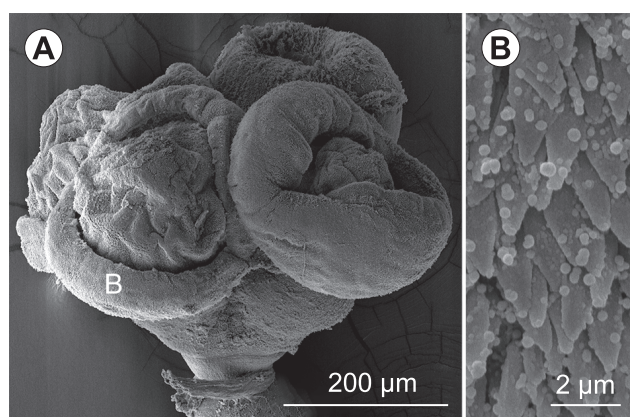
Fig. 7. Scanning electron micrographs of *Ambitalveolus penghuensis* sp. n. from *Orectolobus japonicus* Regan. **A** – Scolex, small letter indicates location of detail shown in Fig. 7B; **B** – gladiate spinitriches on proximal surface of bothridium.

E t y m o l o g y : This species is named for its type locality, the island of Penghu, in the Penghu Island archipelago in the Taiwan Strait west of Taiwan.

Remarks. Despite the small number of specimens of this species available for study, this new species differs conspicuously from both of its congeners. It has fewer proglottids (38 *vs.* 74–89 and 56–65) and fewer testes (182 *vs.* >200 and 200–258) than both *A. costelloae* and *A. kempi*, respectively. It further differs from *A. costelloae* in having fewer marginal loculi (38–42 *vs.* 52–59). It further differs from *A. kempi* in its possession of a scolex that is much narrower (1,042 *vs.* 1,899–2,638). In general, it is a much more delicate species than either of its congeners.

DISCUSSION

The decision to erect a new genus for the three new species described herein was based on a combination of morphological and molecular evidence. Given close similarities in proglottid anatomy, we initially entertained the idea of assigning these species to the genus *Carpobothrium*. The problem with this placement is that all three new species lack one of the key diagnostic features of *Carpobothrium*; rather than bothridia with characteristic anterior and posterior flap-like structures, the anterior of which bears a sucker, the bothridia of the new species are circular. Based on this bothridial configuration, we also considered placing the new species in *Caulopatera*. However, all three new species exhibit vitelline follicles that are arranged in two lateral bands, rather than circum-medullary. In addition, all three possess, rather than lack, conspicuous loculi on their bothridial margins. In fact, the new species exhibit a blend of a subset of the distinctive features of both genera. This led us to consider a third option, which was to synonymise *Caulopatera* with *Carpobothrium* (the latter being the older name) and place the three new species in *Carpobothrium*. The problem with this option is that the resulting revised generic diagnosis would be so general as to make it difficult to distinguish *Carpobothrium* from a number of other genera of 'tetraphyllideans'.

Fortunately, the tree resulting from analysis of our molecular data provided support for the fourth, and in our opinion best, solution which was to erect a new genus for the three new species. In doing so, the generic diagnoses of all three genera in Clade 3 are sufficiently distinctive to allow them to be distinguished not only from one another, but also from the other 21 valid genera in the nine other clades of 'tetraphyllideans'. A key to the three genera is provided below.

1a. Bothridia with anterior and posterior flap-like extensions .... *Carpobothrium*
1b. Bohridia lacking anterior and posterior flap-like extensions 2
2a. Bothridia with conspicuous marginal loculi; vitelline follicles arranged in two lateral bands .... ***Ambitalveolus* gen. n.**
2b. Bothridia lacking conspicuous marginal loculi; vitelline follicles circum-medullary .... *Caulopatera*

The host associations of the genera in Clade 3 are intriguing when considered in the context of the diversity and phylogenetic relationships of the seven families of orectolobiform sharks. To date, only the monotypic Rhincodontidae Müller et Henle (i.e., the whale shark) has not been examined for cestodes. In contrast, relatively extensive work has been done on the cestodes of members of three of the other orectolobiform families. This work has yielded species in a variety of 'tetraphyllidean' genera, but has shown no evidence of members of Clade 3. The Ginglymostomatidae Gill host *Pachybothrium* (see Southwell 1911, Baer and Euzet 1962), *Pedibothrium* (see Caira 1992, Caira and Rasolofonirina 1998, Caira and Euzet 2001, Caira et al. 2004), *Spiniloculus* (see Deshmukh and Shinde 1980), and *Yorkeria* (see Deshmukh 1979). The Stegostomatidae Müller et Henle host *Balanobothrium* (see Hornell 1912, Khambata and Bal 1954, Yamaguti 1954), and *Pedibothrium* (see Shinde et al. 1980). The Parascylliidae Gill host *Pentaloculum* (see Eudy et al. 2019). In contrast, all known species of Clade 3 parasitise members of the remaining three orectolobiform families. Species of *Carpobothrium* and *Caulopatera* parasitise the Hemiscylliidae Gill, and within this family specifically species of *Chiloscyllium*. Species of *Ambitalveolus* parasitise members of the Brachaeluridae and Orectolobidae, which is especially interesting given these two families grouped as sister taxa in the molecular phylogeny of Naylor et al. (2012).

Predictions as to where to look for additional diversity in Clade 3 is informed, at least in part, by these host associations. As noted by Caira et al. (2021b), the species of *Chiloscyllium* that have not yet been examined for cestodes are the most likely candidates as hosts of additional diversity of *Carpobothrium*. Additional species of *Ambitalveolus* are most likely be found in species of the Brachaeluridae and Orectolobidae that have not yet been examined for 'tetraphyllidean' cestodes. In the ditypic Brachaeluridae, the only remaining option is *Brachaelurus colcloughi* Ogilby. In the Orectolobidae the cestode faunas of *Eucrossorhinus dasypogon* (Bleeker) and the nine species of *Orectolobus*

beyond *O. japonicus* would be most interesting to investigate. The most productive strategy for discovering additional novelty in *Caulopatera* is less clear. Of the seven species of *Chiloscyllium* examined for cestodes to date (see Caira et al. 2021b), this genus has only ever been reported from the undescribed species *Chiloscyllium* cf. *punctatum* in Moreton Bay, Australia (Cutmore et al. 2010). Nonetheless, in the absence of other data, the species of *Chiloscyllium* that have not yet been examined for cestodes would seem to be the most viable candidates as hosts for other species of *Caulopatera*.

The results of the molecular phylogenetic analysis have substantially improved our understanding of affinities within Clade 3, especially given the relatively dense taxon sampling of this clade (i.e., all four described and one undescibed species of *Carpobothrium*, the only species of *Caulopatera*, and one of the three species of *Ambitalveolus*) on which the analysis was based. The tree resulting from the analysis indicates that *Ambitalveolus* is the earliest diverging lineage of Clade 3. The grouping of *Caulopatera* and *Carpobothrium* as sister taxa supports the hypothesis advanced by Cutmore et al. (2010), and later endorsed by Caira et al. (2017) when establishing Clade 3, that these genera are close relatives. Within *Carpobothrium*, the earliest diverging species is *Carpobothrium megaphallum* from *Chiloscyllium griseum*. *Carpobothrium marjorieae* from the undescribed species of *Chiloscyllium* sp. 1 (see Fernando et al. 2019) is sister taxon to a subclade that contains the remaining three species. Within this subclade, an undescribed species of *Carpobothrium* from *Chiloscyllium plagiosum* is sister taxon to *Carpobothrium chiloscyllii* from *Chiloscyllium indicum*. This subclade is sister to *Carpobothrium eleanorae* from *Chiloscyllium hasseltii*.

In terms of morphological features, the members of all three genera in Clade 3 share testes that are restricted to the region anterior to genital pore on poral and aporal sides of proglottid. Among other 'tetraphyllidean' genera, this feature is seen only in some, but not all, members of the hook-bearing family Balanobothriidae.

Nonetheless, the phylogenetic affinities of Clade 3 among the acetabulate genera of cestodes remain unresolved. Caira et al. (2014) found *C. eleanorae* (included in their analysis as *Carpobothrium* n. sp. 1) to group away from the other elasmobranch-hosted genera as sister to a clade consisting of the representatives of the orders Cyclophyllidea, Tetrabothriidea, and Nippotaeniidea included in their analysis. However, support for this relationship was poor. In the future, resolution of the phylogenetic relationships of Clade 3 more definitively is likely to involve analyses based on a greater number of molecular markers.

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