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A new genus of rhinebothriidean cestodes from batoid elasmobranchs, with the description of five new species and two new combinations

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Abstract: Survey work of batoid elasmobranchs in the eastern Atlantic and Indo-Pacific revealed multiple species of a new genus of cestode. *Stillabothrium* Healy et Reyda gen. n. (Rhinebothriidea: Escherbothriidae) is unique in its possession of an even number of non-medial longitudinal septa in the posterior portion of the bothridia, resulting in a series of loculi that are longer than wide (i.e. vertically oriented) and are arranged in columns. Five new species of *Stillabothrium* are described, *S. ashleyae* Willsey et Reyda sp. n., *S. davidcynthiaorum* Daigler et Reyda sp. n., *S. campbelli* Delgado, Dedrick et Reyda sp. n., *S. hyphantoseptum* Herzog, Bergman et Reyda sp. n., *S. jeanfortiae* Forti, Aprill et Reyda sp. n., and two species are formally transferred to the genus, *S. amuletum* (Butler, 1987) comb. n., and *S. cadenati* (Euzet, 1954) comb. n., the latter of which is redescribed. The species differ in the configuration of the other bothridial septa and in proglottid anatomy. Species of *Stillabothrium* were found parasitising a total of 17 species of batoid elasmobranchs of the genera *Dasyatis* Rafinesque, *Glaucostegus* Bonaparte, *Himantura* Müller et Henle, *Pastinachus* Rüppell, *Rhinobatos* Linck and *Zanobatus* Garman, including several host species that are likely new to science. A phylogenetic hypothesis based on Bayesian analysis of 1 084 aligned positions of the D1–D3 region of 28S rDNA for 27 specimens representing 10 species of *Stillabothrium* and two outgroup species supported the monophyly of *Stillabothrium*. These results also supported morphologically determined species boundaries in all cases in which more than one specimen of a putative species was included in the analysis. Host specificity appears to vary across species of *Stillabothrium*, with the number of host species parasitised by each species of *Stillabothrium* ranging from one to four. The geographic distribution of species of *Stillabothrium* spans the eastern Hemisphere, including the eastern Atlantic (coastal Senegal) and several locations in the Indo-Pacific (coastal Vietnam, Borneo and Australia). In addition, *Phyllobothrium biacetabulatum* Yamaguti, 1960 is formally transferred into family Escherbothriidae, although its generic placement remains uncertain (species *incertae sedis*).

Keywords: tapeworms, taxonomy, Rhinebothriidea, Escherbothriidae, *Stillabothrium*, stringrays, biodiversity, species boundaries

In recent years, global scale efforts to survey, inventory and describe tapeworms from vertebrates not previously examined for parasites have led to the discovery of hundreds of new species (Caira et al. 2012). Studies of newly collected material have also revealed new genera (Eyring et al. 2012, Schaeffner and Beveridge 2012, Jensen et al. 2014) and have facilitated recognition of new families (Ruhnke et al. 2015) and new orders (Kuchta et al. 2008, Healy et al. 2009, Caira et al. 2014) of cestodes.

The use of DNA sequence data has played a key role in these discoveries, but it also presents a new challenge in that the taxonomic treatments of new species often lag behind the publication of phylogenies that include such undescribed taxa. For example, in their proposal for the tapeworm order Rhinebothriidea, Healy et al. (2009) presented a phylogenetic hypothesis that included a total of 36 rhinebothriidean species, 27 of which were new to science, as well as four clades considered to represent new genera.

This paper focuses on species assigned to 'Rhinebothriinae New genus 3' by Healy et al. (2009). In addition to formal generic designation of Healy et al.'s (2009) New Genus 3, five new species are described and two described species are transferred to the new genus.

The present study includes a large collection of specimens of the new genus from Senegal, Malaysian Borneo, Indonesian Borneo, Vietnam and Australia. In a number of

cases, determination of species boundaries based solely on morphological criteria was initially challenging. Sequence data for the partial 28S rDNA gene were therefore found to be of great use in helping to resolve species boundaries.

The present study contributes to a growing body of knowledge of elasmobranch cestodes by reporting on species of Rhinebothriinae New genus 3 from 17 species of elasmobranchs, nearly all of which have been reported as hosts for other cestode genera and species (see Euzet 1954, Butler 1987, Reyda and Caira 2006, Twohig et al. 2008, Ivanov and Caira 2012, Schaeffner and Beveridge 2012, 2014, Mojica et al. 2013, Cielocha et al. 2014, Jensen and Russell 2014, Jensen et al. 2014, Ruhnke et al. 2015). Most such reports are fairly recent and were the result of the same survey efforts that made the present study possible. Here we characterise a large amount of interspecific morphological variation, especially in bothridial morphology, among the seven species taxonomically treated here, emphasising the need for the continued application of molecular data in combination with morphological data for effectively delineating species.

MATERIALS AND METHODS

The cestode specimens examined here were obtained from a total of 38 elasmobranch specimens representing 17 species from the coasts of Senegal, Vietnam, Malaysian Borneo, Indonesian Borneo, and Australia. Elasmobranchs were collected by trawler, gill net, seine or bought directly from local fisherman or at fish markets. Each host was identified in the field, assigned a Collection Code and unique Collection Number, photographed and relevant information (e.g. sex, size) was recorded. A tissue sample was also collected for subsequent DNA analysis. Images and detailed collection data for each host can be accessed at the Global Cestode Database (Caira et al. 2012) at www.elasmobranchs.tapewormdb.uconn.edu by entering its assigned Collection Code and Collection Number (e.g. SE-289). Elasmobranch classification follows Naylor et al. (2012a); elasmobranch taxonomy follows Naylor et al. (2012b).

Host field identifications were verified using NADH2 sequence data for each host (see Naylor et al. 2012b). Hosts and their unique Collection Code and Collection Numbers obtained from each country are as follows: *Rhinobatos rhinobatos* (Linnaeus) (SE-289) and *Zanobatus schoenleinii* (Müller et Henle) (SE-28, SE-201, SE-299) from Senegal; *Dasyatis zugei* (Müller et Henle) (VN-23, VN-34) from Vietnam; *Dasyatis biasa* (Last, White et Naylor) (BO-477), *Urogymnus lobistomus* (Manjaji-Matsumoto et Last) (BO-247), *Himantura* cf. *pastinacoides* (BO-61, BO-79, BO-98, BO-100, BO-119, BO-168), *Himantura uarnacoides* (Bleeker) (BO-118), *Himantura uarnak* 3 (BO-47), *Himantura undulata* (Bleeker) (BO-24), *Himantura heterura* (Bleeker) (BO-19, BO-66, BO-67, BO-141, BO-170, BO-237, BO-238) and *Pastinachus solocirostris* Last, Manjaji et Yearsley (BO-267) from Malaysian Borneo; *Dasyatis biasa* (KA-182, KA-184, KA-378), *Himantura macrura* (Bleeker) (KA-111), *Himantura gerrardi* (Gray) (KA-145), *Himantura oxyrhyncha* (Sauvage) (KA-252), *H. heterura* (KA-99) and *P. solocirostris* (KA-148) from Indonesian Borneo; *Himantura leoparda* Manjaji-Matsumoto et Last (NT-117), *Himantura australis* Last, White et Naylor (CM03-3, CM03-25, CM03-65) and *Glaucostegus ty-*

pus [Anonymous (Bennett)] (AU-56) from Australia. Additional locality data for each host are provided below in the relevant taxonomic treatment for each cestode species.

In the case of each host specimen, the spiral intestine was removed and opened with a longitudinal incision. A subsample of worms was removed, washed in seawater, and sorted into two sets. The first set was fixed in 10% seawater-buffered formalin and subsequently stored in 70% ethanol, the other set was fixed in 95% ethanol. Spiral intestines were fixed in 10% seawater-buffered formalin and additional worms were removed under a dissecting microscope upon returning to the laboratory. Worms to be prepared as whole mounts were hydrated in a graded series of ethanols, stained in Delafield's hematoxylin, destained in 70% acid ethanol, neutralised in 70% basic ethanol, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted on glass slides in Canada balsam.

Specimens for histological sectioning were embedded in paraplast and sectioned at 8 or 10 μm intervals using an Olympus CUT 4060 retracting rotary microtome. Sections were mounted on glass slides flooded with 2.5% sodium silicate and dried on a slide warmer for 4–8 hr. Cross sections of mature proglottids and longitudinal or cross sections of scoleces were prepared for each species described here. Sections were stained with Delafield's hematoxylin and eosin according to conventional techniques. A portion of each worm sectioned was prepared as a whole mount as described above, and retained as a voucher.

For examination with scanning electron microscopy (SEM), selected worms were first cut in half and the strobila of each worm was prepared as a whole mount, as above, and kept as a voucher and the scolex was examined with SEM. Scoleces were hydrated in a graded ethanol series, placed in 1% osmium tetroxide overnight, dehydrated in a graded ethanol series, transferred to hexamethyldisilazane for 15 min in an exhaust hood and allowed to air dry. Dried worms were mounted on carbon tabs (Ted Pella, Inc., Redding, California) on aluminium stubs, placed in a dessicator overnight, sputter coated with 250–300 Å of gold/palladium and examined with a LEO/Zeiss DSM982 Gemini or FEI Nova Nano 450 (University of Connecticut) field emission scanning electron microscope. Microthrix terminology follows Chervy (2009).

Measurements of whole mounted cestodes were obtained using an ocular micrometre on an Olympus CX31 compound microscope or taken with the aid of LAS V3.8 (Leica Application Suite, Leica microsystems, Switzerland) digital microscopy software connected to a Leica DSC295 digital camera on a Leica DM2500 compound microscope. All measurements are reported in micrometres, unless otherwise stated, and are provided in descriptions as the range followed in parentheses by the mean, standard deviation and number of worms measured. Scolex morphological shape terminology follows Clopton (2004). Drawings were made with the aid of a drawing tube. Museum abbreviations are as follows: IPCAS – Institute of Parasitology of the Biology Centre of the Czech Academy of Science, České Budějovice, Czech Republic; LRP – Lawrence R. Penner Parasitology Collection, Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA; MNHN – Muséum National d'Histoire Naturelle, Paris, France; MZB – Museum Zooligum Bogoriense, Center for Biology, Indonesian Institute of Science, Cibinong, Jakarta-Bogo, Java, Indonesia; MZUM (P) – Muzium Zoologi,

Universiti Malaya, Kuala Lumpur, Malaysia; QM, Queensland Museum, Queensland, Australia; SBC – Sarawak Biodiversity Center, Kuching, Sarawak, Malaysia; USNM – United States National Museum, Smithsonian Institution, Washington, D.C. USA. Nomenclatural acts in this manuscript are registered at Zoobank. org. Not all authors of this work are the authors of the individual generic and species descriptions. The authors of each individual taxonomic action are listed after the first use of the taxon name in the description.

Cestode specimens included in the molecular analyses, with taxon names, hosts, collection localities and museum voucher numbers for hologenophores, are provided in Table 1. Specimens for DNA sequencing that were originally fixed in 95% ethanol were digitally photographed using a Luminera Infinity 2 digital microscope camera on an Optivision SZ 6745 stereomicroscope. Digital images of those specimens were deposited in LRP. Scoleces and/or terminal proglottids were removed and prepared as whole mounts as described above and hologenophores (*sensu* Pleijel et al. 2008) were deposited in the LRP.

Total genomic DNA was extracted from the specimens using a Qiagen® DNEasy tissue kit. Kit protocol was followed with the following two exceptions: in the final elution of DNA, columns were allowed to stand for 15–20 min per elution, as opposed to one minute, and final elution was performed in nuclease free water as opposed to the kit buffer. The D1–D3 regions of the large nuclear ribosome regions (28S DNA) were amplified and sequenced using the forward primers ITS4f (5'-GCATATCAATAAGCGGAG-GA-3'), LSU-5 (5'-TAGGTCGACCCGCTGAAYTTAAGC-3') and 330f (5'-CAAGTACCGTGAGGGAAAGTTG-3') and the reverse primers ECD2 (5'-CCTTGGTCCGTGTTTCAAGACG-GG-3'), Tylo-1357 (5'-TCGATTTGCACGTCAGAATCG-3') and 1500R (5'-GCTATCCTGAGGGAAACTTCG-3'). The D1–D3 region of 28S rDNA was amplified using Promega® GoTaq Master Mix (Promega Corp., Madison, Wisconsin, USA) in a Bio-rad® iCycler version 4006 (Bio-Rad Laboratories, Inc., Hercules, California, USA) under the following conditions: denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 2 min 30 s, repeated for 45 cycles. PCR amplicons were loaded into a 1% molecular grade agarose gel with 1X TAE buffer. Gels were stained using ethidium bromide and were visualised using a FluorChem™ 8900 system (Protein Simple, San Jose, California, USA) as well as an ultra violet lamp tray. Bands of the correct length were excised and purified using a Promega Wizard® SV Gel and PCR Clean-Up System. Kit protocol was followed with one exception: final incubation time with elution buffer was extended from 1 min to 15–30 min DNA yield was analysed for yield by gel electrophoresis. DNA was sequenced using ABI Big Dye 3.1v chemistry.

Sequencing product was cleaned using EDTA/ethanol precipitation and analysed by an ABI 3130x/genetic analyser. Contiguous sequences of 28S rDNA were assembled using or Geneious 6.1.5. Alignments were performed using the Muscle option in MEGA v. 6. Sequences were aligned with MUSCLE in Geneious v. 5.6.4 with default settings. Phylogenetic analyses were conducted on sequences from a total of 27 cestode specimens, including 19 specimens from which sequences were generated *de novo* for this study (Table 1). *Anthocephalum michaeli* Ruhnke et Seaman, 2009 and *Escherbothrium* sp. were used as outgroup species.

Bayesian inference was conducted using MrBayes version 3.2 (Ronquist and Huelsenbeck 2003) with the following setttings: lset nst = 6 rates = invgamma ngammacat = 4; ngen = 5 000 000; samplefreq = 1 000. Fifty percent of the samples were discarded on burnin. A parsimony bootstrap analysis was also conducted using PAUP* version 5.4.0b (Swofford 2000). One thousand replicates (1 000) were performed, with 10 step-wise 6 addition heuristic searches per replicate.

RESULTS

Phylogenetic analyses. The Bayesian phylogram topology is given in Fig. 1. Strong support for the monophyly of replicate specimens of each species of *Stillabothrium* gen. n. was found both as a result of the Bayesian and Bootstrap analyses. The ten species of *Stillabothrium* gen. n. were grouped in two principle clades, each with five species. Clade 1 (see Fig. 1) consisted of *Stillabothrium jeanfortiae* sp. n., *Stillabothrium cadenati* (Euzet, 1954) comb. n., and three undescribed species (*Stillabothrium* spp. n. 1, 2, 4). Four of these five species conspicuously lack marginal septa on the posterior region of the bothridia, but the scolex voucher (LRP 3899) of the fifth species, *Stillabothrium* sp. n. 2, bears marginal septa in the anterior region of the bothridia that appear to extend into the posterior region. Clade 2 comprises *Stillabothrium amuletum* (Butler, 1987) comb. n., *Stillabothrium hyphantoseptum* sp. n., *Stillabothrium ashleyae* sp. n., *Stillabothrium davidcynthiaorum* sp. n., and *Stillabothrium campbelli* sp. n. Four of the five species of that clade possess marginal septa in the posterior region of the bothridia, in contrast, *S. hyphantoseptum* sp. n., does not (Fig. 1).

The results of the phylogenetic analyses also provide support for the morphologically-based species boundaries employed here. For each species of *Stillabothrium* gen. n. for which two or more specimens were sequenced (i.e. *S. ashleyae* sp. n., *S. davidcynthiaorum* sp. n., *S. campbelli* sp. n., *S. hyphantoseptum* sp. n., *S. cadenati* [Euzet, 1954] comb. n.), the replicate specimens of morphologically circumscribed species were found to be monophyletic (Table 1, Fig. 1). However, among the five species, only the three replicates of *S. ashleyae* sp. n. (all from KA-182) were identical with one another. Two samples of *S. davidcynthiaorum* sp. n. from two different stingray species (KA-145.3 from *H. gerrardi* and KA-111.1 from *Himantura macrura*) differed by one base pair (bp) from one another, as well as from the four specimens from *H. heterura* (BO-237.1, BO-237.2, BO-237.3, and LRP 3926), and from the single specimen from *Himantura uarnak* 3 (BO-47.2). All four specimens of *S. campbelli* sp. n. were obtained from the same individual *Himantura* cf. *pastinacoides*. One of those four specimens (BO-100.8) differed by one bp from the other three. The two specimens of *S. hyphantoseptum* sp. n. (KA-148.1, KA-148.3), both from the same individual *P. solocirostris*, differed by two bp. Two specimens of *S. cadenati* comb. n. (SE-299.1, SE-299.2), both from the same individual of *Z. schoenleinii*, differed from one another by one bp. One of those (SE-299.1) was identical to a specimen (LRP 3924) from another individual of

Table 1. Cestode specimens included in the molecular analyses, with taxon names, hosts, localities, field and specimen codes, museum voucher numbers for hologenophores and GenBank numbers.

Species†	Host species	Locality	Field code	Specimen number	Voucher Acc. No.	GenBank Acc. No.**	References
Anthocephalum michaeli Ruhnke et Seaman, 2009	*Dasyatis longa* (Garman)	Gulf of California, San Jose del Cabo, Mexico	BJ-423	BJ-423	LRP 8515	KM658204	Ruhnke et al. 2015
Escherbothrium sp.	*Urotrygon* sp. 1	Eastern Pacific Ocean, Costa Rica	CRP-50	CRP-50	LRP 8519	KM658197	Ruhnke et al. 2015
Stillabothrium amuletum (Butler, 1987) comb. n.*	*Glaucostegus typus*‡	Timor Sea, off Dundee Beach, Northern Territory, Australia	AU-56	AU-56	LRP 3917	FJ177117	Healy et al. 2009
Stillabothrium ashleyae Willsey et Reyda sp. n.	*Dasyatis biasa* (Last, White et Naylor)	Java Sea (Pacific Ocean), off Selakau, West Kalimantan, Indonesian Borneo	KA-182	KA-182.3	LRP 8992	KX826838	Present study
			KA-182	KA-182.4	LRP 8993	KX826837	Present study
			KA-182	KA-182.5	LRP 8994	KX826836	Present study
Stillabothrium cadenati (Euzet, 1954) comb. n.	*Rhinobatos rhinobatos* (Linnaeus)	Eastern Atlantic Ocean, off Kafountine, Senegal	SE-289	SE-289.1	LRP 9000	KX826839	Present study
	Zanobatus schoenleinii (Müller et Henle)	Eastern Atlantic Ocean, off Joal, Senegal	SE-299	SE-299.1	LRP 9001	KX826840	Present study
			SE-299	SE-299.2	LRP 9002	KX826841	Present study
		Eastern Atlantic Ocean, off Soumbedioune, Senegal	SE-201	SE-201	LRP 3924	FJ177110	Healy et al. 2009
Stillabothrium campbelli Delgado, Dedrick et Reyda sp. n.	*Himantura* cf. *pastinacoides*	Sulu Sea (Pacific Ocean), off Kampung Tetabuan, Sabah, Malaysian Borneo	BO-100	BO-100.2	LRP 8995	KX826842	Present study
			BO-100	BO-100.6	LRP 8996	KX826843	Present study
			BO-100	BO-100.7	LRP 8997	KX826844	Present study
			BO-100	BO-100.8	LRP 9149	KX826845	Present study
Stillabothrium davidcynthiaorum Daigler et Reyda sp. n.	*Himantura heterura* (Bleeker)	South China Sea, off Mukah, Sarawak, Malaysian Borneo	BO-237	BO-237.1	LRP 8986	KX826847	Present study
			BO-237	BO-237.2	LRP 8987	KX826848	Present study
			BO-237	BO-237.3	LRP 8988	KX826849	Present study
	Himantura heterura‡	South China Sea, off Mukah, Sarawak, Malaysian Borneo	BO-237	BO-237	LRP 3926	FJ177116	Healy et al. 2009
	Himantura uarnak 3	South China Sea, off Mukah, Sarawak, Malaysian Borneo	BO-47	BO-47.2	LRP 8989	KX826851	Present study
	Himantura macrura (Bleeker)	Java Sea (Pacific Ocean), off Kalapseban, Central Kalimantan, Indonesian Borneo	KA-111	KA-111.1	LRP 8990	KX826846	Present study
	Himantura gerrardi (Gray)	Java Sea (Pacific Ocean), off Singkawang, West Kalimantan, Indonesian Borneo	KA-145	KA-145.3	LRP 8991	KX826850	Present study
Stillabothrium hyphantoseptum Herzog, Bergman et Reyda sp. n.	*Pastinachus solocirostris* Last, Manjaji et Yearsley	Java Sea (Pacific Ocean), off Singkawang, West Kalimantan, Indonesian Borneo	KA-148	KA-148.1	LRP 9003	KX826853	Present study
			KA-148	KA-148.3	LRP 9004	KX826852	Present study
Stillabothrium jeanfortiae Forti, Aprill et Reyda sp. n.	*Himantura australis* Last, White et Naylor	Gulf of Cerpentaria, Weipa, Australia	CMO3-3	CMO3-3.5	LRP 8999	KX826854	Present study
Stillabothrium sp. n. 1†	*Fontitrygon margaritella*‡ (Compagno et Roberts)	Eastern Atlantic Ocean, off Mbour, Senegal	SE-125	SE-125	LRP 3898	FJ177111	Healy et al. 2009
Stillabothrium sp. n. 2†	*Fontitrygon margaritella*‡	Eastern Atlantic Ocean, off Mbour, Senegal	SE-125	SE-125	LRP 3899	FJ177112	Healy et al. 2009
Stillabothrium sp. n. 4†	*Himantura astra*‡ Last, Manjaji-Matsumoto et Pogonoski	Wessel Islands, Arafura Sea, Australia	NT-26	NT-26	LRP 3906	FJ177114	Healy et al. 2009

† undescribed rhinebothriine species follow the naming scheme used by Healy et al. (2009); ‡ denotes hosts that were previously referred to under different names by Healy et al. (2009); * referred to by Healy et al. (2009) as Rhinebothriinae New genus 3 n. sp. 7; ** (D1–D3 28S rDNA).

the same host species, *Z. schoenleinii*. The other specimen (SE-299.2) was identical to a specimen (SE-289.1) from a different host species, *R. rhinobatos*.

Stillabothrium Healy et Reyda gen. n.

ZooBank number for genus:
urn:lsid:zoobank.org:act:B2B660B1-E30D-4208-935E-F89C06A2D16D

Diagnosis: Rhinebothriidea. Worms euapolytic, small. Scolex consisting of scolex proper and 4 bothridia; cephalic peduncle absent; short germinative zone present; apical organ absent. Bothridia stalked, consisting of anterior and posterior regions with distinctly different arrangement of loculi and septa; bothridial margins with thin rim. Anterior region with horizontally oriented loculi (i.e. loculi wider than long) with two (Figs. 2B, 4B) or more (Figs. 6B, 8B, 10B, 12B, 15) complete transverse septa, with (Figs. 2B, 4B) or without (Figs. 6B, 8B, 10B, 12B, 15) single partial medial longitudinal septum. Posterior region lacking medial longitudinal septum, divided into odd number of vertically oriented loculi (i.e. loculi longer than wide) by even number of nonmedial longitudinal septa; nonmedial longitudinal septa all incomplete (Figs. 6B, 8B, 10B, 12B, 15) or a combination of incomplete and complete (Figs. 2B, 4B); incomplete nonmedial longitudinal septa either abut posteriormost transverse septum of anterior region of bothridia (Figs. 2B, 4B, 10B,), or overlap one or more posteriormost transverse septa (Figs. 6B, 8B, 12B, 15). Lateral margins of posterior region of bothridium divided into additional loculi by marginally (Figs. 2B, 4B) or diagonally (Figs. 6B, 15) oriented septa in some species.
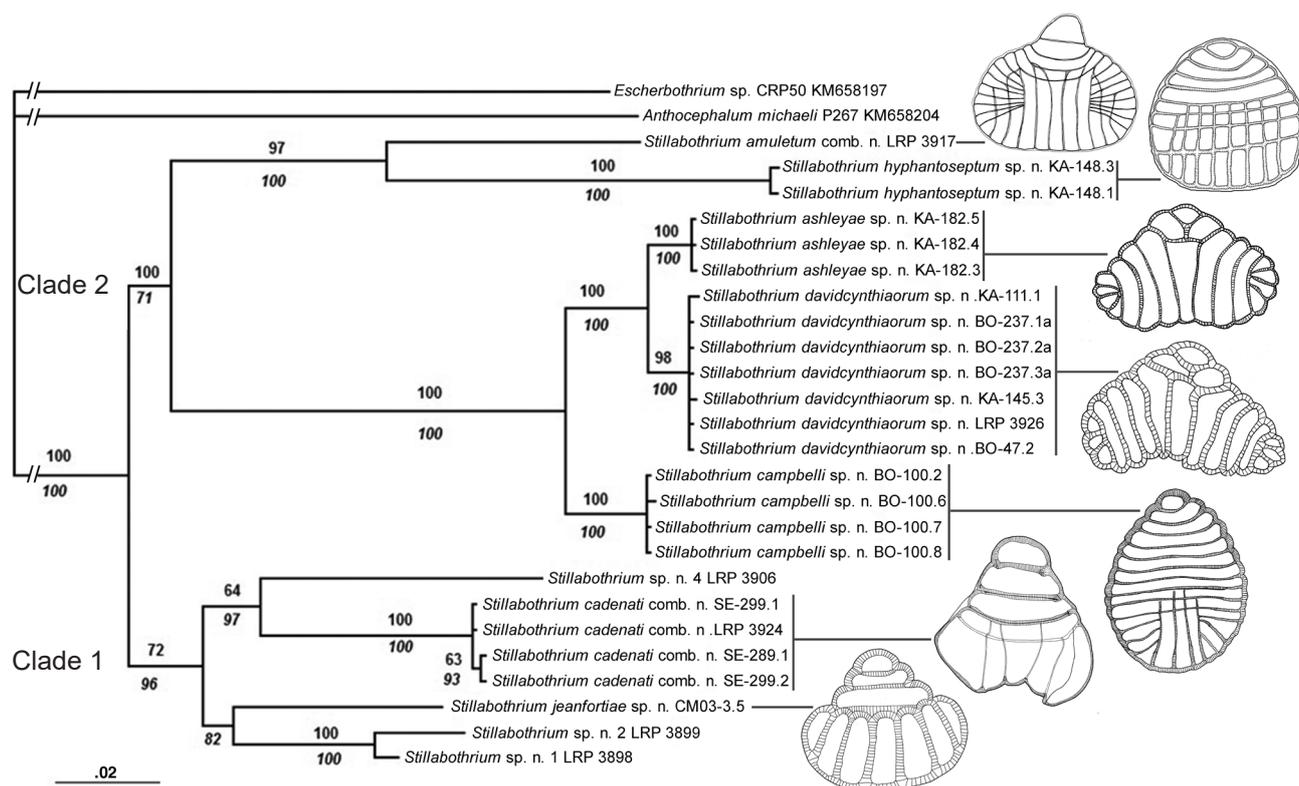
Fig. 1. Phylogram based on Bayesian analysis of 1 084 bp region of D1–D3 28S rDNA. Numbers above branches indicate Bayesian posterior probabilities, numbers below indicate Bootstrap percentage values, based on 1 000 replicates. Scale indicates expected number of substitutions per site. Diagrammatic line drawings of fully opened bothridia of the seven described species mapped onto the clades representing those respective species. Host species information in Table 1.

Longitudinal septa of posterior region appear as ridges in section (Fig. 13) with proximal and distal portions different; proximal portion of septa formed by underlying bothridial wall, consisting of radial muscles oriented with proximal ends of fibres adjacent to each other; distal portion of septa formed by separate muscle bundle; proximal and distal portions of septa separated by triangular gap.

Testes numerous, arranged in two columns, one layer deep in cross section, restricted to pre-poral region of proglottid. Cirrus sac extending medially to or past midline of proglottid. Cirrus spinitriches present. Vas deferens extending posteriorly to ovarian isthmus, entering cirrus sac at anterior margin. Vagina opening anterior to cirrus sac; vaginal sphincter absent. Ovary H-shaped in dorsoventral view, tetralobed in cross section. Vitellarium follicular; follicles in 2 lateral bands; bands interrupted by terminal genitalia and usually also by ovary. Uterus saccate, medial, extending from posterior margin of proglottid or ovarian isthmus, anteriorly to near anterior margin of proglottid. Parasites of batoid elasmobranchs (Rhinobatidae, Zanobatidae and Dasyatidae); Indo-Pacific and coastal Afro-tropics.

I n f o r m a l   s y n o n y m s : Rhinebothriinae New genus 3 Healy et al. (2009), Caira et al. (2014), Ruhnke et al. (2015), and Marques and Caira (2016).

T y p e   s p e c i e s : *Stillabothrium ashleyae* sp. n.

A d d i t i o n a l   s p e c i e s : *Stillabothrium amuletum* (Butler, 1987) comb. n.; *Stillabothrium cadenati* (Euzet, 1954) comb. n.; *Stillabothrium campbelli* sp. n.; *Stillabothrium davidcynthiaorum* sp. n.; *Stillabothrium hyphantoseptum* sp. n.; *Stillabothrium jeanfortiae* sp. n.

E t y m o l o g y : From the Latin 'stilla', meaning drop, for the teardrop shape of the bothridia of species the genus.

Remarks. *Stillabothrium* gen. n. is generally consistent with the diagnosis of the order Rhinebothriidea as given by Healy et al. (2009): Species of *Stillabothrium* possess facially loculated bothridia borne on stalks and possess a vas deferens that enters the cirrus sac at the anterior, rather than the medial, margin. *Stillabothrium* can be distinguished from all rhinebothriidean genera except *Escherbothrium* Berman and Brooks, 1994, *Phormobothrium* Alexander, 1963 and *Tritaphros* Lönnberg, 1889 in its possession of bothridia that are fully facially loculate, with posterior loculi that are longer than wide. *Stillabothrium* differs from *Phormobothrium* and *Tritaphros* in lacking an apical organ on the scolex. *Stillabothrium* is most similar to *Escherbothrium*, but *Stillabothrium* can be distinguished from the latter genus in lacking a medial longitudinal septum in the posterior region of the bothridium, thereby possessing an odd number of loculi. In *Escherbothrium*, the posterior region of the bothridium includes a short medial longitudinal septum (see fig. 6 in Berman and Brooks 1994) and an even number of loculi. In addition, *Escherbothrium* was described as possessing an apical sucker and rounded protrusions on its distal bothridial surfaces (see both in fig. 8 in Berman and Brooks 1994). Conversely, in *Stillabothrium* the feature on the anteriormost portion of the bothridium is considered to be a loculus, rather than a sucker, and

no rounded protrusions were observed on the scolex of any surfaces of any of the six species of *Stillabothrium* examined with SEM in this study. Based on the recent designation of families within Rhinebothriidea by Ruhnke et al. (2015), *Stillabothrium* belongs to family Escherbothriidae Ruhnke, Caira et Cox, 2015.

Species of *Stillabothrium* have appeared in previous works under different temporary names. The genus was first recognised by Healy (2006) in her dissertation, under a preliminary name which, as recommended by Article 8 of the ICZN (1999), she disclaimed. In addition to providing preliminary morphological characterisation of species, Healy (2006) included partial 28S rDNA sequence data for eight species of *Stillabothrium*. Subsequently, four molecular phylogenetic studies (Healy et al. 2009, Caira et al. 2014, Ruhnke et al. 2015, Marques and Caira 2016) have included the sequence data originally generated by Healy (2006). Each study, which refers to *Stillabothrium* as 'Rhinebothriinae New genus 3', supported recognition of those eight species as an independent, novel, genus.

Stillabothrium ashleyae Willsey et Reyda sp. n.

Figs. 1–3, 16A

ZooBank number for species:
urn:lsid:zoobank.org:act:B316EE69-A04B-4A09-9AB5-658752FDC431

Description (based on whole mounts of 25 complete mature worms and 14 free proglottids, cross sections of 2 strobila, longitudinal sections of 1 scolex, and 3 scoleces prepared for SEM): Worms (Fig. 2A) euapolytic, acraspedote, 1.11–2.78 mm (1.66 ± 0.39; n = 23) long, greatest width 353–590 (455 ± 70; n = 24) at level of scolex; 6–10 (7.7. ± 1; n = 25) proglottids per worm. Cephalic peduncle lacking; darkly staining germinative zone 17–217 (54 ± 37; n = 24) long.

Scolex (Fig. 2B) consisting of scolex proper bearing 4 stalked bothridia. Stalks 46–164 (76 ± 31; n = 13) long by 53–100 (75 ± 17; n = 13) wide, attached slightly posterior to middle of bothridia. Bothridia (Fig. 2B) varying in shape with degree of contraction, from shallowly-deltoid (Fig. 3A) to deeply-deltoid (Fig. 3B), facially loculated, 140–227 (175 ± 26; n = 20) long by 190–334 (270 ± 44; n = 24) wide; bothridial margins with thin rim. Anterior region of bothridia (Fig. 2B) with 3 (n = 18) horizontally oriented loculi (i.e. loculi wider than long) with 2 complete transverse septa and one partial medial longitudinal septum. Anteriormost loculus 30–46 (39 ± 5; n = 15) long by 41–72 (59 ± 11; n = 18) wide. Posterior region of bothridia with 8 (n = 21) nonmedial longitudinal septa dividing bothridia into 9 primary loculi longer than wide; outermost primary loculi on each side subdivided by 3, or occasionally 2 (2.94 ± 0.2; n = 16) relatively short marginal septa into 3–4 small subloculi; longitudinal septa of posterior region not overlapping transverse septa of anterior region.

Loculi (Fig. 3D) and septa of distal bothridial surfaces bearing capilliform filitriches and coniform spinitriches. Proximal bothridial rim (Fig. 3E) bearing capilliform filitriches greater in length than those on distal bothridial sur-

faces (Fig. 3D). Proximal bothridial surfaces (Fig. 3E,F) away from rim bearing acicular filitriches and coniform spinitriches. Isolated cilia observed on proximal bothridial surfaces. Bothridial stalks (Fig. 3G) and strobila (Fig. 3H) bearing capilliform filitriches only.

Strobila with 2–4 (3.2 ± 0.8; n = 25) proglottids wider than long followed by 3–7 (4.5 ± 1; n = 25) proglottids longer than wide. Strobila widest at terminal proglottid; terminal proglottid 320–880 (591 ± 125; n = 25) long by 68–150 (101 ± 20; n = 24) wide; genital pore located 35–46% (40 ± 3; n = 25) of proglottid length from proglottid posterior margin. Immature proglottids 4–9 (6.4 ± 1.2; n = 25) in number. Mature proglottids 1–2 (1.2 ± 0.4; n = 25) in number, including 0–1 (0.3 ± 0.5; n = 25) vas deferens-mature proglottids.

Testes in mature proglottids 18–27 (22 ± 2; n = 25) in total number, 1 layer deep in cross section (Fig. 2D), arranged in 2 columns (Fig. 2C); columns extending from anterior margin of proglottid to anterior margin of cirrus sac, 19–37 (25 ± 4; n = 24) long by 20–45 (31 ± 6; n = 22) wide. Vas deferens coiled, entering anterior margin of cirrus sac, extending from level of ovarian isthmus to overlap posteriormost testes (Fig. 2C). Cirrus sac thin-walled, oval, extending medially to near midline of proglottid; cirrus sac in terminal mature proglottid 26–42 (33 ± 5; n = 17) long by 26–47 (32 ± 6; n = 17) wide; cirrus sac in vas deferens-mature proglottids 28–38 (34 ± 4; n = 8) long by 36–50 (43 ± 5; n = 8) wide. Cirrus spinitriches present.

Vagina (Fig. 2C) thick-walled, weakly sinuous, somewhat overlapping anterior margin of cirrus sac (Fig. 16A), extending along midline of proglottid from ootype region to anterior margin of cirrus sac, then laterally to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Seminal receptacle present. Ovary near posterior end of proglottid, H-shaped in frontal view, tetralobed in cross section (Fig. 2E); ovarian lobes asymmetrical; poral and aporal ovarian lobes in terminal mature proglottids 80–215 (161 ± 33; n = 16) and 85–220 (163 ± 34; n = 16) long, respectively. Poral and aporal ovarian lobes in vas deferens-mature proglottids 146–265 (194 ± 47; n = 7) and 141–282 (190 ± 51; n = 7) long, respectively. Maximum width of ovary 35–96 (56 ± 17; n = 17). Ovarian isthmus at or anterior to midpoint of ovary; poral lobe of ovary stopping 26–63 (41 ± 12; n = 23) short of genital pore. Mehlis' gland well posterior to ovarian isthmus, 19–35 (26 ± 5; n = 21) long by 14–30 (18 ± 4; n = 21) wide. Vitellarium follicular; vitelline follicles arranged in 1 dorsal and 1 ventral column on each side of proglottid; columns extending from anterior to posterior margin of proglottid, interrupted by terminal genitalia, and mostly interrupted by ovary (Fig. 2C). Uterus ventral, sacciform, extending from near isthmus of ovary to near anterior margin of proglottid.

Free proglottids with greatly expanded vas deferens and atrophied testes, 521–919 (724 ± 136; n = 12) long by 134–202 (165 ± 21; n = 12) wide. Free gravid proglottids 750 (n = 1) long by 210–280 (245 ± 50; n = 2) wide; unembryonated eggs oval, without filaments, 14–17 (15 ± 1; n = 6; from same proglottid) long.
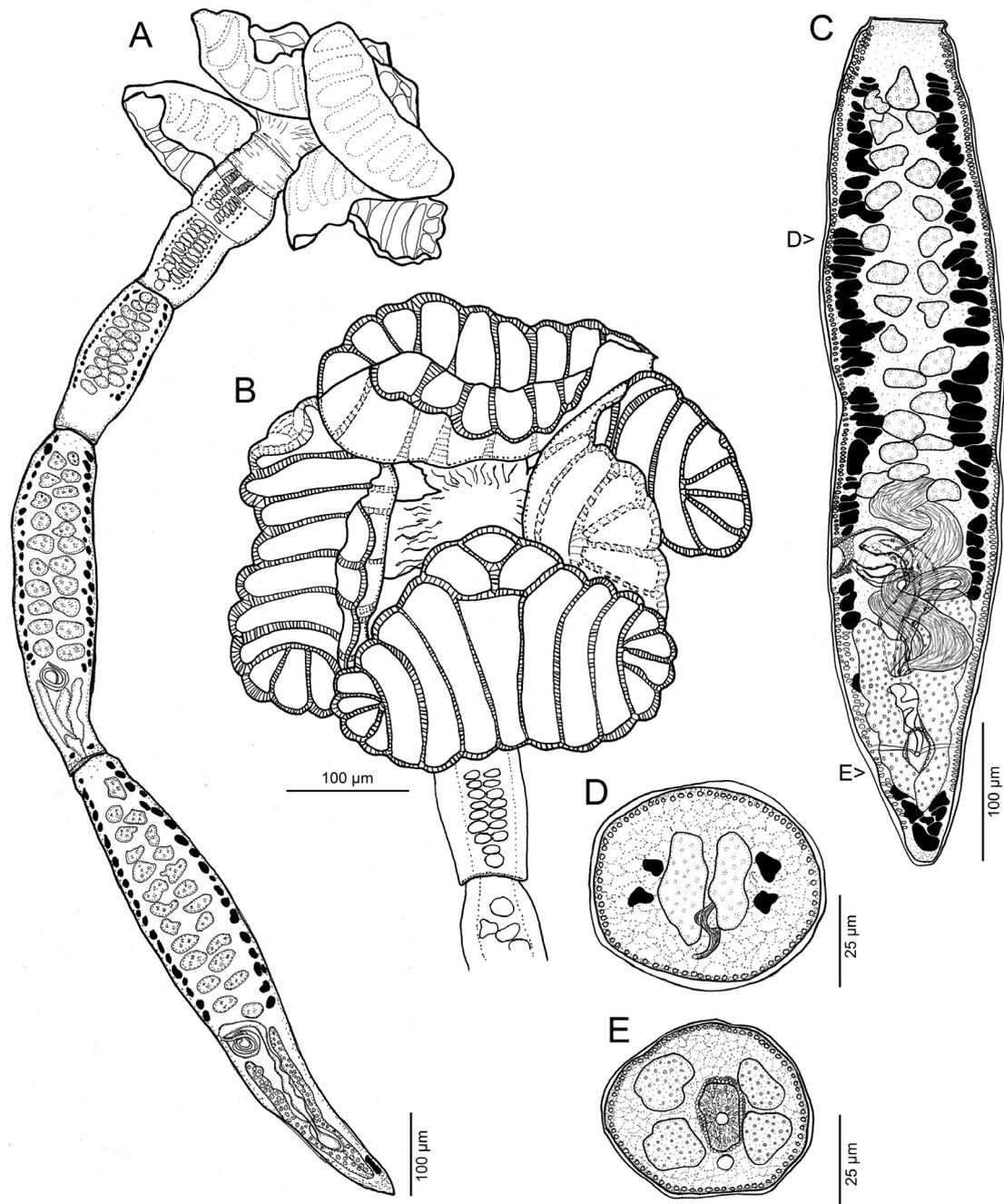
Fig. 2. Line drawings of *Stillabothrium ashleyae* sp. n. from *Dasyatis biasa* (Last, White et Naylor). **A** – whole worm (holotype; MZUM [P] 2016.6 [H]); **B** – scolex (LRP 9011); **C** – terminal proglottid (LRP 9010) with position of sections labeled (uterus not shown); **D** – section of mature proglottid at level of testes (LRP 9037); **E** – section of mature proglottid posterior to ovarian bridge (LRP 9030).

T y p e   h o s t : *Dasyatis biasa* (Last, White et Naylor) (Myliobatiformes: Dasyatidae).

A d d i t i o n a l   h o s t : None.

T y p e   l o c a l i t y : South China Sea off Mukah (02°53'52"N; 112°05'44"E), Sarawak, Malaysian Borneo (BO-477).

A d d i t i o n a l   l o c a l i t i e s : Java Sea off Selakau (01°03'31"N; 108°58'26"E), West Kalimantan, Indonesian Borneo (KA-182, KA-184). Java Sea off Sukadana (01°14'33"S; 109°57'00"E), West Kalimantan, Indonesian Borneo (KA-378).

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 MZUM (P) No. 2016.6 (H). Paratypes: IPCAS No. C-737; LRP Nos. 8992–8994; 9005–9037 (including molecular vouchers, cross sections and SEM specimens); MZB Nos. Ca197–Ca199; MZUM (P) Nos. 2016.7 (P)–2016.8 (P); SBC No. P-00069; USNM Nos. 1420453–1420460.

E t y m o l o g y : This species is named in honour of Ashley Willsey Attoma, sister of D.D. Willsey, for her support of her sister's studies.

Remarks. *Stillabothrium ashleyae* sp. n. is the type and first described species of the genus. The distribution of *S. ashleyae* includes both the Malaysian and Indonesian portions of the island of Borneo. *Stillabothrium ash-*
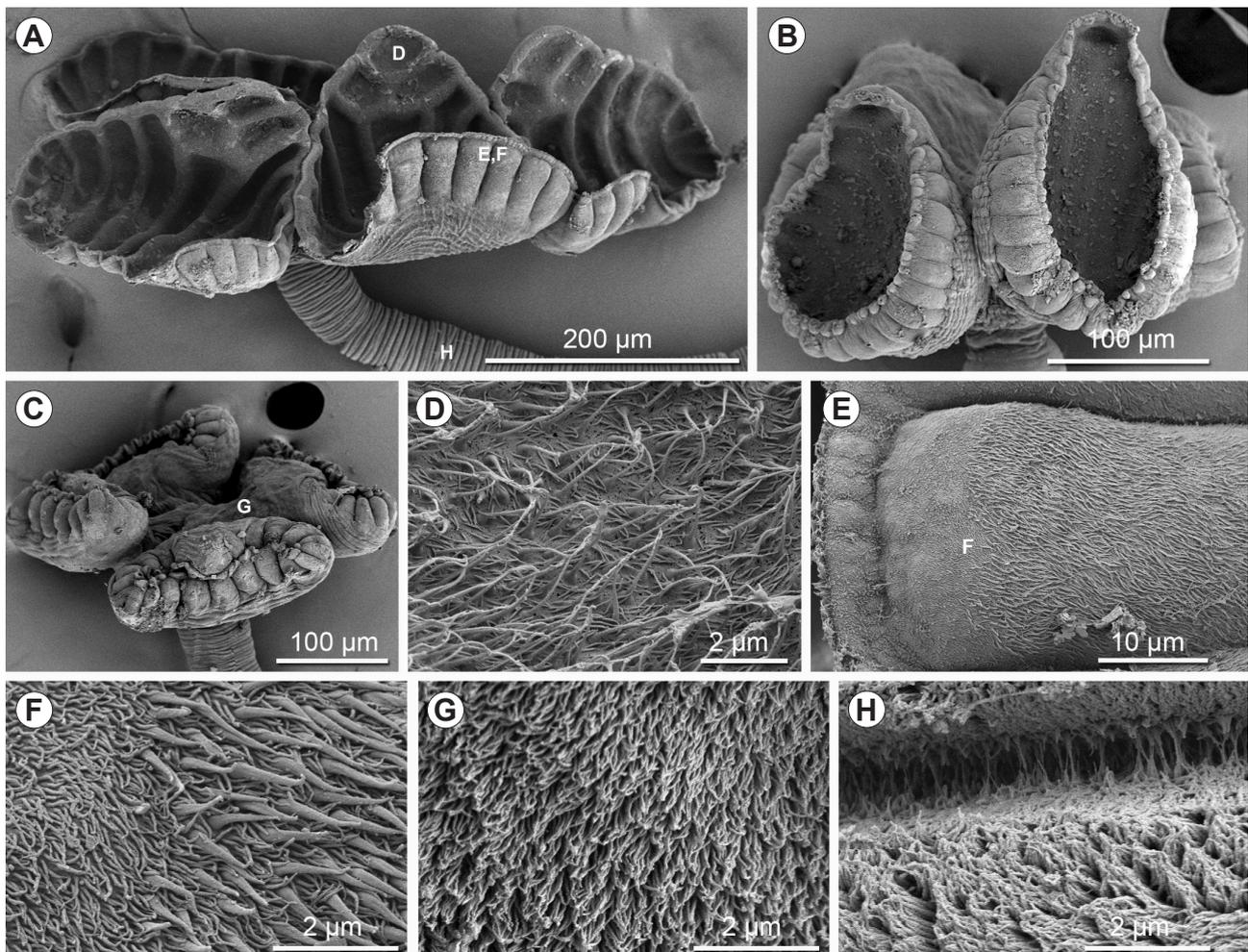
Fig. 3. Scanning electron micrographs of *Stillabothrium ashleyae* sp. n. from *Dasyatis biasa* (Last, White et Naylor). **A–C** – scoleces, letters indicate locations of other SEMs; **D** – distal bothridial surface at anterior loculus; **E** – proximal bothridial surface and rim, letter indicates location of F; **F** – proximal bothridial surface; **G** – stalk; **H** – strobila.

leyae appears to be highly host specific, as it was only encountered in specimens of *D. biasa*. It was not found parasitising any of the multiple other species of dasyatids (e.g. *Dasyatis* Rafinesque and *Himantura* Müller et Henle) examined during our survey work. We note that specimens with a morphology similar to *S. ashleyae* were found in *D. zugei* (VN-23, VN-34) from Vietnam. However, preliminary molecular and morphological data suggest that the specimens from Vietnam represent a distinct species of *Stillabothrium* that awaits formal study and description.

Three specimens of *S. ashleyae* were included in the phylogenetic analysis (Fig. 1, Table 1).

Stillabothrium davidcynthiaorum Daigler et Reyda sp. n.
Figs. 1, 4, 5, 16B

ZooBank number for species:
urn:lsid:zoobank.org:act:D36AE2D9-C4A6-4C4D-8F82-E9C0FAF1F92C

Description (based on whole mounts of 32 complete mature worms, cross sections of 1 strobila and longitudinal sections of 1 scolex and 3 scoleces prepared for SEM): Worms (Fig. 4A) euapolytic, acraspedote, 0.62–2.59 mm

(1.29 ± 0.57; n = 30) long, greatest width 195–561 (332 ± 84.7; n = 32) at level of scolex; 4–10 (7 ± 2.1; n = 32) proglottids per worm. Cephalic peduncle lacking; darkly staining germinative zone 12–40 (22 ± 7; n = 29) long.

Scolex (Fig. 4B) consisting of scolex proper bearing 4 stalked bothridia. Stalks 25–100 (52 ± 20; n = 23) long by 27–83 (55 ± 17; n = 23) wide, attached slightly posterior to middle of bothridia. Bothridia (Fig. 4B) varying in shape with degree of contraction, from shallowly-deltoid (Fig. 5A) to deeply-deltoid (Figs. 4B, 5B), facially loculated, 72–175 (108 ± 26; n = 20) long by 140–325 (202 ± 42; n = 32) wide; bothridial margins with thin rim. Anterior region of bothridia (Fig. 4B) with 3 (n = 28) horizontally oriented loculi (i.e. loculi wider than long) with 2 complete transverse septa and one partial medial longitudinal septum. Anteriormost loculus 22–49 (31 ± 8; n = 18) long and 37–78 (47 ± 9; n = 25) wide. Posterior region of bothridia with 10 (n = 30) nonmedial longitudinal septa dividing bothridia into 11 primary loculi longer than wide, outermost primary loculi on each side subdivided by 2, or occasionally 3 (2.04 ± 0.2; n = 24) relatively short marginal septa into 3–4 small subloculi; longitudinal septa of posterior region not overlapping transverse septa of anterior region.
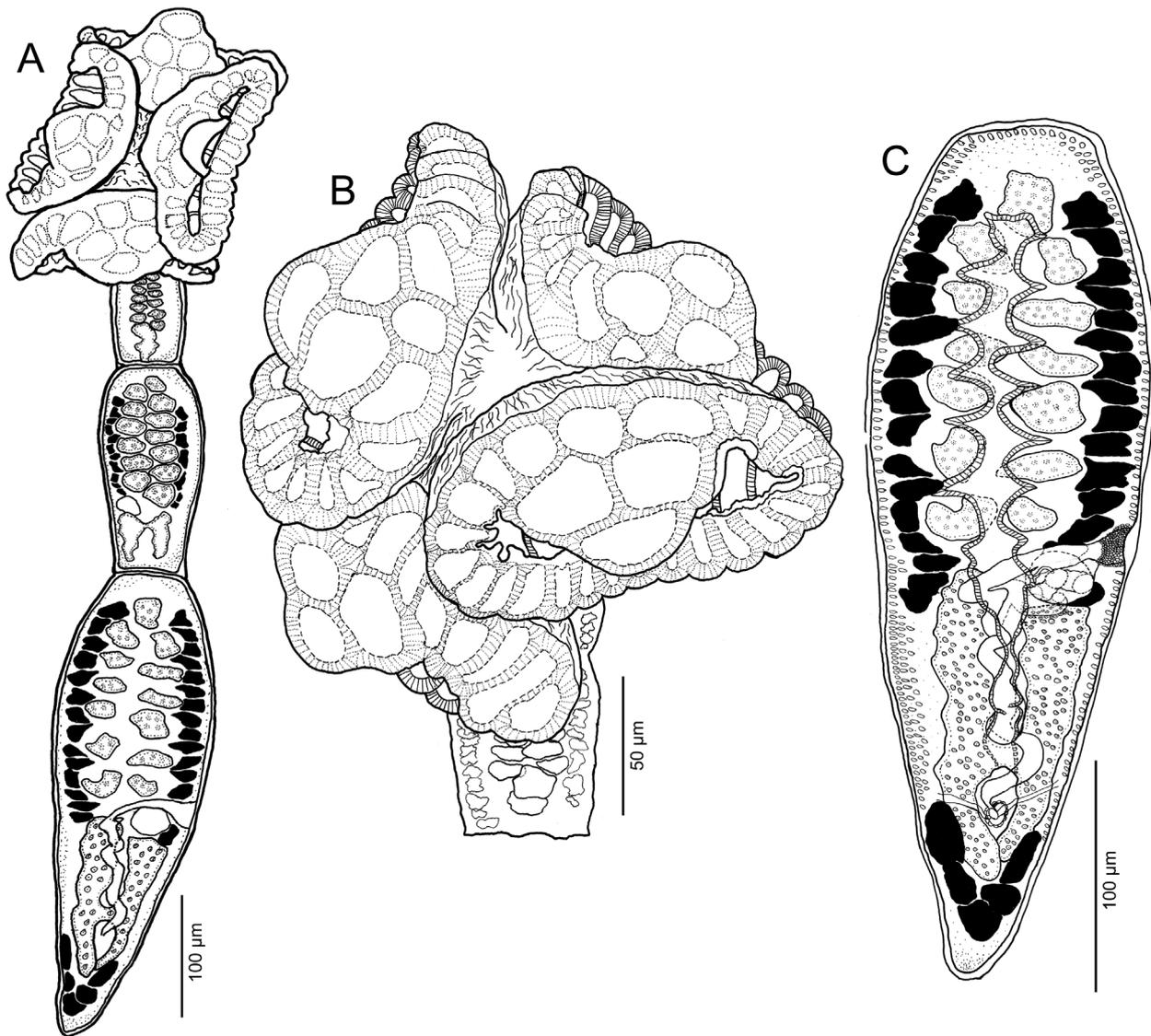
Fig. 4. Line drawings of *Stillabothrium davidcynthiaorum* sp. n. from *Himantura heterura* (Bleeker). **A** – whole worm (LRP 9046); **B** – scolex (holotype, MZUM [P] 2016.9 [H]); **C** – terminal proglottid (LRP 9046).

Loculi and septa of distal bothridial surfaces (Fig. 5C) bearing capilliform filitriches and coniform spinitriches. Proximal bothridial rim (Fig. 5D) bearing capilliform filitriches greater in length than those on distal bothridial surfaces (Fig. 5C). Proximal bothridial surfaces (Fig. 5D,E) away from rim bearing acicular filitriches and coniform spinitriches. Isolated cilia observed on proximal bothridial surfaces. Bothridial stalks (Fig. 5F) bearing capilliform filitriches and coniform spinitriches; strobila (Fig. 5G) bearing capilliform filitriches only.

Strobila with 1–5 (2.6 ± 0.9; n = 32) proglottids wider than long followed by 2–8 (4.1 ± 1.8; n = 32) proglottids longer than wide. Strobila widest at terminal proglottid; terminal proglottid 255–790 (438 ± 124; n = 31) long by 60–144 (108 ± 18; n = 32) wide; genital pore located 42–54% (48 ± 3.5; n = 21) of proglottid length from proglottid posterior margin. Immature proglottids 3–9 (5.5 ± 1.9; n = 32) in number. Mature proglottids 1–3 (1.2 ± 0.5; n = 32) in number, including 0–2 (0.2 ± 0.5; n = 32) vas deferens-mature proglottids.

Testes in mature proglottids 11–21 (16 ± 3; n = 32) in total number, 1 layer deep in cross section, arranged in 2 columns (Fig. 4C); columns extending from anterior margin of proglottid to anterior margin of cirrus sac, 13–49 (25 ± 8; n = 32) long by 24–50 (35 ± 6; n = 32) wide. Vas deferens coiled, entering anterior margin of cirrus sac, extending from level of ovarian isthmus to overlap posteriormost testes. Cirrus sac thin-walled, oval, extending medially to near midline of proglottid; cirrus sac in terminal mature proglottid 18–35 (26 ± 5; n = 26) long by 24–38 (31 ± 4; n = 24) wide; cirrus sac in vas deferens-mature proglottids 32–45 (39 ± 5; n = 5) long by 33–46 (40 ± 5; n = 5) wide. Cirrus spinitriches present.

Vagina (Fig. 4C) thick-walled, weakly sinuous, somewhat overlapping anterior margin of cirrus sac (Fig. 16B), extending along midline of proglottid from ootype region to anterior margin of cirrus sac, then laterally to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Seminal receptacle present. Ovary near posterior end of proglottid, H-shaped in frontal view, tetralobed in
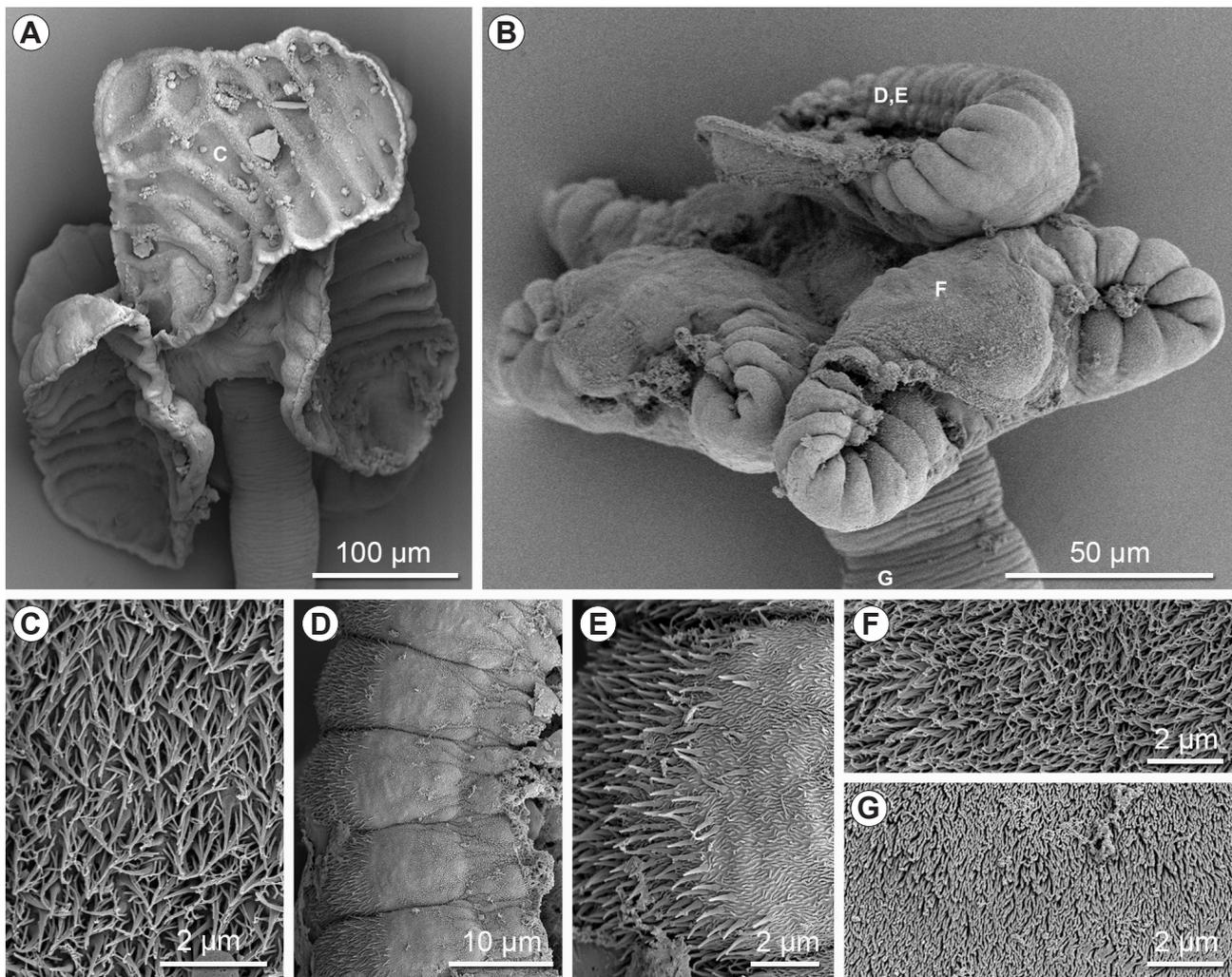
Fig. 5. Scanning electron micrographs of *Stillabothrium davidcynthiaorum* sp. n. from *Himantura uarnak* 3 (A, C, F) and *Himantura heterura* (Bleeker) (B, D, E, G). **A**, **B** – scoleces, letters indicate locations of other SEMs; **C** – distal bothridial surface in center of loculus; **D** – proximal bothridial surface with rim; **E** – proximal bothridial surface near rim; **F** – proximal bothridial surface; **G** – strobila.

cross section; ovarian lobes asymmetrical; poral and aporal ovarian lobes in terminal mature proglottids 75–254 (144 ± 40; n = 24) and 75–272 (152 ± 42; n = 24) long, respectively. Poral and aporal ovarian lobes in vas deferens-mature proglottids 145–269 (198 ± 48; n = 5) and 152–270 (206 ± 46; n = 5) long, respectively. Maximum width of ovary 39–139 (65 ± 21; n = 32). Ovarian isthmus near midpoint of ovary; poral lobe of ovary stopping 19–85 (31 ± 16; n = 28) short of genital pore. Mehlis' gland well posterior to ovarian isthmus, 12–43 (27 ± 8; n = 29) long by 10–35 (20 ± ; n = 29) wide. Vitellarium follicular, vitelline follicles arranged in 1 dorsal and 1 ventral column on each side of proglottid; columns extending from anterior to posterior margin of proglottid, interrupted by genital genitalia, and mostly interrupted by ovary (Fig. 4C). Uterus ventral, sacciform, extending from near isthmus of ovary to near anterior margin of proglottid.

I n f o r m a l s y n o n y m s : Rhinebothriinae New genus 3 sp. n. 6 of Healy et al. (2009), Caira et al. (2014), Ruhnke et al. (2015), Marques and Caira (2016).

T y p e h o s t : *Himantura heterura* (Bleeker), dwarf whipray

(Myliobatiformes: Dasyatidae).

A d d i t i o n a l h o s t s : *Himantura macrura*, *Himantura gerrardi*, *Himantura uarnak* 3.

T y p e l o c a l i t y : South China Sea off Sematan (01°48'15"N; 109°46'47"E), Sarawak, Malaysian Borneo (hosts BO-19, BO-141, BO-170).

A d d i t i o n a l l o c a l i t i e s : South China Sea off Mukah (02°53'52"N; 112°05'44"E), Sarawak, Malaysian Borneo (BO-47, BO-66, BO-67, BO-237, BO-238). Java Sea off Kalapseban (03°14'30"S; 112°54'52"E), Central Kalimantan, Indonesian Borneo (KA-99, KA-111). Java Sea off Singkawang (00°55'06"N; 108°58'60"E), West Kalimantan, Indonesian Borneo (KA-145).

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 MZUM (P) No. 2016.9 (H). Paratypes: IPCAS No. C-739; LRP Nos. 8986–8991; 9038–9062 (including molecular vouchers, cross sections and SEM specimens); MZB Nos. Ca200–Ca201; MZUM (P) No. 2016.10 (P); SBC No. P-00070; USNM Nos. 1420461–1420466.

E t y m o l o g y : This species is named in honour of David and Cynthia Daigler, parents of A.L. Daigler, for their support of his education and his interests.

Remarks. *Stillabothrium davidcynthiaorum* sp. n. can be distinguished from *S. ashleyae* in its possession of 10, rather than eight, longitudinal septa on the central posterior region of the bothridium. While *S. davidcynthiaorum* and *S. ashleyae* are similar in possessing a range of 2–3 marginal septa on both of the lateral sides on the posterior region of the bothridium, the former differs from the latter in mean number (2.04 [n = 24] *vs* 2.94 [n = 16]).

In the present study we report specimens of *S. davidcynthiaorum* from potentially four species of *Himantura*, *H. heterura*, *H. macrura*, *H. gerrardi* and *H. uarnak* 3. Although *S. davidcynthiaorum* was encountered in four potential species of *Himantura,* we point out having encountered specimens morphologically similar to *S. davidcynthiaorum* in four additional species of *Himantura* that were examined during the survey work in Borneo, including *Himantura* cf. *pastinacoides* (BO-61, BO-168), *H. undulata* (BO-24), *U. lobistomus* (BO-247) and *H. oxyrhyncha* (KA-252). Further examination of the *Stillabothrium* specimens from those additional four species of *Himantura* is needed, in combination with DNA sequence data, to establish whether those cestodes are conspecific with *S. davidcynthiaorum* or represent an undescribed species of the genus.

Seven specimens of *S. davidcynthiaorum* were included in the phylogenetic analysis (Fig. 1, Table 1). One of the seven specimens (LRP 3926) was previously included in the analysis provided by Healy et al. (2009) as 'Rhinebothriinae New genus 3 sp. n. 6'.

Stillabothrium campbelli Delgado, Dedrick et Reyda sp. n.   Figs. 1, 6, 7, 16C

ZooBank number for species:
urn:lsid:zoobank.org:act:8EA1CC15-6A59-4FDC-96DE-7B8389B40F7C

Description (based on whole mounts of 14 complete mature worms, cross sections of 2 strobila and longitudinal sections of 3 scoleces and 3 scoleces prepared for SEM): Worms (Fig. 6A) euapolytic, acraspedote, 1.06–2.60 mm (1.68 ± 0.4; n = 14) long, greatest width 170–360 (261 ± 55; n = 14) at level of scolex; 5–8 (6 ± 1.0; n = 14) proglottids per worm. Cephalic peduncle lacking; small darkly staining germinative zone present.

Scolex (Fig. 6B) consisting of scolex proper bearing 4 stalked bothridia. Stalks 20–90 (49 ± 2; n = 13) long by 25–110 (68 ± 25; n = 13) wide, attached slightly posterior to middle of bothridia. Bothridia varying in shape with degree of contraction; ovoid, broadly ovoid, finely ovoid (Fig. 6B), deeply ovoid (Fig. 7B), very deeply ovoid, to finely deltoid (Fig. 7A), facially loculated, 166–240 (205 ± 22; n = 15) long by 110–235 (150 ± 32; n = 14) wide; bothridial margins with thin rim. Anterior region of bothridia (Fig. 6B) with 10–12 (10.6 ± 0.7; n = 11) horizontally oriented loculi (i.e. loculi wider than long) with 10–12 complete transverse septa. Anteriormost loculus 15–25 (20 ± 4; n = 13) long and 24–35 (31 ± 3; n = 12) wide. Posterior region of bothridia with 4 (n = 11) nonmedial longitudinal septa dividing bothridia into 5 primary loc-

uli longer than wide; outermost primary loculi on each side subdivided by 3–4 (3.7 ± 0.5; n = 9) diagonal septa into 4–5 small subloculi; central 2 longitudinal septa of posterior region overlapping 3–4 (3.3 ± 0.5; n = 6) posteriormost transverse septa of anterior region.

Loculi of distal bothridial surfaces (Fig. 7C) bearing acicular and capilliform filitriches; septa on distal bothridial surfaces (Fig. 7C) bearing acicular and capilliform filitriches and coniform spinitriches. Proximal bothridial rim (Fig. 7D) bearing capilliform filitriches greater in length than those on distal bothridial surfaces (Fig. 7C). Proximal bothridial surfaces away from rim bearing acicular filitriches throughout (Fig. 7D). Posterior margin of proximal bothridial surfaces bearing patch of coniform spinitriches (Fig. 7E) near, but not extending to, bothridial rim. Isolated cilia observed on distal and proximal bothridial surfaces. Bothridial stalks and strobila (Fig. 7F) bearing capilliform filitriches only.

Strobila with 1–4 (2.3 ± 1.0; n = 14) proglottids wider than long followed by 3–5 (3.8 ± 0.6; n = 14) proglottids that are longer than wide. Strobila widest at terminal proglottid; terminal proglottid 490–1 250 (798 ± 209; n = 14) long by 78–180 (125 ± 31; n = 14) wide, genital pore located 41–55% (49 ± 4.7; n = 14) of proglottid length from proglottid posterior margin. Immature proglottids 3–6 (4.5 ± 0.9; n = 14) in number. Mature proglottids 1–2 (1.6 ± 0.5; n = 14) in number, including 0–1 (0.7 ± 0.5; n = 14) vas deferens-mature proglottid.

Testes in mature proglottids 12–19 (15 ± 2.1; n = 14) in total number, 1 layer deep in cross section, arranged in 2 columns; columns extending from anterior margin of proglottid to anterior margin of cirrus sac, 23–42 (33 ± 6; n = 13) long by 28–50 (37 ± 8; n = 13) wide. Vas deferens coiled, entering anterior margin of cirrus sac, extending from level of ovarian isthmus to overlap posteriormost testes. Cirrus sac thin-walled, oval, extending medially well past midline of proglottid; cirrus sac in terminal mature proglottid 42–80 (66 ± 17; n = 4) long by 50–70 (62 ± 9; n = 4) wide; cirrus sac in vas deferens-mature proglottids 45–90 (71 ± 13; n = 9) long by 50–100 (75 ± 18; n = 9) wide. Cirrus spinitriches present.

Vagina (Fig. 6C) thick-walled, sinuous, somewhat overlapping anterior margin of cirrus sac (Fig. 16C), extending from ootype past midline to aporal side of proglottid to anterior margin of cirrus sac, then laterally to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Seminal receptacle present. Ovary near posterior end of proglottid, H-shaped in frontal view, tetralobed in cross section, overlapping cirrus sac; ovarian lobes symmetrical; poral ovarian lobe somewhat overlapping cirrus sac; poral and aporal ovarian lobes in terminal mature proglottids 135–270 (219 ± 61; n = 5) and 140–275 (219 ± 60; n = 5) long, respectively. Poral and aporal ovarian lobes in vas deferens-mature proglottids 135–425 (258 ± 103; n = 8) and 155–450 (264 ± 108; n = 8) long, respectively. Maximum width of ovary 52–110 (78 ± 22; n = 13). Ovarian isthmus at or anterior to midpoint of ovary; poral lobe of ovary stopping 45–150 (81 ± 21; n = 13) short of genital pore. Mehlis' gland posterior to ovarian isthmus, 30–45
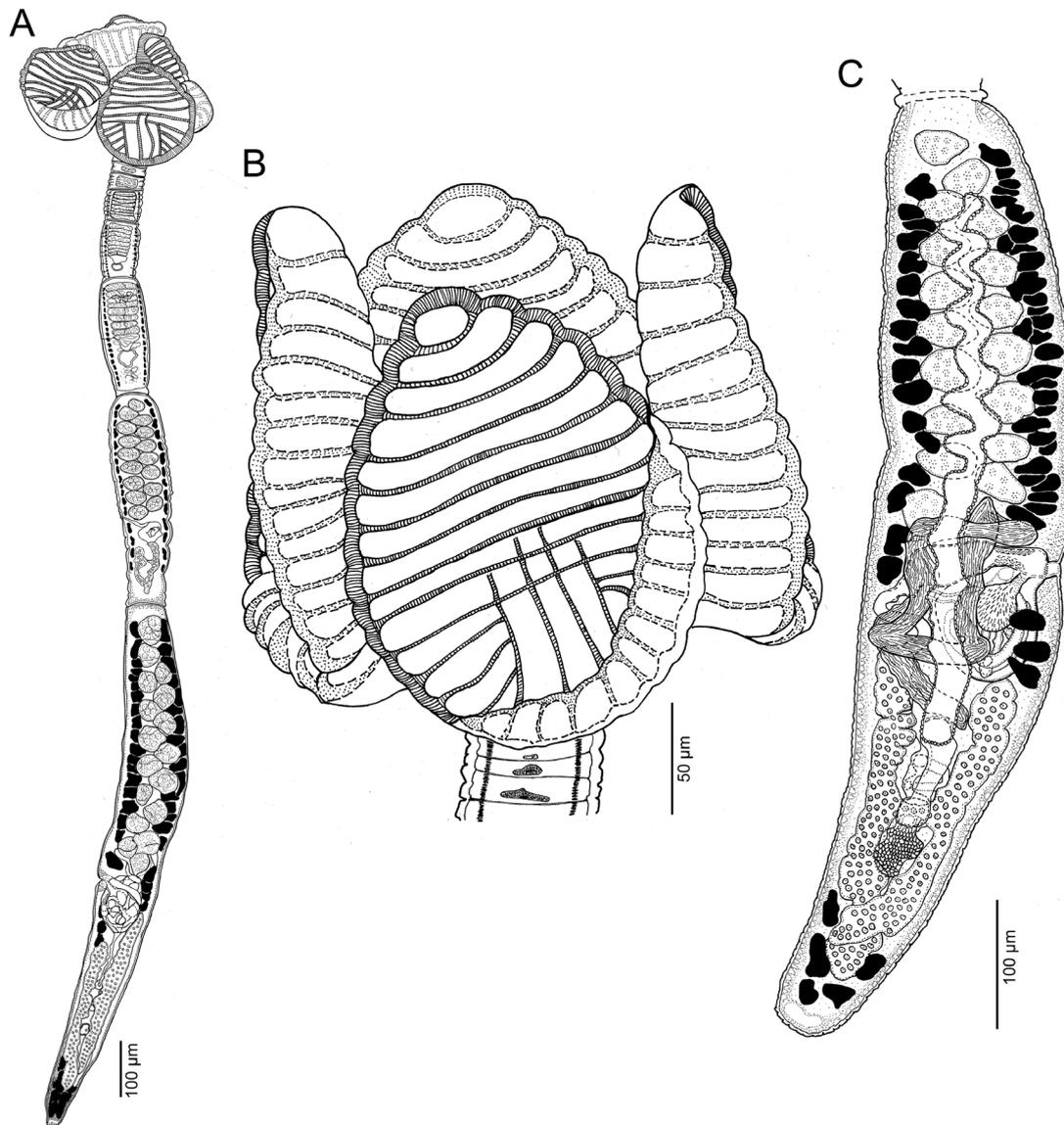
Fig. 6. Line drawings of *Stillabothrium campbelli* sp. n. from *Himantura* cf. *pastinacoides*. **A** – whole worm (holotype; MZUM [P] 2016.11 [H]); **B** – scolex (LRP 9066); **C** – terminal proglottid (LRP 9067).

(39 ± 7; n = 7) long by 25–37 (29 ± 4; n = 7) wide. Vitellarium follicular; vitelline follicles arranged in 1 dorsal and 1 ventral column on each side of proglottid; columns extending from anterior to posterior margin of proglottid, interrupted by terminal genitalia, and mostly interrupted by ovary (Fig. 6A,C). Uterus ventral, sacciform, extending from near isthmus of ovary to near anterior margin of proglottid.

Type and only known host: *Himantura* cf. *pastinacoides* (Myliobatiformes: Dasyatidae).

Type locality: Sulu Sea off Kampung Tetabuan (06°01'10"N; 117°42'15"E), Sabah, Malaysian Borneo (BO-98, BO-100, BO-119).

Additional localities: Sulu Sea off Sandakan (05°50'20"N; 118°07'16"E), Sabah, Malaysian Borneo (BO-79). South China Sea off Sematan (01°48'15"N; 109°46'47"E), Sarawak, Malaysian Borneo (BO-168).

Site of infection: Spiral intestine.

Type material: Holotype MZUM (P) No. 2016.11 (H).

Paratypes: IPCAS No. C-738; LRP Nos. 8995–8997; 9063–9082; 9149 (including molecular vouchers, sections and SEM specimens); MZUM (P) No. 2016.12 (P); SBC No. P-00071; USNM Nos. 1420467–1420470.

Etymology: This species is named in honour of Dr. William Campbell, Nobel laureate, for his role in the development of drugs to fight parasitic infections.

Remarks. *Stillabothrium campbelli* sp. n. is the only *Stillabothrium* species treated in this study that possesses a greater number of anterior loculi than posterior loculi on the bothridia. *Stillabothrium campbelli* can be distinguished from both *S. ashleyae* and *S. davidcynthiaorum* in the unique configuration of the septa and loculi on its bothridia. In terms of the anterior region of the bothridia, *S. campbelli* possesses a total of 10–12 loculi (Fig. 6B) whereas both *S. ashleyae* and *S. davidcynthiaorum* each possess only 3 loculi (Figs. 2B, 4B). In the posterior region of the bothridia, *S. campbelli* possesses 5 loculi that are longer than wide whereas both *S. ashleyae* and *S. da-*
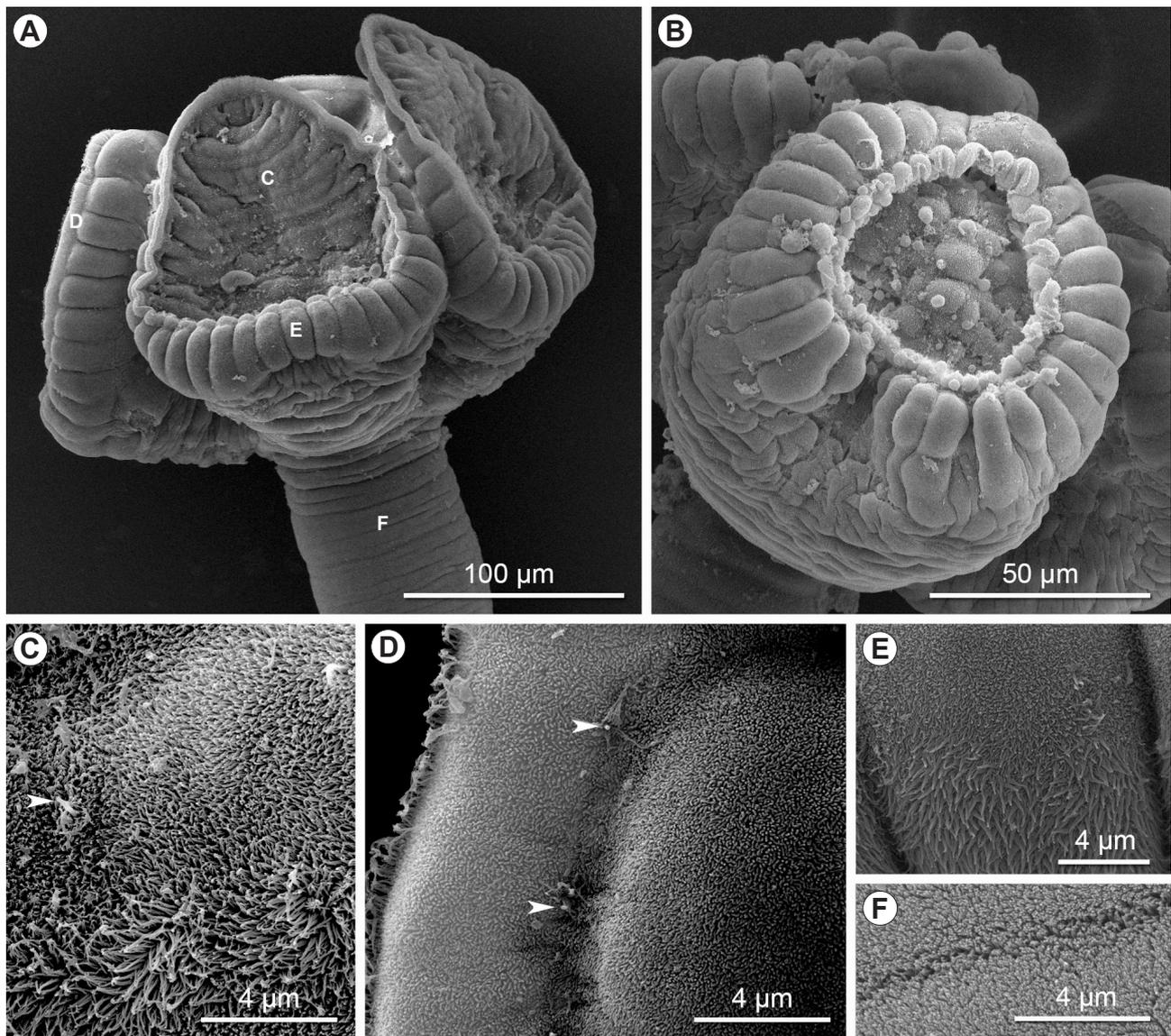
Fig. 7. Scanning electron micrographs of *Stillabothrium campbelli* sp. n. from *Himantura* cf. *pastinacoides*. **A** – scolex; letters indicate locations of other SEMs; **B** – bothridium; **C** – distal bothridial surface at third loculus (upper half) and transverse septum (lower half); **D** – proximal bothridial surface with rim; **E** – proximal bothridial surface near rim; **F** – strobila. White arrowheads indicate cilia in Fig. 7C,D.

vidcynthiaorum respectively possess 7 or 9 loculi that are longer than wide (Figs. 2B, 4B). In addition, the longitudinal septa in the posterior bothridia of *S. campbelli* extend anteriorly such that they overlap with the 3–4 posteriormost transverse septa of the anterior region of the bothridia. In *S. ashleyae* and *S. davidcynthiaorum*, the septa do not overlap.

Scolex microtriches can also be used to distinguish *S. campbelli* from *S. ashleyae* and *S. davidcynthiaorum*. The posterior proximal surface of bothridia in *S. campbelli* possess a patch of coniform spinitriches (in addition to acicular filitriches throughout) near the rim, that is restricted in distribution, whereas the proximal bothridial surface of *S. ashleyae* and *S. davidcynthiaorum* is evenly covered with coniform spinitriches and acicular filitriches. Finally, proglottid morphology can also be used to distinguish *S. campbelli* from *S. ashleyae* and *S. davidcynthiaorum*.

The cirrus sac of *S. campbelli* sp. n. is larger (45–90 μm long by 50–100 μm wide) than those of *S. ashleyae* (26–42 μm long by 26–47 μm wide) and of *S. davidcynthiaorum* (18–35 μm long by 24–38 μm wide), and it extends further across the midline (Figs. 6C, 16C), than in the latter two species (Figs. 2C, 4C, 16A,B).

Stillabothrium campbelli is reported from *H.* cf. *pastinacoides* from three different localities in Malaysian Borneo. It should be noted that the stingray host specimens of *S. campbelli* in this study, BO-61, BO-79, BO-98, BO-100, BO-119 and BO-168, are identified as *H.* cf. *pastinacoides* (Caira et al. 2012), but that identification awaits expert verification. Given that Naylor et al. (2012b) showed that two species of stingray similar to *H. pastinacoides* (i.e. *H.* cf. *pastinacoides* 1 and *H.* cf. *pastinacoides* 2) co-occur in the region sampled, it is possible that *S. campbelli* sp. n. parasitises either or both. Additional specimens
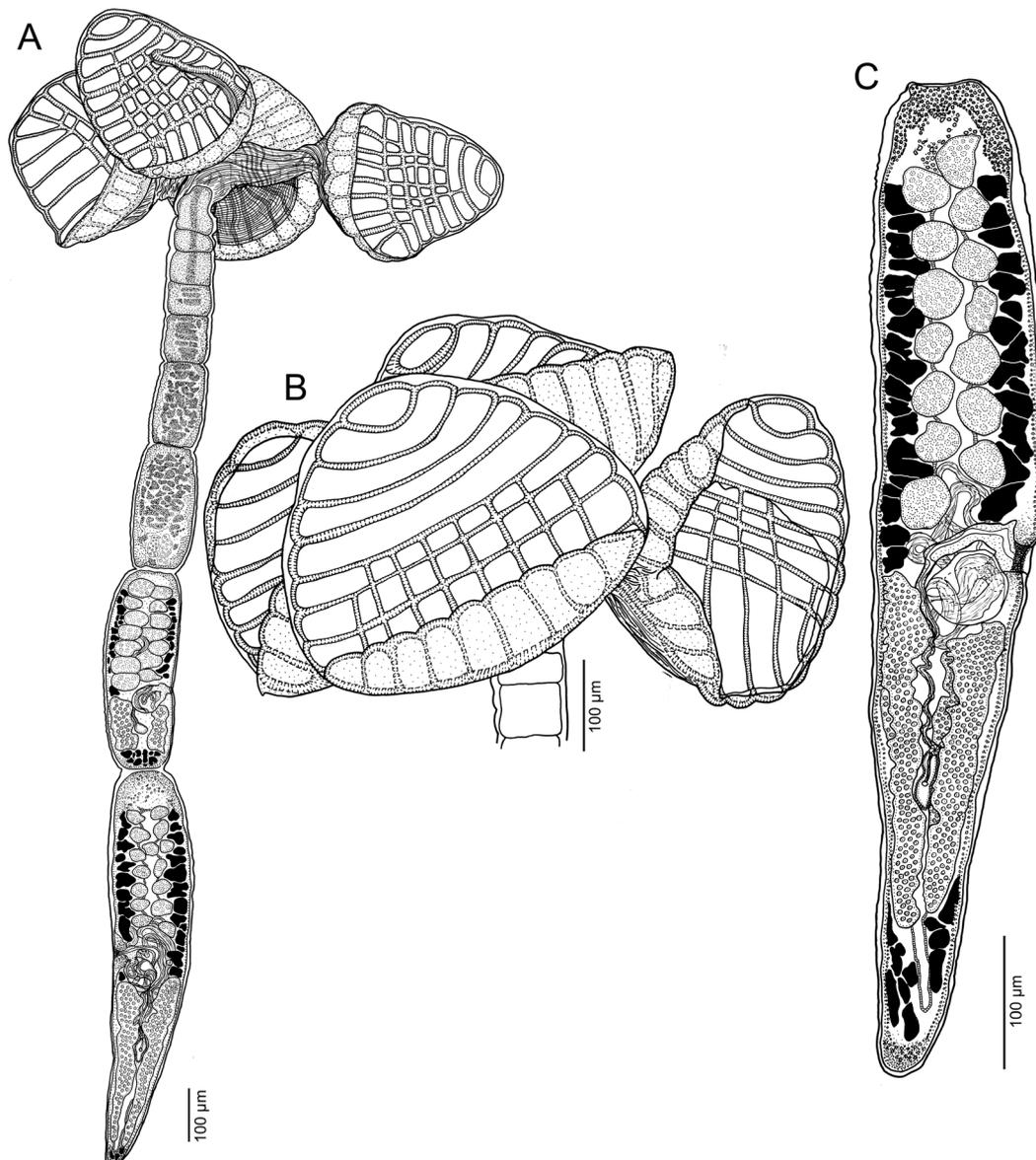
Fig. 8. Line drawings of *Stillabothrium hyphantoseptum* sp. n. from *Pastinachus solocirostris* Last, Manjaji et Yearsley. **A** – whole worm (holotype; MZUM [P] 2016.13 [H]); **B** – scolex (LRP 9092); **C** – terminal proglottid (LRP 9088).

morphologically similar to *S. campbelli* were encountered in *H. uarnacoides* (BO-118). Further studies are needed to determine whether *Stillabothrium* specimens from *H. uarnacoides* are conspecific with *S. campbelli* or represent an undescribed species of *Stillabothrium*.

Four specimens of *S. campbelli* were included in the phylogenetic analysis (Fig. 1, Table 1).

Stillabothrium hyphantoseptum Herzog, Bergman et Reyda sp. n.   Figs. 1, 8, 9, 16D

ZooBank number for species:
urn:lsid:zoobank.org:act:97362AA0-762D-4C87-B5ED-0A3664D69E98

Description (based on whole mounts of 29 complete mature worms, cross sections of 2 strobila and longitudinal sections of 2 scoleces and 2 scoleces prepared for SEM): Worms (Fig. 8A) euapolytic, acraspedote, 1.10–2.28 mm

(1.64 ± 0.3; n = 29) long, greatest width 368–933 (601 ± 140; n = 29) at level of scolex; 5–9 (7.3 ± 1.1; n = 29) proglottids per worm. Cephalic peduncle lacking; small darkly staining germinative zone present.

Scolex (Fig. 8B) consisting of scolex proper bearing 4 stalked bothridia. Stalks 49–270 (119 ± 54; n = 22) long by 48–101 (73 ± 15; n = 27) wide, attached slightly posterior to middle of bothridia. Bothridia varying in shape with degree of contraction, from finely ovoid (Fig. 8A), broadly ovoid (Fig. 8B), to broadly deltoid (Fig. 9A), facially loculated, 247–390 (319 ± 35; n = 24) long by 213–360 (288 ± 37; n = 24) wide; bothridial margins with thin rim. Anterior region of bothridia (Figs. 8A,B, 9B) with 6–8 horizontally oriented loculi (i.e. loculi wider than long) with 6–8 complete transverse septa (7.2 ± 0.5; n = 26). Anteriormost loculus 30–51 (40 ± 6; n = 27) long by 54–90 (71 ± 10; n = 29) wide. Posterior region of bothridia with 8 (n = 21) nonmedial longitudinal septa dividing bothrid-
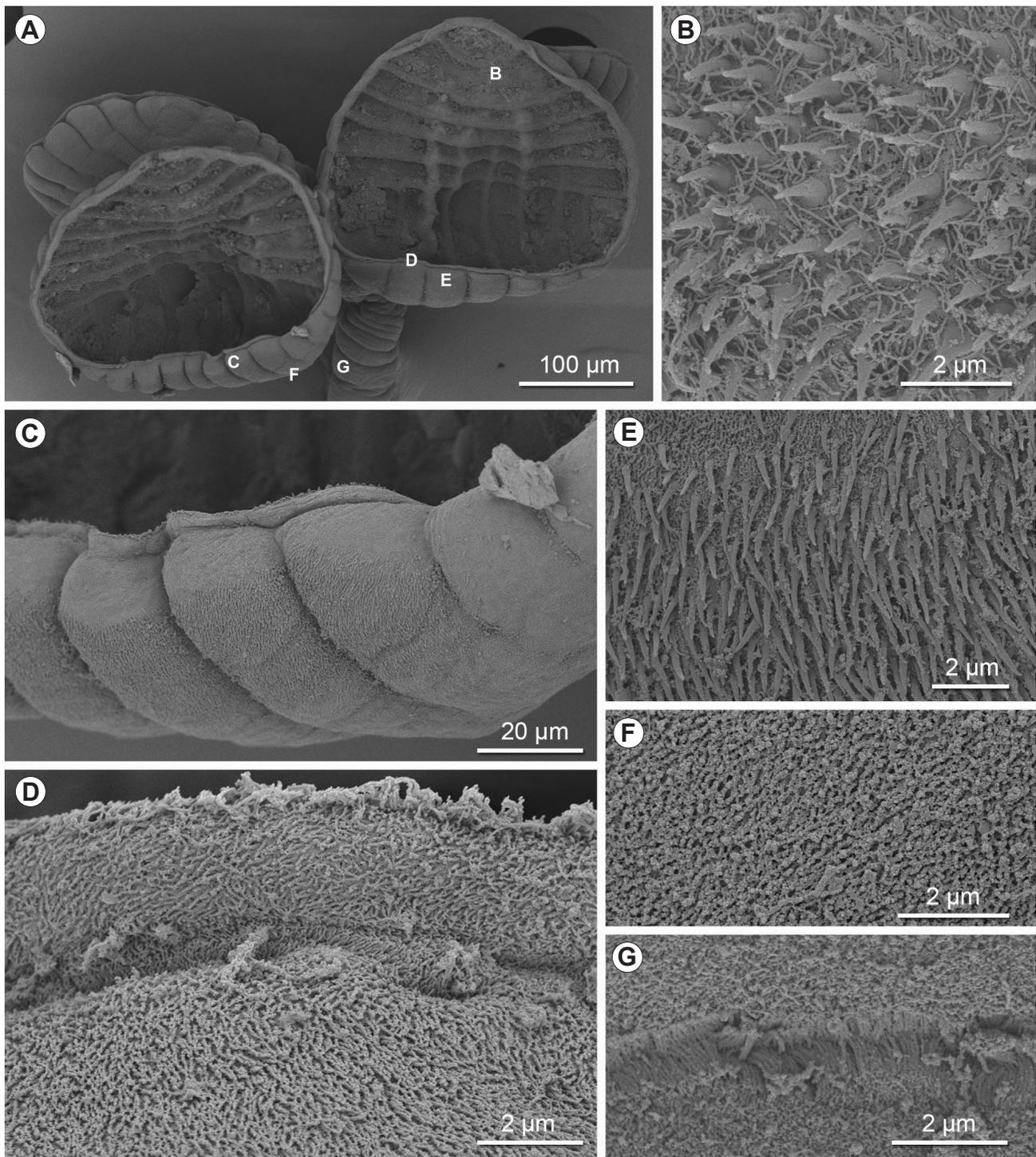
Fig. 9. Scanning electron micrographs of *Stillabothrium hyphantoseptum* sp. n. from *Pastinachus solocirostris* Last, Manjaji et Yearsley. **A** – scolex; letters indicate locations of other SEMs; **B** – distal bothridial surface at second loculus and transverse septum; **C**, **D** – proximal bothridial surface with rim; **E** – proximal bothridial surface near rim; **F** – proximal bothridial surface; **G** – strobila.

ia into 9 loculi longer than wide; majority of longitudinal septa overlapping posteriormost 3–6 (4.3 ± 0.9; n = 13) transverse septa of anterior region of bothridia, resulting in a grid-like pattern of septa and loculi in the centre of bothridium (Fig. 8A,B).

Loculi and septa of distal bothridial surfaces (Fig. 9B) bearing capilliform filitriches and coniform spinitriches. Proximal bothridial rim (Fig. 9C,D) bearing capilliform filitriches (Fig. 9B). Proximal bothridial surfaces away

from rim bearing acicular filitriches throughout bothridium (Fig. 9D,E,F). Posterior margin of proximal bothridial surfaces bearing a patch of coniform spinitriches (Fig. 9C,E) near, but not extending to, bothridial rim. Bothridial stalks and strobila (Fig. 9G) bearing capilliform filitriches only.

Strobila with 2–5 (3.5 ± 0.9; n = 29) proglottids wider than long followed by 2–6 (3.9 ± 0.8; n = 29) proglottids longer than wide. Strobila widest at terminal proglottid; terminal proglottid 381–840 (601 ± 127; n = 29) long

by 78–155 (12 ± 18; n = 29) wide; genital pore located 50–61% (55 ± 3.6; n = 29) of proglottid length from proglottid posterior margin. Immature proglottids 4–8 (6.0 ± 1.1; n = 29) in number. Mature proglottids 1–2 (1.3 ± 0.5; n = 29) in number, including 0–1 (0.6 ± 0.5; n = 29) vas deferens-mature proglottid.

Testes in mature proglottids 9–16 (13 ± 1.9; n = 28) in total number, 1 layer deep in section, arranged in 2 columns; columns extending from anterior margin of proglottid to anterior margin of cirrus sac, 23–57 (33 ± 7; n = 25) long by 27–49 (39 ± 7; n = 25) wide. Vas deferens coiled, entering anterior margin of cirrus sac, extending from level of ovarian isthmus to overlap posteriormost testes. Cirrus sac thin-walled, oval, extending medially past midline of proglottid; cirrus sac in terminal mature proglottid 45–69 (57 ± 6; n = 12) long by 38–60 (49 ± 7; n = 12) wide; cirrus sac in vas deferens-mature proglottids 52–79 (63 ± 8; n = 16) long by 45–63 (54 ± 5; n = 16) wide. Cirrus spinitriches present.

Vagina (Figs. 8C, 16D) thick-walled, weakly sinuous, somewhat overlapping medial portion of cirrus sac (Fig. 8A), extending along midline of proglottid from ootype region to anterior margin of cirrus sac, then laterally to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Seminal receptacle present. Ovary near posterior end of proglottid, H-shaped in frontal view, tetralobed in cross section. Ovarian lobes asymmetrical; poral ovarian lobe somewhat overlapping cirrus sac; poral and aporal ovarian lobes in terminal mature proglottids 125–201 (155 ± 28; n = 9) and 140–213 (165 ± 27; n = 9) long, respectively. Poral and aporal ovarian lobes in vas deferens-mature proglottids 175–290 (216 ± 27; n = 15) and 175–290 (228 ± 34; n = 15) long, respectively. Maximum width of ovary 68–114 (92 ± 12; n = 24). Ovarian isthmus at or anterior to midpoint of ovary; poral lobe of ovary stopping 30–80 (53 ± 14; n = 24) short of genital pore. Mehlis' gland posterior to ovarian isthmus, 24–70 (40 ± 11; n = 20) long by 14–30 (21 ± 5; n = 18) wide. Vitellarium follicular, 1 dorsal and 1 ventral column on each side of proglottid; columns extending from anterior to posterior margin of proglottid, interrupted by terminal genitalia, and interrupted by ovary (Fig. 8A,C). Uterus ventral, sacciform, extending from posterior margin of proglottid to near anterior margin of proglottid.

T y p e   a n d   o n l y   k n o w n   h o s t : *Pastinachus solocirostris* Last, Manjaji et Yearsley, Roughnose stingray (Myliobatiformes: Dasyatidae).

T y p e   l o c a l i t y : South China Sea off Mukah (02°53'52"N; 112°05'44"E), Sarawak, Malaysian Borneo (BO-267).

A d d i t i o n a l   l o c a l i t i e s : Java Sea off Singkawang (Pasar Bringin) (00°55'06"N; 108°58'58"E), West Kalimantan, Indonesian Borneo (KA-148).

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 MZUM (P) No. 2016.13 (H). Paratypes: IPCAS No. C-741; LRP Nos. 9003–9004; 9083–9117 (including molecular vouchers, sec-tions, and SEM specimens); MZUM (P) No. 2016.14 (P)–2016.15 (P); SBC No. P-00072; USNM Nos. 1420471–1420477.

E t y m o l o g y : This species is named for the grid-like pattern of

septa in the centre of the bothridium. The name is a combination of the Latin 'hyphantos', meaning woven, and the Latin 'septum'.

Remarks. The most striking characteristic of *S. hyphantoseptum* sp. n. is the extensive grid-like appearance of the septa on the centre of the bothridia (Fig. 8A,B). This configuration results from the extensive overlap of transverse (anterior) septa and longitudinal (posterior) septa. This feature readily distinguishes *S. hyphantoseptum* from *S. ashleyae* and *S. davidcynthiaorum*, both of which lack overlapping septa. The only other described species of *Stillabothrium* with overlapping transverse and longitudinal septa is *S. campbelli* (Fig. 6A,B). Although the centre of bothridia of *S. campbelli* is grid-like, the grid-like area is less extensive than in *S. hyphantoseptum* (compare Figs. 6B and 8B). In *S. campbelli*, the posteriormost 3–4 transverse septa are overlapped by its central 2 longitudinal septa, whereas in *S. hyphantoseptum* the posteriormost 3–6 transverse septa are overlapped by most of its eight longitudinal septa. The number of loculi that are wider than long in the anterior region of bothridia is also different between the two species (6–8 in *S. hyphantoseptum* vs 10–12 in *S. campbelli*).

In terms of proglottid morphology, *S. hyphantoseptum* sp. n. can be distinguished from *S. ashleyae*, *S. davidcynthiaorum* and *S. campbelli* in its possession of a uterus that extends to the posterior margin of the proglottid (Fig. 8C), whereas in the latter three species the uterus only extends posteriorly to the ovarian isthmus (Figs. 4C, 6C; not illustrated for *S. ashleyae*, but observed).

Stillabothrium hyphantoseptum is the only species of *Stillabothrium* reported from a species of *Pastinachus* Rüppell. The host species, *P. solocirostris* (roughnose stingray) was described by Last et al. (2005) as part of the same survey work that made the current study possible.

Two specimens of *S. hyphantoseptum* were included in the phylogenetic analysis (Fig. 1, Table 1).

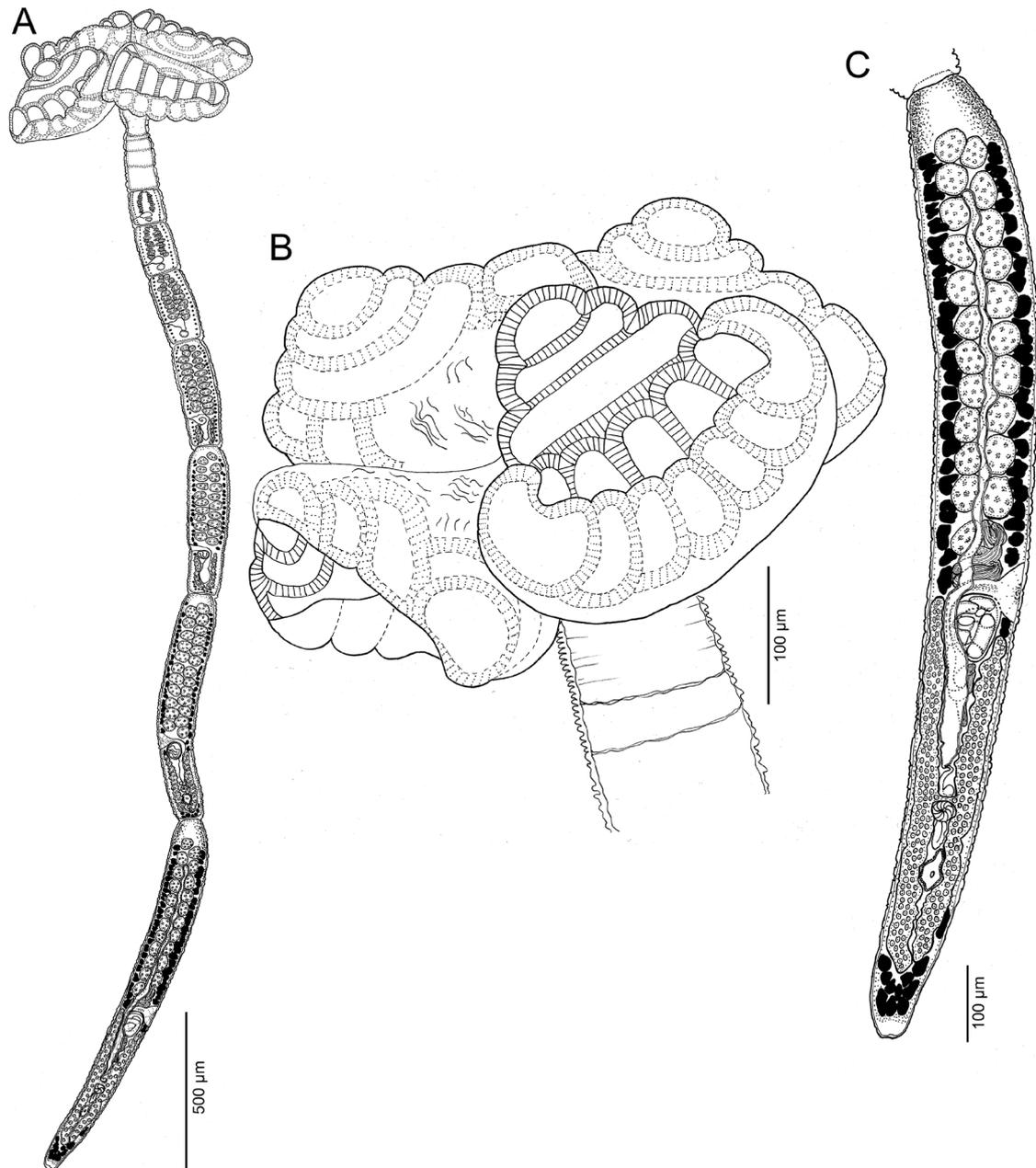***Stillabothrium jeanfortiae*** Forti, Aprill et Reyda sp. n.

Figs. 1, 10, 11, 16E

ZooBank number for species:
urn:lsid:zoobank.org:act:D2B25027-058D-4E3D-B880-F1FA5AB763DB

Description (based on whole mounts of 20 complete mature worms, cross sections of 1 strobila and longitudinal sections of 1 scolex and 2 scoleces prepared for SEM): Worms (Fig. 10A) euapolytic, acraspedote, 1.7–3.9 mm (2.5 ± 0.6; n = 20) long, greatest width 428–822 (588 ± 121; n = 20) at level of scolex; 5–10 (7.1 ± 1.2; n = 20) proglottids per worm. Cephalic peduncle lacking, small darkly staining germinative zone present.

Scolex (Fig. 10B) consisting of scolex proper bearing four stalked bothridia. Stalks 30–125 (77 ± 25; n = 16) long by 65–140 (94 ± 18; n = 16) wide, attached slightly posterior to middle of bothridia. Bothridia (Figs. 10A,B, 11A) always constricted but varying in shape with degree of contraction, broadly (Fig. 10B), shallowly (Fig. 10A), very shallowly (Fig. 11A), or depressed deltoid, facially loculat-

Fig. 10. Line drawings of *Stillabothrium jeanfortiae* sp. n. from *Himantura australis* Last, White et Naylor. **A** – whole worm (holotype; QM G235198); **B** – scolex (LRP 9123); **C** – terminal proglottid (holotype; QM 235198).

ed, 218–305 (259 ± 21; n = 20) long by 255–460 (339 ± 58; n = 20) wide. Anterior region of bothridia (Figs. 10A,B, 11A) with 3 horizontally oriented loculi (i.e. loculi wider than long) with 3 complete complete transverse septa (n = 20). Anteriormost loculus 35–60 (46 ± 7; n = 18) long by 64–115 (81 ± 16; n = 18) wide. Posterior region of bothridia (Figs. 10A,B, 11A) with 6 (n = 20) nonmedial longitudinal septa dividing bothridia into 7 loculi longer than wide; longitudinal septa not overlapping transverse septa in anterior region of bothridia.

Loculi and septa of distal bothridial surfaces (Fig. 11B) bearing coniform spinitriches and, less conspicuously, capilliform filitriches. Proximal bothridial rim (Fig. 11C) bearing capilliform filitriches greater in length than those on distal bothridial surfaces (Fig. 11B). Proximal bothridial surfaces

away from rim bearing densely arranged capilliform filitriches throughout bothridium (Fig. 11E). Proximal bothridial surfaces near bothridial rim bearing coniform spinitriches (Fig. 11D) that do not extend to stalks. Bothridial stalks and strobila (Fig. 11F) bearing capilliform filitriches only.

Strobila with 2–5 (3.2 ± 0.8; n = 20) proglottids wider than long followed by 3–7 (3.9 ± 1.0; n = 20) proglottids longer than wide. Strobila widest at terminal proglottid; terminal proglottid 724–1 260 (1 013 ± 185; n = 20) long by 92–192 (132 ± 24; n = 20) wide; genital pore located 42–56% (48 ± 4; n = 20) of proglottid length from proglottid posterior margin. Immature proglottids 4–8 (5.8 ± 1.0; n = 20) in number. Mature proglottids 1–2 (1.4 ± 0.5; n = 20) in number, including 0–1 (0.3 ± 0.5; n = 20) vas deferens-mature proglottid.
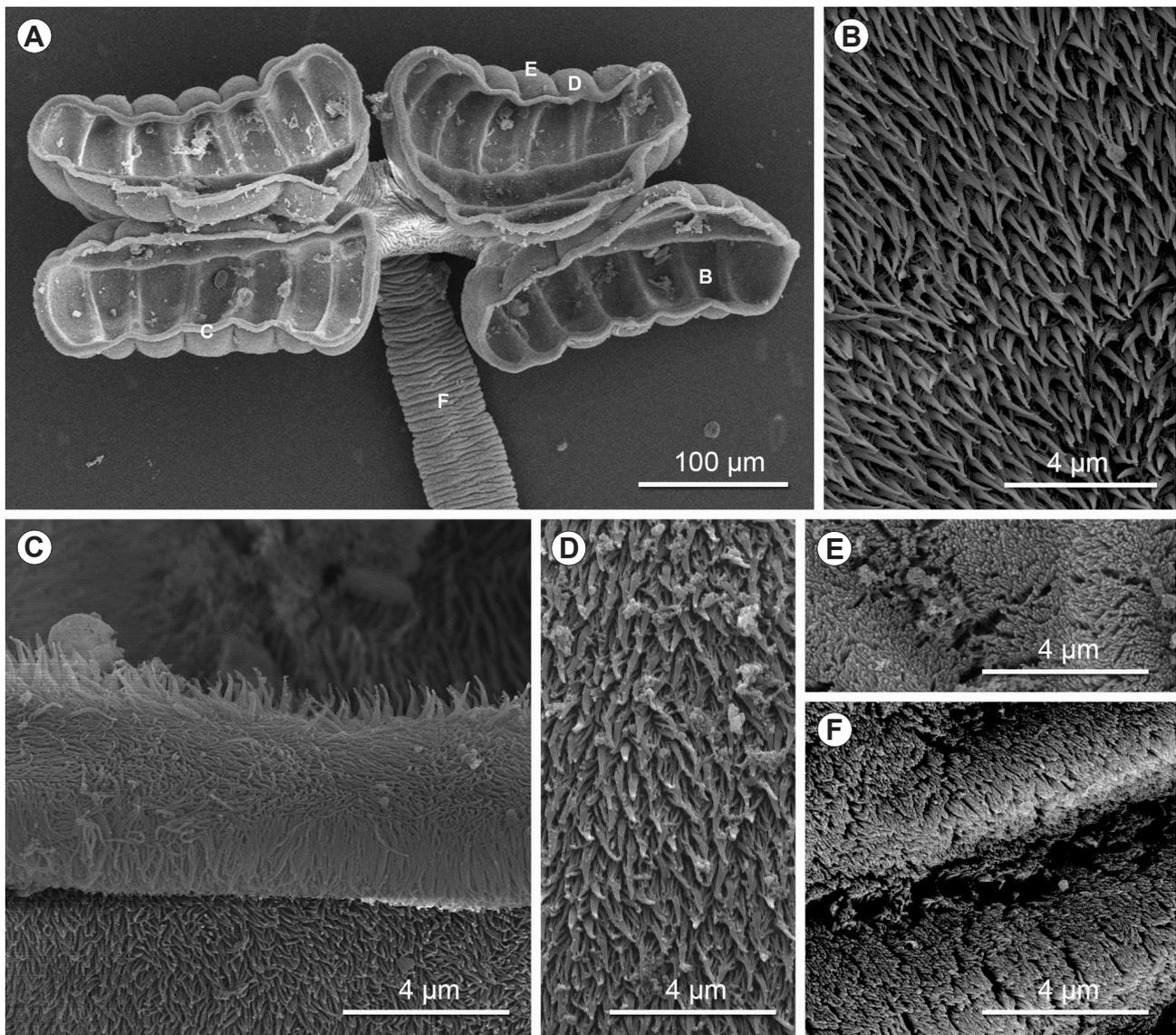
Fig. 11. Scanning electron micrographs of *Stillabothrium jeanfortiae* sp. n. from *Himantura australis* Last, White et Naylor. **A** – scolex; letters indicate locations of other SEMs; **B** – distal bothridial surface in posterior borthridium; **C** – proximal bothridial surface with rim; **D** – proximal bothridial surface near rim; **E** – proximal bothridial surface; **F** – strobila.

Testes in mature proglottids 20–29 (23 ± 2; n = 20) in total number, in 1 layer deep in section, arranged in 2 columns (Fig. 10C), columns extending from anterior margin of proglottid to anterior margin of cirrus sac, 26–52 (38 ± 8; n = 16) long by 29–55 (42 ± 7; n = 16) wide. Vas deferens coiled, entering anterior margin of cirrus sac, extending from level of ovarian isthmus anteriorly to overlap posteriormost testes. Cirrus sac thin-walled, oval, extending medially past midline of proglottid. Cirrus sac in terminal mature proglottid 55–81 (72 ± 8; n = 15) long by 47–73 (59 ± 8; n = 15) wide; cirrus sac in vas deferens-mature proglottids 80–87 (84 ± 3; n = 5) long by 62–80 (72 ± 7; n = 5) wide. Cirrus spinitriches present.

Vagina (Fig. 10C) thick-walled, non-sinuous, somewhat overlapping medial portion of cirrus sac (Fig. 10C), extending along midline of proglottid from ootype region to anterior margin of cirrus sac, then laterally to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Seminal receptacle present. Ovary near posterior end of proglottid, H-shaped in frontal view, tetralobed in cross section; ovarian lobes somewhat asymmetrical; poral and aporal ovarian lobes in terminal mature proglottids 238–396 (304 ± 47; n = 12) and 265–394 (318 ± 45; n = 12) long, respectively. Poral and aporal ovarian lobes in vas deferens-mature proglottids 355–486 (412 ± 58; n = 6) and 370–488 (434 ± 48; n = 6) long, respectively. Maximum width of ovary 60–122 (91 ± 19; n = 17). Ovarian isthmus at or posterior to midpoint of ovary; poral lobe of ovary stopping 18–70 (51 ± 14; n = 17) short of genital pore. Mehlis' gland well posterior to ovarian isthmus, 32–72 (47 ± 13; n = 16) long by 22–35 (28 ± 4; n = 16) wide. Vitellarium follicular; vitelline follicles arranged in 1 dorsal and 1 ventral column on each side of proglottid; columns extending from anterior to posterior margin of proglottid, interrupted by terminal genitalia and mostly interrupted by ovary (Fig. 10A,C). Uterus ventral, sacciform extending

from near ovarian isthmus to near anterior margin of proglottid.

Type and only known host: *Himantura australis* Last, White et Naylor, Reticulate whipray (Dasyatidae: Myliobatiformes).

Type and only known locality: Gulf of Carpentaria (Indian Ocean) off Weipa (12°35'11"S; 141°42'34"E), Queensland, Australia (CM03-3, CM03-25, CM03-65).

Site of infection: Spiral intestine.

Type material: Holotype QM No. G235198. Paratypes: LRP Nos. 8999; 9118–9133 (including molecular vouchers, sections, and SEM specimens); QM Nos. G235199– G235200; USNM Nos. 1420478–1420483; IPCAS No. C-740.

Etymology: This species is named in loving memory of Jean Forti, mother of K.S. Forti, for her support of her daughter's education.

Remarks. *Stillabothrium jeanfortiae* sp. n. is the fifth new *Stillabothrium* species described in this study. Its configuration of bothridial septa is unique relative to its four described congeners. *Stillabothrium jeanfortiae* is distinguished from *S. ashleyae* and *S. davicynthiae* in that it lacks, rather than possesses, marginal septa in the posterior region of bothridia. It also differs from *S. ashleyae* and *S. davicynthiae* in that the three loculi in its anterior region of bothridia are oriented in tandem, instead of occurring as a row of one and then two loculi. *Stillabothrium jeanfortiae* can be distinguished from *S. campbelli* and *S. hyphantoseptum* in that it lacks, rather than posseses, septa that overlap one another.

Stillabothrium jeanfortiae is described from the stingray *Himantura australis* from the Gulf of Carpentaria in northern Australia. Specimens similar in morphology to *S. jeanfortiae* were encountered in *H. leoparda* (NT-117), also from the Gulf of Carpentaria, but confirmation of the identity of this material awaits further study. Specimens with a similar bothridial morphology to that of *S. jeanfortiae* were also encountered in a diversity of species of *Himantura* during survey work in Borneo. Those specimens can be readily distinguished from *S. jeanfortiae* based on proglottid morphology, but their taxonomic treatment also awaits further study.

One specimen of *S. jeanfortiae* was included in the phylogenetic analysis (Fig. 1, Table 1). It grouped with two undescribed species of *Stillabothrium* from Senegal.

Stillabothrium cadenati (Euzet, 1954) Healy et Reyda, 2016 comb. n.
Figs. 1, 12–14, 16E

Synonym: *Rhinebothrium cadenati* Euzet, 1954

Informal synonyms: Rhinebothriinae New genus 3 *cadenati* of Healy et al. (2009), Caira et al. (2014), Ruhnke et al. (2015), Marques and Caira (2016).

ZooBank number for species:

urn:lsid:zoobank.org:act:9274C612-40F1-4F66-839C-DE145BC0C01C

Redescription (based on specimens collected from *Z. schoenleinii* consisting of whole mounts of 6 complete mature worms, 5 strobilae and 2 scoleces; cross sections of 1 strobila and longitudinal sections of 2 scoleces [including 1 *in situ* scolex], and 1 specimen prepared for SEM): Worms euapolytic, slightly craspedote (Fig. 12A), 1.04–1.77 mm (1.35 ± 0.3; n = 6) long, greatest width 439–694 (568 ± 96; n = 7) at level of scolex; 5–7 (5.7 ± 0.8; n = 6) proglottids per worm. Cephalic peduncle lacking; small darkly staining germinative zone present. Scolex (Figs. 12A, 14A) consisting of scolex proper bearing four stalked bothridia. Stalks 39–128 (86 ± 33; n = 8) long by 40–77 (60 ± 13; n = 8) wide, attached slightly posterior to middle of bothridia. Bothridia (Figs. 12A,B, 14A,B) varying in shape with degree of contraction, deeply (Fig. 12A), finely deltoid or broadly deltoid (Figs. 12B, 14A,B), facially loculated, 285–378 (328 ± 38; n = 6) long by 201–355 (274 ± 64; n = 7) wide. Anterior region of bothridia (Figs. 12A,B) with 3 horizontally oriented loculi (i.e. loculi wider than long) with 3–4 (3.8 ± 0.4; n = 6) complete transverse septa; fourth complete but reduced transverse septum (Fig. 12B) observed in 5 of 6 specimens. Anteriormost loculus 58–105 (77 ± 14; n = 8) long and 74–118 (108 ± 14; n = 8) wide. Posterior region of bothridia (Fig. 12A,B) with 4 (n = 8) nonmedial longitudinal septa dividing bothridia into 5 loculi longer than wide; longitudinal septa in posterior region of bothridia overlapping posteriormost (fourth) transverse septum in anterior region of bothridia (Fig. 12B). Muscle fibres other than septa but parallel to bothridial surface also observed on bothridia (Fig. 12A). All septa appear as ridges in section with proximal and distal portions different (Fig. 13); proximal portion of septa formed by underlying bothridial wall, consisting of radial muscles oriented with proximal ends of fibres adjacent to each other; distal portion of septa formed by separate muscle bundle; proximal and distal portions of septa separated by a triangular gap.

Loculi (Fig. 14D) and septa (Fig. 14E) of distal bothridial surfaces bearing coniform spinitriches and capilliform filitriches; spinitriches lacking on distal bothridial margin and rim (Fig. 14C). Proximal bothridial rim (Fig. 14C) bearing capilliform filitriches greater in length than those on distal bothridial surfaces (Fig. 14D,E). Proximal bothridial surfaces away from rim and stalks bearing coniform spinitriches and capilliform filitriches (Fig. 14F,G). Strobila bearing capilliform filitriches only.

Strobila with 3–4 (3.2 ± 0.4; n = 6) proglottids wider than long followed by 2–4 (2.5 ± 0.8; n = 6) proglottids longer than wide. Strobila widest at terminal proglottid; terminal proglottid 467–1 005 (n = 11) long by 103–139 (n = 11) wide; genital pore located 64–73% (n = 11) of proglottid length from proglottid posterior margin. Genital atrium expansive, 38–68 (51 ± 10; n = 11) long by 31–49 (40 ± 7; n = 11) wide, with convoluted, muscular walls. Immature proglottids 4–5 (4.5 ± 0.5; n = 6) in number. Mature proglottids 1–2 (1.4 ± 0.5; n = 11) in number, including 0–1 (0.7 ± 0.5; n = 11) vas deferens-mature proglottids.

Testes in mature proglottids 7–13 (10.8 ± 2; n = 12) in total number, 1 layer deep in section, arranged in 1–3 (2 ± 0.4; n = 11) though usually 2, columns (Fig. 12C); columns extending from near anterior margin of proglottid to anterior margin of vagina, 22–36 (30 ± 4; n = 11) long by 29–50 (38 ± 6; n = 11) wide. Vas deferens coiled, entering
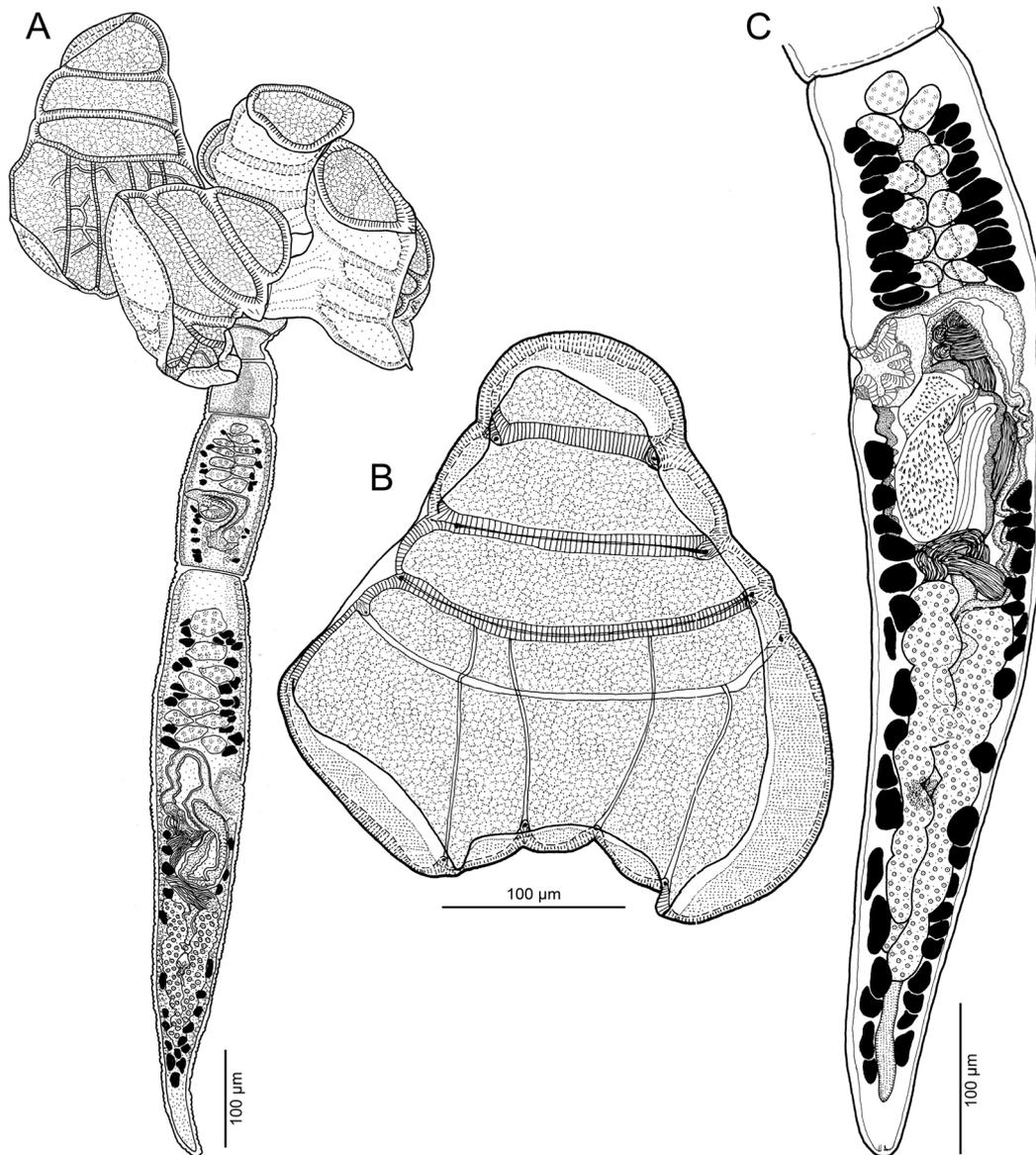
Fig. 12. Line drawings of *Stillabothrium cadenati* comb. n. from *Zanobatus schoenleinii* (Müller et Henle). **A** – whole worm (LRP 9135); **B** – bothridium (LRP 9134); **C** – terminal proglottid (LRP 9136).

anterior margin of cirrus sac, extending anterior to ovarian isthmus to near anterior vagina. Cirrus sac thick-walled and relatively large, oval and bent posteriorly, extending medially past midline of proglottid; cirrus sac in terminal mature proglottid 85–152 (119 ± 47; n = 2) long by 58–62 (60 ± 3; n = 2) wide; cirrus sac in vas deferens-mature proglottids 103–171 (136 ± 23; n = 8) long by 50–75 (67 ± 8; n = 8) wide. Cirrus spinitriches present, 3.5–6.1 (4.3 ± 0.9; n = 10) long by 2.0–3.7 (2.8 ± 0.5; n = 10) wide at base.

Vagina (Fig. 12A,C) thick-walled, sinuous, not overlapping cirrus sac, recurved (Figs. 12C, 16F); poral portion expanded; medial and posterior portion abruptly narrowed; extending from ootype past midline to aporal side of proglottid to anterior margin of cirrus sac, then laterally to open into genital atrium anterior to cirrus sac; vaginal sphincter absent. Seminal receptacle present. Ovary near posterior end of proglottid, lobulated, H-shaped in frontal view, tetralobed in cross section, not

overlapping cirrus sac; ovarian lobes asymmetrical; poral and aporal ovarian lobes in terminal mature proglottids 125–215 (170 ± 64; n = 2) and 125–225 (175 ± 71; n = 2) long, respectively. Poral and aporal ovarian lobes in vas deferens-mature proglottids 120–350 (222 ± 71; n = 9) and 125–350 (218 ± 69; n = 9) long, respectively. Maximum width of ovary 48–87 (62 ± 13; n = 10). Ovarian isthmus at or anterior to midpoint of ovary; poral lobe of ovary stopping 90–190 (145 ± 35; n = 10) short of genital pore. Mehlis' gland posterior to ovarian isthmus, 25–40 (33 ± 5; n = 10) long by 16–25 (20 ± 3; n = 10) wide. Vitellarium follicular; vitelline follicles arranged in 1 dorsal and 1 ventral column on each side of proglottid; columns extending from anterior to posterior margin of proglottid, interrupted by terminal genitalia, not interrupted by ovary (Fig. 12A,C). Uterus ventral, sacciform, extending from posterior margin of proglottid to near anterior margin of proglottid.
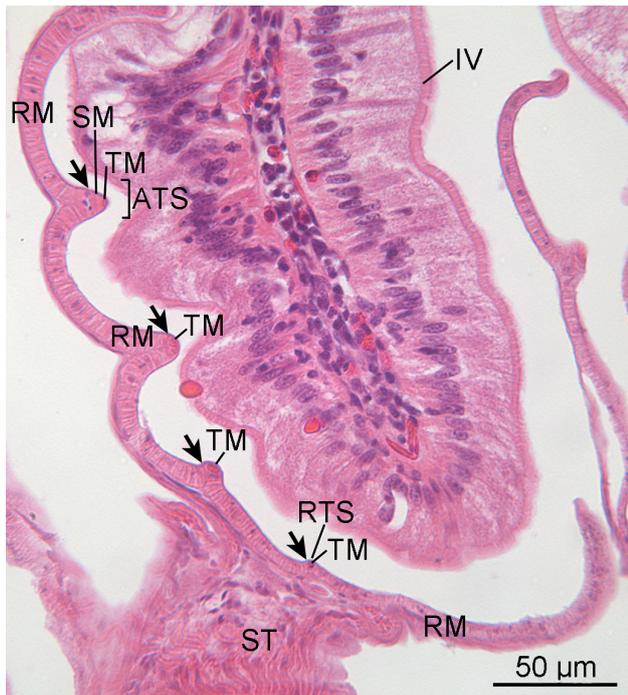
Fig. 13. Histological sections through bothridium of *Stillabothrium cadenati* (Euzet, 1954) comb. n. from *Zanobatus schoenleinii* (Müller et Henle). Arrows indicate divisions between proximal and distal portions of transverse septa. *Abbreviations*: ATS – anteriormost transverse septum; IV – intestinal villus; RM – radial musculature; RTS – reduced transverse septum; SM – septal musculature; ST – stalk; TM – transverse muscle bundle.

T y p e h o s t : *Zanobatus schoenleini* [sic!] (=*schoenleinii*) (Müller et Henle), striped panray (Rhinopristiformes: Zanobatidae).

A d d i t i o n a l h o s t : *Rhinobatos rhinobatos* (Linnaeus), Common guitarfish (Rhinopristiformes: Rhinobatidae).

T y p e l o c a l i t y : Gorée Island, Senegal.

A d d i t i o n a l l o c a l i t i e s : Atlantic Ocean off Ouakam (14°42'54"N; 17°29'28"W) (SE–28) and Soumbédioune (14°40'42"N; 17°27'42"W) (SE–201) in Dakar, and Joal (14°10'30"N; 16°51'12"W) (SE–299) and Kafountine (12°55'41"N; 16°45'10"W) (SE–289), Senegal.

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 : Unknown.

M a t e r i a l e x a m i n e d (v o u c h e r s) : LRP Nos. 9000–9002; 9134–9148 (including molecular vouchers, sections and SEM specimens); MNHN Nos. HEL580–HEL581; USNM No. 1420484.

Remarks. Type specimens of *S. cadenati* comb. n. were not available for this study. In the original description of *S. cadenati*, Euzet (1954) did not provide specimen deposition information. It is possible that the specimens were in the personal collection of the late Louis Euzet, a collection now transferred to the Museum National d'Histoire Naturelle (MNHN) in Paris, France. Examination of that collection by the curator, however, did not reveal the presence of any specimens of *S. cadenati*. The material on which this redescription was based were newly collected, topotypic specimens of *S. cadenati* The specimens were collected from the type host at Ouakam and Soumbédioune on the west coast of Dakar, Senegal, approximately 12 and seven

km, respectively away from Gorée island, the type locality of this species. Gorée lies 2.5 km off the eastern coast of Dakar. Specimens of *S. cadenati* were also obtained from the type host in Joal, Senegal.

Two specimens of *S. cadenati* were included in the phylogenetic analysis (Fig. 1, Table 1). One was from the type host, *Z. schoenleinii*, and one was from *R. rhinobatos* (the common guitarfish). Given that *R. rhinobatos* and the type host, *Z. schoenleinii*, belong to different orders of elasmobranchs, this host record requires verification, ideally by the examination of additional specimens.

The bothridial morphology of *S. cadenati* was not comprehensively described by Euzet (1954). While he noted the presence of 3 transverse septa, Euzet (1954) stated that the bothridia of the specimens he examined were tightly folded and clamped in four scallops. Subsequently, when Euzet and co-authors (Ball et al. 2003) erected the genus *Scalithrium* Ball, Neifar et Euzet, 2003, for species of *Rhinebothrium* that lack a median longitudinal septum, they concluded that the scolex morphology of *S. cadenati* was too poorly known to allow them to confidently place it in their new genus at that time.

The work conducted here enabled comprehensive characterisation of the bothridial morphology of *S. cadenati* for the first time. A fourth, less muscular transverse septum (Figs. 12B, 13) lies posterior to the three transverse septa noted by Euzet (1954), and this reduced transverse septum is crossed by some, but not all, non-medial longitudinal septa (Fig. 12B). We believe the scalloped appearance in the posterior part of the bothridium of specimens with folded bothridia noted by Euzet (1954) is due to the presence of these non-medial longitudinal septa.

This redescription also provides additional data on the reproductive morphology and microthrix distribution patterns of this species. Some measurements reported by Euzet (1954) suggest that his specimens of *S. cadenati* were somewhat larger than those examined here. For example, Euzet's (1954) specimens were 4–6 mm long in total length and possessed 13–16 proglottids, whereas the specimens examined here were 1.04–1.77 mm long and possessed 5–7 proglottids. In spite of these differences, we believe that the specimens we obtained for the current study are conspecific with those examined by Euzet (1954), based on the illustrations he provided, and considering that that the specimens we used for this study are from the same host, and within 11 km of the type locality.

The bothridia of *S. cadenati* are unlike any previously described *Stillabothrium* species in that they possess septa that differ in thickness. In *S. ashleyae*, *S. davidcynthiaorum*, *S. campbelli*, *S. hyphantoseptum* and *S. jeanfortiae*, all transverse and longitudinal septa appear uniform in thickness. In *S. cadenati* the 3 anteriormost transverse septa are thicker than the fourth transverse septa and the longitudinal septa (Fig. 12B).

Stillabothrium cadenati can also be distinguished from each of its five congeners in its conspicuously different proglottid morphology. Unlike those of its congeners, the genital atrium in *S. cadenati* has convoluted walls that are more muscular in appearance (compare Fig. 12C with
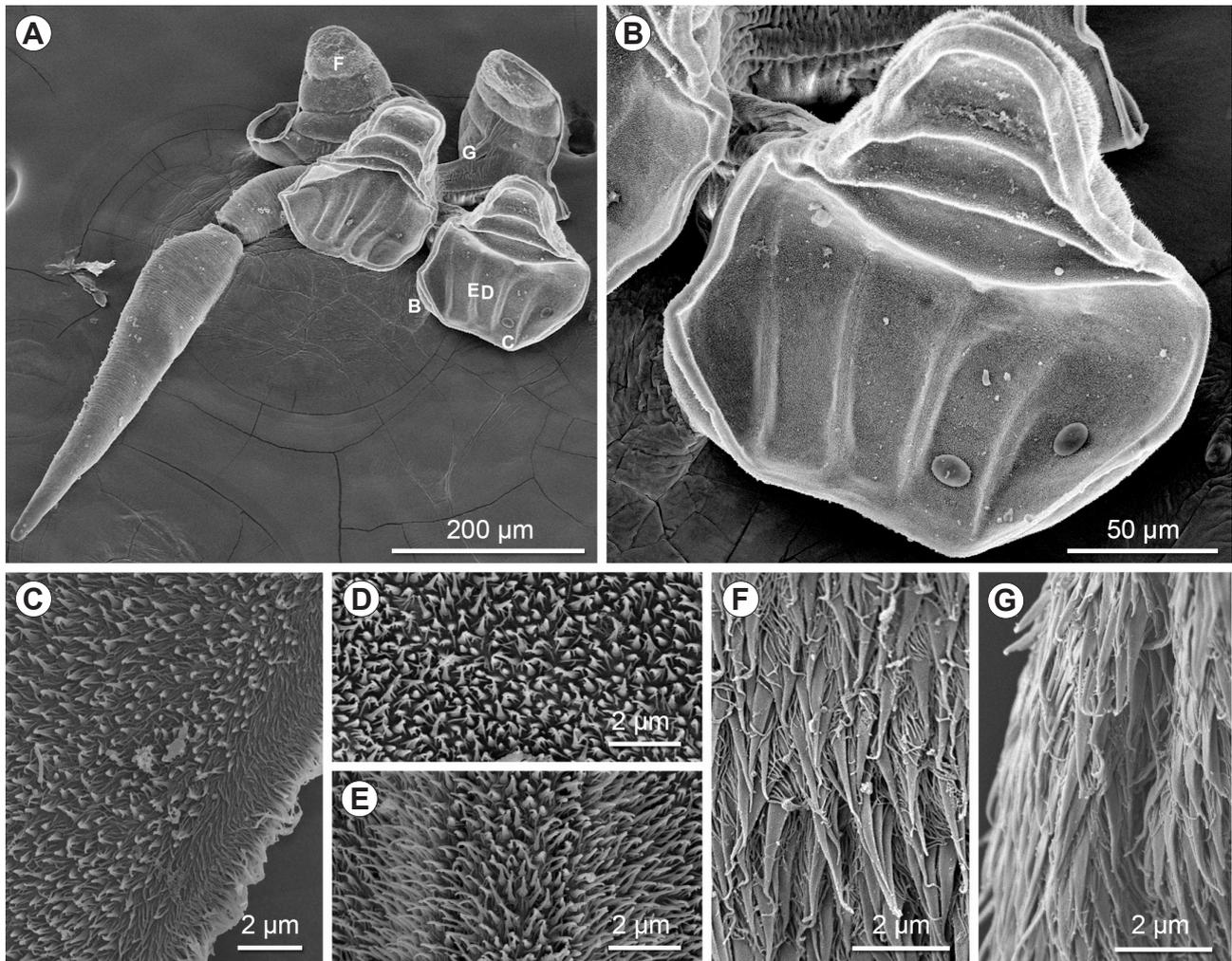
Fig. 14. Scanning electron micrographs of *Stillabothrium cadenati* (Euzet, 1954) comb. n. from *Zanobatus schoenleinii* (Müller et Henle). **A** – scolex, letters indicate locations of other SEMs; **B** – bothridium; **C** – distal bothridial surface at rim; **D** – distal bothridial surface within loculus; **E** – distal bothridial surface within septa; **F** – proximal bothridial surface; **G** – strobila.

Figs. 2C, 4C, 6C, 8C, 10C), and the genital pore is located more anteriorly in *S. cadenati* (64–73% of proglottid length from proglottid posterior margin) than it is in *S. ashleyae*, *S. davidcynthiaorum*, *S. campbelli*, *S. hyphantoseptum* and *S. jeanfortiae* (35–46%, 42–54%, 41–55%, 50–61% and 42–56%, respectively). In *S. cadenati*, the vitellarium (Fig. 12C) occurs along the length of the ovary, whereas in *S. ashleyae*, *S. davidcynthiaorum*, *S. campbelli*, *S. hyphantoseptum* and *S. jeanfortiae*, the vitelline columns are interrupted by the ovary (Figs. 2C, 4C, 6C, 8C, 10C). The cirrus sac is relatively larger in *S. cadenati* when compared to its 5 congeners (compare Fig. 12C to Figs. 2C, 4C, 6C, 8C, 10C), and reaches a greater length (103–171 μm *vs* 28–38 μm, 32–45 μm, 45–90 μm, 52–79 μm and 80–87 μm, respectively).

Stillabothrium cadenati is the only species of the genus reported from the Atlantic Ocean. Specimens that appear to represent at least two additional species of *Stillabothrium* were encountered in stingrays of the genus *Fontitrygon* Last, Naylor et Manjaji-Matsumoto over the course of survey work in Senegal. These remain to be examined in detail.

Four specimens of *S. cadenati* were included in the phylogenetic analysis (Fig. 1, Table 1). One of the four specimens (LRP 3924) was previously included in the analysis of Healy et al. (2009) as 'Rhinebothriinae New genus 3 *cadenati*'.

Stillabothrium amuletum (Butler, 1987) Healy et Reyda comb. n.   Figs. 1, 15

ZooBank number for species:
urn:lsid:zoobank.org: act:DFCD1070-9190-4890-ADEE-5474390DB2BF

S y n o n y m : *Anthobothrium amuletum* Butler, 1987.

I n f o r m a l s y n o n y m s : Rhinebothriinae New genus 3 sp. n. 7 of Healy et al. (2009), Caira et al. (2014), Ruhnke et al. (2015), Marques and Caira (2016).

T y p e a n d o n l y k n o w n h o s t : *Glaucostegus typus* [Anonymous (Bennett)], Giant shovelnose ray (Rhinopristiformes: Rhinobatidae) (= *Rhinobatos armatus*).

T y p e l o c a l i t y : Moreton Bay, Northern Territory, Australia.

A d d i t i o n a l l o c a l i t y : Fog Bay, Timor Sea (Indian Ocean) off Dundee Beach (12°45'33"S; 130°21'7"E), Queensland, Australia (AU-56).
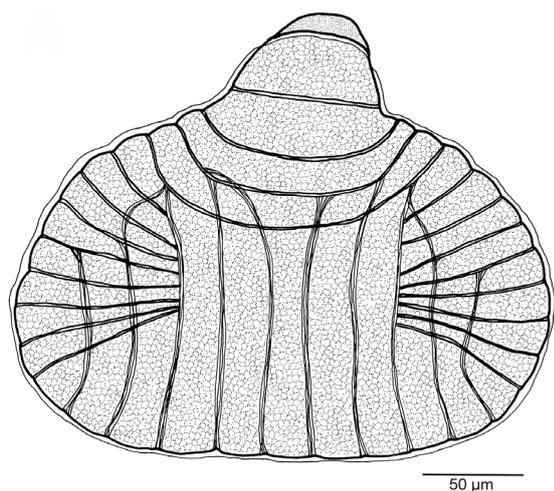
Fig. 15. Line drawing of bothridium of the holotype (QM GL4621) of *Stillabothrium amuletum* (Butler, 1987) comb. n. from *Glaucostegus typus* [Anonymous (Bennett)].

Site of Infection: Spiral intestine.

Specimens examined: AU-56, QM GL4621 (holotype), GL4622 (paratype).

Remarks. Butler (1987) provided a comprehensive description of this species, which included scanning electron micrographs and drawings, and represents the only treatment of this species, to date. Although examination of the holotype for the present study confirmed many of Butler's (1987) observations, it refuted some of her observations, and revealed additional details missing from the original description. According to Butler (1987), the anterior region of the bothridia lack transverse septa. However, at least 4 transverse septa are visible in the anterior region of the scolex of this holotype (Fig. 15). These septa, and other septa not described by Butler (1987), are best visible using differential interference contrast microscopy (DIC). Examination of additional material of this species and, ideally, histological sections of the scolex, would be very useful in determining the exact number and extent of the septa (and the loculi) in this species. Not stated in the description, but visible in the holotype, are the following features: the vas deferens joins the cirrus sac at the middle of its anterior margin; the genital atrium has internally convoluted margins; when at least partially expanded with spermatozoa, the vas deferens extends from between the anterior lobes of the ovary to the level of the recurvature of the vagina.

The morphology of the ovary, posterior vitelline follicles and posterior part of the uterus as drawn by Butler (1987) differ from that observed in the terminal proglottid of the holotype: the ovary is tetralobed and H-shaped; vitellarium is interrupted at the level of the ovary and some vitelline follicles are present in the lateral margins of the proglottid, posterior to the ovary; uterus extends posterior to the ovary.

This species possesses transverse septa and non-medial longitudinal septa, as well as other characteristics consistent with its placement in *Stillabothrium*, such as a posterior row of loculi that are longer than wide (Fig. 15). Thus, this species is herein transferred into this genus as *Stillabothrium amuletum* comb. n.

Stillabothrium amuletum possesses diagonal septa on the sides of the posterior bothridia, which overlap several longitudinal septa (Fig. 15). This feature distinguishes it from *S. ashleyae* (Fig. 2B) and *S. davidcynthiaorum* (Fig. 4B), and from *S. campbelli* (Fig. 6B), which possess marginal (*S. ashleyae* and *S. davidcynthiaorum*) or diagonal (*S. campbelli*) septa that abut but do not overlap longitudinal septa, and from *S. hyphantoseptum* (Fig. 8B), *S. jeanfortiae* (Fig. 10B) and *S. cadenati* (Fig. 12B) which each lack marginal or diagonal septa.

One sequence of *Stillabothrium* from *G. typus*, the type host of *S. amuletum*, was included in the phylogenetic analysis in this study (Table 1, Fig. 1). It was from Healy et al. (2009), who referred to it as 'Rhinebothriinae New genus 3 sp. n. 7'.

DISCUSSION

The integration of morphological and molecular data facilitated species delimitation in the present study. The molecular data were especially helpful in delimiting *Stillabothrium ashleyae* and *S. davidcynthiaorum*. *Stillabothrium davidcynthiaorum* and *S. ashleyae* possess relatively similar bothridial morphologies (i.e. three loculi in the anterior region and longitudinal and marginal loculi in the posterior region; see Fig. 1). These species also have similar proglottid anatomies and are sympatric at least in part in that both occur off Mukah in Malaysian Borneo. These two species differ, however, in the number of longitudinal loculi and septa (eight longitudinal septa in *S. ashleyae* vs ten longitudinal septa in *S. davidcynthiaorum*).

The molecular data provided support for our hypothesis that the variation in number of longitudinal septa is interspecific rather than intraspecific. The three specimens of *S. ashleyae* that were sequenced, all of which were from the same host individual, had identical sequences. The seven specimens of *S. davidcynthiaorum* that were sequenced formed a clade in which no two individuals differed by more than two bp. The clade of *S. ashleyae* was the sister clade to *S. davidcynthiaorum* (Fig. 1) and the members of the two clades differed from each other by 15–19 bp.

The relatively relaxed degree of host specificity seen in *S. davidcynthiaorum*, which appears to parasitise four (species of *Himantura* is unusual for a rhinebothriidean species. Such a relatively relaxed pattern of host specificity contrasts a widely demonstrated pattern of strict host specificity in rhinebothriideans and other elasmobranch cestodes in which cestode species typically occur in only a single host species. This is termed an oioxenous level of host specificity (*sensu* Euzet and Combes 1980) and has been demonstrated in many (Williams 1964, Caira and Jensen 2001, 2014, Jensen 2005, Tyler 2006, Ruhnke et al. 2015) but not all (Palm and Caira 2008, Reyda and Marques 2011) elasmobranch cestode species.

To examine this observation in detail, we included specimens morphologically consistent with *S. davidcynthiaorum* from all four potential host species in the molecular analyses and multiple specimens from each host species
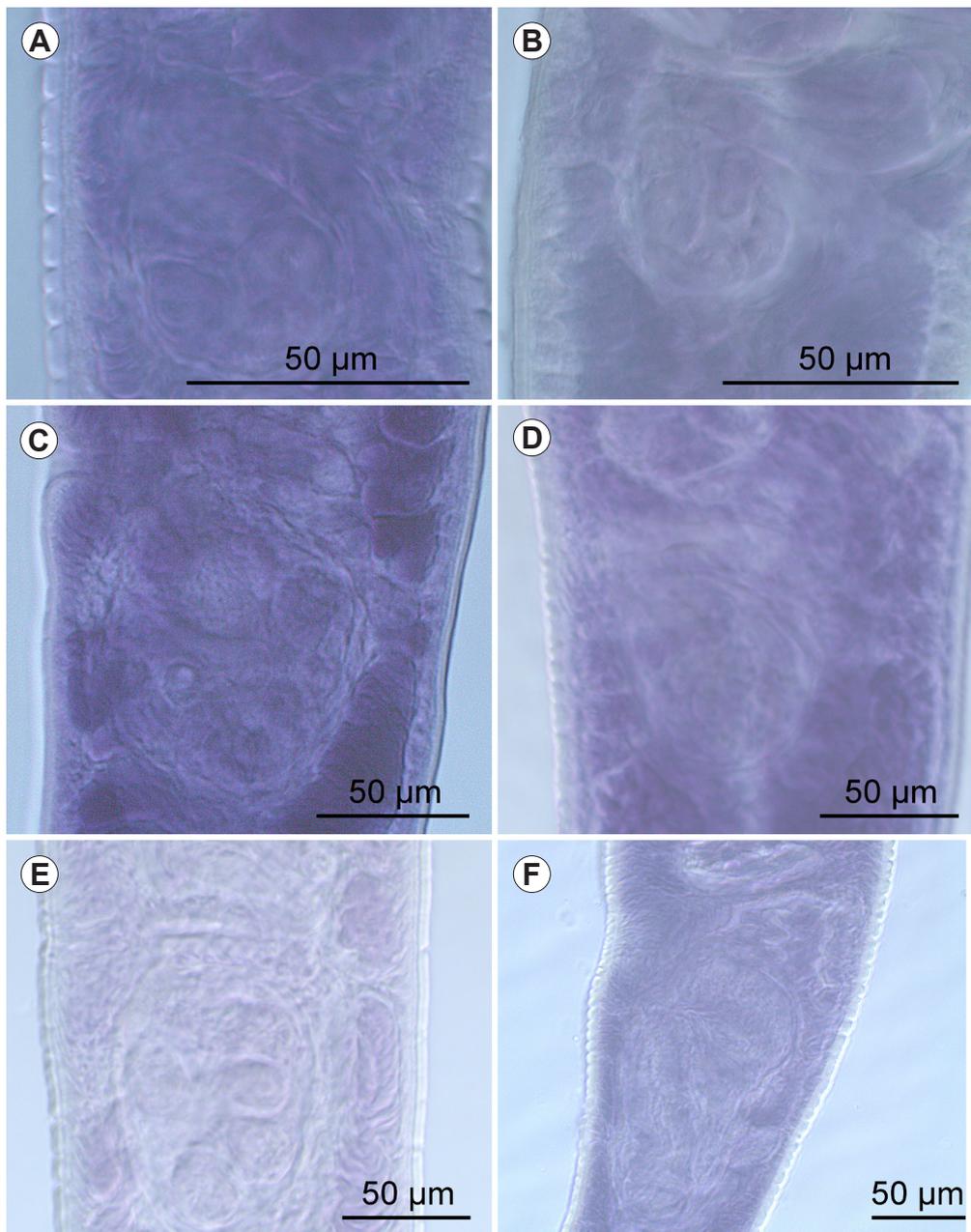
Fig. 16. Light micrographs of terminal genitalia of *Stillabothrium* species, each with genital pore oriented left. **A** – *Stillabothrium ashleyae* sp. n. from *Dasyatis biasa* (Last, White et Naylor); **B** – *Stillabothrium davidcynthiaorum* sp. n. from *Himantura gerrardi* (Gray); **C** – *Stillabothrium campbelli* sp. n. from *Himantura* cf. *pastinacoides*; **D** – *Stillabothrium hyphantoseptum* sp. n. from *Pastinachus solocirostris* Last, Manjaji et Yearsley; **E** – *Stillabothrium jeanfortiae* sp. n. from *Himantura australis* Last, White et Naylor; **F** – *Stillabothrium cadenati* comb. n. from *Zanobatus schoenleinii* (Müller et Henle).

were examined in detail morphologically. However, examination of nine specimens from *H. uarnak* 3, six specimens from *H. gerrardi* and 17 specimens from *H. heterura* revealed no clear pattern of anatomical variation beyond the fact that specimens from *H. heterura* were generally smaller (0.6–1.1 mm; n = 15) than those from *H. gerrardi* (1.0–1.6 mm; n = 6) and *H. uarnak* 3 (1.4–2.6 mm; n = 9). That variation was ultimately considered to represent intra-specific variation; all specimens, regardless of host species, possessed the key feature of *S. davidcynthiaorum*, i.e. 10 longitudinal septa (and 11 longitudinal loculi) on

the posterior bothridia. The molecular data supported this result that given across host species, specimens of *S. davidcynthiaorum* differed by no more than two bp.

Host specificity in *S. ashleyae*, *S. campbelli*, *S. jeanfortiae*, *S. hyphantoseptum* and *S. amuletum* was much more strict and also much more in line with that seen in other non-trypanorhynch orders of elasmobranch-hosted cestodes (see Caira and Jensen 2014), for each was found to parasitise only a single host species. Host specificity in *S. cadenati* is also slightly more relaxed in that is has been reported from two species, *Zanobatus schoenleinii* and *Rh-*

inobatos rhinobatos, both in Senegal. However, its occurrence in the latter species requires verification considering that only a single specimen was encountered in that host.

Beyond their possession of transverse septa and an even number of longitudinal septa, the bothridia across species of *Stillabothrium* vary greatly in the septal arrangement. *Stillabothrium jeanfortiae* could be viewed as having the simplest bothridial morphology in that it possesses only transverse septa in the anterior region and only longitudinal septa in the posterior region, and the two types of septa do not overlap. The bothridia of the other six described species are more complicated in that they possess additional features with respect to septa. Four of the other six described species, all of which are from Clade 2, each possess other septa in addition to transverse and longitudinal septa (Fig. 1); in *S. ashleyae*, *S. davidcynthiaorum*, *S. campbelli* and *S. amuletum* the longitudinal primary loculi on the left and right side are divided by additional marginal or diagonal septa into subloculi. Species also vary in whether septa overlap one another. *Stillabothrium campbelli*, *S. hyphantoseptum*, *S. cadenati* and *S. amuletum* each possess longitudinal septa that overlap transverse septa.

The proglottid anatomy also varies greatly among species of *Stillabothrium*. The size of the cirrus sac relative to proglottid width differs among species (Fig. 16) from relatively small in *S. ashleyae* and *S. davidcynthiaorum* (Figs. 2C, 4C) to relatively large in *S. cadenati* and *S. amuletum* (Fig. 12C and fig. 22 in Butler 1987). The vagina is strongly recurved in *S. cadenati* and *S. amuletum* (Fig. 12C and fig. 22 in Butler 1987), whereas those of the other five species are not. In *S. hyphantoseptum*, *S. cadenati* and *S. amuletum* the uterus extends to the posterior margin of the proglottid (Figs. 8C, 12C), while the uterus in each of the other four species extends posteriorly only to the level of the ovarian isthmus. The extent to which the columns of vitelline follicles are interrupted by the ovary also varies. In all but one species the vitelline columns are mostly or entirely interrupted by the ovary (Figs. 2C, 4C, 6C, 8C, 10C). The exception is *S. cadenati* (Fig. 12C), which bears columns that are not interrupted by the ovary.

The morphological features shared by all species of *Stillabothrium* constitute a very short list. The bothridia of the seven described as well as the three undescribed members of the genus included in the molecular analysis conducted here (i.e. *Stillabothrium* sp. n. 1, *Stillabothrium* sp. n. 2 and *Stillabothrium* sp. n. 4) possess one conspicuous key feature that could be considered a synapomorphy for the genus. In all 10 species, the longitudinal septa in the posterior portion of bothridia are vertically oriented (i.e. longer than wide) and are even, rather than odd, in number. There is one other feature of the scolex that is potentially possessed by all 10 members of the genus. The six *Stillabothrium* species observed with SEM here (i.e. *S. ashleyae*, *S. davidcynthiaorum*, *S. campbelli*, *S. hyphantoseptum*, *S. jeanfortiae* and *S. cadenati*) possess a limited distribution of coniform spinitriches on the proximal bothridial surfaces, though the extent varies. Additional SEM work is needed to verify whether this feature is possessed by the other

four species of *Stillabothrium* included in the molecular analysis.

There is no question that species diversity in *Stillabothrium* is substantially greater than that characterised here. To begin, as mentioned above, this study included three species (*Stillabothrium* sp. n. 1, *Stillabothrium* sp. n. 2, and *Stillabothrium* sp. n. 4) which await formal study. Two of those are from Senegal, *Stillabothrium* sp. n. 1 and *Stillabothrium* sp. n. 2, each parasitising *Fontitrygon margaritella* (Compagno et Roberts). The voucher specimens of each of those two species (LRP 3898 and LRP 3899, respectively) reveal that the scoleces of both display variations on bothridial septa configuration that are entirely unlike those described here. There is also at least one additional species of *Stillabothirum* from Australia, *Stillabothrium* sp. n. 4, from *Himantura astra* Last, Manjaji-Matsumoto et Pogonoski. Study of additional specimens acquired during our survey work also suggest that there is additional species diversity of *Stillabothrium*. As noted in the Remarks sections of the descriptions of *S. ashleyae*, *S. davidcynthiaorum*, *S. campbelli* and *S. jeanfortiae*, additional specimens from other host species with similar bothridial morphologies to those respective species were encountered here. Those specimens may represent new species, but they await further study. Finally, given that the stingray genus *Himantura*, the host genus for five of the ten *Stillabothrium* species in our analysis, is comprised of over 50 species, it seems likely that additional survey work on species of *Himantura* previously unexamined for cestodes will result in the discovery of other, potentially many, new species of *Stillabothrium*.

Stillabothrium is a member of the globally distributed family Escherbothriidae. The two genera in the family, *Escherbothrium* and *Stillabothrium*, possess vertically oriented septa and loculi in the posterior region of the bothridia. The results of the analysis of Ruhnke et al. (2015) revealed a sister group relationship between the species referred to as *Escherbothrium* sp., collected from *Urotrygon aspidura* Jordan et Gilbert from the Pacific Ocean off Costa Rica and *Stillabothrium* species (referred to in their study as Rhinebothriinae New genus 3). However, the two genera have intriguingly different distributions. *Escherbothrium molinae* Berman et Brooks, 1994 and the species of *Escherbothrium* included by Ruhnke et al. (2015) are both from the Pacific coast of Costa Rica (i.e. the Western Hemisphere). In spite of extensive recent survey work in the Western Hemisphere in recent years, no species of *Stillabothrium* has been encountered there. Rather, the seven described species of *Stillabothrium*, as well as the multiple undescribed species, are all from the Eastern Hemisphere, specifically the Indo-Pacific and eastern Atlantic. Thus the family Escherbothriidae consists of a clade of species of *Escherbothrium* from the Western Hemisphere, and a clade of species of *Stillabothrium* from the Eastern Hemisphere.

Ruhnke et al. (2015) stated that a remaining problematic issue for the diagnosis of the family Escherbothriidae is whether only some, or all, species of the group possess an apical sucker. All evidence suggests that both known spe-

cies of *Escherbothrium* have an apical sucker (Berman and Brooks 1994, Ruhnke et al. 2015). This, however, does not appear to be the case for *Stillabothrium*. Instead, all evidence suggests that the anteriormost feature of the bothridia is a loculus, rather than a sucker. In general this structure is somewhat triangular, rather than round. We see no structural evidence to suggest that the anteriormost loculus differs from any of the other loculi in the anterior region of bothridium. For example, in cross section the musculature of the anteriormost transverse septum and the second transverse septum of *S. cadenati* have the same appearance (compare ATS in Fig. 13 with the transverse septum below it).

Phyllobothrium biacetabulatum Yamaguti, 1960, described from *Rhinobatos schlegelii* Müller et Henle from the Inland Sea of Japan by Yamaguti (1960), is a rhinebothriidean that potentially belongs within the genus *Stillabothrium*, but a lack of information on this species prevents its transfer to *Stillabothrium* at this time. In his monograph on the Phyllobothriidae, Ruhnke (2011) considered *Phyllobothrium biacetabulatum* as *incertae sedis* owing to its lack of conforming to the features of the family. In fact, this species has several features that are consistent with the diagnosis of *Stillabothrium*. The description and accompanying micrographs of Yamaguti (1960) clearly show that each of the bothridia of this species is stalked and divided into an anterior and a posterior region, with the anterior region bearing two transverse septa and the posterior region bearing "about 40 elongate loculi," (Yamaguti 1960, p. 42). Furthermore, its testes do not extend posterior to the cirrus sac, and the vas deferens enters the cirrus sac on its anterior margin close to the genital pore. Those features are sufficient to support formal placement of *P. biacetabulatum* within the Rhinebothriidea as we have done here. Unfortunately, the description is ambiguous with regard to whether or not a medial longitudinal septum is present in the posterior region of the bothridia. It is this feature that distinguishes *Stillabothrium* from *Escherbothrium* and from New genus 2 and New genus 4 of Healy et al. (2009). *Phyllobothrium biacetabulatum*, however, must remain *incertae sedis* with regard to its generic placement at this time, until further study and examine of type specimens.

Parallel to keeping pace with the growth in knowledge of cestode species diversity over the last two or so decades, is a growth in the knowledge of elasmobranch species diversity, with many new species being discovered and/or recognised in recent years (Naylor et al. 2012b, Last et al. 2016). This has implications for our study. We report seven species of *Stillabothrium* from a total of 11 elasmobranch species, and we note additional *Stillabothrium* specimens and additional potential species from six other elasmobranch species. Among these 17 species of elasmobranchs, two were considered by Naylor et al. (2012b) either to be unique undescribed species (and hence given numerical designations) or to be species that may be new to science but whose identifications require further study (and hence were given 'cf.' designations): *H.* cf. *pastinacoides*, and *H. uarnak* 3. Both of these species are from Borneo, a region considered to be a centre of elasmobranch endemism

and diversification (Naylor et al. 2012b, White and Last 2012). We have employed the Naylor et al. (2012b) designations here so that as these host species are formally described in the future, the elasmobranch collection information provided here can be linked to the Global Cestode Database (Caira et al. 2012) where the ray names would be updated.

We suggest that future studies of *Stillabothrium* continue to use a combination of molecular and morphological data for species delimitation and understanding of host specificity and that, whenever possible, those analyses include multiple specimens of each putative *Stillabothrium* species. This has also been done in species of other rhinebothriidean genera, such as *Rhinebothrium* (see Reyda and Marques 2011). Considering the importance of bothridial septa in defining species of *Stillabothrium* and given that septa can vary in their visibility (e.g. the fourth transverse septum of *S. cadenati* was not visible in all specimens; compare Figs. 12A and 12B), we also emphasise the importance of using DIC (differential interference contrast) microscopy in future studies of the genus. In addition, we caution against using bothridial shape in contracted specimens in delineating *Stillabothrium* species.

REFERENCES

Ball D., Neifar L., Euzet L. 2003: [Description of *Scalithrium* n. gen. (Cestoda, Tetraphyllidea), with *Scalithrium minimum* (Van Beneden, 1850) n. comb., a parasite of *Dasyatis pastinaca* (Elasmobranchii, Dasyatidae), as type species] Parasite 10: 31–37. (In French.)

Berman R., Brooks D.R. 1994: *Escherbothrium molinae* n. gen. et n. sp. (Eucestoda: Tetraphyllidea: Triloculariinae) in *Urotrygon chilensis* (Chondrichthyes: Myliobatiformes: Urolophidae) from the Gulf of Nicoya, Costa Rica. J. Parasitol. 80: 775–780.

Butler S.A. 1987: Taxonomy of some tetraphyllidean cestodes from elasmobranch fishes. Aust. J. Zool. 35: 343–371.

Caira J.N., Jensen K. 2001: An investigation of the co-evolutionary relationships between onchobothriid tapeworms and their elasmobranch hosts. Int. J. Parasitol. 31: 960–975.

Caira J.N., Jensen K. 2014: A digest of elasmobranch tapeworms. J. Parasitol. 100: 373–391.

Caira J.N., Jensen K., Barbeau E. 2012: Global Cestode Database. University of Connecticut, Storrs, CT, USA. World Wide Web electronic publication, http://www.tapewormdb.uconn.edu, 12/2015.

Caira J.N., Jensen K., Waeschenbach A., Olson P.D., Littlewood D.T.J. 2014: Orders out of chaos – molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships. Int. J. Parasitol. 44: 55–73.

Chervy L. 2009: Unified terminology for cestode microtriches: a proposal from the International Workshops on Cestode Systematics in 2002–2008. Folia Parasitol. 56: 199–230.

Cielocha J.J., Jensen K., Caira J.N. 2014: *Floriparicapitus*, a new genus of lecanicephalidean tapeworm (Cestoda) from sawfishes (Pristidae) and guitarfishes (Rhinobatidae) in the Indo-West Pacific. J. Parasitol. 100: 485–499.

Clopton R.E. 2004: Standard nomenclature and metrics of plane shapes for use in gregarine taxonomy. Comp. Parasitol. 71: 130–140.

Euzet L. 1954: Parasites de poisons de mer ouest africains récoltés par J. Cadenat. I. Cestodes tetraphylllides de sélaciens. (Note préliminaire). Bull. Ins. Fran. d'Afrique Noire. Série A 16: 126–138.

Euzet L., Combes C. 1980: Les problémes de l'espéce chez les animaus parasites. Mem. S. Zool. F. 3: 239–285.

Eyring K.L. Healy C.J., Reyda F.B. 2012: A new genus and species of cestode (Rhinebothriidea) from *Mobula kuhlii* (Rajiformes: Mobulidae) from Malaysian Borneo. J. Parasitol. 98: 584–591.

Healy C.J. 2006: A revision of selected Tetraphyllidea (Cestoda): *Caulobothrium*, *Rhabdotobothrium*, *Rhinebothrium*, *Scalithrium*, and *Spongiobothrium*. PhD Dissertation, University of Connecticut, Storrs, Connecticut, 382 pp.

Healy C.J., Caira J.N., Jensen K., Webster B.L., Littlewood, D.T.J. 2009: Proposal for a new tapeworm order, Rhinebothriidea. Int. J. Parasitol. 39: 497–511.

ICZN 1999: International Code of Zoological Nomenclature, Fourth Edition. The International Trust for Zoological Nomenclature, London, 306 pp.

Ivanov V.A., Caira J.N. 2012: Description of three new species of *Echinobothrium* (Cestoda: Diphyllidea) from Indo-Pacific elasmobranchs of the genus *Glaucostegus* (Rajiformes: Rhinobatidae). J. Parasitol. 98: 365–377.

Jensen K. 2005: Tapeworms of Elasmobranchs (Part I). A Monograph on the Lecanicephalidea (Platyhelminthes, Cestoda). Bull. Univ. Nebr. State Mus. 18 (i–viii), 241 pp.

Jensen K., Russell S.L. 2014: *Seussapex*, a new genus of lecanicephalidean tapeworm (Platyhelminthes: Cestoda) from the stingray genus *Himantura* (Myliobatiformes: Dasyatidae) in the Indo-West Pacific with investigation of mode of attachment. Folia Parasitol. 61: 231–241.

Jensen K., Mojica K.R., Caira J.N. 2014: A new genus and two new species of lecanicephalidean tapeworms from the striped panray, *Zanobatus schoenleinii* (Rhinopristiformes: Zanobatidae), off Senegal. Folia Parasitol. 61: 432–440.

Kuchta R., Scholz T., Brabec J., Bray R.A. 2008: Suppression of the tapeworm order Pseudophyllidea (Platyhelminthes: Eucestoda) and the proposal of two new orders, Bothriocephalidea and Diphyllobothriidea. Int. J. Parasitol. 38: 49–55.

Last P.R., Manjaji B. M., Yearsley G.K. 2005: *Pastinachus solocirostris* sp. nov., a new species of stingray (Elasmobranchii: Myliobatiformes) from the Indo-Malay Archipelago. Zootaxa 1040: 1–16.

Last P.R., Naylor G., Manjaji-Matsumoto B.M. 2016: A revised classification of the family Dasyatidae (Chondrichthyes: Myliobatiformes) based on new morphological insights. Zootaxa 4139: 345–368.

Marques F.P.L., Caira J.N. 2016: Pararhinebothroides – neither the sister taxon of Rhinebothroides nor a valid genus. J. Parasitol. 102: 249–259.

Mojica K.R., Jensen K., Caira J.N. 2013: Revision of *Anteropora* (Cestoda: Lecanicephalidea) and descriptions of five new species from stingrays (Myliobatiformes: Dasyatidae) in Borneo. Raffles Bull. Zool. 61: 491–506.

Naylor G., Caira J.N., Jensen K., Rosana K.A., Straube N., Lakner C. 2012a: Elasmobranch phylogeny: a mitochondrial estimate based on 595 species. In: J.C. Carrier, J.A. Musick and M.R. Heithaus (Eds.), Biology of Sharks and Their Relatives. CRC Press, Boca Raton, pp. 31–56.

Naylor G., Caira J.N., Jensen K., Rosana K.A., White W.T. 2012b: A DNA sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. Bull. Am. Mus. Nat. Hist. 367: 215–240.

Palm H.W., Caira J.N. 2008: Host specificity of adult versus larval cestodes of the elasmobranch tapeworm order Trypanorhyncha. Int. J. Parasitol. 38: 381–388.

Pleijel R., Jondelius U., Norlinder E., Nygeren A., Oxelman B., Schander C., Sundberg P., Thollesson M. 2008: Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. Mol. Phylogenet. Evol. 48: 369–371.

Reyda F.B., Caira J.N. 2006: Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from *Himantura uarnacoides* (Myliobatiformes: Dasyatidae) in Malaysian Borneo. Comp. Parasitol. 73: 49–71.

Reyda F.B., Marques F.P.L. 2011: Diversification and species boundaries of *Rhinebothrium* (Cestoda; Rhinebothriidea) in South American freshwater stingrays (Batoidea; Potamotrygonidae). PLoS ONE 6: e22604.

Ronquist F., Huelsenbeck J.P. 2003: MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19: 1572–1574.

Ruhnke T.R. 2011: Tapeworms of Elasmobranchs (Part III). A Monograph on the Phyllobothriidae (Platyhelminthes, Cestoda). Bull. Univ. Nebr. State Mus. 25 (i–xii), 208 pp.

Ruhnke T.R., Caira J.N., Cox A. 2015: The cestode order Rhinebothriidea no longer family-less: a molecular phylogenetic investigation with erection of two new families and description of eight new species of *Anthocephalum*. Zootaxa 3904: 051–081.

Schaeffner B.C., Beveridge I. 2012: *Cavearhynchus*, a new genus of tapeworm (Cestoda: Trypanorhyncha: Pterobothriidae)

from *Himantura lobistoma* Manjaji-Matsumoto & Last, 2006 (Rajiformes) off Borneo, and new records of species of *Pterobothrium* Diesing, 1850. Syst. Parasitol. 82: 147–166.

Schaeffner B.C., Beveridge I. 2014: The trypanorhynch cestode fauna of Borneo. Zootaxa 3900: 021–049.

Swofford D.L. 2000: PAUP*: Phylogenetic Analysis Using Parsimony, Version 4.0b, Sinauer Assoc., Sunderland, Massachusetts.

Twohig M.E., Caira J.N., Fyler C.A. 2008: Two new cestode species from the dwarf whipray, *Himantura walga* (Batoidea: Dasyatidae), from Borneo, with comments on site and mode of attachment. J. Parasitol. 94: 1118–1127.

Tyler G.A. 2006: Tapeworms of Elasmobranchs (Part II). A Monograph on the Diphyllidea (Platyhelminthes, Cestoda). Bull. Univ. Nebr. State Mus. 20 (i–xiii), 142 pp.

White W.T., Last P.R. 2012. A review of the taxonomy of chondrichthyan fishes: a modern perspective. J. Fish Biol. 80: 901–917.

Williams H. 1964: Some new and little known cestodes from Australian elasmobranches with a brief discussion on their possible use in problems of host taxonomy. Parasitology 54: 737–748.

Yamaguti S. 1960: Studies on the helminth fauna of Japan. Part 56. Cestodes of fishes, III. Publ. Seto Marine Biol. Lab. 8: 41–50.

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