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NOVELTY AND PHYLOGENETIC RELATIONSHIPS WITHIN THE SERENDIPEIDAE (CESTODA: "TETRAPHYLLIDEA")

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KEY WORDS

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ABSTRACT

Nanoduplicibothrium n. gen. is erected for the subgroup containing the smallest members of the "tetraphyllidean" family Serendipeidae with bothridia fused lengthwise in 2 pairs that lack both a distinct row of posterior loculi and a cephalic peduncle. Two new species in this genus are described. These are *Nanoduplicibothrium leanneae* n. gen. n. sp. from *Rhinoptera bonasus* off South Carolina and *Nanoduplicibothrium megaphallum* n. sp. from *Rhinoptera jayakari* off Mozambique. Two species currently assigned to *Duplicibothrium* are transferred to the new genus as *Nanoduplicibothrium paulum* n. comb and *Nanoduplicibothrium jillae* n. comb. and the diagnosis of *Duplicibothrium* is emended so that it aligns with the revised membership of the group. *Duplicibothrium bilai* n. sp. is also described from *R. jayakari* off Mozambique. The description of these species provides formal names for 3 species included in previously published molecular phylogenetic work under the provisional names *Duplicibothrium* n. sp. 2, *Duplicibothrium* n. sp. 4, and *Duplicibothrium* n. sp. 5, respectively. Erection of the new genus substantially reduces the number of instances of congeners in the family parasitizing the same host species because in most instances the pairs of species now represent 1 species each in *Nanoduplicibothrium* and *Duplicibothrium*. Sequence data for the D1–D3 region of the *28S* rDNA gene were generated for *Serendip* for the first time from an undescribed species from *Aetomylaeus asperrimus* collected off Panama. This finding also expands the known host associations of the Serendipeidae beyond the Rhinopteridae to include a species of Myliobatidae. A maximum-likelihood phylogenetic analysis of all species of serendipeids for which data for the D1–D3 region of the *28S* rDNA gene are available confirms the reciprocal monophyly of *Nanoduplicibothrium*, *Duplicibothrium*, and *Serendip*. The phylogenetic placement of the fourth genus in the family—the monotypic *Glyphobothrium*—remains to be determined.

The cestode family Serendipeidae Brooks and Evenhuis, 1995 remains 1 of the more intriguing clades of "Tetraphyllidea." All 9 described species are known only from cownose rays in the genus *Rhinoptera* Cuvier. The group is morphologically cohesive. Similarities in proglottid anatomy among members of its 3 genera (i.e., *Duplicibothrium* Williams and Campbell, 1978; *Glyphobothrium* Williams and Campbell, 1977, and *Serendip* Brooks and Barriga, 1995) are particularly striking. Recent work by Stephan and Caira (2022) revealed the existence of a considerable amount of undescribed novelty in species of cownose rays not previously examined for this family of cestodes. These authors also observed a curious scolex morphology in 1 of their new species of *Duplicibothrium* that raises questions about the reciprocal monophyly of the latter genus and *Serendip*.

The current study builds on the work of Stephan and Caira (2022) to further our understanding of the diversity and phylogenetic relationships of the Serendipeidae. It has 3 main goals. The first is to formally describe 3 of the 6 species, previously known solely by provisional names, for which sufficient material is now available for further study (i.e., *Duplicibothrium* n. sp. 2 of Jensen and Bullard [2010], and *Duplicibothrium* n. sp. 4 and *Duplicibothrium* n. sp. 5 of Stephan and Caira [2022]). The second is to revise the generic classification of the family to align with the morphological features and phylogenetic relationships of its members. The result was the erection of a new genus to house a morphologically cohesive and molecularly divergent subset of the species formally assigned to *Duplicibothrium*. The third is to explore the question about the reciprocal monophyly of *Serendip* and *Duplicibothrium* raised by

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Stephan and Caira (2022) based on a phylogenetic analysis that includes sequence data generated for the D1–D3 region of the *28S* rDNA gene for a member of *Serendip* for the first time.

MATERIALS AND METHODS

Collection of specimens

Each host specimen was assigned a unique collection code and number, and photographs and measurements were taken. More detailed information for these specimens can be accessed in the Global Cestode Database (Caira et al., 2022) by unique collection code and number (e.g., CH-40). Cestodes examined in this study were obtained from 2 specimens of *Rhinoptera bonasus* (CH-40, CH-42) collected off Charleston, South Carolina, in June 2015, and 3 specimens of *Rhinoptera jayakari* (MZ-1, MZ-3, MZ-4) collected off Tofo, Inhambane, Mozambique in June 2016. In all instances, the body cavity was opened with a midventral incision, and a small sample of liver tissue was taken and preserved in 95% ethanol for molecular verification of host identity. The spiral intestine was then removed, opened with a longitudinal incision, rinsed in seawater, and examined for cestodes. A subset of the cestodes found was fixed in 95% ethanol for molecular sequencing; the remaining specimens were fixed in 10% seawater-buffered formalin (9:1) for examination with light and scanning electron microscopy (SEM). The spiral intestine of each ray was then fixed in either 95% ethanol or seawater-buffered formalin. After approximately 1 wk, cestodes and spiral intestines fixed in seawater-buffered formalin were transferred to 70% ethanol for storage. Cestodes and spiral intestines fixed in 95% ethanol were transferred to new 95% ethanol and stored in a –20 C freezer. Spiral intestines were examined under an Olympus SZ-30 dissecting microscope (Olympus, Center Valley, Pennsylvania), and any additional cestodes were removed and transferred to either 70% or 95% ethanol.

Morphological methods

Cestodes prepared for light microscopy were hydrated in a graded ethanol series, stained for 20–60 min in a working solution of Delafield's hematoxylin (1:9 mixture of hematoxylin: distilled water), differentiated in tap water, destained in acidic 70% ethanol, neutralized in basic 70% ethanol, dehydrated in a graded ethanol series, cleared in methyl salicylate, mounted in Canada balsam on glass slides under glass coverslips, and left to dry in an oven set to 55 C for 1 wk. Measurements were taken with a Zeiss Axioskop 2 Plus compound microscope (Zeiss, Thornwood, New York) using a SPOT Diagnostic Instrument Digital Camera System and SPOT software (version 4.6; SPOT Imaging Solutions, Sterling Heights, Michigan). Measurements are given in the text as the range (in micrometers unless stated otherwise). In instances in which measurements were taken from 5 or more specimens, the range is followed in parentheses by the mean, standard deviation, number of specimens measured, and total number of measurements in instances in which more than 1 measurement was made per worm. In all other cases, the range is followed in parentheses by the number of specimens measured. Shape terminology follows Clopton (2004).

Scoleces prepared for SEM were hydrated in a graded ethanol series, transferred to a 1% solution of osmium tetroxide overnight, dehydrated in a graded ethanol series, placed in hexamethyldisilazane in a fume hood for 30 min, and then allowed to air dry. The specimens were then mounted on double-sided PELCO carbon tabs (Ted Pella Inc., Redding, California) on aluminum stubs, sputter-coated with 45 nm of gold/palladium, and examined with an FEI Nova NanoSEM 450 field emission SEM (FEI, Hillsboro, Oregon) at the Bioscience Electron Microscopy Laboratory, University of Connecticut (Storrs, Connecticut). Microthrix terminology follows Chervy (2009).

Museum abbreviations used are as follows: LRP, Lawrence R. Penner Parasitology Collection, University of Connecticut, Storrs, Connecticut; NMB-P, National Museum, Bloemfontein, South Africa, Parasite Collection; USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Molecular methods and phylogenetic analysis

Sequence data were generated for the D1–D3 region of the *28S* rDNA gene for 2 adult specimens of an undescribed species of *Serendip* (henceforth referred to as *Serendip* n. sp. 1) from *Aetomylaeus asperrimus* (Gilbert) collected off Mariato, Veraguas, Panama. The middle portion of each cestode specimen was removed and allowed to air dry for approximately 5 min at room temperature before extraction. The scoleces were prepared as whole mounts (as described above) to serve as vouchers for the specimens sequenced. These hologenophores (sensu Pleijel et al., 2008) were deposited in the Lawrence R. Penner Parasitology Collection (LRP 9827 and LRP 9828). Extraction, amplification, and Sanger sequencing of DNA followed Bueno and Caira (2017). The primer pairs used for amplification were LSU-5 (5′-TAGGTCGACCCGCTGAAYTTA-3′; Littlewood et al., 2000) and LSU-1500R (5′-GCTATCCTGGAGGGAAACTTCG-3′; Tkach et al., 2003). The primer pairs used for sequencing were LSU-55F (5′-AACCAGGATTCCCCTAGTAACGGC-3′) (Bueno and Caira, 2017) and LSU-1200R (5′-GCATAGTTCACCATCTTTCGG-3′; Littlewood et al., 2000).

The 2 newly generated sequences of the undescribed species of *Serendip* were combined with sequence data for the D1–D3 region of the *28S* rDNA gene from GenBank for all 43 specimens of the Serendipeidae comprising the ingroup and both species of *Caulobothrium* used as members of the outgroup in the phylogenetic analysis of Stephan and Caira (2022). GenBank and LRP voucher numbers for all specimens are provided on the phylogenetic tree following the host, life-cycle stage, and unique host specimen number. When possible, a unique cestode specimen code is also provided.

Sequences were aligned and trimmed using the MAFFT (Katoh and Standley, 2013) multiple alignment plug-in in Geneious Prime 2022.0.1® (www.geneious.com). The aligned data matrix was 1,235 base pairs (bp) in length. A maximum-likelihood phylogenetic analysis was performed on the cluster in the Bioinformatics facility of the Institute of Systems Genomics at the University of Connecticut using IQ-TREE 1.6.10 (Nguyen et al., 2015). GTR+I+G with empirical base frequencies was selected as the best-ranked model of molecular evolution according to the corrected Akaike information criterion (AICc) as implemented in ModelFinder (Kalyaanamoorthy et al., 2017) in IQ-TREE and followed by tree reconstruction and 200 nonparametric bootstrap replicates with the command *iqtree -s datamatrix.nex -m MFP -merit AICc -b 200*.

DESCRIPTIONS

Nanoduplicibothrium n. gen.

Serendipeidae: Worms protandrous, euapolytic, tiny; proglottids weakly craspedote. Scolex with 4 bothridia, each with apical sucker; dorsal and ventral bothridia fused lengthwise in 2 pairs. Bothridial surfaces with continuous band of loculi extending throughout bothridial margins and single central column of loculi stopping short of locular band; distinct row of posterior loculi and small, circular loculi flanking apical sucker lacking. Cephalic peduncle absent. Surfaces of bothridia with papilliform filitriches; spinitriches lacking. Testes extending into postovarian field, in 2 irregular dorso-ventral fields. Vas deferens not observed. Genital pores in anterior third of proglottid, submarginal, irregularly alternating. Vagina opening into genital atrium anterior to cirrus sac. Ovary digitiform, radiating from central isthmus. Uterus ventral, sacciform, extending to cirrus sac. Vitellarium follicular; dorsal vitelline follicles in single extensive field, interrupted or not by cirrus sac and ovary; ventral vitelline follicles lateral. Parasites of cownose stingrays (Rhinopteridae); cosmopolitan in distribution.

Type species: Nanoduplicibothrium leanneae n. gen. n. sp.

Additional species: Nanoduplicibothrium jillae (Stephan and Caira, 2022) n. comb. (note that USNM number 1009862 for 1 of the paratypes is incorrect, the correct number is USNM 1660862), *Nanoduplicibothrium megaphallum* n. sp., *Nanoduplicibothrium paulum* (Ruhnke, Curran, and Holbert, 2000) n. comb.

Taxon with provisional name: Nanoduplicibothrium n. sp. 3 (formerly *Duplicibothrium* n. sp. 3 of Stephan and Caira, 2022).

ZooBank registration: urn:lsid:zoobank.org:act:38D8932A-0711-4026-B64E-EC8695CC7CD3

Etymology: The name of this genus reflects the small (*nano*; L.) size of its members relative to species of *Duplicibothrium*.

Remarks

Nanoduplicibothrium differs from *Duplicibothrium* in that, as the name suggests, members of this genus are relatively tiny worms; none of the 4 species exceed 3 mm in total length, whereas all 5 species of *Duplicibothrium* reach a total length of 4.6–29.2 mm. Furthermore, its species lack, rather than possess, a distinct row of posterior loculi on each of their bothridia and a cephalic peduncle. We also believe the interpretation of the bothridia of this species as possessing 5 posterior loculi is incorrect; these loculi are more appropriately assigned to the continuous band of loculi extending throughout the bothridial margins. *Nanoduplicibothrium* n. gen. conspicuously differs from *Serendip* in that the bothridia of its members each bear transverse and longitudinal septa, rather than radially diverging septa, marginal loculi, and a marginal velum. The new genus conspicuously differs from the monotypic *Glyphobothrium* in that its scolex consists of dorsal and ventral bothridia fused lengthwise in 2 pairs with essentially no scolex proper, rather than being globular with 4 bothridia fused to the outer surface of a sizeable scolex proper as illustrated by the longitudinal section of a scolex presented by Caira et al. (1999: fig. 59). Our attention was first drawn to the potential novelty of this genus when we mapped morphology onto the phylogenetic tree in Stephan and Caira (2022: fig. 1) and noticed that the distinguishing features outlined above were consistent with the 2 major subclades that emerged in the tree resulting from their phylogenetic analysis.

Nanoduplicibothrium leanneae n. gen. n. sp.

(Figs. 1, 2A–E)

Description (based on 6 mature worms, and 2 scoleces examined with SEM): Worms weakly craspedote, euapolytic, 630–884 (741 ± 97; 5) long; maximum width at level of scolex. Proglottids 3–4 (3 ± 1; 5) in total number. Scolex consisting of 4 bothridia arranged in 2 dorso-ventral fused pairs (Figs. 1A, 2A). Cephalic peduncle absent. Bothridia each with oval apical sucker, 198–217 (n = 3) long, 76–100 (n = 3) wide, free anteriorly, sessile posteriorly, with at least 84 loculi arranged as continuous band extending throughout bothridial margins and single central column stopping short of locular band. Distal (Fig. 2B) and proximal (Fig. 2C) bothridial surfaces covered with papilliform filitriches; strobila immediately behind scolex (Fig. 2E) and more posteriorly (Fig. 2D) covered with densely arranged capilliform filitriches.

Immature proglottids wider than long, becoming longer than wide with maturity (Fig. 1C). Mature proglottids 1 (n = 5) in number; terminal mature proglottid (Fig. 1B) 211–377 (291 ± 59; 5) long, 121–186 (161 ± 27; 5) wide, length:width ratio 1.58– 2.05 (1.8 ± 0.18; 5):1. Testes 16–21 (n = 3) in number, arranged in 2 irregular columns extending throughout proglottid length, 2 irregular rows deep, oblong, 21–40 (n = 4) long, 29–45 (38 ± 4; 4, 15) wide. Vas deferens not observed. Cirrus sac ovoid (Fig. 1D), 50–106 (n = 2) long, 33–45 (n = 4) wide; containing coiled cirrus. Cirrus weakly developed in terminal proglottids, armed with spinitriches. Genital pores slightly submarginal, 67–76% (n = 4) of proglottid length from posterior end of proglottid, irregularly alternating. Vagina extending from ovarian bridge along midline of proglottid to anterior margin of cirrus sac then along anterior margin to open into genital atrium anterior to cirrus. Ovary terminal in position, highly digitiform, 64–99 (n = 4) long, 88–112 (n = 4) wide. Vitellarium follicular; dorsal vitelline follicles arranged in single extensive field, interrupted by terminal genitalia and ovary; ventral vitelline follicles arranged in 2 lateral bands interrupted by terminal genitalia. Uterus median, ventral, sacciform, extending from ovarian bridge to level of cirrus sac. Excretory ducts 4 in number, arranged in 1 dorsal and 1 ventral pair. Gravid proglottids not observed.

Taxonomic summary

Type and only known host: American cownose ray, *R. bonasus* Mitchill (Myliobatiformes; Rhinopteridae).

Type locality: Off Charleston (32°42′18.08″N, 79°53′18.77″W) South Carolina, Atlantic Ocean.

Additional locality: Gulf of Mexico (30°14′16.90″N, 89°13′35.13″W), Mississippi (based on larval stage data).

Site of infection: Spiral intestine.

Specimens deposited: Holotype (USNM 1678883) and 2 paratypes (USNM 1678884, 1678885); 3 paratypes (LRP 11015, 11016, 11019) and SEM vouchers (LRP 11017, 11018); scoleces prepared for SEM retained with JNC at the University of Connecticut.

ZooBank registration: urn:lsid:zoobank.org:act:B842F328-A15E-42C3-8929-44D497C070E9.

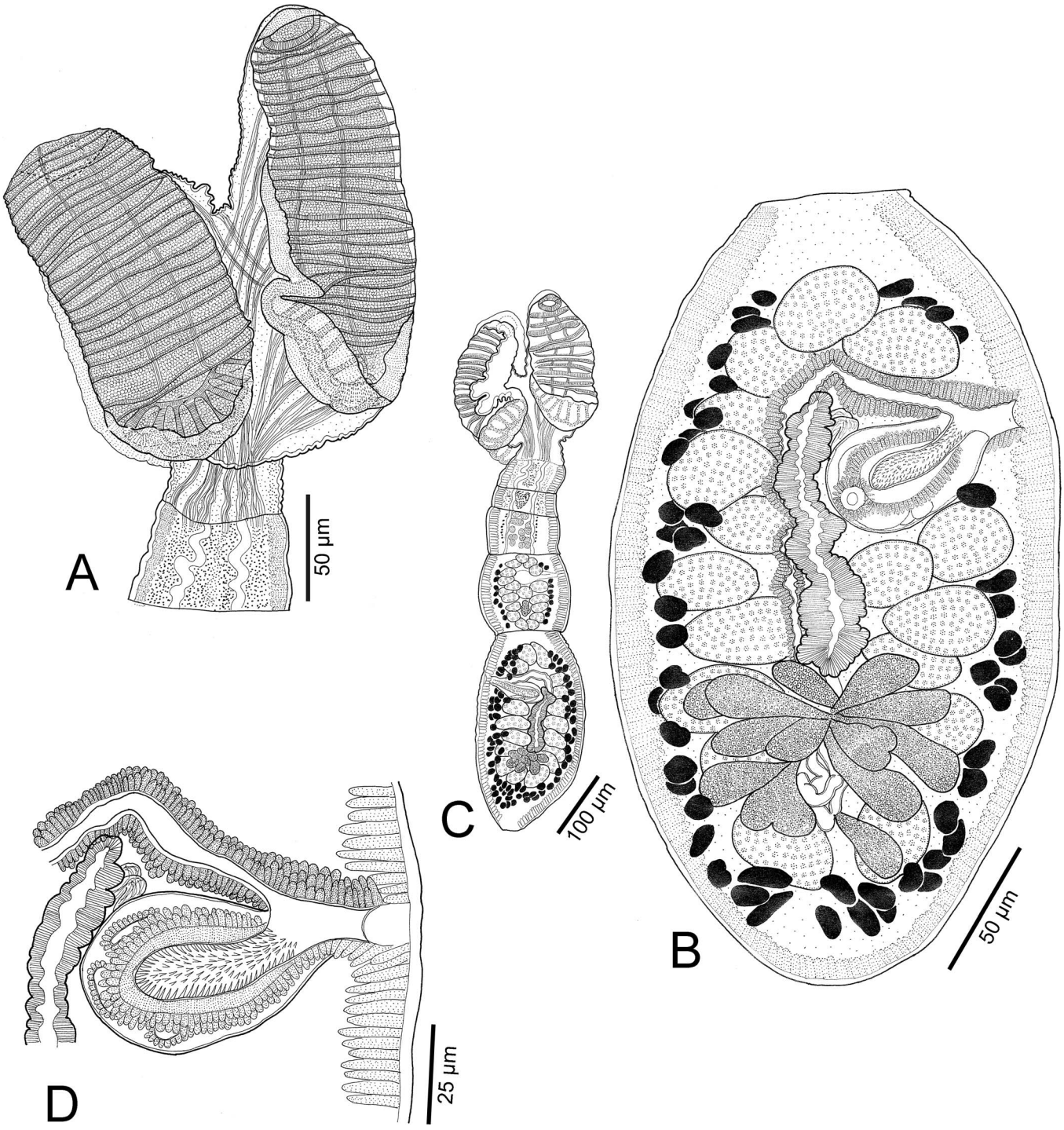

Figure 1. Line drawings of *Nanoduplicibothrium leanneae* n. gen. n. sp. (**A**) Scolex (paratype, Lawrence R. Penner Parasitology Collection, 11019). (**B**) Terminal proglottid (holotype, National Museum of Natural History, Smithsonian Institution [USNM] 167883), ventral view. (**C**) Whole worm (paratype, USNM 1678885), ventral view. (**D**) Detail of terminal genitalia (holotype, USNM 1678883).

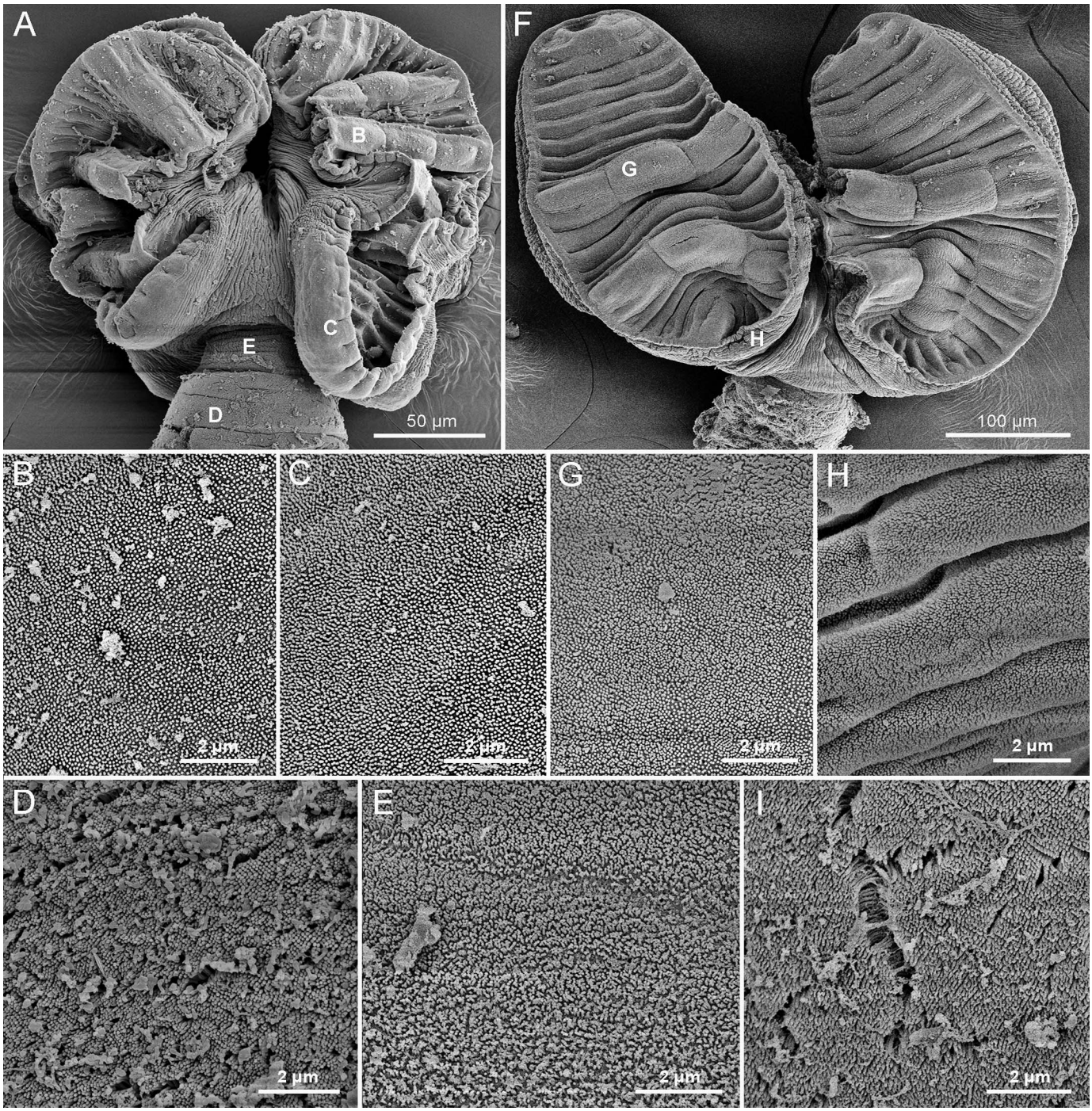

Figure 2. Scanning electron micrographs of *Nanoduplicibothrium leanneae* n. gen. n sp. (**A–E**) and *Nanoduplicibothrium megaphallum* n. sp. (**F–I**). (**A**) Scolex of *N. leanneae* n. gen. n. sp.; small letters indicate locations of details in micrographs **B**–**E**. (**B**) Papilliform filitriches on distal surface of bothridium of *N. leanneae* n. gen. n. sp. (**C**) Papilliform filitriches on proximal surface of bothridium of *N. leanneae* n. gen. n. sp. (**D**) Densely arranged capilliform filitriches on more posterior region of strobila of *N. leanneae* n. gen. n. sp. (**E**) Densely arranged capilliform filitriches on more anterior region of strobila of *N. leanneae* n. gen. n. sp. (**F**) Scolex of *N. megaphallum* n. sp.; small letters indicate locations of details in micrographs **G** and **H**. (**G**) Papilliform filitriches on distal surface of bothridium of *N. megaphallum* n. sp. (**H**) Papilliform filitriches on proximal surface of bothridium of *N. megaphallum* n. sp. (**I**) Densely arranged capilliform filitriches on strobila of *N. megaphallum* n. sp.

Etymology: The name of this tiny worm honors Leanne Kennedy Harty in recognition of her enthusiastic assistance with multiple aspects of laboratory support over the past several years.

Remarks

Nanoduplicibothrium leanneae n. gen. n. sp. differs from both *N. paulum* and *N. jillae* in terms of the number of total loculi per bothridia (84 vs. 57–63 and 59, respectively). This new species further differs from *N. paulum* in that it is a smaller worm (630–884 vs. 700–2,900) and it possesses a shorter terminal proglottid (211–295 vs. 350–950). *Nanoduplicibothrium leanneae* n. gen. n. sp. further differs from *N. jillae* in its possession of a narrower terminal proglottid (81–87 vs. 112–126). The description of *N. leanneae* n. gen. n. sp. provides a formal name for the species provisionally referred to as *Duplicibothrium* n. sp. 2 by Jensen and Bullard (2010) and Stephan and Caira (2022). A large number of loculi and the tendency for the bothridia to fold makes the exact number of loculi in *N. leanneae* n. gen. n. sp. difficult to determine.

Nanoduplicibothrium megaphallum n. sp.

(Figs. 2F–I, 3)

Description (based on 9 mature worms, and 2 scoleces examined with SEM): Worms weakly craspedote, euapolytic, 1,880–3,000 (2,350 ± 390; 9) long; maximum width at level of scolex. Proglottids 5–7 (6 ± 1; 9) in total number. Scolex consisting of 4 bothridia arranged in 2 dorso-ventral fused pairs (Figs. 2F, 3A). Cephalic peduncle absent. Bothridia each with oval apical sucker, 411 (n = 1) long, 182 (n = 1) wide, free anteriorly, sessile posteriorly, with at least 66 loculi; loculi arranged as continuous band extending throughout bothridial margins and single central column stopping short of locular band (Fig. 3B). Distal (Fig. 2G) and proximal (Fig. 2H) bothridial surfaces covered with papilliform filitriches; strobila covered with densely arranged capilliform filitriches (Fig. 2I).

Immature proglottids wider than long, becoming longer than wide with maturity (Fig. 3E). Mature proglottids (Fig. 3C) 1 (n = 9) in number; terminal mature proglottid (Fig. 3D) 763–1,088 (960 ± 115; 9) long, 275–375 (319 ± 43; 9) wide, length:width ratio 2.67– 3.61 (3.12 ± 0.31; 9):1. Testes 24–28 (n = 4) in number, arranged in 2 irregular columns extending throughout proglottid length, 2 irregular rows deep, oblong, 22–40 (n = 4) long, 25–37 (n = 4) wide. Vas deferens not observed. Cirrus sac enormous, ovoid, conspicuously tilted posteriorly, 277–419 (325 ± 44; 8) long, 119–174 (143 ± 18; 8) wide; containing coiled cirrus. Cirrus armed with spinitriches. Genital pores slightly submarginal, 67–76% (72 ± 3; 9) of proglottid length from posterior end of proglottid, irregularly alternating. Vagina extending from ovarian bridge along midline of proglottid to anterior margin of cirrus sac then along anterior margin to open into genital atrium anterior to cirrus. Ovary terminal in position, highly digitiform, 201–308 (253 ± 38; 8) long, 170–229 (197 ± 22; 8) wide. Vitellarium follicular; dorsal vitelline follicles arranged in single extensive field, interrupted by cirrus sac, distal portion of vagina and ovary; ventral vitelline follicles arranged in 2 lateral bands interrupted by terminal genitalia. Uterus median, ventral, sacciform, extending from ovarian bridge to level of cirrus sac. Excretory ducts 4 in number, arranged in 1 dorsal and 1 ventral pair. Gravid proglottids not observed.

Taxonomic summary

Type and only known host: Oman cownose ray, *R. jayakari* Boulenger (Myliobatiformes; Rhinopteridae).

Type locality: Off Tofo, (23°47′33.02″S, 35°31′16.38"E), Mozambique, Indian Ocean.

Additional locality: None.

Site of infection: Spiral intestine.

Specimens deposited: Holotype (NMB-P 925) and 2 paratypes (NMB-P 926, NMB-P 927); 3 paratypes (LRP 11022–11024) and 2 SEM vouchers (LRP 11020, 11021); 3 paratypes (USNM 1678886–1678888); scoleces prepared for SEM retained with JNC at the University of Connecticut.

ZooBank registration: urn:lsid:zoobank.org:act:C83F6419-1749-4AB8-A89E-FEFC7DB79708.

Etymology: This species is named for the conspicuously large size (*mega*; L.) of its cirrus (*phallum*; L.) compared to its congeners.

Remarks

Nanoduplicibothrium megaphallum n. sp. differs from *N. paulum*, *N. jillae*, and *N. leanneae* in its possession of a cirrus sac that is conspicuously longer (277–419 vs. 64–144, 33–36, and 50–68, respectively) and wider (119–174 vs. 36–110, 28–37, and 39–40, respectively) as well as in its total number of loculi per bothridium (66 vs. 57–63, 59, and 84, respectively). This new species further differs from *N. jillae* and *N. leanneae* in that it is a larger worm (1,880–3,000 vs. 700–1,000 and 630–884, respectively) and has a greater number of proglottids (5–7 vs. 2–4 and 3–4, respectively). The description of this species provides a formal name for the species provisionally referred to as *Duplicibothrium* n. sp. 4 by Stephan and Caira (2022).

Duplicibothrium Williams and Campbell, 1978 emended

Serendipeidae: Worms protandrous, euapolytic, moderate in size; proglottids weakly craspedote. Scolex with 4 bothridia, each with apical sucker; dorsal and ventral bothridia fused lengthwise in 2 pairs. Bothridial surfaces divided into loculi by transverse septa, longitudinal septa, or a combination of both types of septa; **distinct posterior row of 5 or 7 loculi** present, occasionally with 4 small loculi flanking apical sucker on each side. **Cephalic peduncle present, scutellate**. Surfaces of **bothridia** with papilliform filitriches; spinitriches lacking. Testes extending into postovarian field, in 2 irregular dorso-ventral fields. Genital pores in anterior third of proglottid, submarginal, irregularly alternating. Vagina opening into genital atrium anterior to cirrus sac. Ovary digitiform, radiating from central isthmus. Uterus ventral, sacciform, extending to cirrus sac. Vitellarium follicular; dorsal vitelline follicles in single extensive field, interrupted or not by cirrus sac and ovary; ventral vitelline follicles arranged in 2 lateral bands, encroaching on midline of proglottid or not. Parasites of cownose stingrays (Rhinopteridae); cosmopolitan in distribution.

Type species: Duplicibothrium minutum Williams and Campbell, 1978

Additional species: Duplicibothrium bilai n. sp., *Duplicibothrium cairae* Ruhnke, Curran, and Holbert, 2000; *Duplicibothrium colossum* Stephan and Caira, 2022 and *Duplicibothrium jeannettae,* Stephan and Caira, 2022.

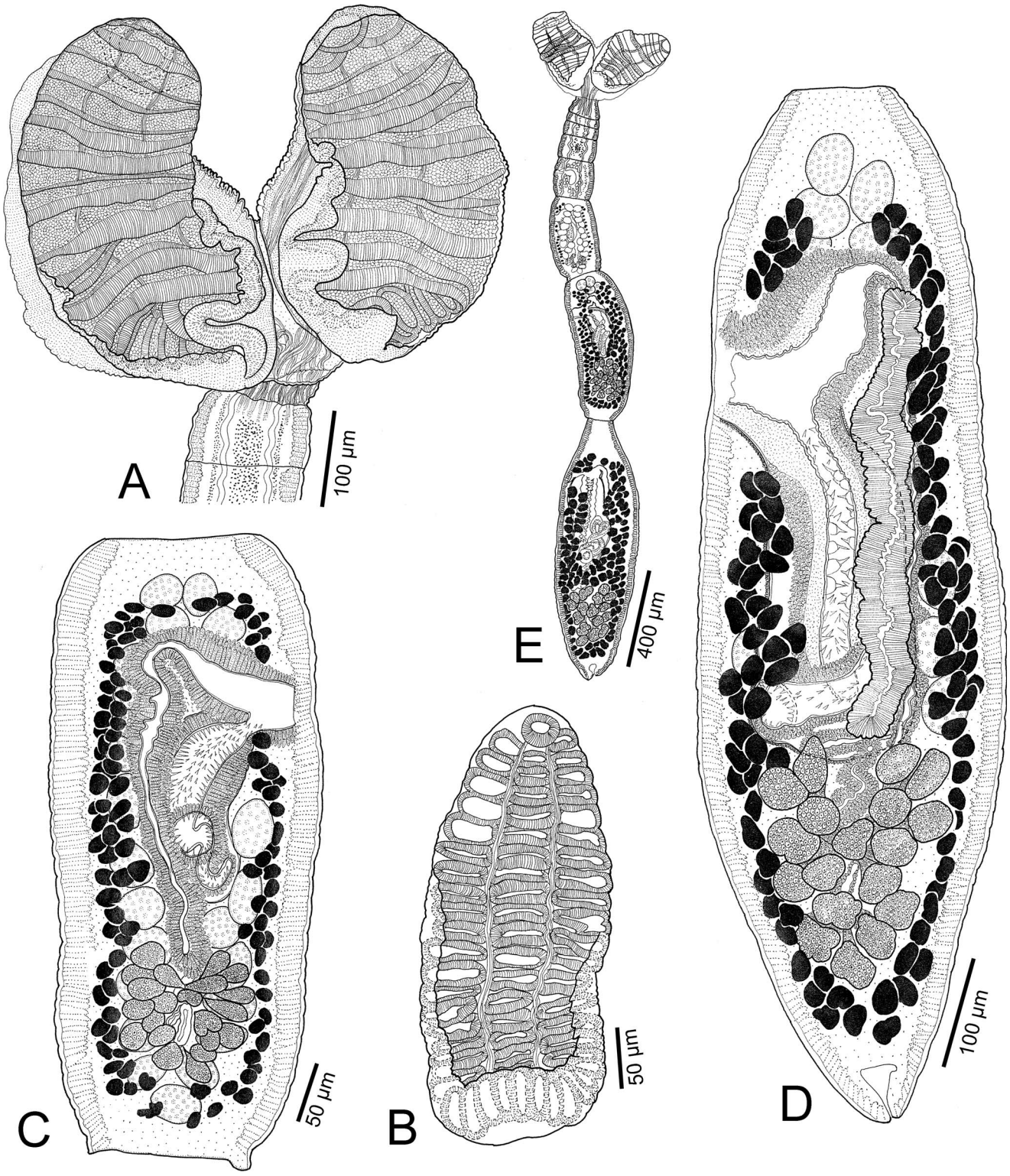

Figure 3. Line drawings of *Nanoduplicibothrium megaphallum* n. gen. n. sp. (**A**) Scolex (holotype, National Museum, Bloemfontein, South Africa, Parasite Collection 925). (**B**) Detail of bothridium (paratype, National Museum of Natural History, Smithsonian Institution [USNM] 1678886). (**C**) Subterminal proglottid (paratype, Lawrence R. Penner Parasitology Collection [LRP] 11022), ventral view. (**D**) Terminal proglottid (paratype, LRP 11022), ventral view. (**E**) Whole worm (paratype, USNM 1678888), dorsal view.

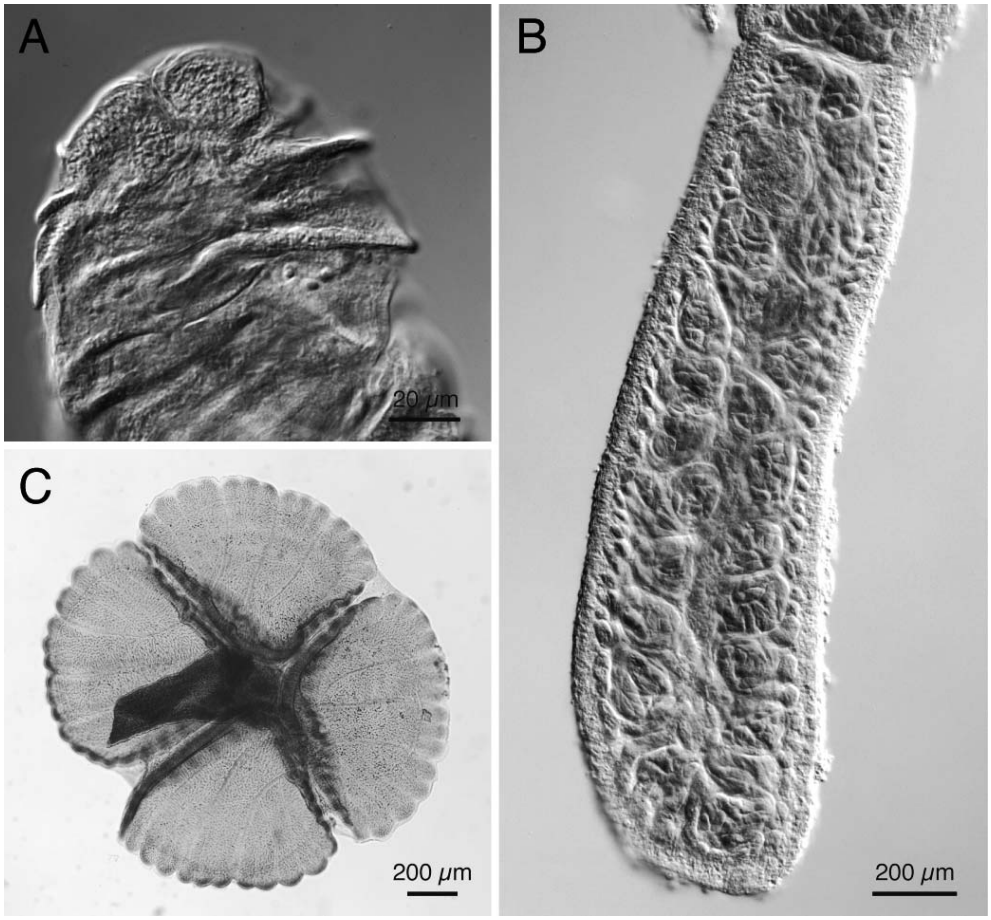

Figure 4. Light micrographs of *Duplicibothrium minutum* and *Serendip* n. sp. 1. (**A**) Apical sucker on bothridium of paratype of *D. minutum* (Lawrence R. Penner Parasitology Collection [LRP] 3610). (**B**) Terminal proglottid of holotype of *D. minutum* (National Museum of Natural History, Smithsonian Institution 1370283). (**C**) Scolex of hologenophore (LRP 9827) of *Serendip* n. sp. 1 from *Aetomylaeus asperrimus* collected off the Pacific coast of Panama. Color version available online.

Taxa with provisional names: Duplicibothrium n. sp. 1 (of Jensen and Bullard, 2010) and *Duplicibothrium* n. sp. 6 (of Stephan and Caira, 2022).

Remarks

The most recent diagnosis of *Duplicibothrium* (see Stephan and Caira, 2022) is emended to reflect the transfer of the species originally assigned to this genus that lack a distinct row of posterior loculi and a cephalic peduncle to *Nanoduplicibothrium* n. gen. This emendation also reflects 3 modifications in the interpretation of the morphology of *Duplicibothrium minutum* made following examination of paratype specimens deposited in LRP (LRP 3551, 3610–3612) and images of the holotype (USNM 1370283). First, the bothridia of *D. minutum*, like those of its congeners, bear, rather than lack, an apical sucker on their anterior margin (Fig. 4A). In addition, the vitelline follicles in the proglottid of the holotype are much larger than illustrated by Williams and Campbell (1978: fig. 3) and, rather than being circum-medullary, do not extend into the ventral portions of the proglottid (Fig. 4B). Major modifications of the diagnosis are indicated in bold. The new species of *Duplicibothrium* described below is distinguished only from the 4 described species now considered to belong to that genus.

***Duplicibothrium bilai* n. sp.**

(Figs. 5, 6)

Description (based on 8 mature and 1 immature worms, and 1 scolex examined with SEM): Worms weakly craspedote, euapolytic, 4,033–5,940 (4,730 ± 680; 8) long; maximum width at level of scolex. Proglottids 11–18 (14 ± 3; 7) in total number. Scolex consisting of 4 bothridia arranged in 2 dorso-ventral fused pairs (Figs. 5A, 6A) and elongate cephalic peduncle (Fig. 5C). Bothridia each with apical sucker, pyriform, 287–384 (327 ± 32; 7, 13) long, 174–260 (206 ± 26; 8, 14) wide, free anteriorly, sessile posteriorly, with 37 loculi; loculi arranged as single apical loculus followed by 2 lateral columns of 10 loculi, 1 medial column of 9 loculi, and 1 row of 7 posteriormost elongate loculi. Cephalic peduncle 1,407–2,127 (1,732 ± 228; 8) long, 118–148 (130 ± 10; 8) wide. Distal (Fig. 6B) and proximal (Fig. 6C) bothridial surfaces covered with papilliform filitriches; cephalic peduncle scutellate; scutes consisting of densely arranged capilliform filitriches (Fig. 6D).

Immature proglottids wider than long, becoming longer than wide with maturity (Fig. 5C). Mature proglottids 1–2 (1 ± 0.4; 7) in number; terminal mature proglottid (Fig. 5B) 730–1,138 (848 ± 151; 6) long, 373–490 (429 ± 42; 6) wide, length:width ratio 1.7–2.8 (2 ± 0.4; 6):1. Testes 38–51 (45 ± 5; 6) in number, arranged in 2 irregular columns extending throughout proglottid length,

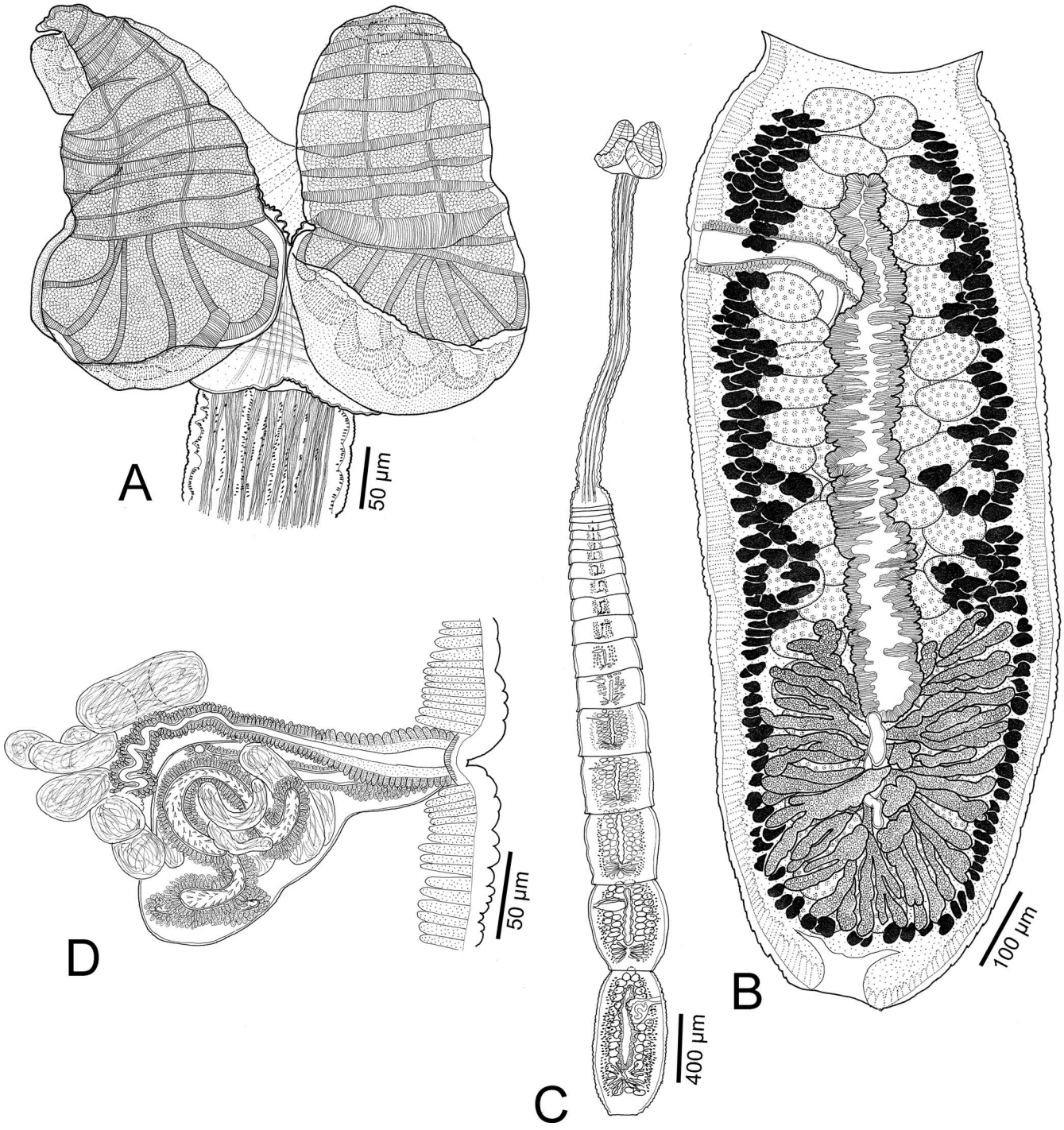

Figure 5. Line drawings of *Duplicibothrium bilai* n. sp. (**A**) Scolex (holotype, National Museum, Bloemfontein, South Africa, Parasite Collection 922). (**B**) Terminal proglottid (paratype, Lawrence R. Penner Parasitology Collection 11013), ventral view. (**C**) Whole worm (paratype, National Museum of Natural History, Smithsonian Institution [USNM] 1678890) dorsal view. (**D**) Detail of terminal genitalia (paratype, USNM 1678890).

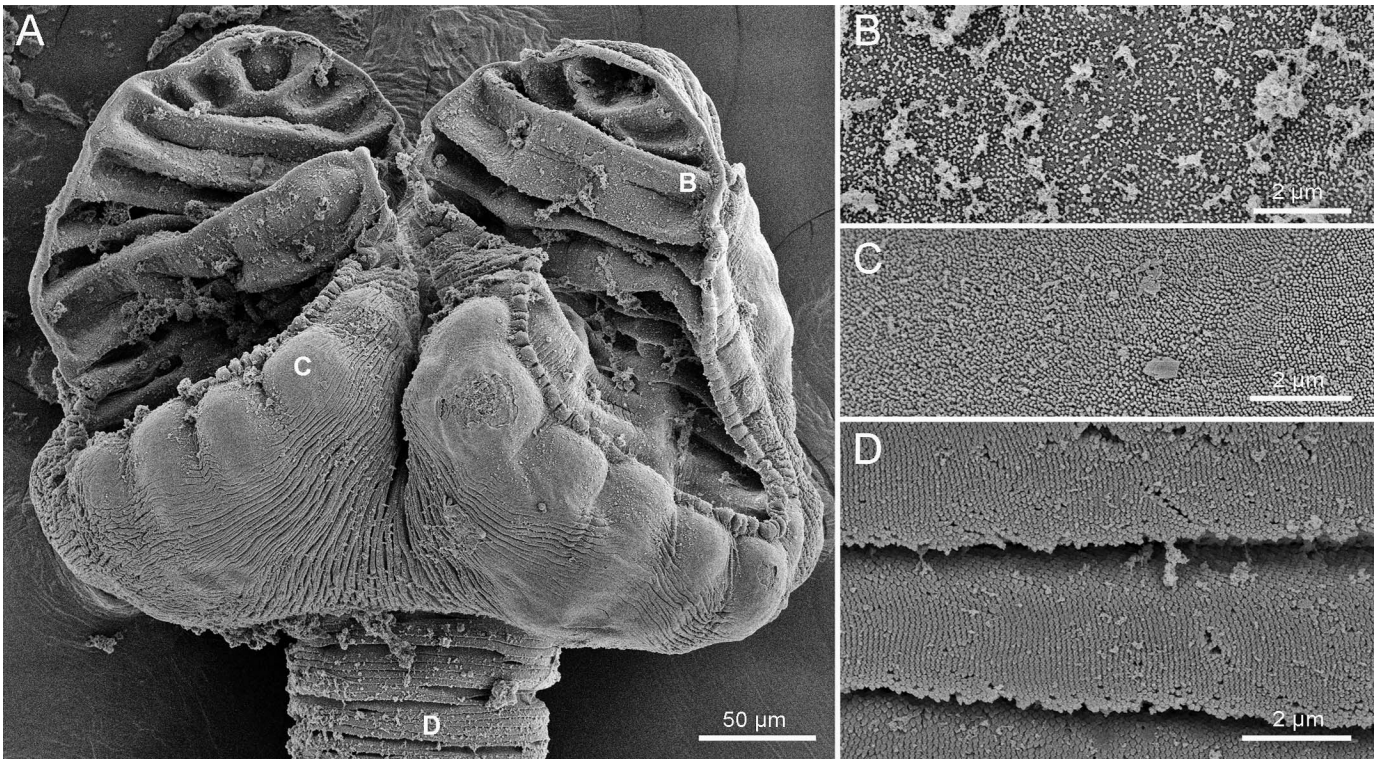

Figure 6. Scanning electron micrographs of *Duplicibothrium bilai* n. sp. (**A**) Scolex; small letters indicate locations of details in micrographs **B**–**D**. (**B**) Papilliform filitriches on distal surface of bothridium. (**C**) Papilliform filitriches on proximal surface of bothridium. (**D**) Densely arranged capilliform filitriches arranged as scutes on cephalic peduncle.

including dorsal to ovary, 2 irregular rows deep, oblong, 33–65 (44 ± 8; 5, 20) long, 50–93 (72 ± 11; 5, 20) wide. Vas deferens minimal, coiled medial and posterior to cirrus sac. Cirrus sac inconspicuous, pyriform (Fig. 5D), 102–164 (n = 4) long, 42–93 (n = 4) wide; containing coiled cirrus. Cirrus armed with spinitriches. Genital pores slightly submarginal, 78–85% (82% ± 2; 5) of proglottid length from posterior end of proglottid, irregularly alternating. Vagina extending from ovarian bridge along midline of proglottid to anterior margin of cirrus sac then along anterior margin to open into common genital atrium anterior to cirrus. Ovary terminal in position, highly digitiform, 215–384 (281 ± 67; 5) long, 279–335 (306 ± 24; 5) wide. Vitellarium follicular; dorsal vitelline follicles arranged in single extensive field, partially interrupted by cirrus sac and ovary; ventral vitelline follicles arranged in 2 lateral bands. Uterus median, ventral, sacciform, extending from ovarian bridge to level of cirrus sac. Excretory ducts 4 in number, arranged in 1 dorsal and 1 ventral pair. Gravid proglottids not observed.

Taxonomic summary

Type and only known host: Oman cownose ray, *R. jayakari* Boulenger (Myliobatiformes; Rhinopteridae).

Type locality: Off Tofo, (23°47′33.02″S, 35°31′16.38"E), Mozambique, Indian Ocean.

Additional locality: None.

Site of infection: Spiral intestine.

Specimens deposited: Holotype (NMB-P 922) and 2 paratypes (NMB-P 923, NMB-P 924); 3 paratypes (LRP 11011–11013) and 1 SEM voucher (LRP 11014); 3 paratypes (USNM 1678889–1678891); scolex prepared for SEM retained with JNC at the University of Connecticut.

ZooBank registration: urn:lsid:zoobank.org:act:834414EA-FED6-44B7-B05B-BEDD22382852.

Etymology: This species honors the intrepid Dr. Sam Bila of the University of Maputo in Mozambique, without whose valuable assistance the collection of the type host of this species would not have been possible.

Remarks

Duplicibothrium bilai n. sp. differs from *D. cairae*, *D. minutum*, and *D. jeannettae* in that it possesses a wider terminal proglottid (413–460 vs. 190–336, 128–240, and 186–316, respectively). *Duplicibothrium bilai* n. sp. further differs from *D. minutum* in that its bothridia bear both longitudinal and transverse septa rather than only transverse septa as well as in its possession of a greater number of testes (38–48 vs. 26–32). This new species further differs from *D. jeannettae* in that it possesses a longer cephalic peduncle (1,407–2,127 vs. 774–1,920), a posteriormost row of 7 rather than 5 loculi, and a longer terminal proglottid (735–1,138 vs. 514–656). This new species differs from *D. colossum* in that it is a smaller worm (4.3–5.9 vs. 10–29 mm) and possesses fewer proglottids (11–22 vs. 85–139). *Duplicibothrium bilai* n. sp. most closely resembles *D. cairae*; however, it differs in possessing fewer proglottids (11–22 vs. 20–35) and a greater number of total loculi per bothridia (37 vs. 27–33). With the description of *D. bilai* n. sp. we are assigning a formal name to

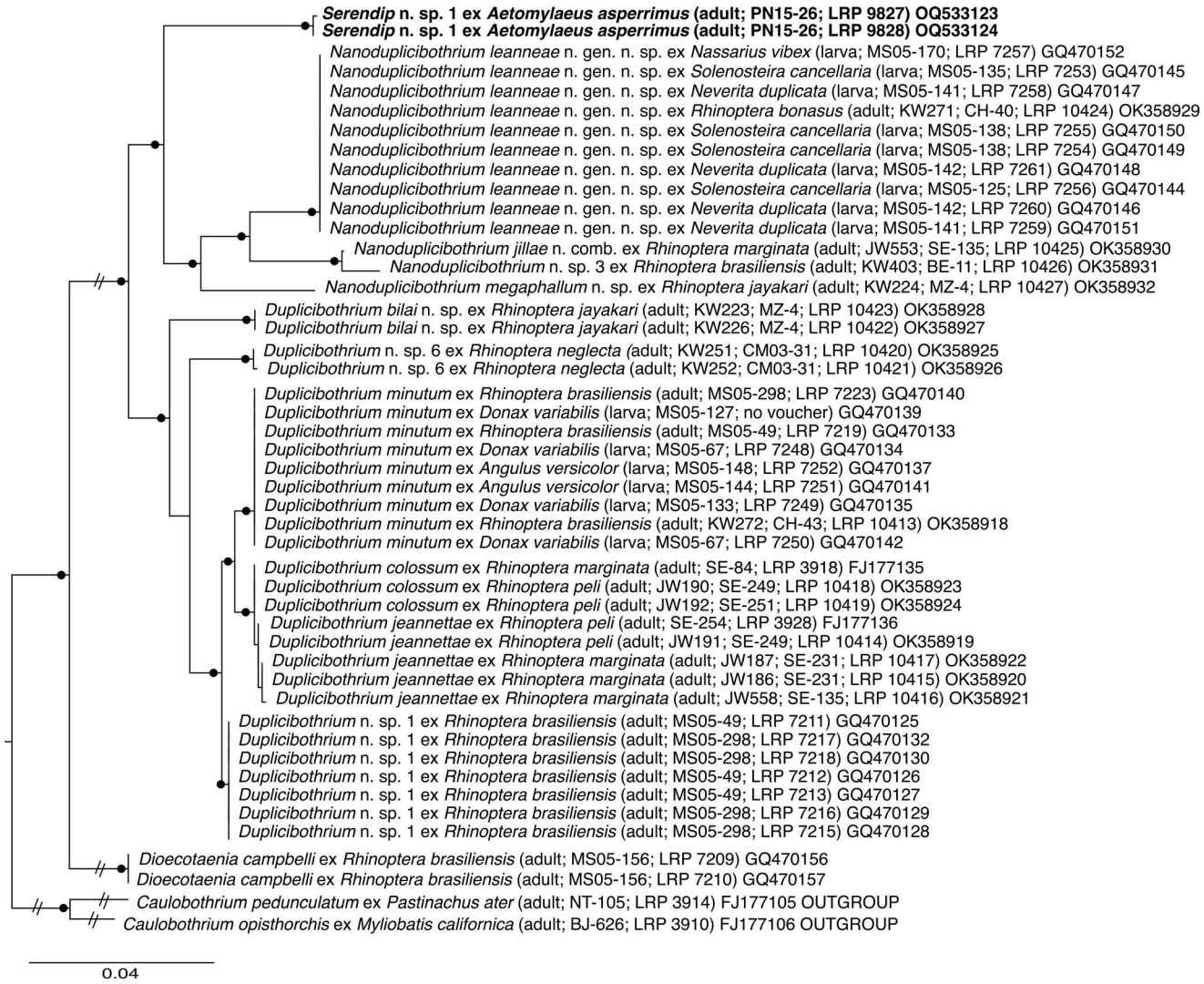

Figure 7. Phylogenetic tree resulting from maximum-likelihood analysis of the D1–D3 region of the *28S* rDNA gene for species in the family Serendipeidae. Scale bar indicates number of substitutions per site. Nodes with bootstrap support values ≥80% are indicated by black dots. Taxon labels are presented as cestode and host names followed in parentheses by developmental stage, cestode specimen number when available, host specimen number, and Lawrence R. Penner Parasitological Collection (LRP) hologenophore accession number, with the GenBank accession number outside of parentheses. Newly generated sequences are indicated in boldface type.

the specimens provisionally identified as *Duplicibothrium* n. sp. 5 in the molecular phylogeny of Stephan and Caira (2022).

Molecular phylogenetic analysis

The discovery of the undescribed species of *Serendip* n. sp. 1 (Fig. 4C) in the rough eagleray, *Aetomylaeus asperrimus*, off the Pacific coast of Panama has advanced our understanding of *Serendip*. Beyond expanding the known host associations of this genus to include a species in the family Myliobatidae, this material provided an opportunity for *Serendip* to be included in a molecular phylogenetic analysis for the first time. In the tree resulting from the present maximum-likelihood phylogenetic analysis of the Serendipeidae (Fig. 7), a subclade consisting of the species of *Nanoduplicibothrium* grouped as the sister taxon to the subclade consisting of the 2 specimens of *Serendip* n. sp. 1. A subclade consisting of the specimens of *Duplicibothrium* grouped as the sister to that clade. All 3 groups were supported with bootstrap support values ≥80%.

DISCUSSION

The results of our molecular phylogenetic analysis have helped address the question of the reciprocal monophyly of *Duplicibothrium* and *Serendip* raised by Stephan and Caira (2022) given the close similarity in bothridial morphology between *D. colossum* and species of *Serendip*. In the tree resulting from our analysis, as was found by Stephan and Caira (2022), *D. colossum* was deeply embedded among species of *Duplicibothrium*. Consistent with recognition of *Serendip* as an independent genus, *Serendip* n. sp. 1 grouped as the sister taxon to the subclade containing species of *Nanoduplicibothrium*.

The tree also supports the morphological differences seen between species of *Duplicibothrium* and those of *Nanoduplicibothr-*

rium. Given the close relationship between the latter species and those of *Serendip*, we initially entertained the idea of assigning these species to *Serendip*. However, the substantial differences in scolex morphology between the 2 groups in combination with unique similarities within the 2 groups, made the establishment of a new genus the more diagnosable option.

It is interesting that, based on the larval work of Jensen and Bullard (2010), it appears that the final larval stage of *Duplicibothrium minutum* parasitizes a variety of bivalves, whereas the final larval stage of *Nanoduplicibothrium leanneae* parasitizes a variety of gastropods. Although these data come from only a single species in each genus, it will be interesting to see if this trend toward differential use of major groups of molluscs as intermediate hosts holds for other members of each genus. Based on these associations and the diet of rhinopterid and myliobatid species, we would predict that species of *Serendip* and *Glyphobothrium* will be similarly found to employ molluscs as their final intermediate hosts.

The erection of a new genus to house a subset of species originally assigned to *Duplicibothrium* led us to rethink our interpretation of the facial loculi of *D. colossum*. Under the revised concept of *Duplicibothrium*, all members of the genus possess a distinct posterior row of 5 or 7 bothridial loculi. In the case of *D. colossum*, the 5 loculi in this row occupy much of the length of the bothridium leaving little space for the longitudinal septa and/or transverse septa forming loculi in the anterior regions of the bothridia of its congeners. The resemblance to species of *Serendip* is thus superficial.

With respect to global diversity, the erection of *Nanoduplicibothrium* brings the total number of genera in the Serendipeidae to 4 (i.e., *Duplicibothrium*, *Glyphobothrium*, *Nanoduplicibothrium*, and *Glyphobothrium*). Description of 3 new species brings the total number of species in the family to 12; with 3 provisionally identified species (i.e., *Duplicibothrium* n. sp. 1 of Jensen and Bullard [2010], and *Duplicibothrium* n. sp. 6 and *Nanoduplicibothrium* n. sp. 3 of Stephan and Caira [2022]) remaining to be formally described. All 15 of these species are known only from cownose rays of the genus *Rhinoptera*. The apparent fidelity of the Serendipeidae for species of *Rhinoptera*, in combination with the fact that most species in this genus of cownose rays have now been examined for cestodes, led Stephan and Caira (2022) to suggest little diversity of serendipeids likely remains to be discovered across the globe. However, the collection of specimens of *Serendip* n. sp. 1 in the rough eagleray, *A. asperrimus*, from Panama expands the potential repertoire of hosts beyond the Rhinopteridae to include the Myliobatidae, suggesting that the global diversity of this family of cestodes may be more extensive than previously thought. Unfortunately, description of this new species awaits the collection of additional material to allow characterization of its morphological features.

The erection of *Nanoduplicibothrium* substantially reduces the number of instances of congeners parasitizing the same host species discussed by Stephan and Caira (2022). *Rhinoptera jayakari* hosts *N. megaphallum* and *D. bilai*, *Rhinoptera steindachneri* hosts *N. paulum* and *D. cairae*, and *R. bonasus* hosts *N. leanneae* and *D. minutum*. Although *Rhinoptera brasiliensis* hosts *D. minutum* and *Duplicibothrium* n. sp. 1 (of Jensen and Bullard, 2010), it also hosts *Nanoduplicibothrium* n. sp. 3 (of Stephan and Caira, 2022). Similarly, *Rhinoptera marginata* hosts *D. colossum* and *D. jeannettae*, but also hosts *N. jillae*. Thus, in most cases, species parasitizing the same host species are not each other's closest relatives; rather, they belong to separate genera.

Our results support the position of Caira et al. (2017) that Glyphobothriidae, which was established by Monks et al. (2015) to house *Duplicibothrium* and *Glyphobothrium* to the exclusion of *Serendip*, is a junior synonym of Serendipeidae. We have provided evidence of the reciprocal monophyly of *Serendip*, *Duplicibothrium*, and *Nanoduplicibothrium* relative to one another, but the phylogenetic affinities of the monotypic *Glyphobothrium* remain unresolved. The somewhat unusual scolex morphology of *Glyphobothrium zwerneri* Williams and Campbell, 1977, which consists of a globular scolex with 4 sessile bothridia fused to the outer surface of the scolex proper, leads us to believe it will be found to represent a phylogenetically divergent group relative to the 3 other genera once it is included in a molecular phylogenetic analysis. However, scolex morphology can be misleading (e.g., Jensen et al., 2016). Further complicating the situation are the depictions of the scolex of specimens identified as *G. zwerneri* by Monks et al. (2015:fig. 1) collected from *R. bonasus* off Campeche, México. These specimens exhibit dorsal and ventral bothridia fused length-wise in 2 pairs and thus resemble *Nanoduplicibothrium* more closely than *Glyphobothrium*. As the oldest of the 4 generic names in the family, if *Glyphobothrium* is found to group among members of any of the other 3 genera of Serendipeidae, the generic assignments of the other members of the genus within which it falls will need to be revised.

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