A New Genus of Tapeworm (Cestoda: Onchoproteocephalidea) from Sawfish (Elasmobranchii: Pristidae)

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A NEW GENUS OF TAPEWORM (CESTODA: ONCHOPROTEOCEPHALIDEA) FROM SAWFISH (ELASMOBRANCHII: PRISTIDAE)

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ABSTRACT: Collections from the dwarf sawfish, *Pristis clavata*, near Darwin, Australia, in 1997 led to the discovery of the new onchoproteocephalidean genus *Matticestus* n. gen.—a taxon that has been referred to in molecular phylogenetic analyses in which it has been included as "New genus 8." Its type species, *Matticestus anneae* n. gen., n. sp., and a second species, *Matticestus kathleenae* n. sp., are described. Placement of this taxon in the Onchoproteocephalidea is supported morphologically in that both species bear a scolex with 4 bothridia each with a pair of bi-pronged hooks and spinitriches that extend throughout the length of the body. Sequence data for the D1–D3 region of the *28S rDNA* gene also place the genus solidly among the other elasmobranch-hosted members of the order. The new genus differs from the other elasmobranch-hosted genera in the order in that its members possess a combination of biloculated bothridia with lateral lappets on the posterior margin of the anterior loculus and a pair of bi-pronged hooks with a distinctive configuration of tubercles and internal channels. Its members are also extremely small. In summary, *Matticestus* n. gen. is an unusually tiny, "spiny," genus of cestode that seems to exclusively parasitize sawfish of the genus *Pristis*.

The past almost 2 decades have seen a remarkable transformation in our global understanding of the tapeworms of sharks and stingrays (i.e., elasmobranchs). In total, 202 genera are now recognized across the 9 orders of cestodes that parasitize elasmobranchs (Caira and Jensen, 2017; Haseli and Malekpour Fard, 2017). A phenomenal 57 (i.e., 28%) of these genera have been erected in the mere 17 yr since the turn of the 21st century. This paper adds another member to this assemblage by formally establishing a genus that has been given the unofficial designation "New genus 8" in the various molecular phylogenetic studies in which it has been included (e.g., Caira et al., 2014, 2017).

This genus was first brought to our attention by the late Dr. Tom Mattis, who had collected material more than 4 decades ago from an elasmobranch specimen he identified as the smalltooth sawfish, *Pristis pectinata* Latham, off New Orleans, Louisiana. Unfortunately, that material consisted of a few macerated specimens that were essentially impossible to describe, particularly given their tiny size. Many years later, working in northern Australia on the parasites of the dwarf sawfish, *Pristis clavata* Garman, in collaboration with Darwin Fisheries, we encountered specimens from *P. clavata* that were similar to, although not identical with, the Mattis material from *P. pectinata*. These specimens were numerous and of sufficiently high quality to allow for the formal erection of the genus. In honor of his many contributions to the field of cestodology, this new genus bears the patronym *Matticestus* n. gen. Our material from *P. clavata* consisted of 2 species belonging to the new genus, both of which are described below.

MATERIALS AND METHODS

Collections

The cestodes described here came from 4 individuals of *P. clavata*. These individuals consisted of 1 female (accession AU-

136) 98 cm in total length (TL) and 3 males (accessions AU-15, AU-36, and AU-138) ranging from 90 to 200 cm in TL. The hosts were collected using gill nets set in Buffalo Creek near Darwin, Australia (12°20′11″S, 130°54′39″E) between 5 and 15 August 1997. A small sample of liver tissue from each sawfish was preserved in 95% ethanol to confirm the specific identity of the hosts using *NADH2* sequence data. The identities of all 4 sawfish specimens have been confirmed by Naylor et al. (2012a). Additional information of each host can be found in the Global Cestode Database (elasmobranchs.tapewormdb.uconn.edu) by searching for the accession number, which is a combination of collection code and collection number (e.g., AU-15). The spiral intestine from each animal was opened with a mid-ventral, longitudinal incision and fixed in 10% seawater-buffered formalin (9:1). Before fixation, a subsample of the worms from 1 of the 4 host specimens (AU-36) was removed and preserved in 95% ethanol for molecular work.

Morphological methods

Whole mounts were prepared following conventional techniques (see Pickering and Caira, 2012). Worms were stained with Delafield's hematoxylin, cleared in methyl salicylate, and mounted in Canada balsam. Measurements were taken with an Axioskop 2 Plus compound microscope (Carl Zeiss Microscopy, Thornwood, New York) by using a SPOT Diagnostic Instrument Digital Camera and SPOT version 4.6 (SPOT Imaging Solutions, Sterling Heights, Michigan). Unless otherwise stated, measurements are presented in micrometers as range values followed parenthetically by the mean ± SD, worm sample size, and total number of observations when more than 1 measurement per specimen was made, as appropriate.

Histological sections were prepared for the strobila of 1 specimen of *Matticestus kathleenae* n. sp. as follows. The strobila was embedded in paraffin and sectioned at 8-μm intervals by using a CUT4060 retracting rotary microtome (Olympus Corporation, Melville, New York). Sections were mounted on glass slides flooded with 2.5% sodium silicate and dried on a slide warmer overnight. Sections were stained with Gill's hematoxylin, counterstained with eosin. The paraffin was removed with xylene,

and then the sections were mounted under coverslips on glass slides in Canada balsam.

The scolex of 3 or 4 specimens and 2 detached proglottids of each species, and a whole worm of 1 species, were prepared for scanning electron microscopy (SEM) as follows. Specimens were hydrated in a graded ethanol series, placed in osmium tetroxide overnight, dehydrated in a graded ethanol series, placed in hexamethyldisilazane (Ted Pella Inc., Redding, California), and allowed to air dry for at least 1 hr in a fume hood. They were then mounted on aluminum stubs on double-sided PELCO carbon tabs (Ted Pella Inc.), sputter coated with 30 nm of gold/palladium, and examined with a LEO/Zeiss DSM 982 Gemini field emission scanning electron microscope (Carl Zeiss Microscopy) or a Nova NanoSEM 450 field emission scanning electron microscope (FEI, Hillsboro, Oregon). Stubs have been retained in the personal collection of C.A.F.

Museum abbreviations used are as follows: LRP, Lawrence R. Penner Parasitology Collection, University of Connecticut, Storrs, Connecticut, U.S.A.; QM, Queensland Museum, Brisbane, Australia; and USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C., U.S.A. Microthrix terminology follows Chervy (2009). Hook measurement lettering system follows Ghoshroy and Caira (2001); proglottid measurements were taken from terminal proglottids unless otherwise indicated.

Molecular methods

Total genomic DNA was extracted from the strobila of 2 or 3 specimens of each species preserved in 95% ethanol from host specimen AU-36 by using a standard phenol–chloroform protocol (Hillis et al., 1996). The D1–D3 region of the *28S rDNA* gene was targeted because of its previously established utility in resolving interspecific relationships in other cestode groups (see Caira and Jensen, 2017, and references therein). For amplification, LSU5 (Littlewood et al., 2000) was used as the forward primer and 1500R (Tkach et al., 2003) was used as the reverse primer. For sequencing reactions, LSU5 was used as the forward primer and 1200R (Lockyer et al., 2003) was used as the reverse primer.

In all cases, the *28S rDNA* (D1–D3) sequence data were generated from specimens of the new genus that were photo vouchered before DNA extraction. These photo vouchers have been deposited in the LRP; accession numbers are included in the taxon labels in the tree provided herein and in the descriptions of each species, along with the corresponding GenBank accession numbers. Polymerase chain reaction (PCR) was performed in 25-µl reactions by using 2–100 ng of template DNA, 0.25 µM of each primer, 1 µM MgCl$_2$, 0.12 µmM of each dNTP, and 1.2 units of Taq DNA polymerase, with annealing temperatures between 50 and 60 C. Cycle sequencing conditions were as follows: initial denaturation for 1 min at 94 C, followed by 40 cycles of 30 sec at 94 C, 30 sec at 50–60 C, 1 min at 72 C, and completed by 5 min at 72 C. PCR products were purified using Nucleospin® extraction kit (BD Biosciences, San Jose, California), cycle sequenced with the appropriate primer using Big Dye® dideoxy terminators version 1.1 and sequenced on an ABI PRISM® 3100 genetic analyzer (Applied Biosystems, Foster City, California).

To assess the phylogenetic relationships of the new species, and thus also to explore the novel nature of the genus, *28S rDNA* sequence data for a diversity of relevant taxa were obtained from GenBank. In addition to the data for a specimen of *Matticestus* referred to as "New genus 8 n. sp. 1" (TE-92) in Caira et al. (2014), these specimens consisted of 13 of the 14 other onchoproteocephalidean species also included in the analyses of Caira et al. (2014). Because it lacks a voucher, *Proteocephalus macrocephalus* (Diesing, 1850) LaRue, 1914, was replaced in our analyses by *Proteocephalus kuyukuyu* Woodland, 1935 from de Chambrier et al. (2015). Also included as members of the ingroup in our analyses here were 24 adult specimens representing 13 species of *Acanthobothrium* Blanchard, 1848 beyond the 2 included by Caira et al. (2014). These data came from Fyler (2011), Fyler et al. (2009), Fyler and Caira (2006, 2010), and Jensen and Bullard (2010). Outgroup taxa consisted of *Caulobothrium opisthorchis* Riser, 1955; *Paraorygmatobothrium exiguum* (Yamaguti, 1935) Ruhnke, 1994; and *Rhoptrobothrium* cf. *gambangi* Jensen and Caira, 2006, all also from Caira et al. (2014). For each specimen, the museum accession number of the hologenophore and the corresponding GenBank accession number are included in the taxon label in the tree presented herein. Specimens for which novel sequence data were generated here are indicated in bold in that figure.

Sequences were assembled, aligned (using MUSCLE Alignment with 100 iterations and default settings otherwise; Edgar, 2004), and trimmed to remove leading and trailing gaps by using Geneious 10.1.3 (Biomatters Inc., Newark, New Jersey). Ambiguously aligned regions were identified and excluded using Gblocks 0.91b (Castresana, 2000) under parameters for less stringent character selection for exclusion (i.e., minimum number of sequences for a conserved position, 25; minimum number of sequences for a flanking position, 25; maximum number of contiguous nonconserved positions, 8; and minimum length of a block, 5).

The number of constant characters was determined using PAUP* version 4.0a157 (Swofford, 2003) from the matrix with the ambiguously aligned regions excluded, both with and without outgroups. GTR+I+Γ was determined to be the most appropriate model of nucleotide evolution for the analyses using the corrected Akaike Information Criterion (AICc) implemented in jModeltest 2.1.7 v20150220 (Darriba et al., 2012) following evaluation of a total of 88 models. Maximum likelihood (ML) analyses were performed using serial implementation of Garli-2.01 (Zwickl, 2006). Twenty independent ML runs were conducted in Garli-2.01 by using default settings with the following adjustments: 'genthreshfortopoterm = 100000' and 'significanttopochange = 0.0001.' Bootstrap support was estimated using serial implementation of Garli-2.01. In total, 200 bootstrap replicates were conducted using the same configuration and model as were used for the ML analyses, with following changes from the default settings: 'genthreshfortopoterm = 10000,' 'significanttopochange = 0.01,' and 'treerejectionthreshold = 20.0.' Bootstrap results were summarized on the optimal tree (i.e., the 'best' tree resulting from the 20 independent ML runs) by using SumTrees 4.0.0 in DendroPy 4.0.3 (Sukumaran and Holder, 2010) available at https://github.com/jeetsukumaran/DendroPy).

DESCRIPTIONS
Matticestus n. gen.

Diagnosis: Scolex consisting of scolex proper and cephalic peduncle. Scolex proper with 4 bothridia; each bothridium with anterior muscular pad bearing 1 accessory sucker and 1 pair of hooks, divided into 2 loculi by transverse septum; anterior loculus longer than posterior loculus; postero-lateral margins of anterior loculus with lappets extending to posterior margin of bothridium. Hooks bi-pronged, hollow; accessory piece between bases of medial and lateral hooks absent; internal channel of hooks not extending into bases of hooks. Distal and proximal bothridial surfaces with acicular filitriches; some small gladiate spinitriches on proximal lateral surfaces of bothridia. Cephalic peduncle and strobila with conspicuous gladiate spinitriches; spinitriches densely arranged on cephalic preduncle becoming more sparse along length of strobila. Proglottids acraspedote, non-laciniate, euapolytic. Genital pores lateral, irregularly alternating. Testes numerous, entirely anterior to ovary, in 2 to 3 columns, 1 layer deep in cross section; post-poral field of testes present. Cirrus armed with spinitriches. Ovary posterior, inverted A-shaped in frontal view, bilobed in cross section. Vagina opening anterior to cirrus sac into genital atrium. Vitellarium follicular; follicles in 2 lateral bands; each band consisting 1 dorsal and 1 ventral column of follicles, extending from near anterior of proglottid, stopping short of posterior margin of ovary, interrupted by terminal genitalia. Uterus saccate, medioventral, beginning at ovarian isthmus, not reaching anterior margin of proglottid. Excretory ducts 4, arranged in 1 dorsal and 1 ventral pair. Eggs spherical. Parasites of sawfish of the genus *Pristis* Linck (Rhinopristiformes: Pristidae Bonaparte); circumglobal in distribution.

Type species: Matticestus anneae n. gen., n. sp.

Additional species: Matticestus kathleenae n. sp.

Etymology: This genus honors the late Dr. Tom Mattis not only for his discovery of the first of its members but also for his remarkable contributions to our knowledge of the life cycles of elasmobranch-hosted cestodes.

Remarks

In the most recent treatment of the elasmobranch-hosted onchoproteocephalideans, Caira et al. (2017) recognized 11 valid genera. *Matticestus* n. gen. is readily distinguished from more than half of these based on the presence and configuration of its hooks. Unlike *Prosobothrium* Cohn, 1902, it bears, rather than lacks, hooks. Each of the hooks in a pair in *Matticestus* n. gen. is bi-pronged, rather than tri-pronged, as in *Phoreiobothrium* Linton, 1889 and *Triloculatum* Caira and Jensen, 2009, both of which, unlike the new genus, also bear a posterior-most loculus that is divided by subloculi. The bi-pronged nature of the hooks of *Matticestus* n. gen. also distinguishes it from *Onchobothrium* de Blainville, 1828 and *Potamotrygonocestus* Brooks and Thorson, 1976, both of which bear uni-pronged hooks. It further differs from these genera in its possession of 2, rather than 3 or 1, loculi per bothridium, respectively. Unlike *Acanthobothrium*, *Acanthobothroides* Brooks, 1977, *Pinguicollum* Riser, 1955, and *Platybothrium* Linton, 1890, the bothridia of *Matticestus*, each bear 2, rather than 3, loculi. It further differs from *Acanthobothrium* in that the internal channel of each hook does not extend into the hook base; it possesses, rather than lacks, postero-lateral bothridial lappets that extend to the posterior margin of the bothridia; and it possesses, rather than lacks, gladiate spinitriches that extend throughout much of the length of the strobila. It further differs from *Pinguicollum* in that it lacks a layer of tissue covering its bothridia and from *Platybothrium* in that the talons of its hooks are in the form of tubercles on the proximal surfaces of the axial prongs, rather than extending freely between the axial and abaxial hook prongs. The new genus most closely resembles *Megalonchos* Baer and Euzet, 1962 and species currently assigned to *Uncibilocularis* Southwell, 1925 (see Jensen and Caira, 2008) in its possession of both bi-pronged hooks and 2 loculi per bothridium. However, unlike the former genus, it possesses, rather than lacks, post-poral testes and its uterus extends well beyond, rather than stops short of, the cirrus sac. Unlike *Uncibilocularis*, species of *Matticestus* n. gen. bear large, gladiate spinitriches throughout the length of their strobila. Furthermore, the hooks of the new genus lack the matrix of dark tissue that surrounds the bases of the hooks of species of *Uncibilocularis*. In summary, *Matticestus* n. gen. is a tiny, "spiny," genus of cestode that seems to exclusively parasitize sawfish of the genus *Pristis*.

Matticestus anneae n. gen., n. sp.
(Figs. 1, 2)

Description (based on 11 complete worms; 1 mature and 2 gravid detached proglottids; and 1 worm, 3 scoleces, and 2 detached proglottids examined with SEM): Worms 860–1,100 (952 ± 83; 11) long, greatest width at level of terminal proglottid, 7–9 (7.8 ± 0.6; 11) proglottids per worm; eupolytic. Scolex consisting of scolex proper and cephalic peduncle. Scolex proper with 4 bothridia, 135–173 (146 ± 11; 11) long by 93–120 (104 ± 9; 11) wide. Bothridia free posteriorly, 45–56 (50 ± 3; 11) wide, each with specialized anterior region in form of muscular pad and 2 loculi; muscular pad 36–50 (44 ± 4; 9; 18) long by 35–55 (44 ± 5; 9; 17) wide, triangular in shape (Fig. 2B), bearing accessory sucker and 1 pair of hooks at posterior margin; accessory sucker 14–25 (17 ± 4; 6; 9) long by 21–30 (27 ± 3; 6; 9) wide; anterior loculus 68–87 (75 ± 5; 11; 20) long at center, with concave posterior margin and postero-lateral lappets; lappets extending to posterior margin of bothridium; posterior loculus 22–26 (24 ± 1; 11; 20) long; posterior-to-anterior loculus length ratio 1:2.8–3.6 (3.2 ± 0.3; 11; 20). Velum between medial margins of dorsal and ventral pairs of bothridia inconspicuous. Hooks bi-pronged, hollow, with tubercle on proximal surface of each axial prong; internal channels of axial and abaxial prongs continuous, smooth, not extending into hook bases; axial prong of lateral hook slightly shorter than abaxial prong; axial and abaxial prongs of medial hook approximately equal in length; lateral and medial hooks approximately equal in size. Lateral hook measurements: A 23–27 (25 ± 1; 11; 21), B 37–51 (43 ± 4; 11; 21), C 40–58 (50 ± 5; 11; 21), D 52–72 (63 ± 5; 11; 21). Medial hook measurements: A′ 22–27 (25 ± 2; 11; 22), B′ 42–61 (50 ± 5; 11; 22), C′ 40–59 (50 ± 5; 11; 22), D′ 60–79 (70 ± 5; 11; 22). Bases of lateral and medial hooks approximately equal in length. Cephalic peduncle 33–120 (55 ± 25; 11) long by 22–44 (34 ± 5; 11) wide at posterior margin.

Muscular pad (Fig. 2F) and distal (Fig. 2G) and proximal (Fig. 2I, H) surfaces of both bothridial loculi covered with acicular filitriches; proximal surface of lateral margin of anterior loculus of
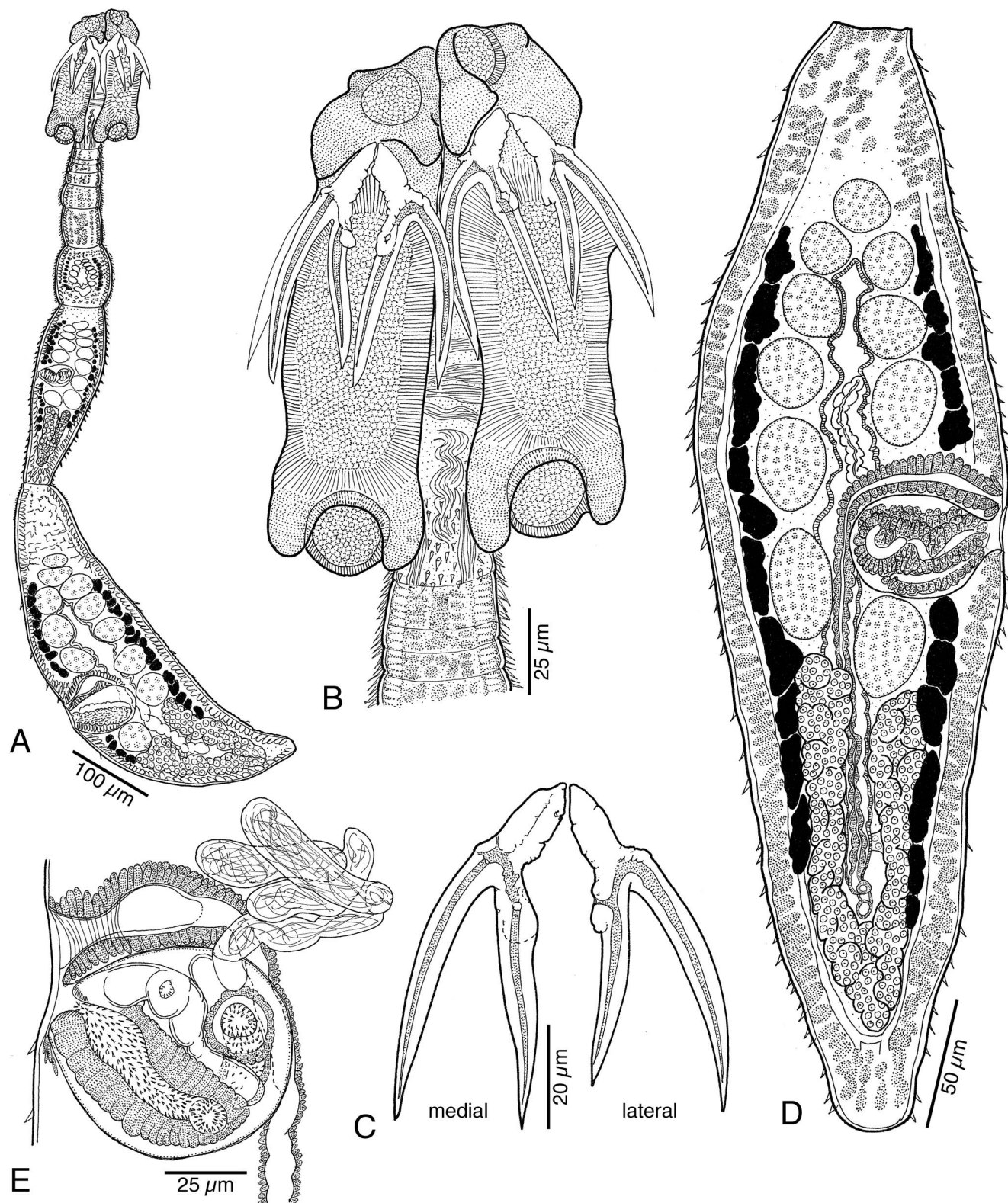
FIGURE 1.—Line drawings of *Matticestus anneae* n. gen., n. sp. (**A**) Whole worm (holotype, QM G236758). (**B**) Scolex (holotype, QM G236758). (**C**) Hooks (paratype, USNM 1470750). (**D**) Terminal proglottid (paratype, USNM 1470750). (**E**) Terminal genitalia from detached mature proglottid (paratype, QM G236763).
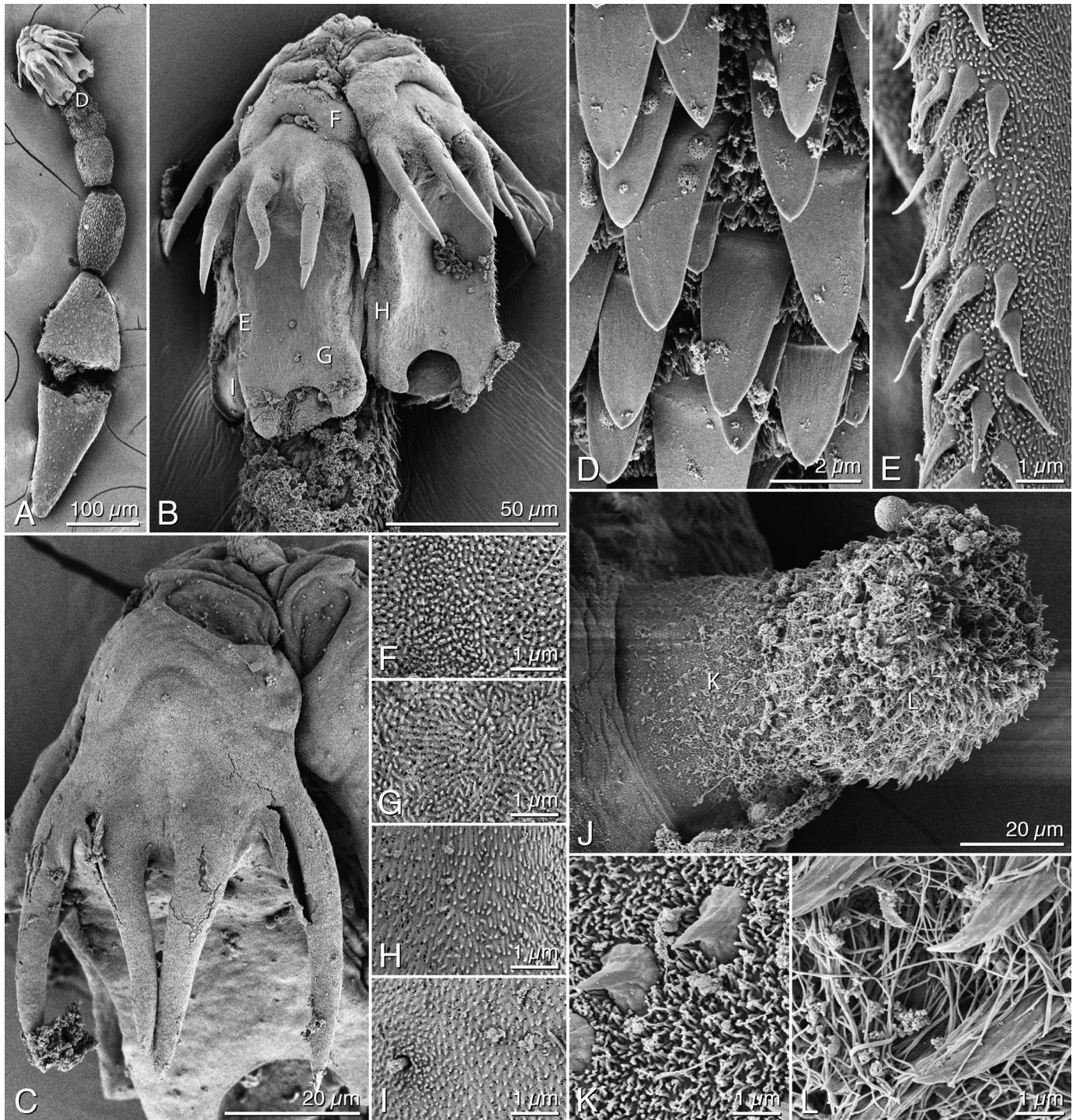
FIGURE 2.—Scanning electron micrographs of *Matticestus anneae* n. gen., n. sp. (**A**) Whole worm; small letter indicates location of detail in D. (**B**) Scolex; small letters indicate locations of details in E–I. (**C**) Detail of apical region of bothridium and hooks. (**D**). Microtriches on cephalic peduncle. (**E**) Narrow band of gladiate spinitriches on proximal lateral surface of anterior loculus. (**F**) Acicular filitriches on muscular pad. (**G**) Acicular filitriches on distal bothridial surface. (**H**) Acicular filitriches on medial proximal bothridial surface rim. (**I**) Acicular filitriches on proximal bothridial surface. (**J**) Partially everted cirrus; small letters indicate locations of details in K and L. (**K**) Rostrate spinitriches and acicular fillitriches on base of cirrus. (**L**) Uncinate spinitriches and capilliform filitriches on more distal portions of cirrus.

bothridia adjacent to rim with narrow band of small gladiate spinitriches (Fig. 2E). Cephalic peduncle (Fig. 2A, B) and strobila covered with gladiate spinitriches and capilliform filitriches; gladiate spinitriches becoming less dense along length of strobila (Figs. 1A, 2A).

Proglottids acraspedote, protandrous. Immature proglottids 6–8 (7 ± 0.7; 11) in number; mature proglottids 1 in number. Terminal proglottid 386–525 (434 ± 58; 11) long by 116–160 (136 ± 15; 11) wide, length-to-width ratio 2.5–4 (3.2 ± 0.5; 11):1; detached mature proglottid (n = 1) 689 long by 162 wide, length-to-width ratio 4.25:1; detached gravid proglottids (n = 2) 608–721 long by 267–284 wide, length-to-width ratio 2.1–2.7:1. Genital pores marginal, irregularly alternating, 51–62% (56 ± 3; 11) of proglottid length from posterior end; 57% in detached mature proglottid (n = 1); 60–62% in detached gravid proglottids (n = 2). Testes irregularly oval in frontal view, 18–50 (36 ± 8; 11; 42) long by 20–50 (35 ± 8; 11; 41) wide, arranged in 2 regular columns anterior to ovary, 1 layer deep, 9–13 (11 ± 1; 11; 21) in total number, 1–2 (1.2 ± 0.4; 11; 21) in post-poral field. Vas deferens coiled at anterio-medial margin of cirrus sac. Cirrus sac spherical, 55–83 (71 ± 10; 11) long by 40–55 (47 ± 6; 1) wide; 78 long by 72 wide in detached mature proglottid (n = 1); 104–110 long by 90–96 wide in detached gravid proglottids (n = 2), containing coiled cirrus; base of cirrus covered with sparsely arranged rostrate spinitriches and acicular filitriches (Fig. 2K), followed distally by robust large, uncinate spinitriches and capilliform filitriches (Fig. 2L). Vagina relatively thick-walled, straight to weakly sinuous, extending along medial line of proglottid from ootype to anterior margin of cirrus sac, then laterally along anterior margin of cirrus sac to open into common genital atrium anterior to cirrus; vaginal sphincter observed only in gravid proglottids; seminal receptacle not seen. Ovary occupying posterior third of proglottid, inverted A-shaped in frontal view, bilobed in cross section, with lobulate margins, 38–90 (63 ± 15; 11) wide at isthmus, asymmetrical, with aporal lobe slightly longer than poral lobe; poral lobe 108–180 (129 ± 22; 11) long; aporal lobe 110–208 (139 ± 27; 11); ovarian isthmus in anterior half of ovary. Mehlis' gland posterior to ovarian isthmus. Vitellarium follicular, in 2 lateral bands; each band consisting of 1 dorsal and 1 ventral column of follicles, extending from near anterior limit of field of testes to near ovarian isthmus, interrupted by terminal genitalia; vitelline follicles irregular in shape. Uterus medio-ventral, saccate, thin-walled, extending along medial line from ovarian isthmus to near anterior limit of field of testes. Excretory ducts 4, arranged in 1 dorsal and 1 ventral pair. Eggs in detached gravid proglottids spherical, 12–13 (12.5 ± 0.2; 2; 8) long by 12–13 (12.5 ± 0.4; 2; 8) wide.

Taxonomic summary

Type and only known host: Pristis clavata Garman, dwarf sawfish (Rhinopristiformes: Pristidae).

Site of infection: Spiral intestine.

Type locality: Buffalo Creek near Darwin (12°20′11″S, 130°54′39″E), Northern Territory, Australia, Timor Sea, Indian Ocean.

Prevalence: Four of 4 hosts examined (100%).

Etymology: This species honors Anne St. Onge in recognition of her decades of service as the remarkable graduate student coordinator of Biological Sciences at the University of Connecticut.

Specimens deposited: Holotype (QM G236758) and 5 paratypes (4 whole worms and 1 detached mature proglottid; QM G236759-G236763), 4 paratypes (3 whole worms and 1 detached gravid proglottid; USNM 1470750-1470753), and 4 paratypes (3 whole worms and 1 detached gravid proglottid; LRP 9315–9318); specimens prepared for SEM retained with J.N.C. at the University of Connecticut. Photographic vouchers of specimens for which *28S rDNA* (D1–D3) sequence data were generated are deposited in the LRP (9313–9314).

Matticestus kathleenae n. sp.
(Figs. 3, 4)

Description (based on 8 complete mature worms, 3 mature detached proglottids, cross section series of 1 mature proglottid, and 4 scoleces and 2 detached proglottids examined with SEM): Worms 1,465–2,925 (1,985 ± 458; 8) long, greatest width at level of terminal proglottid, 10–18 (16 ± 3; 8) proglottids per worm; eupolytic. Scolex consisting of scolex proper and cephalic peduncle. Scolex proper with 4 bothridia, 180–208 (191 ± 11; 8) long by 160–186 (172 ± 11; 7) wide. Bothridia free posteriorly, 70–88 (81 ± 6; 8) wide, each with specialized anterior region in form of muscular pad and 2 loculi; muscular pad 35–55 (45 ± 6; 8; 12) long by 48–73 (64 ± 7; 8; 12) wide, triangular in shape (Figs. 3B, 4A, 4B), bearing accessory sucker and 1 pair of hooks at posterior margin; accessory sucker 23–28 (26 ± 2; 6; 10) long by 26–40 (33 ± 4; 10) wide; anterior loculus 98–118 (106 ± 6; 8; 15) long at center, with concave posterior margin and postero-lateral lappets; lappets extending to posterior margin of bothridium; posterior loculus 33–47 (40 ± 4; 8; 15) long; posterior-to-anterior loculus length ratio 1:2.23–3.38 (2.69 ± 0.3; 8; 15). Velum between medial margins of dorsal and ventral pairs of bothridia inconspicuous. Hooks bi-pronged, hollow, with tubercle on proximal surface of each axial prong; internal channels of axial and abaxial prongs continuous, smooth, not extending into hook bases; abaxial prong of lateral hook slightly shorter than axial prong; axial and abaxial prongs of medial hook approximately equal in length; lateral and medial hooks approximately equal in size. Lateral hook measurements: A 29–34 (31 ± 1; 8; 16), B 50–63 (57 ± 3; 8; 16), C 44–62 (51 ± 5; 8; 16), D 73–87 (80 ± 4; 8; 15). Medial hook measurements: A′ 27–34 (30 ± 2; 8; 17), B′ 55–68 (60 ± 4; 8; 15), C′ 45–62 (52 ± 5; 8; 17), D′ 72–90 (81 ± 5; 8; 14). Bases of lateral and medial hooks approximately equal in length. Cephalic peduncle 108–238 (198 ± 41; 8) long by 42–61 (51 ± 6; 8) wide at posterior margin.

Muscular pad, distal (Fig. 4E), and proximal (Fig. 4F, G) surfaces of both bothridial loculi covered with acicular filitriches; proximal surface of lateral margin of posterior region of anterior loculus also with restricted patch of small gladiate spinitriches (Fig. 4D). Cephalic peduncle (Fig. 4C) and stobila covered with gladiate spinitriches and capilliform filitriches; gladiate spinitriches dense on cephalic peduncle becoming very sparse towards posterior of strobila (Fig. 1D).

Proglottids acraspedote, protandrous. Immature proglottids 10–17 (15 ± 2; 8) in number; mature proglottids 1 in number. Terminal proglottid 327–732 (516 ± 140; 8) long by 140–285 (213 ± 44; 8) wide, length-to-width ratio 1.7–3.8 (2.5 ± 0.8; 8):1; detached mature proglottids (n = 3) 729–810 long by 259–308 wide, length-to-width ratio 2.5–2.9:1. Genital pores marginal,
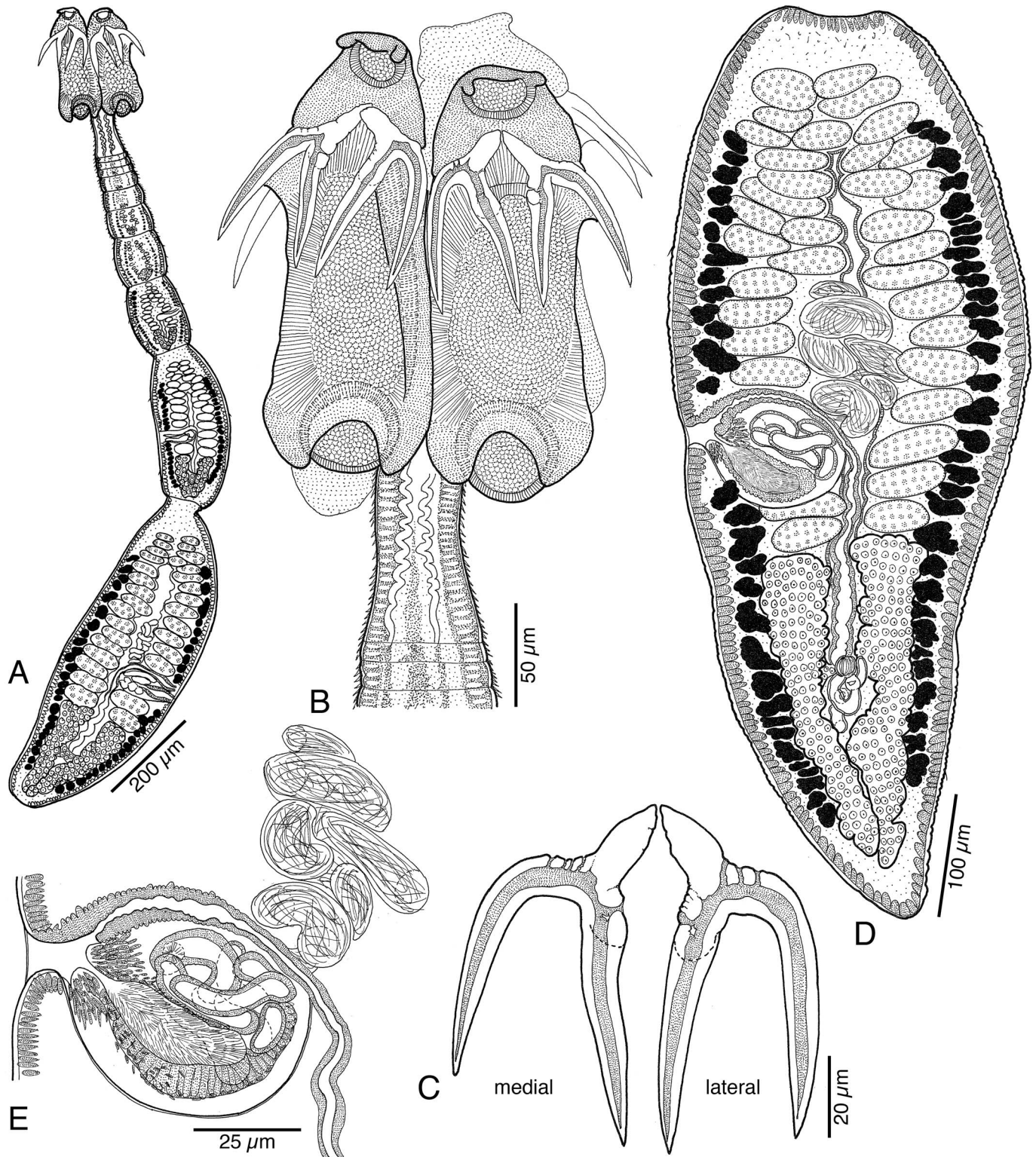
FIGURE 3.—Line drawings of *Matticestus kathleenae* n. sp. (**A**) Whole worm (holotype, QM G236764). (**B**) Scolex (holotype, QM G236764). (**C**) Hooks (paratype, QM G236765). (**D**) Terminal proglottid (paratype, USNM 1470754). (**E**) Terminal genitalia (paratype, USNM 1470754).
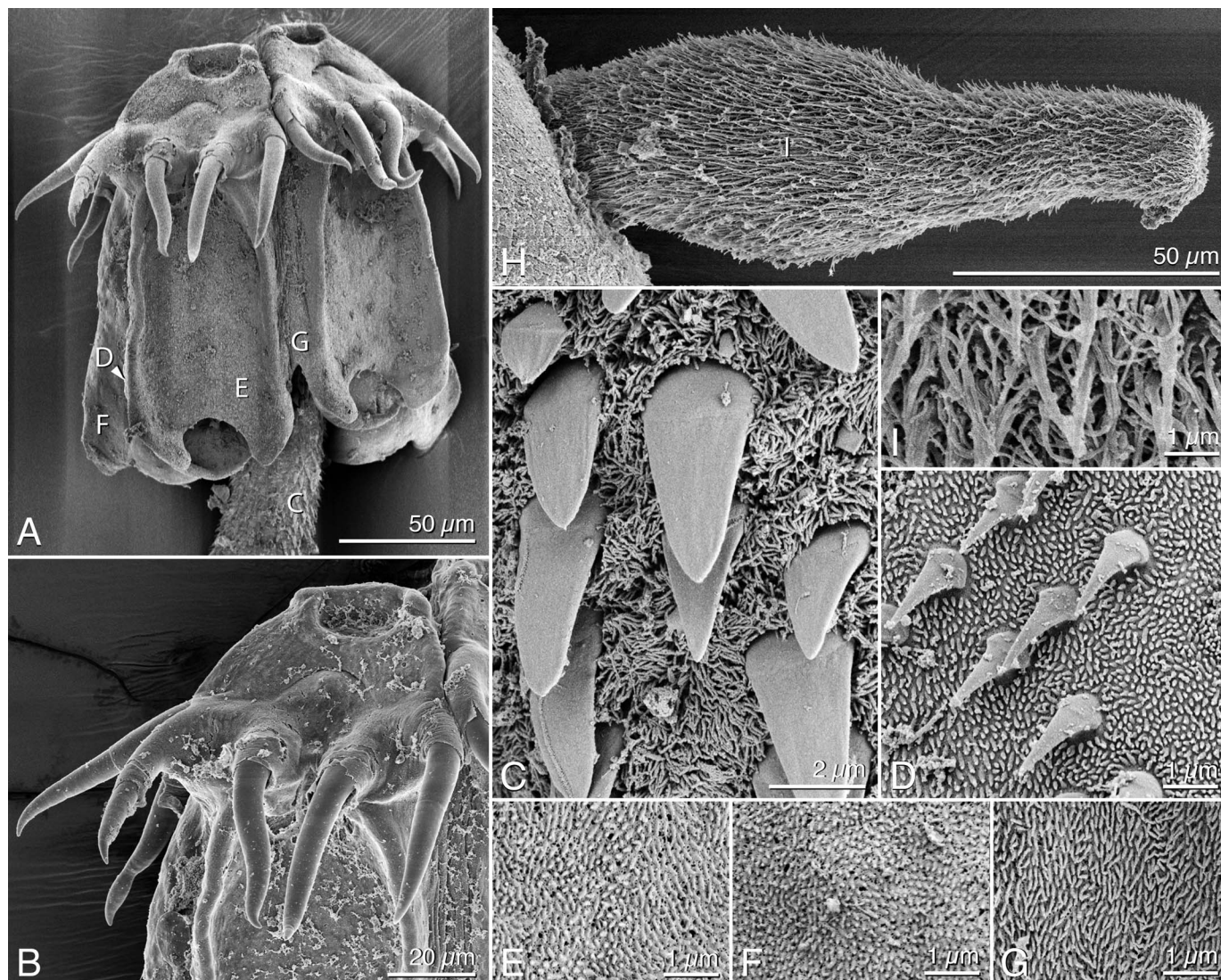
FIGURE 4.—Scanning electron micrographs of *Matticestus kathleenae* n. sp. (**A**) Scolex; small letters indicate locations of details in C–G. (**B**) Detail of apical region of bothridium and hooks. (**C**) Microtriches on cephalic peduncle. (**D**) Narrow band of gladiate spinitriches on proximal lateral bothridial surface. (**E**) Acicular filitriches on distal bothridial surface. (**F**) Acicular filitriches on proximal lateral bothridial surface. (**G**) Acicular filitriches on proximal medial bothridial surface. (**H**) Partially everted cirrus; small letter indicates location of details in I. (**I**) Narrow uncinate spinitriches and capilliform filitriches on cirrus.

irregularly alternating, 47–57% (52 ± 4; 8) of proglottid length from posterior end; 54–61% in detached mature proglottids (n = 3). Testes irregularly oval in frontal view, 15–35 (25 ± 6; 8; 32) long by 36–76 (59 ± 12; 8; 32) wide, arranged in 2–3 irregular columns anterior to ovary, 1 layer deep in cross section, 27–34 (30 ± 2; 8; 21) in total number, 2–3 (2.6 ± 0.5; 8; 21) in post-poral field. Vas deferens coiled at anterio-medial margin of cirrus sac. Cirrus sac spherical, 80–125 (111 ± 15; 8) long by 43–85 (56 ± 13; 8) wide, containing coiled cirrus; cirrus densely covered with narrow, uncinate spinitriches and capilliform filitriches throughout its length (Fig. 4H, I). Vagina relatively thick-walled, straight to weakly sinuous, extending along medial line of proglottid from ootype to anterior margin of cirrus sac, then laterally following anterior margin of cirrus sac to open into common genital atrium anterior to cirrus; vaginal sphincter absent; seminal receptacle not seen. Ovary occupying posterior third of proglottid, inverted A-shaped in frontal view, bilobed in cross section, with weakly lobulate margins, 73–125 (100 ± 23; 8) wide at isthmus, essentially symmetrical; poral lobe 85–250 (160 ± 56; 8) long; aporal lobe 95–258 (161 ± 51; 8) long; ovarian isthmus in anterior half of ovary. Mehlis' gland posterior to ovarian isthmus. Vitellarium follicular, consisting of 2 lateral bands; each band consisting of 1 dorsal and 1 ventral column of follicles, extending from near anterior limit of field of testes to near posterior of ovary, interrupted by terminal genitalia; vitelline follicles somewhat irregular in shape. Uterus medio-ventral, saccate, thin-walled, extending along medial line from ovarian isthmus to near anterior limit of field of testes. Excretory ducts 4, arranged in 1 dorsal and 1 ventral pair. Eggs not seen.
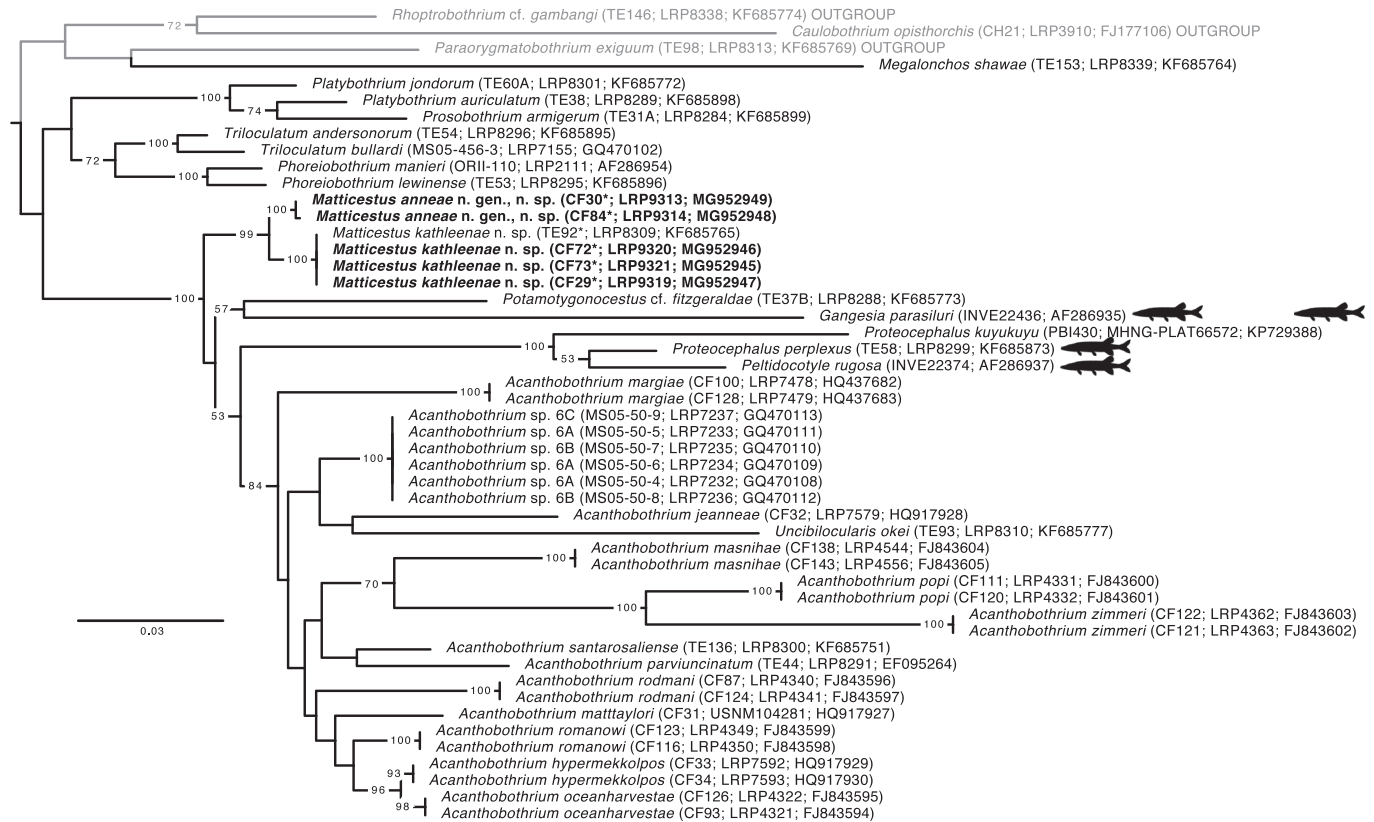
FIGURE 5.—Topology of optimal tree (i.e., with a likelihood score of −8945.7066) resulting from ML analysis; non-elasmobranch hosted onchoproteocephalideans indicated with fish icons. Nodal support given as ML bootstrap values; only bootstrap values >50% are presented. Taxon labels include (in parentheses) specimen numbers, accession numbers of vouchers, and GenBank numbers; * indicates specimens for which only photo vouchers have been deposited; specimens for which novel sequence data were generated are indicated in bold. Scale bar length indicates substitutions per site.

Taxonomic summary

Type and only known host: Pristis clavata Garman, dwarf sawfish (Rhinopristiformes: Pristidae).

Site of infection: Spiral intestine.

Type locality: Buffalo Creek near Darwin (12°20′11″S, 130°54′39″E), Northern Territory, Australia, Timor Sea, Indian Ocean.

Prevalence: Four of 4 hosts examined (100%).

Etymology: This species honors Kathleen Tebo for her more than 2 decades of phenomenal service as Administrative Assistant for the Department of Ecology and Evolutionary Biology at the University of Connecticut.

Specimens deposited: Holotype (QM G236764) and 3 paratypes (2 whole worms and 1 detached mature proglottid; QM G236765-G236767); 3 paratypes (2 whole worms, 1 detached mature proglottid; USNM 1470754-1470756); and 7 paratypes (3 whole worms and 1 detached mature proglottid [LRP 9322–9325]; 2 SEM vouchers [LRP 9326–9327]; and cross section series of mature proglottid and its scolex voucher [LRP 9328–9330]); specimens prepared for SEM retained with J.N.C. at the University of Connecticut. Photographic vouchers of specimens for which *28S rDNA* (D1–D3) sequence data were generated are deposited in the LRP (9319–9321).

Remarks

Matticestus kathleenae n. sp. is easily distinguished from its only described congener, *M. anneae*, in its larger size (1,465–2,925 vs. 860–1,100 in total length); greater number of proglottids (10–18 vs. 7–9); and, most conspicuously, in its greater number of testes (27–34 vs. 9–13). Assuming the base of the cirrus was visible in both species examined with SEM (Figs. 2J, 4H), the cirrus armature of the 2 species also appears to differ substantially in both the type and arrangement of spinitriches (Fig. 2J–L vs. Fig. 4H, I). Finally, the hooks of the 2 species differ somewhat in shape; whereas in *M. anneae* the abaxial prongs are straight, in *M. kathleenae* the abaxial prongs are slightly curved.

Molecular phylogeny

The matrix of sequences from all 49 specimens was 1,406 bp in length, of which 124 bp were excluded using Gblocks. The reduced data matrix comprised 765 constant and 517 variable characters, 377 of which were parsimony informative. In the ingroup alone, there were 790 constant characters and 492 variable characters, 349 of which were parsimony informative. The 4 specimens of *M. kathleenae* were identical in sequence. The 2 specimens of *M. anneae* differed from 1 another by 2 bp. The tree resulting from phylogenetic analysis of the partial *28S rDNA* sequence data is shown in Figure 5.

The analysis yielded 2 highly supported clades of *Matticestus* specimens, each of which consisted of the replicate specimens of the 2 new species. In combination, these 2 groups composed a well-supported clade that grouped among, but independently from, the 8 other genera of elasmobranch-hosted onchoproteocephalideans included in the analysis.

DISCUSSION

Our assignment of *Matticestus* to the order Onchoproteocephalidea is well supported from a morphological standpoint. Its species resemble most of the other elasmobranch-hosted species in the order in their possession of a scolex with 4 bothridia, each of which is armed with a pair of hooks, and proximal bothridial surfaces and cephalic peduncle with large, gladiate spinitriches that extend throughout the length of the stroblia (Figs. 1A, D, 2A, 3A, D). From a molecular standpoint, consistent with the results of Caira et al. (2014), the tree resulting from our analysis of *28S rDNA* data place the genus among the elasmobranch-hosted onchoproteocephalideans. That said, here we found it to group as sister to a clade consisting of the Proteocephalidae + the single representatives of *Potamotrygonocestus* + species of *Acanthobothrium* and the single species of *Uncibilocularis*, rather than as sister to a clade consisting of only the latter 2 genera as was found by Caira et al. (2014). Clearly, the affinities of *Matticestus* within the onchoproteocephalideans remain uncertain given the hypothesized sister-group of the genus was not well supported in the trees resulting from either the Caira et al. (2014) or our analyses.

Our work does nothing to help inform the affinities of *Megalonchos*, which was included here as an ingroup for the sake of complete representation of putative genera of elasmobranch-hosted onchobothriideans for which comparable sequence data are available. Based on its conspicuous morphological resemblance to the elasmobranch-hosted onchoproteocephalideans, Caira et al. (2014, 2017) persist in considering it a member of that order. That ordinal placement is, however, not supported by the results of existing molecular analyses, in which it is positioned at various points across the cestode tree, often outside of the Onchoproteocephalidea. Resolution of this conundrum awaits the application of sequence data for additional molecular markers.

The unusually small size of species of *Matticestus*, given the size of their definitive hosts, which may attain a total length of more than 3 m (Last et al., 2016), raises interesting questions about the possible final intermediate hosts of members of this genus. There is no reason to believe the biology of *Matticestus* differs from that of essentially all other marine cestodes (Caira and Reyda, 2005). Thus, we hypothesize that the life cycle of species of *Matticestus* involves a sequence of several different hosts, but also that the metacestodes are trophically transmitted among these hosts. In this context, the diet data available for *P. clavata* are interesting to consider. In the Kimberley region of Western Australia, Thorburn et al. (2008) reported the popeye mullet (*Rhinomugil nasutas* [De Vis]) as the major prey item they were able to identify in the stomach contents of *P. clavata*, although evidence of prawns was also found. In his unpublished Master's thesis, Peverell (2009) reported the stomach contents of *P. clavata* in northern Australia to include sciaenids such as *Nibea squamosa* Sasaki, leptobramids such as *Leptobrama*

muelleri Steindachner, clupeids, and mugilids. Beyond these teleosts, he also reported finding crustaceans such as the banana shrimp, *Penaeus merguiensis* de Man, in the stomachs of some specimens. As a consequence, all of the above should be considered possible intermediate hosts for the metacestodes of species of *Matticestus*.

The global survey work we have conducted over the past several decades (see Caira and Jensen, 2017) leads us to believe that species of *Matticestus* are exclusively parasites of sawfishes. This highly simplifies estimation of the global diversity of this cestode genus because the number of sawfish species inhabiting the planet is extremely limited. In the most recent taxonomic treatment of the rays of the world, Last et al. (2016) following the work of Faria et al. (2013), recognized the following valid species of sawfishes: *Anoxypristis cuspidata* (Latham), *P. clavata*, *P. pectinata*, *Pristis pristis* (L.), and *Pristis zijsron* Bleeker. Recognition of *Pristis microdon* Latham as a sixth valid species has yet to be resolved. *NADH2* sequence data generated by Naylor et al. (2012a) provide evidence that the Australian and Atlantic specimens attributed to *P. pristis* are distinct; thus, *P. microdon* should perhaps be considered valid rather than a synonym of *P. pristis*. Regardless, the global total of pristids would not exceed 6 species. Beyond the 2 species of *Matticestus* described here from *P. clavata*, the specimens from Dr. Tom Mattis confirm the existence of a third member of the genus in *P. pectinata*, and our collections over the past several decades from *P. zijsron* and *P. pristis* (or *P. microdon*) from Australian waters have yielded yet additional species of the new genus, all of which await formal description. Thus, we predict that species of *Pristis* will ultimately be found to host, at a maximum, 10 species of *Matticestus* in total.

In contrast, we predict that the monotypic *Anoxypristis* White and Moy-Thomas is not an appropriate host of species of *Matticestus*. Since 1991, we have had the opportunity to examine 7 specimens of *A. cuspidata* from several different localities in Australia. Unlike species of *Pristis*, none of these specimens was found to host *Matticestus*, suggesting that, among sawfishes, *Matticestus* is likely restricted to the genus *Pristis*. This is consistent with the results of the phylogenetic analyses of Naylor et al. (2012b), providing evidence that *Anoxypristis* is the sister taxon of the guitarfish genus *Glaucostegus* Bonaparte, rather than of *Pristis*—and thus the sawfishes do not represent a monophyletic group. Further supporting the restricted host associations of *Matticestus* is the fact that we have also examined 3–19 specimens of all 6 species of *Glaucostegus* recognized by Last et al. (2016) and none were found to host the new genus.

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