Journal of Parasitology 2020 106(6) 818–827

Published 21 December 2020

DOI: 10.1645/20-15

Contents and archives available through www.bioone.org or www.jstor.org

Journal of Parasitology

journal homepage: www.journalofparasitology.org

A NEW SPECIES OF *ACANTHOBOTHRIUM* (CESTODA: ONCHOPROTEOCEPHALIDEA) FROM THE SMALLEYE PYGMY SHARK, *SQUALIOLUS ALIAE* (CHONDRICHTHYES: SQUALIFORMES: DALATIIDAE), FROM TAIWAN

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KEY WORDS

Acanthobothrium katherineae
Onchoproteocephalidea
Squaliolus aliae
Dalatiidae
Squaliformes
Taiwan
Molecular Phylogeny

ABSTRACT

The cestode fauna of *Squaliolus aliae* was examined for the first time following the collection of elasmobranch specimens from Taiwan in 2005, 2013, and 2017. This small shark was found to host 2 tapeworm species. These consist of *Acanthobothrium katherineae* n. sp., which is new to science and is described herein, and a second species, in the genus *Scyphophyllidium*, which also appears to be new, but which is represented by insufficient material for formal description. *Acanthobothrium katherineae* is a category 5 species. It can be distinguished from 5 of the 19 other category 5 species in that it is apolytic, retaining proglottids on its strobila until they are gravid. This new species differs from the remaining 14 category 5 species in its combination of the following features: It is a smaller worm, has fewer than 100 proglottids, has a relatively short cephalic peduncle, and differs in bothridial size and loculus ratio. Sequence data for the D1–D3 region of the *28S rDNA* gene were generated for one specimen of *A. katherineae*. This sequence, along with comparable sequence data for adults of 14 described and 2 undescribed species as well as specimens of 6 undescribed larval members of the genus, was included in a maximum likelihood phylogenetic analysis. The resulting tree places the shark-hosted *A. katherineae* within a clade of stingray-hosted species, with *Acanthobothrium romanowi* as its sister taxon. *Acanthobothrium katherineae* is 1 of only 19 *Acanthobothrium* species known to parasitize sharks. The tree resulting from this study, which is preliminary given the relatively poor taxon sampling of the diversity in the genus, included 3 of the shark-parasitizing *Acanthobothrium* species and suggests that all 3 represent host-switching events. This is the first report of an *Acanthobothrium* species from the family Dalatiidae and the first report of a *Scyphophyllidium* species from the order Squaliformes. These findings suggest that other members of the Squaliformes, many of which have not previously been examined for parasites, may host additional novel cestode taxa.

A substantial amount of research over the last decade has focused on the discovery and description of cestodes in sharks (Caira and Jensen, 2017). Nonetheless, the cestodes parasitizing sharks of the order Squaliformes remain poorly known. Our knowledge of the cestodes of members of the squaliform family Dalatiidae Gray is especially deficient. This fauna is particularly intriguing because, with some species reaching a total length (TL) of less than 30 cm, the group includes some of the world's smallest sharks. The data-deficient status of these taxa is largely a result of the fact that as small, deep-water sharks, they are of limited commercial value and are thus not targeted by fishers (Compagno et al., 2005). As a consequence, they are rarely encountered in fish markets, which typically serve as one of the primary sources of elasmobranchs for parasite work. This makes the cestodes of these sharks especially challenging to collect.

During visits to fish markets in Taiwan in 2005, 2013, and 2017, we were fortunate to encounter relatively fresh specimens of the Taiwan smalleye pygmy shark, *Squaliolus aliae* Teng, among the by-catch of local trawling vessels. Here, we provide the first insights into the cestode fauna of this species, the smallest known species of shark in the world (Compagno et al., 2005), which resulted from examination of the spiral intestines of these shark specimens. A new species of *Acanthobothrium* is described herein. Sequence data for the D1–D3 region of the *28S rDNA* gene were generated to examine this taxon's affinities to the other members

Version of Record, first published online with fixed content and layout, in compliance with ICZN Arts. 8.1.3.2, 8.5, and 21.8.2 as amended, 2012. ZooBank publication registration: urn:lsid:zoobank.org:pub:0172B8D1-D8FF-4909-B4F4-BB04C725E11E.

of the genus for which comparable molecular data are available. One of the sharks examined also hosted juvenile specimens of what likely represents a new species of the phyllobothriidean genus *Scyphophyllidium* Woodland, 1927 (sensu Caira et al., 2020). Although the juvenile state of these specimens prevents the formal description of this species at this time, its existence is reported here to encourage future work on this species.

MATERIALS AND METHODS

Specimen collection

In total, 13 specimens of *Squaliolus aliae* that were landed in fish markets in Taiwan as by-catch of trawling vessels in the Taiwan Strait were examined for cestodes. Each shark was assigned a unique combination of collection code and collection number, and photographs and measurements were taken. More detailed information for these specimens can be accessed in the Global Cestode Database (Caira et al., 2019) by searching for the collection code and collection number. The sharks examined came from fish markets at the following localities: Dasi on 11 May 2005 (1 specimen, TW-6), and Donggang on 7 November 2013 (2 specimens, TW-89, TW-90), 6 January 2017 (6 specimens, TW-110, TW-111, TW-112, TW-115, TW-116, TW-117), 9 January 2017 (1 specimen, TW-180), and 10 January 2017 (3 specimens, TW-201, TW-205, TW-206). Nine of the sharks examined were female, 10.5–22.2 in total length (TL), and 4 were male, 13.6–21.5 in TL. The abdominal cavity of each shark was opened with a mid-ventral incision, and a small sample of liver was taken and preserved in 95% ethanol for molecular verification of host identity. The spiral intestine was then removed and opened with a mid-ventral longitudinal incision. A specimen of each of the 2 species of cestodes was preserved in 95% ethanol for molecular sequencing; the remaining worms were preserved in 10% seawater-buffered formalin (9:1) for examination with light and scanning electron microscopy (SEM). The spiral intestine of each shark was also preserved in either 95% ethanol or seawater-buffered formalin. After approximately 1 wk, cestodes and spiral intestines preserved in seawater-buffered formalin were transferred to 70% ethanol for storage. Cestodes and spiral intestines preserved in 95% ethanol were transferred to new 95% ethanol and stored in a –20 C freezer. Preserved spiral intestines were subsequently examined under an Olympus SZ-30 dissecting microscope, and any additional cestode material was removed.

Morphological methods

Cestodes were prepared for light microscopy as follows: Tapeworms were hydrated in a graded ethanol series, stained for 20 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). All measurements, unless otherwise stated, are presented in micrometers as ranges followed in parentheses by the mean, standard deviation, total number of specimens measured, and total number of measurements taken when more than 1 measurement per specimen was made. One scolex was prepared for SEM as follows. It was 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. It was then mounted on a double-sided PELCO carbon tab (Ted Pella Inc., Redding, California) on an aluminum stub, sputter-coated with 35 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). Hook measurements follow Ghoshroy and Caira (2001). Museum abbreviations used are as follows: LRP, Lawrence R. Penner Parasitology Collection, University of Connecticut, Storrs, Connecticut; NMNS, National Museum of Natural Science, Zoology Department, Taichung, Taiwan; USNM, U.S. National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Molecular methods

Total genomic DNA was extracted from 1 free proglottid (as a consequence, there is no voucher) of the new species of *Acanthobothrium* using a MasterPure™ DNA Purification Kit (EpiCentre Technologies, Madison, Wisconsin) following the manufacturer's instructions. Extractions were left at 65 C with gentle shaking overnight to allow the DNA to go into solution. The quality of the extraction was assessed using a NanoDrop 2000 micro-volume spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). The D1–D3 region of the *28S rDNA* gene was amplified using the LSU-5 (5′-TAGGTCGACCCGCTGAAYTTA-3′) (Littlewood et al., 2000) and LSU-1500R (5′-GCTATCCTGGAGGGAAACTTCG-3′) (Tkach et al., 2003) primers. Amplification was achieved in a solution of 1 µl of DNA as a template, 0.1 µl of 10 M solution of each primer, 5 µl of GoTaq® Green Master Mix (Promega, Fitchburg, Wisconsin), and 3.8 µl of water. Polymerase chain reaction (PCR) was completed using the following conditions: Initial denaturation for 1 min at 94 C, followed by 40 cycles of annealing for 30 sec at 94 C, 1 min at 56 C, and 1 min at 72 C, and elongation for 5 min at 72 C. PCR product was cleaned with 1 µl of ExoSAP-IT.7 (Affymetrix, Inc., Santa Clara, California). Sequencing was performed with the internal primers LSU-55F (5′-AACCAGGATTCCCCTAGTAACGGC-3′) (Bueno and Caira, 2017) and LSU-1200R (5′-GCATAGTTCACCATCTTTCGG-3′) (Littlewood et al., 2000) on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, California) using ABI Big Dye™ dideoxy terminators (version 3.1).

Phylogenetic analysis

Sequence data for the D1–D3 region of the *28S rDNA* gene were generated de novo from 1 specimen of the new species of *Acanthobothrium* and were deposited in GenBank (accession no. MT395344). These data were combined with comparable data for adult and larval specimens of *Acanthobothrium* available in GenBank. In many cases, sequence data available in GenBank consisted solely of data for the D2 (i.e., most variable) region of the *28S rDNA* gene. In other cases, data for the D1–D3 region of

the *28S rDNA* gene were available. We conducted a preliminary analysis using only D2 data for all specimens as well as an analysis using D2 and D1–D3 data if available. Given no differences were found in tree topology, the results presented are for the larger data set, which includes both D2 and D1–D3 data. Based on the results of Caira et al. (2014), *Potamotrygonocestus* cf. *fitzgeraldae* was included to serve as the outgroup taxon. Details of the specimens included in our phylogenetic analysis are provided in Table I. Contigs were assembled and sequences were trimmed in Geneious 8.0.5 (Biomatters, Newark, New Jersey). Sequences were aligned in Geneious 8.0.5 using MUSCLE. jModelTest v. 2.1.10 (Guindon and Gascuel, 2003; Darriba et al., 2012) was used to determine the best-fitting model of evolution based on the evaluation of 88 models on the CIPRES Science Gateway (Miller et al., 2012). Goodness of fit was evaluated with sample-size-corrected Akaike information criterion (AICc) values. A maximum likelihood (ML) analysis was conducted using Garli v. 2.01 (Zwickl, 2006) on the CIPRES Science Gateway models. Default Garli configuration settings were used, with the following exceptions: The starting tree topology was set to "random," the number of attachment branches evaluated per terminal was set to 104 (i.e., twice the number of terminals in the matrix), and the number of independent search replicates was set to 100. Based on the results of the jModelTest analysis, the model of evolution employed was GTR + I + γ. Bootstrap (BS) values resulting from 1,000 bootstrap replicates were generated with Garli v. 201 using the above configuration settings. SumTrees v. 4.0.0 in DendroPy v. 4.0.3 (Sukumaran and Holder, 2010) was used to display the bootstrap values on the best tree.

DESCRIPTIONS

Acanthobothrium katherineae n. sp.

(Figs. 1, 2)

Description (based on 1 complete gravid worm, 1 partial submature worm, 1 pre-gravid strobila [i.e., the SEM voucher], 4 free gravid proglottids, and 1 scolex observed with SEM): Worms 21 mm (n = 1) long, greatest width at level of terminal gravid proglottid, 90 (n = 1) proglottids per worm, apolytic. Scolex consisting of scolex proper and cephalic peduncle. Scolex proper 698–718 (708 ± 14; 2; 2) long by 580–646 (612 ± 47; 2; 2) wide with 4 muscular bothridia. Bothridia 590–659 (630 ± 29; 2; 4) long by 265–329 (311 ± 31; 2; 4) wide, free posteriorly, each with 3 loculi and specialized anterior region in form of muscular pad; muscular pad 50–62 (57 ± 6; 2; 4) long by 148–183 (162 ± 16; 2; 4) wide, bearing apical sucker and 1 pair of hooks; apical sucker 30–35 (33 ± 2; 2; 4) long by 54–127 (81 ± 33; 2; 4) wide; anterior loculus substantially longer than other 2 loculi, 336–417 (374 ± 44; 2; 4) long; middle loculus 52–103 (80 ± 21; 2; 4) long; posterior loculus 68–93 (78 ± 12; 2; 4) long; loculus ratio (anterior:middle:posterior) 1:0.21:0.21; velum present between medial margins of adjacent dorsal and ventral bothridia. Hooks bipronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, smooth; axial prongs longer than abaxial prongs. Lateral hooks slightly larger than medial hooks. Lateral hook measurements: A 46–65 (58 ± 8; 3; 4), B 86–119 (100 ± 17; 2; 3), C 48–62 (58 ± 7; 2; 3), D 116–158 (140 ± 20; 2; 4). Medial hook measurements: A′ 51–62 (58 ± 4; 3; 4), B′ 76–102 (92 ± 13; 3; 4), C′ 43–63 (53 ± 10; 2; 3), D′ 133–148 (140 ± 7; 3; 4); bases of lateral and medial hooks closely associated. Cephalic peduncle 276 (n = 1) long by 379 (n = 1) wide at mid-level.

Muscular pad (Fig. 2B) and proximal surfaces of bothridial rims (Fig. 2D) with papilliform filitriches; proximal bothridial surfaces immediately adjacent to rim with acicular filitriches (Fig. 2E); proximal bothridial surfaces away from rim densely covered with mixture of small and large gladiate spinitriches, with spinitriches becoming denser as distance from rim increases (Fig. 2F, G), filitriches not observed; gladiate spinitriches of conspicuously different sizes on regions nearer rims (Fig. 2F), more similar in size on middle of proximal surfaces (Fig. 2G) and between adjacent pairs of bothridia (Fig. 2H). Distal bothridial surfaces with acicular filitriches (Fig. 2C). Cephalic peduncle densely covered with gladiate spinitriches and some acicular filitriches (Fig. 2I).

Proglottids acraspedote, protandrous. Immature proglottids 38–80 (53 ± 23; 3) in number, wider than long; mature/pre-gravid proglottids 6–7 (6.5 ± 0.7; 2) in number, longer than wide; gravid proglottids 1 (n = 1) in number, longer than wide. Terminal pre-gravid proglottid 721 (n = 1) long by 875 (n = 1) wide, length:width ratio 0.8:1; terminal gravid proglottid 1,150 long by 813 wide (n = 1), length:width ratio 1.4:1; detached gravid proglottids 1,928–2,645 (2,346 ± 373; 3) long by 845–935 wide (904 ± 51; 3), length:width ratio 2.5:1. Genital pores marginal, irregularly alternating, 36% (n = 1) of proglottid length from posterior end in terminal pre-gravid proglottid; 44% (n = 1) of proglottid length from posterior end in terminal gravid proglottid; 33–41% (38 ± 5; 3) of proglottid length from posterior end in detached gravid proglottids. Testes arranged in 4 to 5 irregular columns anterior to ovary, 1 layer deep, oval in frontal view, 16–35 (23 ± 6; 3; 10) long by 34–65 (50 ± 11; 3; 10) wide, 55–69 (62 ± 5; 2; 7) in total number, 3 (3; 10) in number in post-poral field. Vas deferens extensive, coiled, extending from near anterior margin of uterus to cirrus sac. Cirrus sac weakly pyriform, 219–224 (222 ± 4; 2; 2) long by 65–83 (74 ± 13; 2; 2) wide in posterior-most pre-gravid proglottids; 205 long by 95 wide in terminal gravid proglottid (n = 1), thin-walled, containing highly coiled cirrus and sperm duct; cirrus armed with small spinitriches in proximal regions. Vagina relatively thick-walled, sinuous, extending along midline of proglottid from ootype to anterior margin of the cirrus sac, then laterally at anterior margin of cirrus sac to open into genital atrium anterior to cirrus sac, vaginal sphincter lacking; seminal vesicle not seen. Ovary at posterior margin of proglottid, lobulated, H-shaped in frontal view, symmetrical, 153–203 (178 ± 36; 2; 2) long by 452–483 (466 ± 23; 2; 2) wide in terminal-most pre-gravid proglottids. Ovarian isthmus slightly posterior to mid-level of ovary; Mehlis' gland posterior to ovarian isthmus. Vitellarium follicular; vitelline follicles ovoid, arranged in 2 lateral bands; each band consisting of 2 columns of follicles extending from near anterior margin of proglottid to slightly overlap anterior margins of ovary, interrupted dorsally and ventrally by terminal genitalia. Uterus medio-ventral, saccate, thin-walled, extending from ovary to near anterior margin of proglottid. Excretory ducts in 2 dorsal and 2 ventral pairs. Eggs in utero in gravid proglottid essentially spherical 18–27 (23 ± 3; 1; 10) long by 16–33 (26 ± 5; 1; 10) wide.

Table I. List of specimens included in the phylogenetic analysis with GenBank accession numbers, host, locality data, and source.

Taxon	GenBank no. (D1–D3 *28S rRNA*)	Host	Locality	Source
Acanthobothrium brevissime	EU170363* EU170364*	*Branchiostoma floridae*	Tampa Bay, Florida	Holland et al. (2009)
Acanthobothrium brevissime	FJ357445*	*Ensis minor*	Tampa Bay, Florida	Holland and Wilson (2009)
Acanthobothrium hypermekkolpos	HQ917929; HQ917930	*Rhynchobatus laevis*	Gulf of Carpentaria, Australia	Fyler and Caira (2010)
Acanthobothrium jeanneae	HQ917928	*Rhynchobatus laevis*	Gulf of Carpentaria, Australia	Fyler and Caira (2010)
Acanthobothrium katherineae n. sp.	MT395344	*Squaliolus aliae*	Gulf of Taiwan, Taiwan	This study
Acanthobothrium margieae	HQ437682; HQ437683	*Orectolobus japonicus*	East China Sea, Taiwan	Fyler (2011)
Acanthobothrium masnihae	FJ843604; FJ843605	*Urogymnus polylepis*†	Kinabatangan River, Malaysian Borneo	Fyler and Caira (2006)
Acanthobothrium matttaylori	HQ917927	*Rhynchobatus laevis*	Gulf of Carpentaria, Australia	Fyler and Caira (2010)
Acanthobothrium oceanharvestae	FJ843594; FJ843595	*Urogymnus acanthobothrium*‡	Arafura Sea, Australia	Fyler et al. (2009)
Acanthobothrium parviuncinatum	EF095264	*Urobatis maculatus*	Gulf of California, México	Waeschenbach et al. (2007)
Acanthobothrium popi	FJ843600; FJ843601	*Urogymnus acanthobothrium*‡	Arafura Sea, Australia	Fyler et al. (2009)
Acanthobothrium rodmani	FJ843596; FJ843597	*Urogymnus acanthobothrium*‡	Arafura Sea, Australia	Fyler et al. (2009)
Acanthobothrium romanowi	FJ843598; FJ843599	*Urogymnus acanthobothrium*‡	Arafura Sea, Australia	Fyler et al. (2009)
Acanthobothrium santarosaliense	KF685751	*Heterodontus mexicanus*	Gulf of California, México	Caira et al. (2014)
Acanthobothrium wedli	MH924011*; MH924012*; MH924013*	*Dipturus nasutus*	Nugget Point and Cape Saunders, New Zealand	Bennett et al. (2019)
Acanthobothrium zimmeri	FJ843602; FJ843603	*Urogymnus acanthobothrium*‡	Arafura Sea, Australia	Fyler et al. (2009)
Acanthobothrium sp. 1	AF286953	*Dasyatis longus*	Gulf of California, México	Olson et al. (2001)
Acanthobothrium sp. 6A	GQ470109	*Dasyatis say*	Gulf of Mexico, Mississippi	Jensen and Bullard (2010)
Acanthobothrium sp. 6B	GQ470110	*Dasyatis say*	Gulf of Mexico, Mississippi	Jensen and Bullard (2010)
Acanthobothrium sp. 6C	GQ470113	*Dasyatis say*	Gulf of Mexico, Mississippi	Jensen and Bullard (2010)
Acanthobothrium sp.	GQ470116; GQ470117; GQ470118; GQ470119; GQ470122; GQ470123	*Diplectrum formosum*	Gulf of Mexico, Mississippi	Jensen and Bullard (2010)
Acanthobothrium sp.	GQ470121	*Cynoscion nebulosus*	Gulf of Mexico, Mississippi	Jensen and Bullard (2010)
Acanthobothrium sp.	GQ470115	*Lagodon rhomboides*	Gulf of Mexico, Mississippi	Jensen and Bullard (2010)
Acanthobothrium sp.	MH924014*	*Netocarcinus antarcticus*	Nugget Point and Cape Saunders, New Zealand	Bennett et al. (2019)
Acanthobothrium sp.	GQ470114; GQ470124	*Paralichthys lethostigma*	Gulf of Mexico, Mississippi	Jensen and Bullard (2010)
Acanthobothrium sp.	MH924015*	*Trachurus novaezelandiae*	Nugget Point and Cape Saunders, New Zealand	Bennett et al. (2019)
Acanthobothrium sp.	FJ843592; FJ843593	*Urogymnus lobistoma*‡	South China Sea, Malaysian Borneo	Fyler et al. (2009)
Potamotrygonocestus cf. *fizgeraldae*	KF685773	*Potamotrygon castexi*	Madre de Dios River, Peru	Caira et al. (2014)

* Sequence data available for only D2 region of *28S rDNA* gene.
† As *Himantura polylepis*.
‡ As *Himantura lobistoma*.

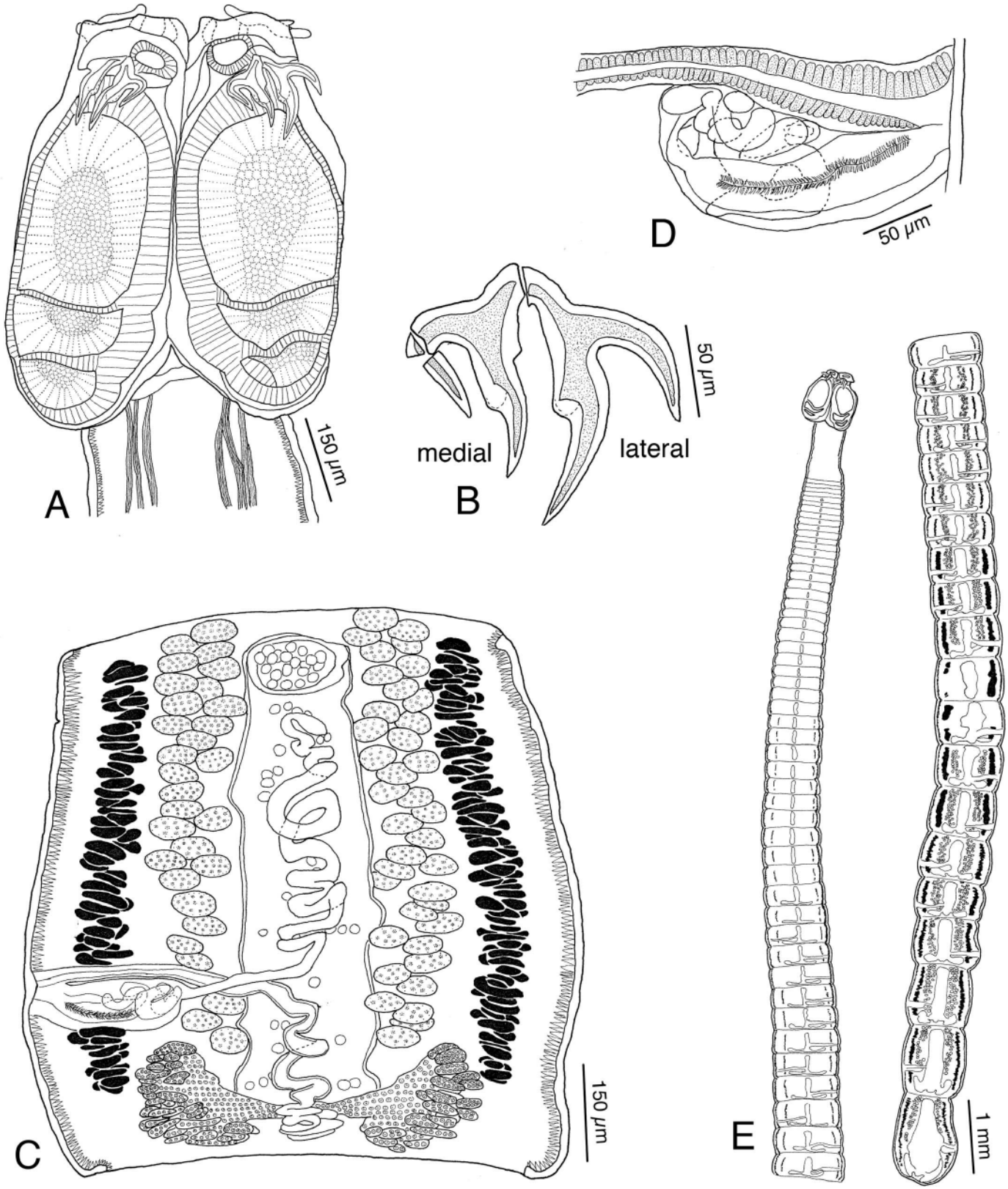

Figure 1. Line drawings of *Acanthobothrium katherineae* n. sp. (**A**) Scolex (paratype, USNM no. 1618754). (**B**) Hooks (paratype, USNM no. 1618754). (**C**) Subterminal gravid proglottid (holotype, NMNS no. 8249-001). (**D**) Terminal genitalia (holotype, NMNS no. 8249-001). (**E**) Whole worm (holotype, NMNS no. 8249-001).

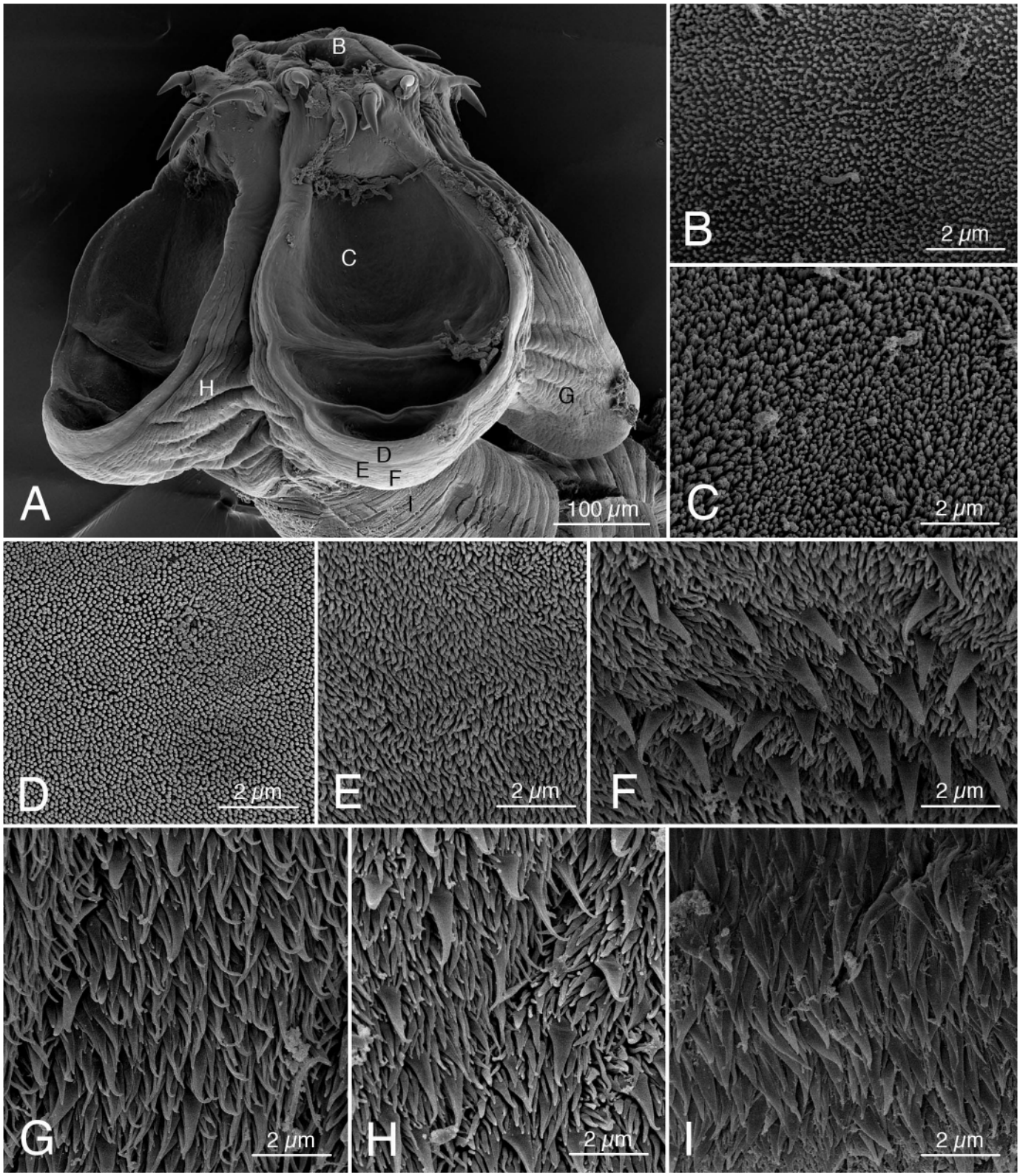

Figure 2. Scanning electron micrographs of *Acanthobothrium katherineae* n. sp. **(A)** Scolex; small letters indicate locations of Figures 2B–I. **(B)** Anterior rim of muscular pad. **(C)** Distal bothridial surface. **(D)** Proximal surface of bothridial rim. **(E)** Proximal surface immediately adjacent to bothridial rim. **(F)** Proximal surface away from bothridial rim. **(G)** Proximal surface on middle of bothridia. **(H)** Surface between adjacent pairs of bothridia. **(I)** Cephalic peduncle.

Taxonomic summary

Type and only known host: Squaliolus aliae Teng, Taiwan smalleye pygmy shark (Squaliformes: Dalatiidae).

Site of infection: Spiral intestine.

Type locality: Taiwan Strait, landed at Donggang (22°21′58.4″N, 120°26′39.1″E), Pingtung Province, Taiwan.

Prevalence: Three of 13 sharks examined (23%).

Intensity: One worm per host.

Specimens deposited: Holotype (gravid whole worm, NMNS no. 8249-001); 1 paratype (free gravid proglottid, LRP no. 10260); 1 paratype (pre-gravid strobila, voucher for SEM, LRP no. 10015); 1 paratype (free gravid proglottid, LRP no. 10016); 1 paratype (partial sub-mature worm, USNM no. 1618754); 2 paratypes (free gravid proglottids, USNM nos. 1618752 and 1618753).

ZooBank registration: urn:lsid:zoobank.org:act:565CEEEB-F6EB-4DA4-A3C0-2153B6F36A85.

Etymology: This species honors Katherine Gallagher, the senior author's mother, for her support and encouragement of the senior author's academic pursuits.

Remarks

Acanthobothrium katherineae n. sp., based on the criteria of Ghoshroy and Caira (2001), is a category 5 species; it is more than 15 mm in total length, possesses more than 50 proglottids, has fewer than 80 testes, and has a symmetrical ovary. According to the information provided in Ghoshroy and Caira (2001), Fyler and Caira (2006), and the original descriptions of *Acanthobothrium* species, *A. katherineae* is 1 of 20 category 5 species. It is easily distinguished from *Acanthobothrium angelae* Campbell and Beveridge, 2002, *Acanthobothrium hispidium* Riser, 1995, *Acanthobothrium indicum* Subhapradha, 1955, *Acanthobothrium maryanskii* Caira and Burge, 2001, and *Acanthobothrium paulum* Linton, 1890 in that it is apolytic, retaining proglottids on its strobila until they are gravid. The new species is much smaller than *Acanthobothrium giganticum* Sanaka, Vijaya, Lakshmi, and Hanumantha Rao, 1993, *Acanthobothrium rajaebatis* (Rudolphi, 1910) Euzet, 1959, and *Acanthobothrium xiamenensis* Yang and Lin, 1994 (21 mm vs. 10–11 cm, 50–60 mm, and 140–160 mm, respectively). *Acanthobothrium katherineae* can be distinguished from *Acanthobothrium confusum* Baer and Euzet, 1962, *Acanthobothrium inbiorium* Marques, Centritto, and Stewart, 1997, and *Acanthobothrium manteri* Hassan, 1983 in its possession of fewer proglottids (90 vs. 250, 156–223, and 120–170, respectively). It differs from *Acanthobothrium amazonensis* Mayes, Brooks, and Thorson, 1978, *Acanthobothrium edmondsi* Campbell and Beveridge, 2002, *Acanthobothrium franus* Marques, Centritto, and Stewart, 1997, *Acanthobothrium lintoni* Goldstein, Henson, and Schlicht, 1968, and *Acanthobothrium regoi* Brooks, Mayes, and Thorson, 1981 in that it has a shorter cephalic peduncle (276 vs. 423–677, 432–880, 1,300–3,600, 1,152, and 900, respectively). The new species has longer bothridia than *Acanthobothrium quinonesi* Mayes, Brooks, and Thorson, 1978 and *Acanthobothrium rhinobati* Alexander, 1953 (590–659 vs. 367–479 and 230–300, respectively). The new species has fewer post-poral testes than *Acanthobothrium goldsteini* Appy and Dailey, 1973 and *Acanthobothrium psammobati* Carvajal and Goldstein, 1969 (2–3 vs. 6–9 and 9, respectively).

An additional 6 species have not officially been assigned to a category but may be category 5 species based on their original descriptions. These species are *Acanthobothrium filicolle* (Zschokke, 1888) Yamaguti, 1959, *Acanthobothrium pintanensis* Wang, 1984, *Acanthobothrium elongatum* Subhapradha, 1955, *Acanthobothrium herdmani* Southwell, 1912, *Acanthobothrium ponticum* Léon Borcea, 1934, and *Acanthobothrium urogymni* (Hornell, 1912) Southwell, 1925. *Acanthobothrium katherineae* can be distinguished from *A. filicolle* in that the axial prongs of its hooks are conspicuously longer than the abaxial prongs, whereas the 2 prongs are of essentially equal length in *A. filicolle*. It has longer bothridia than *A. pintanensis* (590–659 vs. 192–240). This new species is a much smaller worm than *A. elongatum*, *A. herdmani*, *A. ponticum*, and *A. urogymni* (21 vs. 160, 63, 150, and 250 mm, respectively).

Phylogenetic analysis

In total, 1,143 bp of sequence data for the D1–D3 region of the *28S rDNA* gene were generated for 1 specimen of *A. katherineae*. The tree resulting from the ML analysis of the 51 terminals in the data matrix, which appear to represent adults of 17 species and larvae of potentially an additional 6 species, is shown in Figure 3. The most striking result of this analysis was that the taxon that grouped as the sister of the shark-hosted *A. katherineae*, albeit with a modest bootstrap value of 80%, was the stingray-hosted *Acanthobothrium romanowi* Fyler, Caira, and Jensen 2009 (i.e., in *Urogymnus acanthobothrium*).

Additional tapeworm specimens

Four immature specimens of a second species of tapeworm were found in the spiral intestine collected from the *S. aliae* specimen TW-6 in the fish market in Dasi. Whole mounts of these 4 specimens suggest they likely represent a new species that appears to belong to *Scyphophyllidium* category 5 of Caira et al. (2020). To our knowledge, this is the first report of this genus from a shark of the order Squaliformes. Unfortunately, the collection of mature specimens is necessary to confirm the identity and novelty of this species. All 4 specimens have been deposited in the LRP (nos. 10017–10020).

DISCUSSION

This is the first report of a species of *Acanthobothrium* from a member of the family Dalatiidae and also of the genus *Squaliolus* Smith. Furthermore, it is only the third report of *Acanthobothrium* from a squaliform shark. Both of the previous reports came from species of the squalid genus *Squalus* L. *Acanthobothrium australis* Robinson, 1965 was reported from *Squalus megalops* (Macleay) off the coast of Australia by Robinson (1965), and *Acanthobothrium polytesticularis* Wang and Yang, 2001 was reported by Wang and Yang (2001) from an unidentified species of *Squalus* off the coast of China. Except for being a much smaller worm (21 vs. 96 mm in TL), the new species closely resembles *A. australis* in several respects, most notably in proglottid anatomy and hook morphology. This raises the interesting question of whether the *Acanthobothrium* species parasitizing squaliform sharks may belong to the same clade of species. However, the answer to that question awaits a phylogenetic analysis with more extensive taxon sampling.

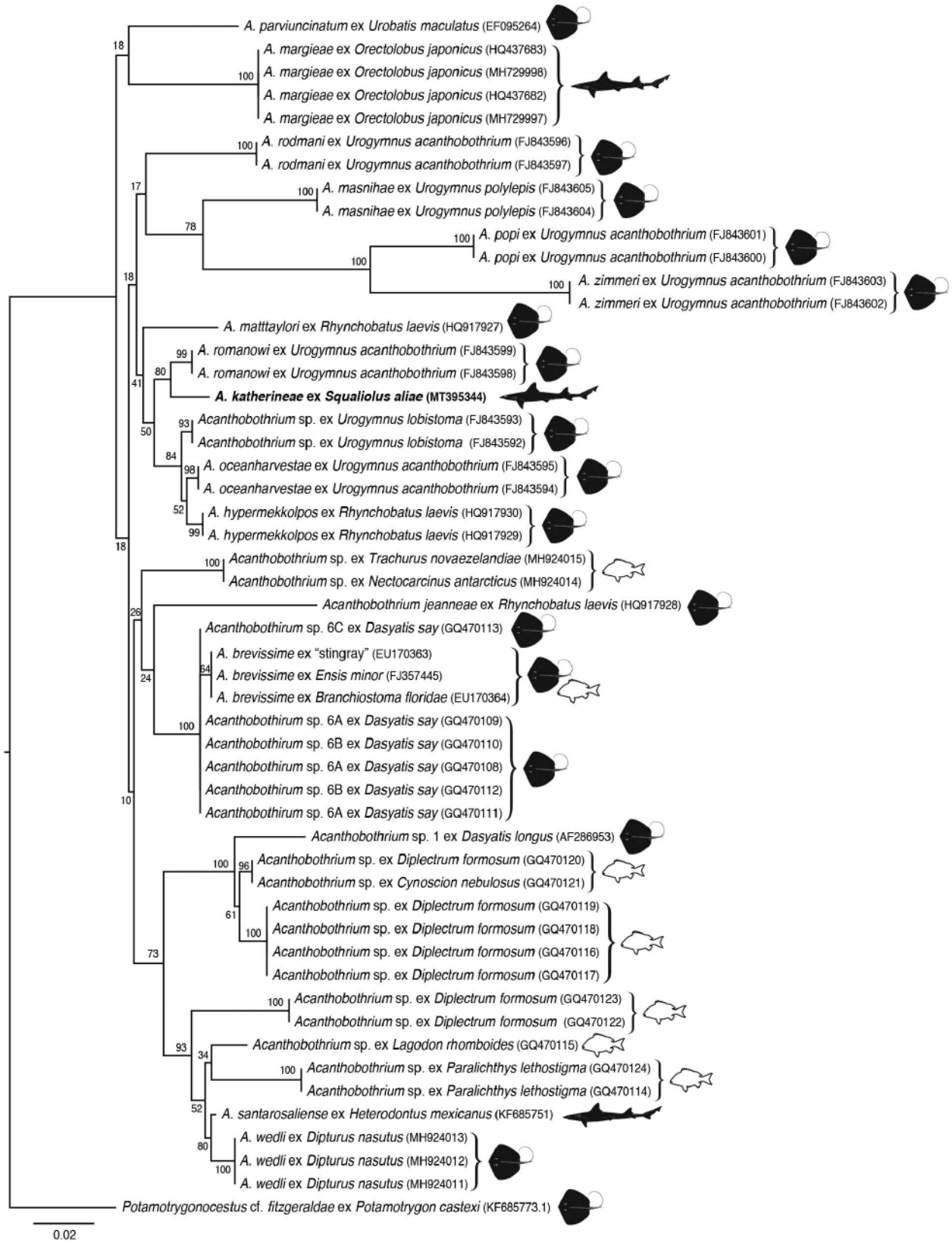

Figure 3. Phylogenetic tree resulting from maximum likelihood (ML) analysis of partial *28S rRNA* gene sequence data for *Acanthobothrium* spp.; placement of *Acanthobothrium katherineae* n. sp., based on new sequence data, is indicated in bold. *Potamotrygonocestus* cf. *fitzgeraldae* was used as the outgroup taxon. Taxon labels consist of published name, followed by host, GenBank number in parentheses, and icons for general categories of hosts. A stingray icon indicates the sequence came from an adult specimen collected from a batoid host; a shark icon indicates the sequence came from an adult specimen collected from a selachian host; a fish icon indicates the sequence came from a larval specimen collected from any type of intermediate host (i.e., teleost, lancelet, or mollusc). Nodal support is presented as ML bootstrap values. Scale bar indicates nucleotide substitutions per site.

Although the immature status of our material prevented its description, this is also the first report of a species of *Scyphophyllidium* from a species of the order Squaliformes. To date, the 45 described members of this genus parasitize sharks of the orders Carcharhiniformes, Lamniformes, and Orectolobiformes, and one stingray (see Caira et al., 2020). The formal description of this species, which will require the collection of additional material from *S. aliae* individuals, is certainly worth pursuing in the future.

In combination, the presence of *Scyphophyllidium* and *A. katherineae* in *S. aliae* individuals suggests that other members of the families Squalidae Bonaparte and Dalatiidae, the vast majority of which have never been examined for cestodes, may be fruitful hosts to explore for additional species of *Acanthobothrium* and *Scyphophyllidium*. Work conducted by Jensen and Bullard (2010), surveying intermediate hosts for larval tapeworms in the Gulf of Mexico, yielded specimens of *Acanthobothrium* only from bony fish. This suggests that members of the above 2 families whose diets include bony fish are likely to be productive candidates for further work, regardless of the small size of some of the shark species in these families. We note that, despite the small size of *S. aliae* individuals, they have been reported to feed on small mesopelagic fishes, among other prey items (Claes et al., 2012), suggesting that the intermediate host for *A. katherineae* may be a small bony fish. This further highlights the fact that host diet, rather than size, is important to consider in future work. Further support for this direction of work comes from the fact that all of the larval stages of species of *Scyphophyllium* reported by Jensen and Bullard (2010) also came from bony fish. Given the poorly sampled nature of the majority of species in the other families of squaliform sharks, these host species are also worth targeting in additional collecting efforts.

The tree resulting from our ML analysis is extremely preliminary because it was based on sequence data for only a portion of the *28S rDNA* gene, it represents only a small subset of the nearly 200 species of *Acanthobothrium*, and nodal support for many clades is weak. Nonetheless, it provides some interesting insights into the host associations of several members of the genus. Although by far the majority of the nearly 200 species of *Acanthobothrium* parasitize batoids, 19 species (including *A. katherineae*) parasitize sharks. The 3 *Acanthobothrium* species from sharks included in our analysis come from 3 of the 5 orders of sharks known to be parasitized by members of the genus. *Acanthobothrium katherineae* parasitizes a squaliform shark, *Acanthobothrium margiae* Fyler, 2011 parasitizes an orectolobiform shark, and *Acanthobothrium santarosaliense* Caira and Zahner, 2001 parasitizes a heterodontiform shark. These 3 species were distributed across the topology of the tree. The fact that each species is deeply nested within different clades of batoid-parasitizing species suggests that all 3 of these shark associations are the result of independent host-switching events. Given the oioxenous (sensu Euzet and Combes, 1980) host specificity of onchoproteocephalidean tapeworms (de Chambrier et al., 2017), and the fact that 2 of the tapeworm specimens were mature, it seems unlikely that this is an instance of accidental infection.

ACKNOWLEDGMENTS

We thank Kirsten Jensen and Kaylee Herzog for their assistance with fieldwork in Taiwan. We are especially grateful to Hsuan-Ching Ho of the National Museum of Marine Biology and the Institute of Marine Biology and Aquarium, National Dong Hwa University, for his support with the logistics and essentially all other aspects of our 3 collecting trips to Taiwan. Elizabeth Jockusch kindly allowed us to conduct the sequencing work in her laboratory at the University of Connecticut. This work was supported by National Science Foundation grants DEB 1921404, 1921411, 1457762, and 1457776. Any opinions, findings, conclusions, or recommendations expressed in this material are those of the authors and do not reflect the views of the National Science Foundation.

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