Article

A revision of the Rhoptrobothriidae (Cestoda: "Tetraphyllidea")

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Abstract

The Rhoptrobothriidae are one of the more enigmatic families of cestodes of elasmobranchs. Opinions on the taxonomic status of the family's three original genera (i.e., *Myzophyllobothrium*, *Rhoptrobothrium*, and *Myzocephalus*) have varied over the 115 years since they were erected. Some authors have considered all three valid, others have considered *Rhoptrobothrium* to be a synonym of *Myzopyllobothrium* or a *genus inquirendum*, yet others have considered *Myzocephalus* to be a synonym of the phyllobothriid genus *Thysanocephalum*. All three genera were established for specimens collected from eagle rays off Sri Lanka. The erection of *Mixophyllobothrium* for two specimens from a cowtail stingray off India three decades ago added additional confusion to the situation, with some authors considering it valid and others a synonym of *Myzocephalus*. These disagreements stem largely from differences in interpretation of the complex morphology of the scolex of members of these genera. Furthermore, with the exception of *Rhoptrobothrium* comprising four species, each genus is monotypic. All but *Rhoptrobothrium* has not been considered in detail for nearly a century, largely because of a lack of available material. The taxonomic status of these genera is assessed here based on light and scanning electron microscopy, and molecular data generated from new material collected from eagle rays off Indonesian and Malaysian Borneo, Japan, Sri Lanka, and Viet Nam. Morphological work indicates that the genera differ largely only in the degree of folding of the four remi that extend from the cephalic peduncle. A molecular phylogeny based on sequence data for the D1–D3 region of the *28S rRNA* gene, which include new data for eight specimens of four species, indicates that *Myzophyllobothrium*, *Myzocephalus*, and *Rhoptrobothrium* are not mutually monophyletic. The latter two genera and *Mixophyllobothrium* are considered synonyms of *Myzophyllobothrium* and five species are transferred to that genus. *Myzophyllobothrium okamuri* **n. comb.** is considered a *species inquirendum*. *Myzophyllobothrium nagasawai* **n. sp.** is described from *Aetobatus narutobiei* off Japan. *Myzophyllobothrium narinari* **n. comb.** is re-described based on newly collected cestodes from the type host and locality (i.e., *Aetobatus ocellatus* off Sri Lanka). Despite consisting of only a single genus, the family status of the group is retained in recognition of the unusual configuration of the scolex, which bears four biloculate bothridia and four remi extending from the cephalic peduncle. The ordinal placement of the family remains uncertain, but affinities with the Phyllobothriidea, rather than "Tetraphyllidea" are considered.

Key words: new species, *Myzophyllobothrium nagasawai* **n. sp.**, *Myzocephalus*, *Rhoptrobothrium*, Rhoptrobothriidae, eagle rays

Introduction

This study aims to resolve uncertainty surrounding the identity, morphology, and validity of a poorly known assemblage of four genera of tapeworms—many of which have not been seen for nearly a century—that parasitize members of the eagle ray genera *Aetobatus* Blainville and *Aetomylaeus* Garman. These tapeworms are unique among genera parasitizing elasmobranchs in their possession of a complex structure posterior to the bothridia consisting of four extensions of the cephalic peduncle referred to as remi (Jensen and Caira 2006). *Myzophyllobothrium* Shipley and Hornell, 1906 is monotypic. To our knowledge, the only collection of specimens beyond the type material of *Myzophyllobothrium rubrum* Shipley and Hornell, 1906 collected by Shipley and Hornell (1906) from the white-spotted eagle ray, *Aetobatus ocellatus* (Kuhl) (as *Aetobatis narinari* [Euphrasen]), off Sri Lanka (as Ceylon),

which also served as the source of the scolex illustrated by Joyeux and Baer (1961; fig. 290), is that of Southwell (1925) who reported collecting two specimens and several fragments of this species from the same host and locality in 1909. *Myzocephalus narinari* Shipley and Hornell, 1906, also the only member of its genus, was collected also from *Aetob. ocellatus* (as *Aetobatis* [sic] *narinari*) off Sri Lanka (as Ceylon) by Shipley and Hornell (1906). This genus was not reported again until almost a century later when Caira *et al.* (2001) included a specimen they identified as *Myzocephalus* sp. from *Aetob. ocellatus* off Australia in their morphological analysis. Caira *et al.* (2014) subsequently included a specimen from the same host and locality, identified as *Myzocephalus* sp. 1, in their molecular phylogenetic analysis. *Mixophyllobothrium okamuri* Shinde and Chincholikar, 1980, the only member of *Mixophyllobothrium* Shinde and Chincholikar, 1980, to our knowledge, has not been reported since it was described by Shinde and Chincholikar (1980) from two specimens collected from a host they identified as *Trygon sephen* (Fabricius) off Ratnagiri, India. *Rhoptrobothrium* Shipley and Hornell, 1906 is the most well-known of the four genera. This genus was established by Shipley and Hornell (1906) for *Rhoptrobothrium myliobatidis* Shipley and Hornell, 1906, which they collected from the mottled eagle ray, *Aetomylaeus maculatus* (Gray) (as *Myliobatis* [sic] *maculata* Gray), off Sri Lanka (as Ceylon). The generic diagnosis was revised by Jensen and Caira (2006) to accommodate three new species from the banded eagle ray, *Aetomylaeus nichofii* (Bloch and Schneider), they collected off Malaysian Borneo. In addition to sharing the presence of extensions of the cephalic peduncle called remi, which vary in width across members of these four genera, each of the four bothridia of *Myzocephalus*, *Mixophyllobothrium*, and *Rhoptrobothrium* were re-interpreted by Jensen and Caira (2006) to bear an anterior region in the form of a loculus followed by a single posterior loculus. However, some uncertainty remains regarding the configuration of this region of the scolex in *Myzophyllobothrium*. In the original description of *Myzop. rubrum*, Shipley and Hornell (1906) considered this region to consist of four suckers, but illustrated both a scolex with four simple suckers (their figs. 19 and 21) as well as one that appears to bear divided bothridia (their fig. 18).

Historically, opinions have differed regarding the validity of these four genera. As examples, Southwell (1925) considered *Rhoptrobothrium* to be a synonym of *Myzophyllobothrium*, and *Myzocephalus* to be a synonym of the phyllobothriid genus *Thysanocephalum* Linton, 1890. These synonymies were accepted by Wardle and McLeod (1952), Yamaguti (1959), and Schmidt (1986). Euzet (1994) considered *Myzocephalus* and *Myzophyllobothrium* to be valid, *Rhoptrobothrium* to be a *genus inquirendum*, and *Mixophyllobothrium* (misspelled as *Myxophyllobothrium*) to be a synonym of *Myzocephalus*. In contrast, Schmidt (1986) considered *Mixophyllobothrium* to be valid. Jensen and Caira (2006) followed Euzet (1994) in considering *Mixophyllobothrium* a synonym of *Myzocephalus* but considered *Myzocephalus*, *Myzophyllobothrium*, and *Rhoptrobothrium* as valid genera. Caira *et al.* (2017) also considered all three of the latter genera valid, but considered *Mixophyllobothrium* as *incertae sedis*.

Examination of new specimens of cestodes bearing remi collected from *Aetob. ocellatus* off Sri Lanka and Indonesian Borneo, and from the Naru eagle ray, *Aetobatus narutobiei* White, Furumitsu, and Yamaguchi, off Viet Nam and Japan, in combination with new molecular data for specimens spanning the range of scolex morphologies seen across these taxa, provide morphological and molecular insights that facilitate assessment of the affinities and validity of these genera at this time.

Materials and methods

Specimen collection

Cestodes were collected from one male specimen of *Aetob. ocellatus* (SL-2), 114 cm in disc width (DW), on March 08, 2018 in the Gulf of Mannar, Sri Lanka (Palkanththura landing site [08°28'34.36"N, 79°51'45.80"E], North Western Province); one female specimen of *Aetob. ocellatus* (KA-304), 75.4 cm in DW, on July 27, 2007 in the South China Sea off Indonesian Borneo (Sepuk Laut [00°12'51.60"S, 109°05'00.30"E], West Kalimantan); two male specimens of *Aetob. narutobiei* (VN-2 and VN-40) collected in the Gulf of Tonkin, Viet Nam (59.5 cm in DW, on March 09, 2010, off Cat Ba Island [20°43'31.1"N, 107°02'54.9"E], and 58.5 cm in DW, on March 11, 2010, off Bach Long Vi Island [20°07'60.00"N, 107°43'60.00"E], respectively); one female specimen of *Aetom. maculatus* (BO-179), 36 cm in DW, on May 15, 2003 in the South China Sea off Malaysian Borneo (Sematan [01°48'15.45"N, 109°46'47.17"E], Sarawak); and one female specimen of *Aetom. nichofii* (KA-96), 34.5 cm in DW, on December 02, 2006 in the Java Sea off Indonesian Borneo (Takisung [03°52'28.00"S, 114°36'37.00"E], South Kalimantan).

All specimens were collected by local fishers. In each case, the eagle ray specimen was assigned a unique speci-

men number consisting of a collection code and collection number (i.e., SL-2), and was measured and photographed. Morphometric and additional collection data are available in the Global Cestode Database (Caira *et al.* 2020a) by searching by the unique specimen number. Host identifications were confirmed using NADH dehydrogenase sub-unit 2 (NADH2) sequence data by Naylor *et al.* (2012) and Fernando *et al.* (2019). In each case, the body cavity was opened with a midventral incision, and the spiral intestine removed and opened with a longitudinal incision. The spiral intestine was rinsed with sea water and worms encountered were saved in either 10% seawater-buffered formalin for morphological work or 95% ethanol for molecular work. The spiral intestine was subsequently fixed in 10% seawater-buffered formalin. All formalin-fixed material was transferred to 70% ethanol one to two weeks post fixation.

Specimens of the new species, which were collected June 19, 2008 from a specimen of *Aetob. narutobiei* of unknown sex and size from the Seto Inland Sea, Japan, were sent to us in 70% ethanol by Kazuya Nagasawa.

Morphological methods

Cestodes from *Aetob. ocellatus* from Sri Lanka and Malaysian Borneo, and from *Aetob. narutobiei* from Japan and Viet Nam prepared was whole mounts were hydrated, stained with Delafield's hematoxylin, dehydrated in a graded ethanol series, cleared with methyl salicylate, and mounted in Canada balsam on glass slides. Scolices and proglottids of cestodes from *Aetob. ocellatus* from Sri Lanka and proglottids from *Aetob. narutobiei* from Japan prepared for histology were superficially stained in Fast Green, dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin. Serial sections were cut at a thickness of 7 µm using an Olympus Cut 4060 retracting rotary microtome (Triangle Biomedical Sciences, Durham, NC). Sections were then floated on a 2.5% sodium silicate solution on glass slides and allowed to dry on a slide warmer at 40 C. Sections were deparaffinized in xylene, hydrated in a graded ethanol series, stained with Delafield's hematoxylin, counter-stained with eosin, differentiated in Scott's solution, dehydrated in a graded ethanol series, cleared in xylene, and mounted on glass slides in Canada balsam. For preparation of semi-permanent mounts of eggs of specimens from *Aetob. narutobiei* from Japan, detached gravid proglottids were placed in a mixture of 70% ethanol and lactophenol (1:1) overnight to clear. Subsequently, proglottids were placed in a drop of lactophenol on a glass slide, broken open with insect pins to release the eggs, and the eggs were mounted in lactophenol under a cover slip. The cover slip was subsequently ringed twice with nail polish to seal. Specimens from *Aetob. ocellatus* from Sri Lanka and from *Aetob. narutobiei* from Japan and Viet Nam for examination with scanning electron microscopy were hydrated, transferred to 1% osmium tetroxide and left overnight at 4 C, dehydrated in a graded ethanol series, and placed in hexamethyldisilizane (HMDS, Electron Microscopy Sciences, Hatfield, PA) for 30 min. Specimens were subsequently allowed to air-dry, mounted on aluminum stubs with double-sided adhesive carbon dots (PELCO tabs, Ted Pella, Inc., Redding, CA), sputter-coated with 30–35 nm of gold, and examined with a Hitachi S4700 field emission scanning electron microscope (Hitachi, Schaumburg, IL) at the Microscopy and Analytical Imaging Research Resource Laboratory, University of Kansas, Lawrence, Kansas. Vouchers of specimens examined as histological sections and with scanning electron microscopy (SEM) were prepared as whole mounts as described above.

Line drawings were made using a camera lucida attached to a Zeiss Axioskop 2 plus compound microscope (Zeiss, Thornwood, NY). Measurements of whole-mounted worms were taken using an Infinity 3-6URC USB camera (Teledyne Lumenera, Ottawa, Ontario, Canada) attached to the same compound microscope and the image analysis program Infinity Analyze Version 7.0.2.920 (Teledyne Lumenera). Measurements are given in micrometers (µm) unless otherwise stated and are reported as ranges followed in parentheses by the mean, standard deviation, number of individual specimens examined, and the total number of measurements if measurements or more than one feature were taken per worm. Images of histological sections and eggs were taken using the camera system described above.

Microthrix terminology follows Chervy (2009). Museum abbreviations used are as follows: BRT-P, Blue Resources Trust Parasitology Collection, Kalkudah, Sri Lanka; LRP, Lawrence R. Penner Parasitology Collection, University of Connecticut, Storrs, USA; MPM, Meguro Parasitological Museum, Tokyo, Japan; USNM, National Museum of Natural History, Smithsonian Institution, Department of Invertebrate Zoology, Washington, D.C., USA.

Molecular methods

Sequence data of the D1–D3 region of the *28S rRNA* gene (hereafter 28S) were generated *de novo* for eight speci-

mens. Based on their original generic assignments, these consisted of three specimens of *Myzoc. narinari*, two that were collected from the type host (*Aetob. ocellatus*) and type locality (Sri Lanka) and one that was collected from *Aetob. ocellatus* off Indonesian Borneo; two specimens superficially resembling *Myzop. rubrum* from *Aetob. narutobiei* in Viet Nam; one specimen of *Rhoptrobothrium gambangi* from *Aetom. nichofii* off Indonesian Borneo; and two specimens of *R. myliobatidis* from the type host *Aetom. maculatus* off Malaysian Borneo. In each case, genomic DNA was extracted from the middle or posterior portions of the strobila, and the remainder of each specimen (i.e., hologenophore sensu Pleijel *et al.* 2008) was prepared as a whole mount and deposited in the LRP.

Sequencing methods differed across specimens. For the three specimens of *Myzoc. narinari*, 28S sequence data were generated using Sanger sequencing. Genomic DNA was extracted from the middle or posterior portions of the strobila using a MasterPure Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI) and eluded in 60 µl TE buffer. For two of these specimens, 28S was amplified using illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare, Chicago, IL) using the primers ZX-1 (5'–ACCCGCTGAATTTAAGCATAT–3') (modified from van der Auwera *et al.* 1994) and 1500R (5'–GCTATCCTGAGGGAAACTTCG–3') (Olson *et al.* 2003; Tkach *et al.* 2003) and the following thermocycler temperature profile: denaturation for 2 min at 94 C, followed by 40 cycles of 30 sec at 94 C, 30 sec at 55 C, and 2 min at 72 C, and elongation for 10 min at 72 C. Unpurified PCR product was sequenced by GENEWIZ (South Plainfield, NJ) using single pass primer extension and PCR primers. For the remaining specimen, amplification, purification, sequencing followed Caira *et al.* (2020b). Contigs were assembled using Geneious Prime 2019 (Biomatters, Inc., San Diego, CA). Data for this same region for the remaining five specimens (i.e., the two specimens from Viet Nam resembling *Myzop. rubrum*, the specimen of *R. gambangi*, and the two specimens of *R. myliobatidis*) were generated and assembled by Hannah Ralicki and Elizabeth Jockusch using MITObim version 1.9.1 (Hahn *et al.* 2013) from Next Generation Sequencing reads for a related project.

Sequence data for the above eight specimens of four species were combined with sequence data for the two specimens of rhoptrobothriids, both from Caira *et al.* (2014), available in GenBank. Based on their original identifications, these are *R.* cf. *gambangi* (KF685774) from *Stegostoma fasciatum* (Hermann) (BO-492) off Malaysian Borneo and *Myzocephalus* sp. 1 (KF685887) from *Aetob. ocellatus* (AU-41) off Australia. The phyllobothriideans *Yamaguticestus* cf. *squali* (KF685897 from Caira *et al.* 2014 [as *Phyllobothrium* cf. *squali*]; see also Caira *et al.* 2021) and *Clistobothrium gabywalterorum* Caira, Hayes, and Jensen, 2020 (MN706183 from Caira *et al.* 2020c) were used as outgroups. Sequence alignment and phylogenetic methods follow Caira *et al.* (2020c). Based on sample size corrected Akaike Information Criterion values used to evaluate goodness of fit generated with jModelTest (Guindon and Gascuel 2003; Darriba *et al.* 2012), TVM ± I was used as the model of evolution.

Results

Phylogenetic analysis

28S sequence data generated for this study have been deposited in GenBank (MZ189002–MZ189009). The sequence alignment obtained using PRANK (Löytynoja and Goldman 2010) was 1,441 bp in length, with the ingroup taxa ranging in alignment length from 1,342 to 1,441 bp. The tree resulting from our phylogenetic analysis is shown in Figure 1. Taxon labels for the rhoptrobothriids include our proposed revised generic assignment and the original generic assignment in parentheses. In the cases of the two specimens for which data were obtained from GenBank, the specific identification has also been revised.

Monophyly of the family, as represented by *Rhoptrobothrium* cf. *gambangi* and *Myzocephalus* sp., was robustly established in the analyses of Caira *et al.* (2014) based on 18S and partial 28S sequence data in a comprehensive framework of elasmobranch-hosted tapeworms. The focus of the phylogenetic analysis conducted here was the relationships of taxa within the Rhoptrobothriidae. This phylogeny suggests that revision of generic assignments is required if generic monophyly is to be achieved. The three species of *Rhoptrobothrium* do not form a monophyletic group relative to the specimens of *Myzocephalus* and those resembling *Myzophyllobothrium*. A clade consisting of the specimens of *R. gambangi* and *R. limae* groups as the sister taxon to a clade consisting of the specimens of *Myzocephalus* and those resembling *Myzophyllobothrium*; *R. myliobatidis* is their sister taxon. To preserve generic monophyly, given the morphology of the taxa involved, the most conservative approach is to synonymize all three genera. Since all three names were established by Shipley and Hornell (1906) in the same paper, the principle of priority (ICZN Article 23.1; ICZN, 1999) does not inform the appropriate nomenclatorial decision in this case. Given that Southwell (1925) considered *Rhoptrobothrium* to be a synonym of *Myzophyllobothrium* we accept the latter as

the valid generic name of the genus based on the Principle of the First Reviser (ICZN Article 24.2.2; ICZN, 1999). *Myzocephalus* and *Rhoptrobothrium* are thus considered synonyms of *Myzophyllobothrium* here. Following Euzet (1994), so too is *Mixophyllobothrium*. However, the identity of its only species, *Mixop. okamuri*, remains in doubt. *Myzophyllobothrium okamuri* (Shinde and Chincholikar, 1980) **n. comb.** is considered as a *species inquirendum*. Based on the information provided by Shinde and Chincholikar (1980), it is possible this is a synonym of *Myzop. narinari*, but this possibility was not addressed at the time of the description. The identity of the type host, *Pastinachus ater* (Mcleay) (as *Trygon sephen*), the only member of *Pastinachus* Rüppell that occurs on the western coast of India (see Last *et al.* 2016), is also highly questionable. This is not only because all other rhoptrobothriids—with the exception of what we believe is an accidental infection in *Stegostoma fasciatum* (see discussion)—parasitize members of the families Aetobatidae Agassiz or Myliobatidae Bonaparte, rather than the Dasyatidae, but also because a substantial amount of work has been done on the cestodes of *Pastinachus* (e.g., Verma 1928; Southwell 1929; Baer and Euzet 1962; Deshmukh 1979; Shinde and Deshmukh 1979; Hassan 1983; Beveridge 1990; Campbell and Beveridge 2002; Jensen and Caira 2008; Kuchta and Caira 2010, Zschoche *et al.* 2011; Schaeffner and Beveridge 2012, 2013; Haseli 2013; Maleki *et al.* 2013, 2018; Haseli and Palm 2015; Reyda *et al.* 2016; Roohi Aminjan and Malek 2017; Coleman *et al.* 2019), none of which has yielded rhoptrobothriid specimens. A revised diagnosis for *Myzophyllobothrium* that accommodates these synonymies is provided below.
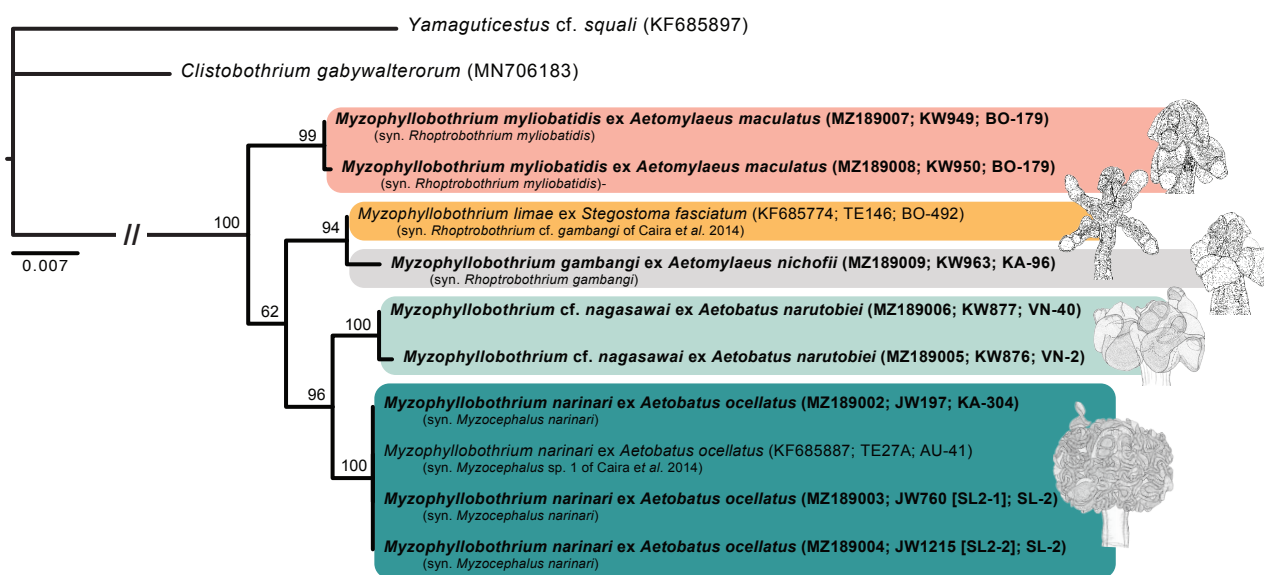

FIGURE 1. Phylogenetic tree showing the interrelationships of the Rhoptrobothriidae based on maximum likelihood analysis of the D1–D3 region of the *28S rRNA* gene. Nodal support, given as bootstrap values, is indicated above the branches. Specimens for which sequence data were generated *de novo* are indicated in bold. Taxon labels for the Rhoptrobothriidae consist of cestode species name with proposed revised generic assignment and host species name, followed by the GenBank accession number, specimen number, and host number; species names with original generic assignments and original identifications are given in parentheses below each taxon label. Scale bar indicates number of nucleotide substitutions per site.

Myzophyllobothrium Shipley and Hornell, 1906

(Syns. *Mixophyllobothrium* Shinde and Chincholikar, 1980; *Myzocephalus* Shipley and Hornell, 1906; *Rhoptrobothrium* Shipley and Hornell, 1906)

Generic diagnosis. Rhoptrobothriidae. Worms euapolytic. Scolex with four acetabula and cephalic peduncle. Acetabulum bothridiate in form, with two loculi; anterior loculus undivided, with or without postero-lateral extensions; posterior loculus undivided. Cephalic peduncle bearing four stalked remi; remus with terminal primary areola, with or without subterminal secondary areola. Neck absent. Proglottids craspedote or acraspedote. Testes numerous, anterior to ovary or ovarian bridge, arranged essentially in single dorso-ventral layer. Vas deferens coiled, medial to cirrus sac. Cirrus sac pyriform, containing coiled cirrus. Cirrus armed with spinitriches. Ovary H-shaped in frontal

view, tetralobed in cross section. Vagina weakly sinuous, opening into genital atrium anterior to cirrus sac; vaginal sphincter absent. Genital pores lateral, irregularly alternating. Uterus medial, saccate, extending to cirrus sac. Vitellarium follicular; follicles arranged in two lateral bands; each band consisting of one to many columns of follicles, extending entire length of proglottid, may be interrupted by ovary. Parasites of Aetobatidae and Myliobatidae. Indian and western Pacific Oceans.

Type species: *Myzophyllobothrium rubrum* Shipley and Hornell, 1906.

Additional species: *Myzophyllobothrium chongi* (Jensen and Caira, 2006) **n. comb.**, *Myzophyllobothrium gambangi* (Jensen and Caira, 2006) **n. comb.**, *Myzophyllobothrium limae* (Jensen and Caira, 2006) **n. comb.**, *Myzophyllobothrium myliobatidis* (Shipley and Hornell, 1906) Southwell, 1925, *Myzophyllobothrium nagasawai* **n. sp.**, and *Myzophyllobothrium narinari* (Shipley and Hornell, 1906) **n. comb.**

***Species inquirendum*:** *Myzophyllobothrium okamuri* (Shinde and Chincholikar, 1980) **n. comb.**

Remarks. The synonymization of *Mixophyllobothrium*, *Myzocephalus*, and *Rhoptrobothrium* with *Myzophyllobothrium* is supported by both morphological and molecular criteria. With respect to morphology, although the original descriptions suggest that the scolices of these genera differ dramatically from one another, examination of material of *M. narinari* and *M. nagasawai* **n. sp.**, the latter of which most closely resembles *M. rubrum*, with light and scanning electron microscopy reveals fundamental commonalities across these taxa. All bear a scolex that consists of four bothridia each of which bears an anterior and a posterior loculus, giving the bothridia a biloculate appearance. Although *Myzophyllobothrium* was described as possessing "four almost terminal suckers" (Shipley and Hornell 1906; pg. 48), the biloculate appearance of the bothridia was illustrated in their fig. 18 and was confirmed by Joyeux and Baer (1961; fig. 290) after examination of a co-type specimen of *M. rubrum*. The remaining differences are in the four remi that extend from the cephalic peduncle posterior to the bothridia. However, these differences can be considered to be essentially just variations in the width and extent of folding of the remi. In *M. narinari* and *M. rubrum* the remi are either much wider than, or nearly as wide as, they are long; in all four species formerly assigned to *Rhoptrobothrium*, the remi are longer than wide. The remi of *M. narinari* are highly folded; those of *M. rubrum* and *M. nagasawai* **n. sp.** are less folded; in all four species formerly assigned to *Rhoptrobothrium*, the remi are weakly or essentially unfolded. These taxa also share a number of notable similarities in proglottid anatomy. In all seven valid species, the cirrus sac is relatively large, extending to the midline of the proglottid, and is located near the middle of the proglottid. All species also possess a post-poral field of testes and a uterus that extends anteriorly in mature proglottids only to the level of the cirrus sac. In all species, the ovary is lobulated, H-shaped in frontal view, and tetralobed in cross section. The main differences among species are in testes number, number of columns of vitelline follicles, and whether the columns are interrupted by the ovary. The microthrix patterns on the scolices of the six species that have been examined with SEM are also very consistent. Little variation exists across surfaces of the scolex; all surfaces of the scolex of all species bear small, slender gladiate spinitriches and capilliform filitriches, albeit of varying relative densities.

The terminology we have applied here to the configuration of the bothridial portion of the scolex differs from that used by Jensen and Caira (2006) for species of *Rhoptrobothrium*. These authors followed the terminology of Caira *et al*. (1999; 2001) and described the bothridia (e.g., Jensen and Caira, 2006, pg. 191) as bearing a "specialized anterior region in form of loculus and undivided main loculus". This terminology was employed to reflect the homology of the different regions of the bothridia of these species with those of other genera. For clarity, we have simplified this terminology here and refer to the bothridia as bearing an anterior and a posterior loculus (i.e., biloculate bothridia). The homologies described by Jensen and Caira (2006) stand; but this revised terminology simplifies comparisons with previous descriptions of members of this group.

Among the 21 valid genera of phyllobothriideans (see Ruhnke *et al*. 2017; Caira *et al*. 2021), *Myzophyllobothrium* most closely resembles *Ruhnkebothrium* Caira, Bueno, and Jensen, 2021 and *Thysanocephalum*. All three genera appear to exhibit a scolex that is divided into two regions—a narrow anterior region bearing four biloculate bothridia and an extensive, often convoluted, posterior region, that also is divided into four parts (see Linton 1892, Caira *et al*. 2021). These similarities are, however, superficial. In *Thysanocephalum* and *Ruhnkebothrium*, the scolex has been determined to consist of four bothridia each of which bears a single loculus at its narrow anterior end and an extensive, convoluted posterior loculus (Caira *et al*. 1999, 2021, respectively). In contrast, the four biloculate bothridia of *Myzophyllobothrium* are completely independent structures from the remi, which are extensions of the cephalic peduncle (see Jensen and Caira, 2006 and Fig. 4C). The independence of these three genera from one another was also supported by the results of the molecular phylogenetic analysis of Caira *et al*. (2014) in which the representatives of *Thysanocephalum*, *Ruhnkebothrium* (referred to as New genus 10), and *Myzophyllobothrium* (referred to as *Rhoptrobothrium* and *Myzocephalus*) grouped with other taxa in different parts of the tree.

Myzophyllobothrium rubrum Shipley and Hornell, 1906
(Fig. 4A)

Type and only known host: Whitespotted eagle ray, *Aetobatus ocellatus* (as *Aetobatis* [sic] *narinari*) (Myliobatiformes: Aetobatidae).

Type locality: Puttalam Lake, Sri Lanka (as Ceylon).

Additional localities: None.

Site of infection: Spiral intestine.

Specimens examined: BMNH nos. 2006.7.14.1–3, three slides identified by Southwell (1925) as *Myzophyllobothrium rubrum* (see Jensen and Caira 2006).

Sequence data: None.

Remarks. Joyeux and Baer (1961) resolved the issue surrounding the two different interpretations of the scolex of this species presented by Shipley and Hornell (1906). In their figure of the scolex of one of the co-types of the species, Joyeux and Baer (1961; fig. 290) illustrated the anterior region of the scolex to clearly consist of four biloculate bothridia rather than four simple suckers. Unfortunately, we have been unable to locate the co-types of this species. The specimens of *M. rubrum* on the three slides examined here were reported by Southwell (1925) as having been collected from the type host and locality. Unfortunately, all of these specimens are in relatively poor condition, and include only one scolex. We did not consider this material to be of sufficient quality to allow re-description of this species. Nonetheless, given the paucity of information available on the proglottid anatomy of *M. rubrum*, a photograph of the posterior region of a mature proglottid of one of these specimens is presented in Fig. 4A.

Myzophyllobothrium narinari (Shipley and Hornell, 1906) n. comb.
(Figs. 2A–C, 3, 4B–E)

(Syn. *Myzocephalus narinari* Shipley and Hornell, 1906; *Myzocephalus* sp. 1 of Caira *et al.* 2014).

Type and only known host: Whitespotted eagle ray, *Aetobatus ocellatus* (as *Aetobatis* [sic] *narinari*) (Myliobatiformes: Aetobatidae).

Type locality: Dutch Bay, Sri Lanka (as Ceylon).

Additional localities: Darwin (12°20'11"S, 130°54'39"E), Northern Territory, Australia (AU-41; see Caira *et al.* 2001, 2014); Sepuk Laut (00°12'51.60"S, 109°05'00.30"E), West Kalimantan, South China Sea, Indonesian Borneo (KA-304).

Site of infection: Spiral intestine.

Specimens deposited: Two mature worms (BRT-P nos. 004 and 005); five mature worms (USNM nos. 1655902–1655906), scolex longitudinal (USNM no. 1655908) and cross-section series (USNM no. 1655907), and proglottid cross-section series (USNM no. 1655909); five mature worms (LRP nos. 10336–10340), scolex longitudinal section series (LRP nos. 10341–10346), and two SEM strobilar vouchers (LRP nos. 10347 and 10348); scolices examined with SEM retained with KJ at the University of Kansas.

Sequence data: KF685887 (hologenophore, LRP no. 8280) ex *Aetobatus ocellatus* (AU-41) of Caira *et al.* (2014) (as *Myzocephalus* sp. 1); MZ189003 and MZ189004 (hologenophores, LRP nos. 10349 and 10350, respectively) ex *Aetobatus ocellatus* (SL-2); MZ189002 (hologenophore, LRP no. 10361) ex *Aetobatus ocellatus* (KA-304).

Re-description. Based on 12 whole mature worms, cross-section series of one scolex, longitudinal-section series of two scolices, one cross-section series of mature proglottids, and two scolices examined with SEM). Worms euapolytic, slightly craspedote, 10.1–19.7 (14.9 ± 3; 12) mm long; proglottids 72–113 (93 ± 13; 12) in total number; maximum width at level of scolex (Fig. 2B). Scolex globular (Figs. 2A, 3A), 748–1,103 (926 ± 113.1; 9) long, 961–1,532 (1,247 ± 179.3; 12) wide, consisting of four acetabula and cephalic peduncle. Scolex proper 350–502 (426 ± 49.7; 12) wide. Acetabulum bothridiate in form, 200–294 (247 ± 30.6; 6; 10) long, biloculate, sessile anteriorly, free posteriorly; anterior loculus undivided, 81–120 (100 ± 11.5; 10; 20) long, 100–160 (130 ± 13.0; 10; 24) wide, without postero-lateral extensions; posterior loculus undivided, 100–197 (149 ± 25.8; 7; 11) long, 88–156 (122 ± 20.1; 9; 13) wide. Cephalic peduncle bearing four stalked remi (Figs. 3B, 4C); region between bothridia and remi, and region posterior to remi short; stalk short; remus voluminous (Fig. 3A, B), highly folded, wider than long, tapering terminally, with terminal primary areola (Fig. 2A, 3A, B); primary areola 56–99 (78 ± 13.6; 8; 11) long, 84–124 (104 ± 13.0; 6; 8); subterminal secondary areola not observed. Neck absent.
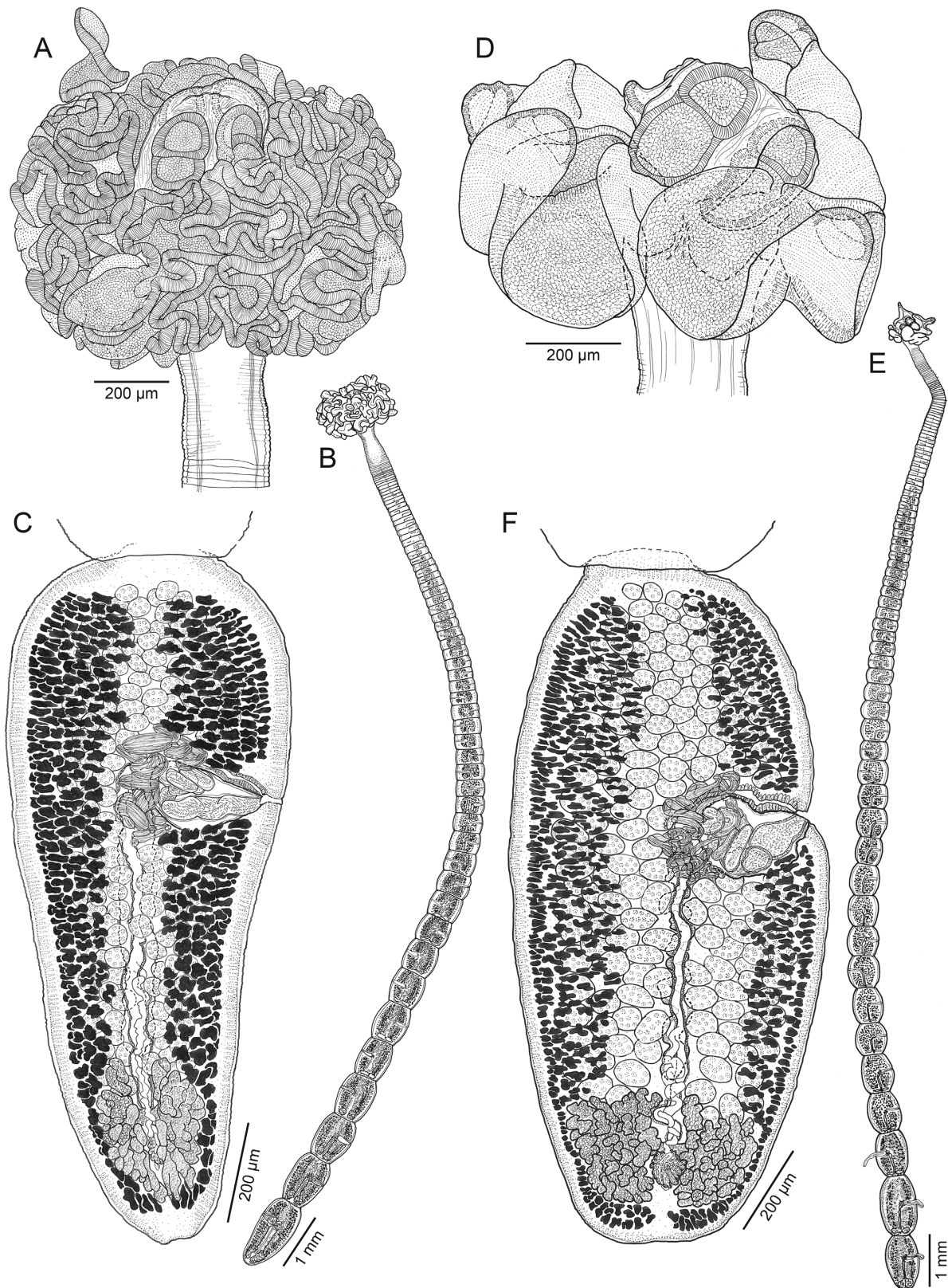
FIGURE 2. Line drawings of *Myzophyllobothrium narinari* (Shipley and Hornell, 1906) **n. comb**. from *Aetobatus ocellatus* from Sri Lanka (**A–C**) and of *Myzophyllobothrium nagasawai* **n. sp.** from *Aetobatus narutobiei* from Japan (**D–F**). **A.** Scolex (USNM no. 1655906, voucher) of *M. narinari*. **B.** Whole worm (USNM no. 1655905, voucher) of *M. narinari*. **C.** Terminal mature proglottid (USNM no. 1655903, voucher) of *M. narinari*. **D.** Scolex (USNM no. 1655912, paratype) of *M. nagasawai*. **E.** Whole worm (MPM no. 21757, holotype) of *M. nagasawai*. **F.** Terminal mature proglottid (USNM no. 1655911, paratype) of *M. nagasawai*.
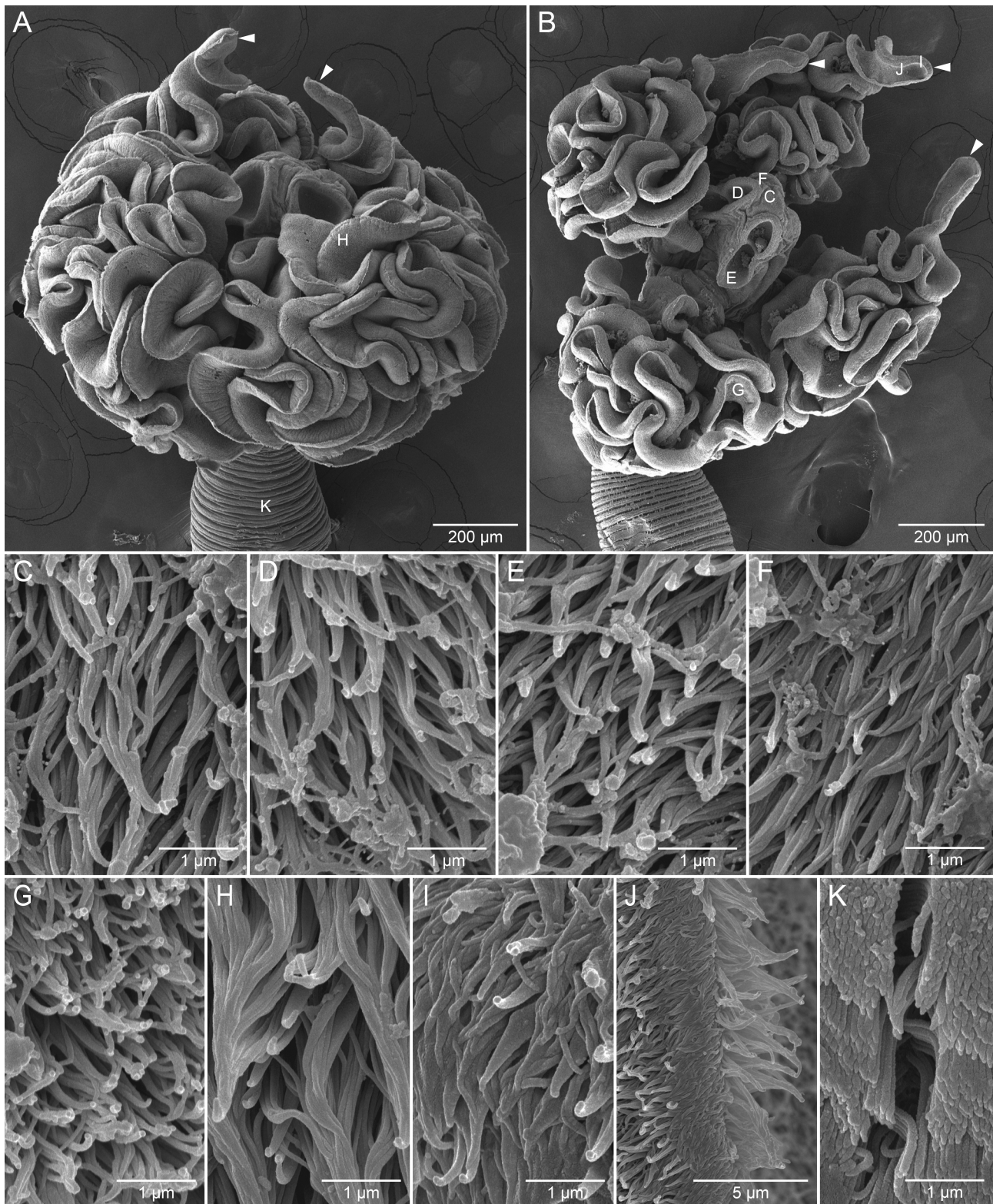
FIGURE 3. Scanning electron micrographs of *Myzophyllobothrium narinari* **n. comb.** from *Aetobatus ocellatus* from Sri Lanka. **A.** Scolex; small letters indicate locations of details in H and K; arrowheads point to primary areolae. **B.** Scolex showing biloculate bothridia and four remi; small letters indicate locations of details in C–G, I, and J; arrowheads point to primary areolae. **C.** Surface of scolex proper. **D.** Distal surface of anterior loculus. **E.** Distal surface of posterior loculus. **F.** Proximal surface of posterior loculus. **G.** Distal surface of remus. **H.** Proximal surface of remus. **I.** Proximal surface of primary areola. **J.** Rim of remus. **K.** Strobilar surface.

Apex of scolex proper (Fig. 3C), distal surface of anterior loculus (Fig. 3D) and posterior loculus (Fig. 3E), proximal surface of anterior loculus and posterior loculus (Fig. 3F), distal (Fig. 3G) and proximal (Fig. 3H) surfaces of remus, and distal surface of primary areola covered with slender gladiate spinitriches and capilliform filitriches of varying densities; proximal surface of primary areola (Fig. 3I) covered with slender gladiate spinitriches and shorter capilliform filitriches; capilliform filitriches on rims of bothridia and remi conspicuous (Fig. 3J). Strobila covered with capilliform filitriches (Fig. 3K).

Immature proglottids wider than long, becoming slightly longer than wide with maturity (Fig. 2B), 66–107 (87 ± 12; 12) in number; last immature proglottid 368–732 (550 ± 109.1; 12) long, 390–648 (519 ± 74.6; 12) wide. Mature proglottids becoming longer than wide posteriorly (Fig. 2C), 2–6 (4 ± 1; 12) in number; terminal mature proglottid 1,010–1,600 (1,222 ± 180.1; 10) long, 360–649 (486 ± 103.2; 10) wide, length to width ratio 1.7–3.5 (2.6 ± 0.5; 10):1. Gravid proglottids not observed. Testes in field extending from anterior margin of proglottid to ovarian bridge, arranged in multiple columns, one irregular layer deep in cross section (Fig. 4D), 98–167 (133 ± 18; 12; 32) in total number, 21–38 (30 ± 4; 12; 32) in number in post-poral field, 19–41 (30 ± 5.4; 12; 36) long, 32–68 (50 ± 8.2; 12; 36) wide. Vas deferens coiled, essentially medial to cirrus sac. Cirrus sac pyriform, 155–267 (211 ± 31.4; 12) long, 108–160 (134 ± 18.9; 12) wide, thin-walled, containing coiled cirrus; cirrus armed with spinitriches. Genital pores irregularly alternating, 58–66% (62 ± 2.6; 10) of proglottid length from posterior end; genital atrium shallow. Vagina sinuous, extending from ootype along midline of proglottid, opening into common genital atrium anterior to cirrus sac. Ovary at posterior margin of proglottid, H-shaped in frontal view, 177–302 (232 ± 45.7; 10) long, 165–315 (229 ± 49.9; 10) wide, tetralobed in cross section (Fig. 4E); ovarian margins lobulated. Vitellarium follicular; follicles 12–43 (27 ± 6.9; 12; 36) long, 28–65 (46 ± 8.5; 12; 36) wide, arranged in two lateral bands; each band consisting of multiple irregular columns of follicles, encroaching in midline of proglottid, extending throughout length of proglottid, interrupted by terminal genitalia, uninterrupted by ovary. Uterus ventral, medial, extending from ootype region to level of cirrus sac; uterine duct sinuous, entering uterus at midpoint. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid.

Remarks. We have been unable to locate the type specimens of this species. Although this species is clearly recognizable based on the original description of the scolex and accompanying illustrations by Shipley and Hornell (1906), these authors were unable to provide a detailed description of the proglottid anatomy due to limitations of the material at hand, and thus presented only measurements of the main features of the species (total length, scolex width, and mature proglottid width). The specimens on which this re-description is based are generally consistent with the original description, however, they are smaller in several respects than those described by Shipley and Hornell (1906) (10.1–19.7 vs. 25 mm in total length; 748–1,103 vs. 2 mm in scolex width; 360–649 vs. 1mm in terminal proglottid width). Nonetheless, given this material came from the type host near the type locality, we have little doubt about its conspecificity with *M. narinari* and attribute the differences observed to intraspecific variation, or differences in fixation and/or variation in measurements taken from live versus preserved material. This re-description expands the concept of this species of Shipley and Hornell (1906) to include new information on scolex morphology. These authors (pg. 47) described this species to bear "four slipper shaped bothridia each divided by a horizontal ridge into two areolas" and a "ruff formed of four immensely crumpled lateral extensions". After viewing these highly mobile portions of the scolex in live worms, they contemplated whether this worm exhibited "a double set of bothridia". The terminology of their otherwise highly accurate account of the scolex of this species is revised here such that the lateral extensions are referred to as remi as defined by Jensen and Caira (2006). Histological sections (Fig. 4B) confirm that these represent extensions of the cephalic peduncle separate from the bothridia. In addition, as seen in other members of the genus, each of the bothridia of this species was found to bear a terminal primary areola. The re-description also provides the first detailed information on the proglottid anatomy and the microthrix pattern of this species.

Myzophyllobothrium narinari differs from *M. rubrum*, *M. chongi*, *M. gambangi*, *M. limae*, and *M. myliobatidis* in possessing remi that are highly folded, rather than moderately or weakly folded. In addition, it is a smaller worm than *M. rubrum* (10.1–19.7 vs. 80 mm in total length [TL]). It is a larger worm than *M. chongi*, *M. gambangi*, *M. limae*, and *M. myliobatidis* (10.1–19.7 vs. 1.4–1.8, 1.1–2.1, 2.4–5, 1.4–3.4 mm in TL, respectively), with a greater number of testes (98–167 vs. 22–37, 22–36, 28–43, and 27–38, respectively). This is the species referred to by Caira *et al.* (2014) in their molecular phylogenetic analysis and in GenBank (KF685887) as *Myzocephalus* sp. 1.

Although not included in the re-description, specimens of this species collected from the type host off West Kalimantan on the island of Borneo (GenBank no. MZ189002) and off the Northern Territory in Australia (KF685887)

were included in our phylogenetic analysis. Despite slight differences in morphology (e.g., total length and scolex size) these specimens were found to be identical in sequence for 28S and are considered herein to be conspecific with the specimens of *M. narinari* from the type locality of Sri Lanka.
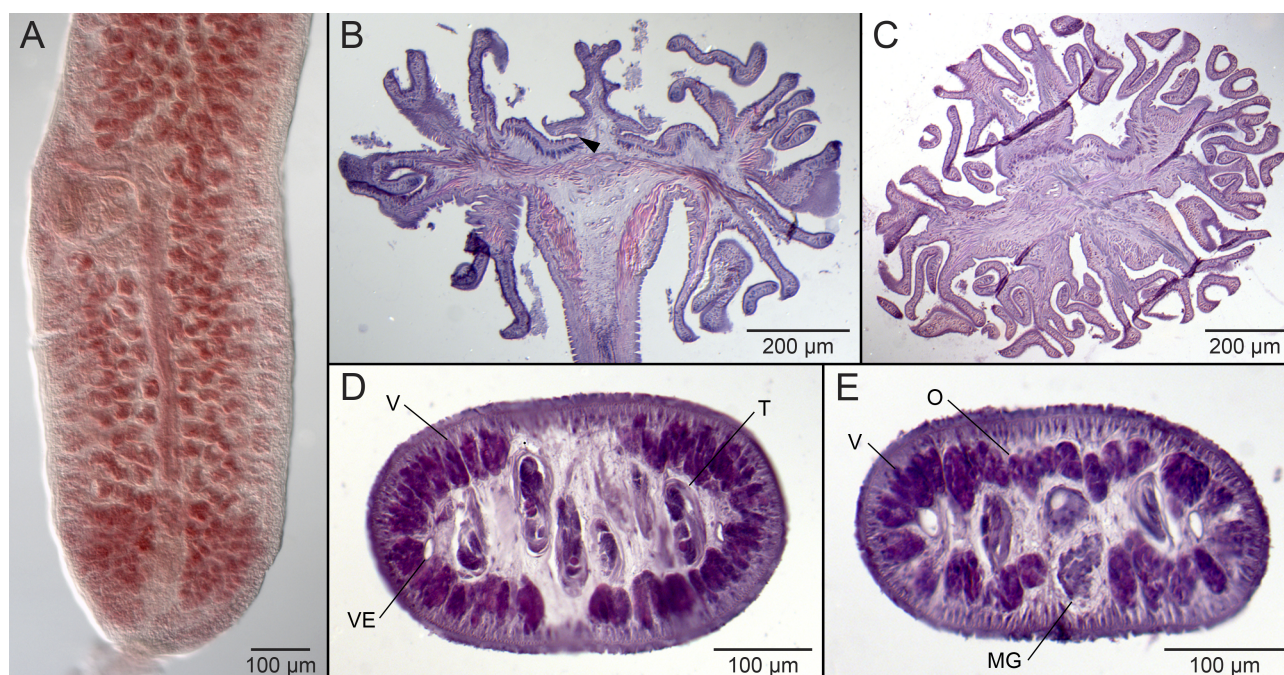

FIGURE 4. Photomicrographs of *Myzophyllobothrium rubrum* Shipley and Hornell, 1906 from *Aetobatus ocellatus* from Sri Lanka (**A**) and *Myzophyllobothrium narinari* (Shipley and Hornell, 1906) **n. comb**. from *Aetobatus ocellatus* from Sri Lanka (**B–E**). **A.** Posterior region of mature proglottid (BMNH nos. 2006.7.14.1–3) of voucher specimen of *M. rubrum* from Southwell (1925). **B.** Longitudinal section through scolex of *M. narinari*; arrowhead points to region of cephalic peduncle between bothridia from remi. **C.** Cross section through scolex of *M. narinari* at level of remi posterior to bothridia. **D.** Cross section through mature proglottid of *M. narinari* at level of anterior to cirrus sac. **E.** Cross section through mature proglottid of *M. narinari* at level of ovary anterior to ovarian bridge. Abbreviations: MG, Mehlis' gland; O, ovary; T, testes; V, vitelline follicles; VE, ventral excretory vessel.

Myzophyllobothrium nagasawai n. sp.

(Figs. 2D–F, 5A–M, 6)

urn:lsid:zoobank.org:act:930A6E47-571D-411F-9377-2DD6E8351AE1

Type and only known host: Naru eagle ray, *Aetobatus narutobiei* (Myliobatiformes: Aetobatidae).

　　Type locality: Seto Inland Sea, Japan.

　　Site of infection: Spiral intestine.

　　Type specimens: Holotype (complete mature worm; MPM no. 21757) and four paratypes (one incomplete mature worm, one detached pre-gravid proglottid, and one detached gravid proglottid [MPM no. 21758], and one egg preparation [MPM no. 21759]); six paratypes (three complete mature worms [USNM nos. 1655910–1655912], two detached gravid proglottids [USNM nos. 1655913 and 1655914], and one proglottid cross-section series [USNM no. 1655915]); five paratypes (two complete mature worms [LRP nos. 10351 and 10352], two detached gravid proglottids [LRP nos. 10353 and 10354], and one proglottid cross-section series [LRP nos. 10355–10358]) and two SEM strobilar vouchers (LRP nos. 10359 and 10360); two scolices and one gravid proglottid examined with SEM retained with KJ at the University of Kansas.

　　Sequence data: None.

　　Etymology: This species is named in honor of Kazuya Nagasawa of Hiroshima University, Japan, for his contributions to marine parasitology and for collecting the specimens on which this description was based.
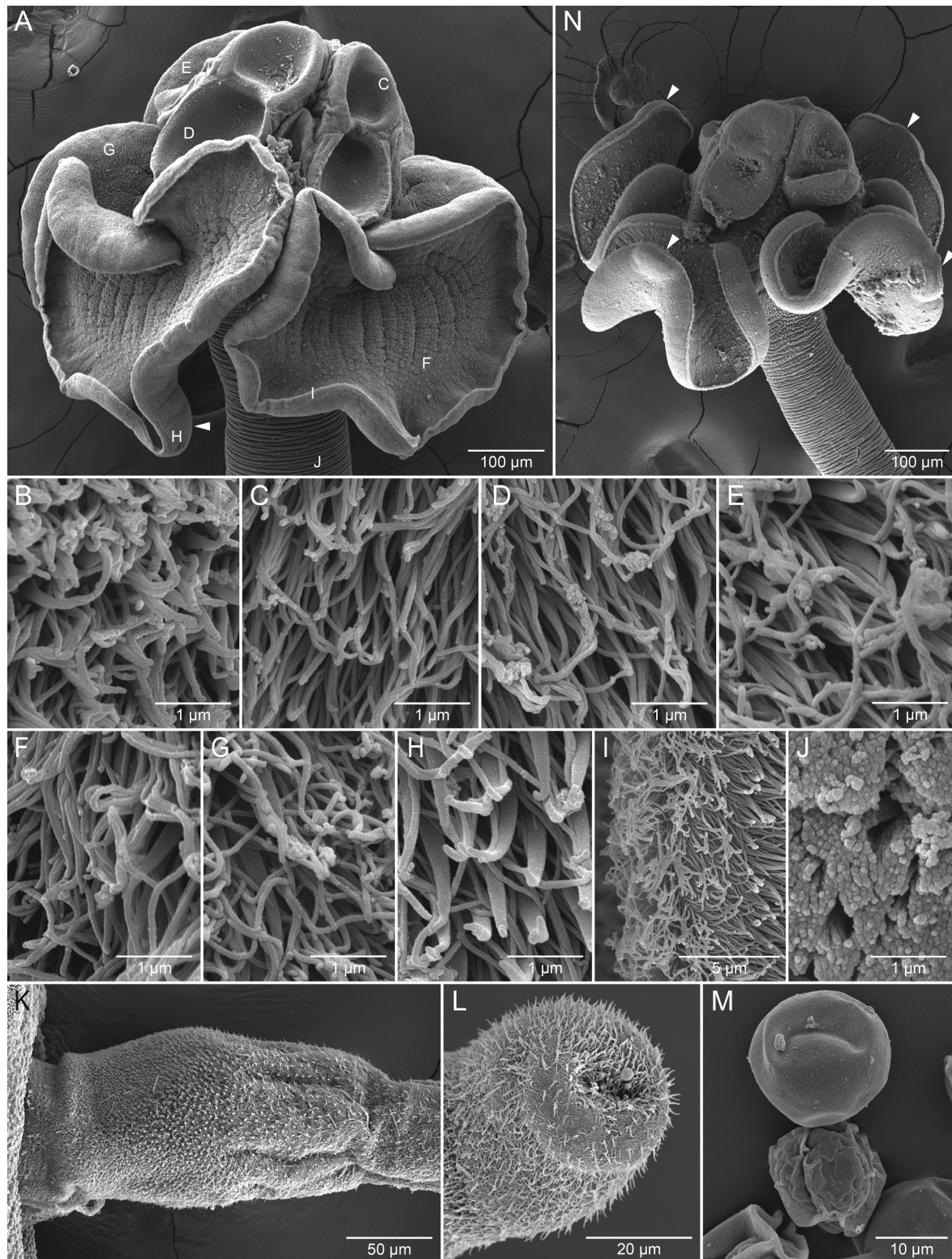
FIGURE 5. Scanning electron micrographs of *Myzophyllobothrium nagasawai* **n. sp.** from *Aetobatus narutobiei* from Japan (**A–M**) and of *Myzophyllobothrium* cf. *nagasawai* from *Aetobatus narutobiei* from Viet Nam (**N**). **A.** Scolex of *M. nagasawai*; small letters indicate locations of details in C–J; arrowhead points to primary areola. **B.** Surface of scolex proper of *M. nagasawai*. **C.** Distal surface of anterior loculus of *M. nagasawai*. **D.** Distal surface of posterior loculus of *M. nagasawai*. **E.** Proximal surface posterior loculus of *M. nagasawai*. **F.** Distal surface of remus of *M. nagasawai*. **G.** Proximal surface of remus of *M. nagasawai*. **H.** Proximal surface of primary areola of *M. nagasawai*. **I.** Rim of remus of *M. nagasawai*. **J.** Strobilar surface of *M. nagasawai*. **K.** Base of everted cirrus of *M. nagasawai*. **L.** Tip of everted cirrus of *M. nagasawai*. **M.** Eggs of *M. nagasawai*. **N.** Scolex of *M.* cf. *nagasawai*; arrowheads point to primary areolae.

Description. Based on six complete mature worms, one incomplete mature worm, one detached pre-gravid proglottid, five detached gravid proglottids, two cross-section series of mature proglottids, two scolices and one detached gravid proglottid examined with SEM, and one egg preparation.

Worms euapolytic, slightly craspedote, 16.6–24.3 (20.0 ± 2.8; 6) mm long; proglottids 90–118 (99 ± 11; 6) in total number; maximum width at level of scolex (Fig. 2E). Scolex 601–1,019 (762 ± 171.9; 7) long, 915–1,299 (1,092 ± 123.7; 7) wide, consisting of four acetabula and cephalic peduncle. Scolex proper 367–405 (390 ± 15.5; 5) wide. Acetabulum bothridiate in form, 255–374 (324 ± 41.7; 6; 12) long, biloculate, sessile anteriorly, free posteriorly; anterior loculus undivided, 98–163 (134 ± 18.3; 7; 14) long, 130–216 (164 ± 31.1; 7; 14) wide, without postero-lateral extensions; posterior loculus undivided, 156–226 (196 ± 24.9; 6; 12) long, 135–201 (164 ± 19.6; 6; 11) wide. Cephalic peduncle bearing four stalked remi (Figs. 2D, 5A); region between bothridia and remi, and region posterior or remi short; stalk 62–179 (116 ± 30.7; 6; 12) long, 144–234 (183 ± 28.5; 6; 11) wide; remus without stalk, weakly folded, slightly longer than wide, 455–880 (597 ± 185.6; 4; 6) long, 386–610 (494 ± 71.1; 6; 11) wide, tapering terminally, with terminal primary areola and inconspicuous subterminal secondary areola; primary areola 48–65 (56 ± 5.5; 6; 11) long, 84–125 (105 ± 14.3; 6; 11) wide. Neck absent.

Apex of scolex proper (Fig. 5B), distal surface of anterior loculus (Fig. 5C) and posterior loculus (Fig. 5D), proximal surface of anterior loculus and posterior loculus (Fig. 5E), distal (Fig. 5F) and proximal (Fig. 5G) surfaces of remus, and distal surface of primary areola covered with slender gladiate spinitriches and capilliform filitriches of varying densities; proximal surface of primary areola (Fig. 5H) covered with slender gladiate spinitriches and shorter capilliform filitriches; filitriches on rims of bothridia and remi conspicuous (Fig. 5I). Strobila covered with capilliform filitriches (Fig. 5J).

Immature proglottids wider than long, becoming approx. as long as wide with maturity (Fig. 2E), 76–111 (89 ± 14; 6) in number; terminal immature proglottid 552–628 (601 ± 27.2; 6) long, 482–670 (567 ± 74.6; 6) wide. Mature proglottids becoming longer than wide posteriorly (Fig. 2F), 5–12 (8 ± 2; 6) in number; terminal proglottid 966–1,707 (1,257 ± 279.3; 6) long, 477–682 (586 ± 85.5; 6) wide, length to width ratio 1.6–3.6 (2.2 ± 0.7; 6):1. Detached gravid proglottids 2,406–3,629 (2,805 ± 483.6; 5) long, 1,000–1,242 (1,110 ± 117.0; 5) wide. Testes in field extending from anterior margin of proglottid to ovarian bridge, arranged in multiple columns, one irregular layer deep in cross section (Fig. 6A), 131–224 (177 ± 28; 6; 18) in total number, 28–50 (38 ± 7; 6; 18) in number in post-poral field, 21–49 (34 ± 7.9; 6; 18) long, 38–90 (57 ± 15.1; 6; 8) wide. Vas deferens coiled, essentially medial to cirrus sac. Cirrus sac pyriform, 204–260 (228 ± 20.5; 6) long, 102–155 (117 ± 19.3; 6) wide, thin-walled, containing coiled cirrus; cirrus armed with coniform spinitriches and capilliform filitriches (Fig. 5L), spinitriches denser at base (Fig. 5K), 77–88 (n=2) wide at base, at least 158 long (n=1). Genital pores irregularly alternating, 57–62% (59 ± 2.1; 6) of proglottid length from posterior end; genital atrium shallow. Vagina weakly sinuous, extending from ootype along midline of proglottid, opening into common genital atrium anterior to cirrus sac. Ovary at posterior margin of proglottid, H-shaped in frontal view, 173–337 (221 ± 65.8; 6) long, 186–405 (324 ± 73.5; 6) wide, tetralobed in cross section (Fig. 6B); ovarian margins lobulated. Vitellarium follicular; follicles 7–20 (12 ± 3.7; 6; 18) long, 16–53 (31 ± 10.1; 6; 18) wide, arranged in two lateral bands; each band consisting of multiple irregular columns of follicles, encroaching in midline of proglottid, extending throughout length of proglottid, interrupted by terminal genitalia, uninterrupted by ovary. Uterus ventral (Fig. 6A), medial, extending from ootype region to level of cirrus sac; uterine duct sinuous, entering uterus approximately at midpoint. Excretory vessels four, arranged in one dorsal and one ventral pair on each lateral margin of proglottid. Eggs spherical (Figs. 5M, 6C), single, 18–23 (21 ± 1.4; 18) in diameter.

Remarks. This new species differs from *M. chongi*, *M. gambangi*, *M. limae*, and *M. myliobatidis* in that its remi are wider than long, rather than longer than wide. It is also a much larger worm than all four of these species (16.6–24.3 vs. 1.4–1.8, 1.1–2.1, 2.4–5, 1.4–3.4 mm in TL, respectively). The remi of *M. nagasawai* **n. sp.** are weakly folded, rather than highly folded and voluminous, as seen in *M. narinari* and, whereas the remi of *M. narinari* taper conspicuously posteriorly, those of *M. nagasawai* **n. sp.** are wider and more bluntly rounded at their posterior-most point. This new species most closely resembles *M. rubrum* in that its remi are both wider than long and weakly folded. It conspicuously differs from the latter species in that it is substantially shorter in total length (4.6–24.3 vs. 80 mm).

Specimens of *M. nagasawai* **n. sp.** from its type host, *Aetob. narutobiei*, off Japan, fixed in ethanol for molecular work were not available for study. Two specimens taken from the type host in the Gulf of Tonkin off Viet Nam were included in our phylogenetic analysis. Although the scolex morphology of the more than 25 specimens from

two host specimens from Viet Nam was highly consistent with that of specimens from Japan (Fig. 5N vs. 5A), the strobila of specimens from Viet Nam was much more delicate and smaller than that of the specimens from Japan. However, the specimens from Viet Nam were also all immature. We have referred to these specimens as *M.* cf. *nagasawai* until mature worms from Viet Nam can be examined and their identity confirmed. The strobila voucher of the scolex of the specimens from Viet Nam examined with SEM was deposited in the LRP (LRP no. 10367).
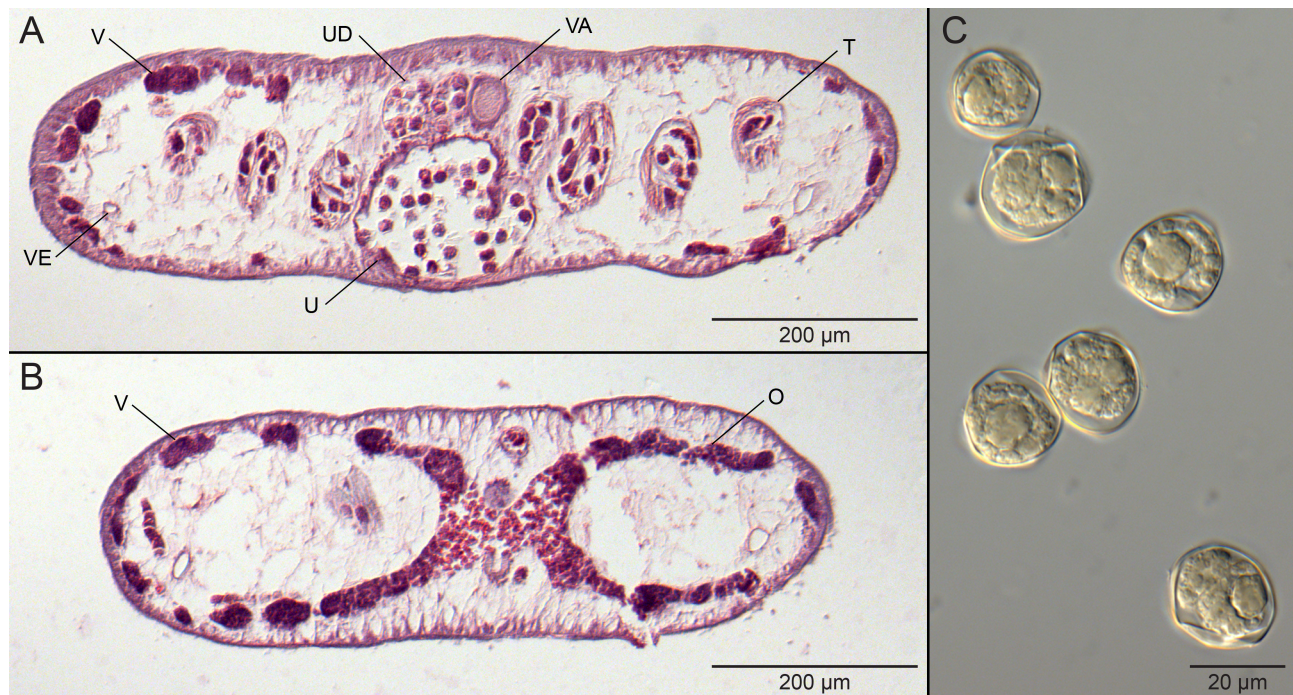
FIGURE 6. Photomicrographs of histological sections of *Myzophyllobothrium nagasawai* **n. sp.** from *Aetobatus narutobiei* from Japan. **A.** Cross section through detached gravid proglottid between cirrus sac and ovary. **B.** Cross section through mature proglottid at level of ovarian bridge. **C.** Eggs. Abbreviations: O, ovary; T, testes; U, uterus; UD, uterine duct; V, vitelline follicles; VA, vagina; VE, ventral excretory vessel.

Discussion

The taxonomic actions taken here to achieve generic monophyly have reduced the number of genera in the family Rhoptrobothriidae from four to one, rendering the family monogeneric. Nonetheless, the bizarre morphology of the members of this genus relative to the other groups of phyllobothriideans, and in particular their possession of remi extending from the cephalic peduncle, seems sufficient to justify retaining their family status. It also seems likely that additional diversity in this group remains to be discovered in the members of the genera *Aetobatus* and *Aetomylaeus* that have not yet been examined for cestodes.

We remain puzzled about the rhoptrobothriid specimen from the zebra shark, *Stegostoma fasciatum* (Orectolobiformes), included in the molecular phylogenetic analysis of Caira *et al*. (2014). In preparing this manuscript we returned to the cestodes we collected from the spiral intestine of that zebra shark specimen (BO-492) and found several additional specimens of this same rhoptrobothriid species. Based on examination of those specimens and the voucher (LRP no. 8338) of the specimen sequenced by Caira *et al*. (2014) we have revised the identification of these specimens from *Rhoptrobothrium* cf. *gambangi* to *Myzophyllobothrium limae*. Their presence in a shark of this order—given the type host is *Aetom. nichofii* collected from that same locality—is puzzling given that we did not find them in any of the other four zebra shark specimens we examined from Borneo, nor have they previously been reported from zebra sharks (Hornell 1912; Khambata and Bal 1954; Yamaguti 1954; Beveridge and Justine 2007). The fact that none of these cestode specimens were fully mature leads us to suspect this may be a case of accidental infection.

Given the proglottid anatomy of the rhoptrobothriids most closely resembles that of a number of phyllobothriid genera, distinguishing *Myzophyllobothrium* from other phyllobothriidean genera in the Remarks section above

seemed appropriate. However, there remains some uncertainty regarding the affinities of *Myzophyllobothrium* relative to other tetrabothridiate genera hosted by elasmobranchs. As a consequence, the ordinal placement of *Myzophyllobothrium* is also uncertain. Historically, its species have been considered members of the family Phyllobothriidae within the order Tetraphyllidea (e.g., Southwell 1925; Wardle and McLeod 1952; Schmidt 1986; Euzet 1994; Jensen and Caira 2006). However, when Caira *et al.* (2014) established the new order Phyllobothriidea, these genera were retained at least temporarily in the non-monophyletic "Tetraphyllidea" because the two representatives included in their analyses (referred to as *Rhoptrobothrium* cf. *gambangi* and *Myzocephalus* sp. 1) failed to group with the Phyllobothriidea. Caira *et al.* (2017) subsequently erected the family Rhoptrobothriidae Caira, Jensen, and Ruhnke, 2017 within the "Tetraphyllidea" to house these genera noting that while the group appears to be highly supported, its affinities relative to other "tetraphyllidean" clades are unclear. Based on Article 40.1 of the ICZN (pg. 46; ICZN, 1999), which states that "when the name of a type genus of a nominal family-group taxon is considered to be a junior synonym of the name of another nominal genus, the family-group name is not to be replaced on that account alone" the family name Rhoptrobothriidae is retained for this clade. Although the two representatives of *Myzophyllobothrium* included by Caira *et al.* (2014) grouped away from the Phyllobothriidea, neither the node of the clade assigned to the Phyllobothriidea nor the node grouping the representatives of *Myzophyllobothrium* with other elasmobranch-hosted genera was well supported. As a consequence, the ordinal affinities of this genus remain unclear.

Acknowledgments

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