Tapeworms of the Mangrove Whipray, *Himantura granulata* Macleay, and an Investigation of Host Size as it Relates to Tapeworm Faunal Composition

by

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ABSTRACT

Since its description by Macleay in 1883, only three tapeworm species have been reported to parasitize the mangrove whipray, *Himantura granulata*. These are the rhinebothriideans *Rhinebothrium himanturi* and a presumably new species referred to as "*Rhinebothrium* sp.", and the trypanorhynch *Prochristianella clarkeae*. Elasmobranch collection efforts in the Solomon Islands and northern Australia from 1997 to 2012 yielded ten specimens of *H. granulata*, all of which were examined for tapeworms. Morphological and molecular data indicate that at least 31 additional species of tapeworms in 13 genera from five orders parasitize *H. granulata* from these localities, bringing the total number of tapeworm species known from this host to 34 species. Included in these 34 species are three new species representing two new lecanicephalidean genera, and at least six new species in the rhinebothriidean genus *Anthocephalum*. Of the ten specimens of *H. granulata* examined, six were small juvenile rays (disk width less than 35 cm) and four were large mature rays (disk width greater than 100 cm), presenting the unique opportunity to assess differences in tapeworm faunal diversity between two size classes of the same host species. Not unexpectedly, host size appears to play an important role, as conspicuous disparities in tapeworm faunal diversity at the specific, generic and ordinal levels were noted between the two host size classes. Ultimately, a combination of variation in both host diet and habitat use between different size classes, as well as the specificity of larval tapeworms within their intermediate hosts, will likely be necessary to explain these observed differences.

Author's Disclaimer

All taxonomic actions in this work are hereby disclaimed for nomenclatural purposes, as recommended in Article 8 of the International Code of Zoological Nomenclature (Ride et al. 1999).

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INTRODUCTION

Since MacArthur and Wilson's (1967) seminal formulation of the theory of island biogeography, ecologists and parasitologists alike have made theoretical forays into the application of the theory to host-parasite systems, with host individuals, populations or species posed as island habitats colonized by immigrating parasites (e.g., Janzen 1968, Dritschilo et al. 1975, Kuris et al. 1980, Poulin and Morand 2004). As two of the major tenants of the theory of island biogeography are the positive relationships between species richness and island size and island age (MacArthur and Wilson 1967), the expectation for a "hosts as islands" framework (coined by Kuris et al. [1980]) would suggest that the larger and/or older an individual is, the more parasite species and/or individuals it is likely to host. Since the proposition of the theory of island biogeography, a number of studies have identified a relationship between parasite species richness and vertebrate host age and size, with larger, older hosts being unsurprisingly parasitized by a greater diversity of parasite species (e.g., Poulin 1995 and citations therein, Gregory et al. 1996).

Qualitative rather than quantitative differences in parasite faunal composition between different age and size classes of hosts have also been noted in particular for various groups of parasites of both marine and freshwater bony fishes. Whether comparing within a single host species (e.g., Grutter and Poulin 1998, Lo et al. 1998, González et al. 2001, Poulin 2001, Timi and Poulin 2003, Johnson et al. 2004) or between multiple sympatric host species (e.g., Guégan et al. 1992, Grutter and Poulin 1998), older and larger fish consistently hosted greater parasite species diversity and were often noted to be parasitized by more individuals than their younger, smaller counterparts. In contrast to the popularity of bony fishes and their parasites as models in which to study patterns between parasitism and host age and size, there are only few studies focused on examining such differences for the parasites of elasmobranchs. For example, in a study of the trematode *Multicalyx cristata* Faust & Tang 1936 and its eagle ray host *Myliobatis freminvillei* Lesueur, Thoney and Burreson (1986) found that only host individuals with a disk diameter greater than 68 cm were infected by *M. cristata*. Additionally,

unpublished work conducted by Dr. T. Mattis documented noticeable turnover in the species of tapeworms parasitizing three size classes of the southern stingray *Dasyatis americana* Hildebrand & Schröder (T. Mattis, pers. comm. in Caira 1990). A similar pattern has also been noted for sharks, as species of tapeworms in the genus *Pedibothrium* Linton 1908 were observed to exhibit differential distributions in terms of relative abundance with respect to host size for nurse sharks in the Florida Keys (i.e., sharks of 103–168 cm in total length hosted the majority of specimens of *Pedibothrium brevispine* [Linton 1908] Caira 1992 and *Pedibothrium manteri* Caira 1992, while the single shark of 230 cm in total length hosted the majority of specimens of *Pedibothrium globicephalum* [Linton 1908] Caira 1992 and all specimens of *Pedibothrium servattorum* Caira 1992) (Caira 1992, Caira, unpublished data in Caira and Euzet 2001). Any conclusions drawn from this last report are, however, tenuous, as only a single large shark was sampled.

Broad meta-analyses of the species diversity of the parasitic endofauna of elasmobranchs such as those conducted by Luque and Poulin (2007) and Randhawa and Poulin (2010)—the latter of which focuses specifically on tapeworms—corroborate these findings, as positive correlations between elasmobranch host size and parasite species richness are consistently uncovered after correcting for sampling bias and the phylogenetic non-independence of host species. Despite the clear evidence for a relationship between host size and parasite community composition revealed by these synthetic data, however, no published works exist which explicitly investigate how the elasmobranch tapeworm faunal composition changes as particular shark or ray host species grows and ages.

Tapeworms are unequivocally the most diverse group of the various metazoans that parasitize elasmobranchs (Caira et al. 2012a). To date, they comprise close to 1,000 described species across nine of the 19 currently recognized tapeworm orders (Caira and Jensen 2014). Perhaps surprisingly, these nine orders do not form a monophyletic group; the elasmobranch tapeworms represent a number of independent lineages within the broader tapeworm phylogeny, and nested within a group of elasmobranch-hosted taxa are several orders that parasitize a

combination of terrestrial birds, marine and terrestrial mammals, and freshwater fishes (Caira and Jensen 2014). Additionally, all nine orders do not parasitize elasmobranchs exclusively as adults, as members of the Onchoproteocephalidea have been reported from sharks, marine and freshwater batoids, freshwater bony fishes, reptiles and amphibians, and a terrestrial mammal (see Caira and Jensen 2014). In fact, the hooked, elasmobranch-hosted species within the Onchoproteocephalidea represent a minority, as the bulk of the diversity of this most specious order lies in the taxa that parasitize teleosts (approximately 200 species vs. over 350 species, respectively) (Caira and Jensen 2014). The remaining eight elasmobranch tapeworm orders are exclusively parasites of sharks and/or batoids, and range widely in their species diversity, from fewer than ten species in each of the Cathetocephalidea (parasites of carcharhiniform sharks) and the monogeneric Litobothriidea (parasites of lamniform sharks), to over 300 species in the Trypanorhyncha, the order with the lowest host specificity and the second greatest species-level diversity, with species described from hosts in nearly all shark and batoid families (Palm 2004, Caira and Jensen 2014). The Lecanicephalidea and Rhinebothriidea both have intermediate levels of diversity, on the order of approximately 100 species each (Caira and Jensen 2014). While rhinebothriideans are found exclusively in batoids in both freshwater and marine habits (Healy et al. 2009, Caira and Jensen 2014), lecanicephalideans are largely marine, but have been described from batoids as well as select species of sharks (see Jensen et al. 2016). The remaining three elasmobranch cestode orders, the Phyllobothriidea (parasites of a variety of sharks and a select few batoids), the Diphyllidea (parasites of sharks and batoids) and the non-monophyletic "Tetraphyllidea" (parasites of a variety of sharks and myliobatiforms) have slightly more modest levels of diversity, each with fewer than 100 species (Caira and Jensen 2014). Collectively, the elamobranch-hosted members of these nine orders (with the exception of many species in the order Trypanorhyncha) are primarily oioxenous (*sensu* Caira et al. 2003) meaning each tapeworm species typically demonstrates extremely strict specificity at the level of their definitive host, and thus one species of tapeworm will only parasitize one species of elasmobranch.

Though to date the elasmobranchs and their tapeworms remain largely underrepresented in the literature examining the relationship between host age and size and parasite faunal composition, they are in fact an ideal host-parasite system in which to study how parasite species assemblages may change as a host grows and matures. This is due to a combination of two distinct features of this system. Firstly, each elasmobranch species is typically parasitized by tapeworms from several orders; for example, the blue shark *Prionace glauca* Linnaeus has been reported to host tapeworms of the orders Onchoproteocephalidea, Phyllobothriidea, Trypanorhyncha, and "Tetraphyllidea" (Robinson 1959, Curran and Caira 1995), and the dwarf whipray *Himatura walga* Müller & Henle has been reported to host tapeworms of the orders Diphyllidea, Lecanicephalidea, Onchoproteocephalidea, Rhinebothriidea and Trypanorhyncha (Shipley and Hornell 1905, 1906; Southwell 1925, Pintner 1928, Euzet 1953, Ivanov and Campbell 2000, Twohig et al. 2008). Since members of each order comprise diverse suites of scolex and proglottid morphologies, intermediate host associations, and geographic distributions, the tapeworm fauna of a single host species represents multiple independent replicates in an examination of the patterns related to host age and size and parasite species diversity.

Secondly, elasmobranch tapeworms have unique and complex life cycles, and are hypothesized to parasitize a variety of intermediate and paratenic hosts. Like all tapeworms, they are transmitted through the food chain (Caira and Reyda 2005) and thus it can be reasonably concluded that host diet is intimately tied to the composition of the community of adult tapeworms within a host (Poulin 1995). This second feature is of principle significance when investigating how tapeworm faunas might change over the life of an elasmobranch host because many elasmobranch species undergo an ontogenetic, or age-driven, shift in diet as they grow and mature. Such diet shifts have been documented in many species of sharks (Hoffmayer and Parsons 2003, Bethea et al. 2006, 2007; Hussey et al. 2011, Newman et al. 2012, Shiffman et al. 2014) as well as in a variety of batoids (Brickle et al. 2003, Farias et al. 2006, Dale et al. 2011, Navia et al. 2011, Espinoza et al. 2013, Šantić et al. 2013, Spath et al. 2013). Given that the most recent investigation into elasmobranch tapeworm faunal turnover suggests relatively short

lifespans for these parasites within their definitive hosts (i.e., less than one year) (Pickering and Caira 2014), it seems likely that juvenile and mature individuals of a shark or ray species would host qualitatively different tapeworm faunas.

Unfortunately, little is known about elasmobranch tapeworm lifecycles or the specificity of these parasites at the level of their intermediate hosts so as not allow for much more than conjecture on the exact role of host diet in tapeworm community composition. To date, only a single complete lifecycle has been described for any elasmobranch tapeworm species; Sakanari and Moser (1989) experimentally replicated the lifecycle of the trypanorhynch *Lacistorhynchus dollfusi* Beveridge & Sakanari 1987 by feeding coracidium larvae hatched from the eggs of gravid proglottids of *L. dollfusi* to copepods. Infected copepods were then fed to mosquitofish, and after a period of three months, plerocercoid larvae were harvested and force-fed to naïve juvenile leopard sharks, *Triakis semifasciata* Girard, which were found upon necropsy nearly two years later to be parasitized by adult *L. dollfusi*.

Various parasitological surveys of teleosts and invertebrates have, however, revealed elasmobranch tapeworm larval stages from multiple potential intermediate hosts. For example, the importance of copepods as first intermediate hosts and teleosts as second intermediate hosts has been demonstrated for multiple species in the trypanorhynch families Aporhynchidae Poche 1926 (parasites of dogfishes as adults), Eutetrarhynchidae Guiart 1927 (parasites of rays and guitarfishes as adults), Lacistorhynchidae Guiart 1927 (parasites of skates and houndsharks as adults), and Otobothriidae Shaeffner, Gasser & Beveridge 2011 (parasites of carchariniform sharks as adults) (Palm 2004). Similarly, work by Chambers et al. (2000) and Jensen and Bullard (2010) identified bivalves and teleosts as hosts of larval rhinebothriideans in the genera *Rhodobothrium* Linton 1889, *Spongiobothrium* Linton 1889 and *Rhinebothrium* Linton 1890 (all parasites of rays as adults). Teleosts were noted as hosts of taxa from across three additional orders of tapeworms: larval onchoproteocephalideans in the genera *Acanthobothrium* Blanchard 1848 (parasites of rays as adults), *Phoreiobothrium* Linton 1889 and *Triloculatum* Caira & Jensen 2009 (both parasites of carchariniform sharks as adults), larval phyllobothriideans in

the genus *Paraorygmatobothrium* Ruhnke 1994 and larval "tetraphyllideans" in the genus *Anthobothrium* van Beneden 1850 (both parasites of carcharhiniform sharks as adults). Gastropods and bivalves were found to be hosts of additional larval "tetraphyllideans" in the genus *Duplicibothrium* Williams & Campbell 1978 (parasites of cownose rays as adults) (Jensen and Bullard 2010). As for the specificity of elasmobranch tapeworms at the level of their intermediate hosts, work by Palm and Caira (2008) suggests that the specificity of larval trypanorhynchs in their penultimate host species is generally more relaxed as compared to that of adults in their definitive elasmobranch hosts. Additionally, Jensen and Bullard (2010) suggest that the tapeworm larvae encountered in their survey—with the exception of members of the genus *Rhodobothrium*—similarly exhibited more relaxed host specificity than their adult counterparts. Though further investigation into these topics is undoubtedly warranted, preliminary results such as these suggest that lifecycle patterns and—by extension, host diet—play a foundational role in determining the adult tapeworm community composition within an elasmobranch.

This study aims to (1) characterize the tapeworm fauna of the mangrove whipray *Himantura granulata* Macleay (family Dasyatidae Jordan) and (2) to identify any differences in the tapeworm species assemblages between small juvenile and large mature individuals of this host species. Prior to this investigation, *H. granulata* was a relatively understudied host for tapeworm species. Only three species of tapeworms have been reported to parasitize *H. granulata*: the rhinebothriideans *Rhinebothrium himanturi* Williams 1964, and a presumably new species referred to as "*Rhinebothrium* sp." known only from scoleces (Williams 1964), and the trypanorhynch *Prochristianella clarkeae* Beveridge 1990*,* reported to parasitize *H. granulata* from northern Australia by Schaeffner and Beveridge (2012).

Unfortunately, the biology and life history of *Himantura granulata* is relatively poorly known. The species is distributed throughout the Indo-West Pacific region, including the coastal and continental shelf waters off of northern Australia, New Guinea, the Solomon Islands, the Phillipines, Viet Nam and Cambodia, as well as the Red Sea (Last and Stevens 2009) (see Fig.

1). Juveniles of this species are noted to prefer shallow-water mangrove and coral reef intertidal habitats, and are approximately 14 cm in disk width at birth (Last and Stevens 2009, Davy et al. 2015). Adults are most often found in shallow hard-bottom habitats, but have been documented at depths of up to 85 m and are known to reach disk widths of up to 140 cm, with males hypothesized to mature between disk widths of 55 cm to 65 cm (Last and Stevens 2009, Ishihara et al. 1993). The diet of *H. granulata* has not been elucidated; however, stomach contents from a portion of the specimens examined for the redescription of the species by Ishihara et al. (1993) were noted to include gobiids, a siganid, a blenniid, a pomacentrid, a labrid, sipunculids, a small octopus and a calappid crab. All teleosts for which standard length (SL) could be estimated were reported to be between 28–86 mm SL.

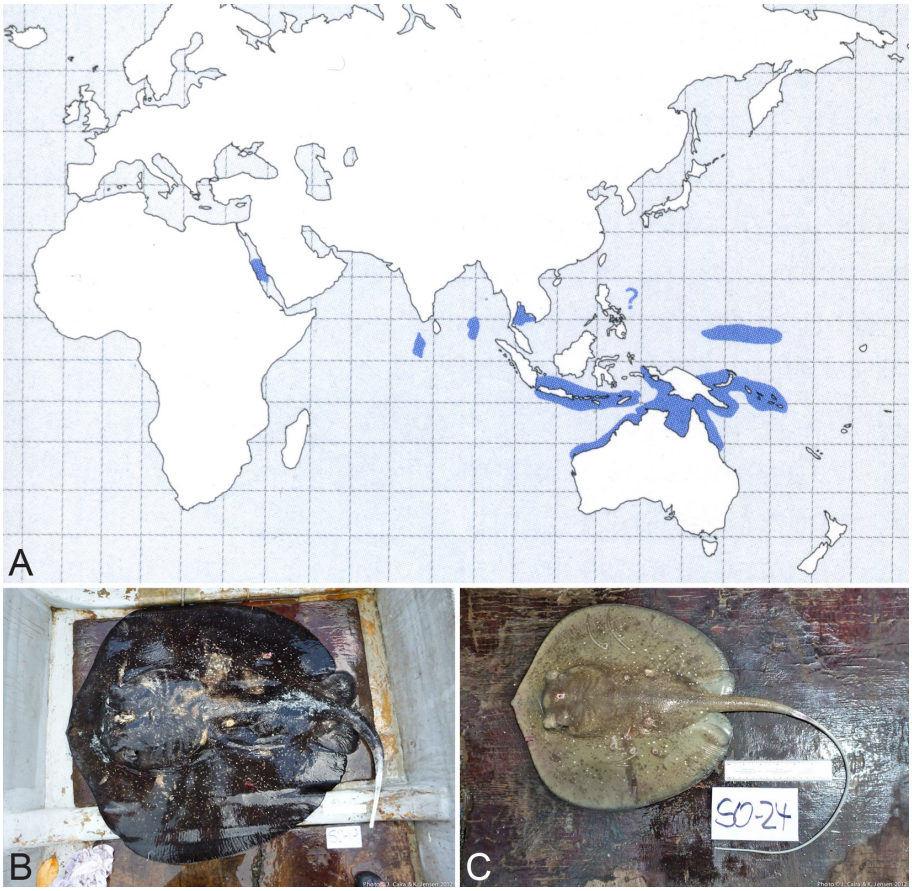

Figure 1. Geographic distribution and images of *Himantura granulata*. (A) Distribution map taken from Last and Stevens (2009). (B) Large mature individual. (C) Small juvenile individual.

For this study, ten specimens of *H. granulata* were collected from localities in northern Australia and the Solomon Islands, and examined for tapeworms. Six individuals were small juvenile rays (less than 35 cm disk width) and four individuals were large mature rays (greater than 100 cm disk width). Specifically, this study aims to characterize the tapeworm fauna of this parasitologically understudied host to the level of species (where possible) and formally describe a subset of those species that are new to science. Additionally, this study aims to identify whether tapeworm species are differentially distributed between the two size/maturity classes represented by the sampled individuals of this host species, and investigate both the quantitative and qualitative differences (if any) in tapeworm species compositions between small juvenile and large mature hosts.

MATERIALS AND METHODS

Specimen Collection

Ten individuals of *Himantura granulata* were collected between 1997 and 2012 from the Solomon Islands and Australia. Eight specimens were collected off of the island of Vonavona, near Rarumana in the Western Province of the Solomon Islands: 1 mature female on March 19, 2012; 1 mature female, 1 juvenile female and 2 mature males on March 22, 2012; and 2 juvenile females and 1 juvenile male on March 23, 2012. One juvenile male was collected from Darwin, Northern Territory, Australia on August 6, 1997 and 1 juvenile male was collected from Weipa, Queensland, Australia on May 16, 2004. Additional collection and specimen data is presented in Table 1, and capture localities are illustrated on a point map in Figure 2. Ray identification follows Last and Stevens (2009); identifications were confirmed using NADH2 sequence data (K. Jensen, pers. comm.). The identity of one specimen (CM03-74) was also confirmed by Naylor et al. (2012a). Host photographs are accessable by searching host codes on the online Global Cestode Database (Caira et al. 2012b).

Table 1. Host size, sex, and capture locality data for the ten individuals of *Himantura granulata* examined in this study.

Host Code	Capture Locality	Coordinates	Date of Collection	Sex	Maturity	Disk Width (cm)
AU-32	Buffalo Creek, Timor Sea, Indian Ocean: Northern Territory, Darwin, Australia	12°20'11"S, 130°54'39"E	6-Aug-97	Male	Juvenile	32
CM03-74*	Gulf of Carpentaria, Indian Ocean: Weipa, Queensland, Australia	12°35'11"S, 141°42'34"E	16-May-14	Male	Juvenile	34
SO-9	Solomon Sea, Pacific Ocean: Rarumana, Western Province, Vonavona, Solomon Islands	8°13'23.8"S, 157°0'2.4"E	19-Mar-12	Female	Mature	105
SO-17		8°13'23.8"S, 157°0'2.4"E	22-Mar-12	Male	Mature	103
SO-18		8°13'23.8"S, 157°0'2.4"E	22-Mar-12	Male	Mature	108
SO-19		8°13'23.8"S, 157°0'2.4"E	22-Mar-12	Female	Mature	115.5
SO-21		8°14'13.4"S, 157°1'53.7"E	22-Mar-12	Female	Juvenile	34
SO-23		8°13'23.8"S, 157°0'2.4"E	23-Mar-12	Female	Juvenile	33
SO-24		8°13'23.8"S, 157°0'2.4"E	23-Mar-12	Male	Juvenile	34
SO-25		8°13'23.8"S, 157°0'2.4"E	23-Mar-12	Female	Juvenile	33

*species identity confirmed in Naylor et al. (2012a)

Mangrove whip rays were captured using gill net or hand spear. The body cavity of each ray was opened with a mid-ventral longitudinal incision, and the spiral intestine was removed and opened with a longitudinal incision. Select worms were removed in the field and fixed in 95% ethanol for later molecular analysis. The remaining worms and the spiral intestines were

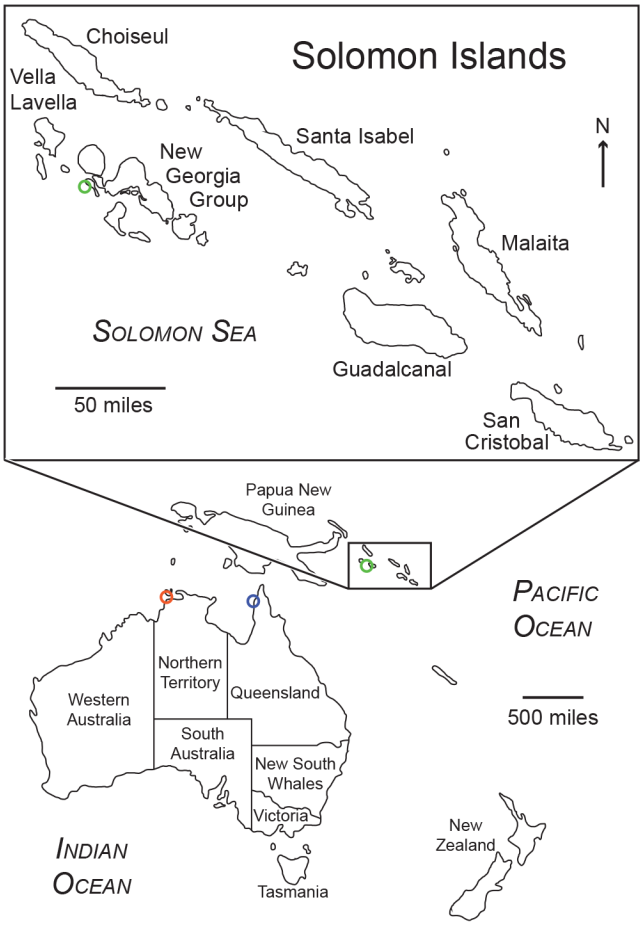

Figure 2. Capture localities for individuals of *Himantura granulata* examined in this study. Green circles denote the locality in the Solomon Islands, the blue circle denotes the locality in Queensland, and the red circle denotes the locality in Northern Territory.

fixed in 10% seawater-buffered formalin and eventually transferred to 70% ethanol at the University of Kansas or the University of Connecticut for permanent storage.

Specimen Preparation

Formalin-fixed specimens were prepared as whole mounts for light microscopy as follows. Worms were hydrated in distilled water, stained in Delafield's hematoxylin, differentiated in tap water, destained in 70% acidic ethanol, alkanized in 70% basic ethanol, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted on glass slides under cover slips in Canada balsam.

Scoleces or whole worms for examination using scanning electron microscopy (SEM)

were prepared as follows. Scoleces were removed from the strobila, and the remaining portion of the strobila was saved and prepared as a permanent whole-mounted voucher. Scoleces or whole worms were hydrated in a graded ethanol series, transferred to 1% osmium tetroxide (OsO_4) and refrigerated at 4° C overnight, rinsed in distilled water, dehydrated in a graded ethanol series, and transferred to hexamethyldisilizane (HMDS) (Ted Pella, Inc., Redding, California, USA) for 30 min. Specimens were then allowed to air-dry before being mounted on aluminum stubs on double-sided adhesive carbon tape. Specimens were sputter-coated with ~35 nm of gold/ palladium and examined with an FEI Versa 3D Dual Beam scanning electron microscope at the Microscopy and Analytical Imaging Laboratory, University of Kansas, Lawrence, Kansas, USA.

Paraffin histological sections of terminal proglottids and scoleces were prepared as follows. Terminal proglottids or scoleces were removed from the strobila, and the remaining portion of the worm was prepared as a permanent whole-mounted voucher. Terminal proglottids and scoleces were then dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin following conventional protocols. Serial sections were cut at 6–7 µm intervals using an Olympus TBS CUT 4060 microtome. Sections were attached to glass slides by floating on 3% sodium silicate (Na_2O_3Si) solution, and allowed to air-dry on a slide warmer. Paraffin was dissolved by transferring sections to xylene. Sections were hydrated in a graded ethanol series, stained with Delafield's hematoxylin, counterstained in eosin, differentiated in Scott's solution, dehydrated in a graded ethanol series, and cleared in xylene. Sections were then mounted on glass slides under cover slips in Canada balsam.

A subset of scoleces embedded in paraffin and sectioned were stained with an adaptation of McManus' periodic acid-Schiff (PAS) reaction (McManus 1948) as follows. Following the affixation of sections to glass slides (as above), paraffin was removed by placing sections in xylene. Subsequently, sections were fully hydrated in a graded ethanol series and distilled water, exposed to 0.5% period acid solution, rinsed with distilled water, stained with Schiff's reagent (Electron Microscopy Sciences, Hatfield, PA), rinsed with warm running tap water or two changes of warm distilled water, counterstained with Delafield's hematoxylin, rinsed with warm

running tap water, dehydrated in a graded ethanol series, and cleared again in xylene. Sections were then mounted on glass slides under cover slips in Canada balsam.

Plastic histological sections of terminal proglottids and scoleces were prepared as follows. Terminal proglottids or scoleces were removed, and the remaining portion of the worm was prepared as a permanent whole-mounted voucher. Terminal proglottids and scoleces were then dehydrated in a graded ethanol series, transferred to a 1:1 solution of 100% ethanol and Technovit® H7100 2-hydroxyethyl methacrylate infiltrating resin (GMA) (Kluzer, Wehrheim, Germany) for two hours, then transferred to pure infiltrating resin and refrigerated at 4° C overnight. Terminal proglottids and scoleces were then embedded in Technovit® H7100 embedding solution in plastic block holders. Serial sections were cut at 4–5 µm intervals on a glass knife using an Olympus TBS CUT 4060 microtome. Sections were attached to Fisherbrand® Superfrost Plus charged microscope slides (Fisherbrand; Fisher, Pittsburgh, Pennsylvania) by floating on ~10 µl drops of distilled water, and allowed to air-dry. Sections were stained with Delafield's hematoxylin, counterstained in eosin, differentiated in Scott's solution, dehydrated in a graded ethanol series, dried for ~2 min in a 60° F oven, and then mounted under cover slips in Canada balsam.

Line drawings were made using a camera lucida attached to a Zeiss Axioskop 2 Plus. Photomicrographs of whole mounts and histological sections were obtained with a Leica DFC420, a Leica DFC480, or a Luminera Infinity 3 camera attached to a Zeiss Axioskop 2 Plus. Measurements were made using Openlab Demo Version 4.0.4, the Leica Application Suite V3 (Leica Application Suite, Leica microsystems, Switzerland), or INFINITY ANALYZE (Lumenera Corporation, Ottawa, Ontario) image analysis software programs. Measurements are reported in micrometers unless otherwise specified, and are given as ranges followed in parentheses by the mean, standard deviation, number of individuals measured, and total number of measurements made if more than one measurement was taken for each individual. Measurements of reproductive organs were made of organs in mature terminal proglottids only. Terminology for microthrix forms follows Chervy (2009). Museum abbreviations used

are as follows: Lawrence R. Penner Parasitology Collection (LRP), University of Connecticut, Storrs, Connecticut, USA; Queensland Museum (QM), South Brisbane, Queensland, Australia; National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C., USA. Statistical analyses of measurement data and host size/tapeworm species associations were performed using R v. 3.2.4 statistical software. Host classification follows Naylor et al. (2012a).

Molecular and Phylogenetic Methods

A subset of specimens originally preserved in 95% EtOH were utilized for DNA sequencing. Prior to processing, the majority of specimens were photographed using a Leica DFC420 or Leica DFC480 camera attached to a Leica MZ16 dissecting scope, or a Leica DFC420, Leica DFC480, or Luminera Infinity 3 camera attached to a Zeiss Axioskop 2 Plus. Scoleces only, or terminal proglottids and scoleces, were removed from each specimen and permanently mounted on slides in Canada balsam to serve as hologenophores (*sensu* Pleijel et al. 2008). Genomic DNA was extracted from each specimen using a QIAGEN® DNEasy® blood and tissue kit (QIAGEN Group). The kit protocol was followed with the following alterations: DNA was eluted in 100 µl Buffer AE and incubated for 10 min at room temperature, then centrifuged for 2 min at 8,000 rpm. The D1–D3 gene region of the large nuclear ribosomal subunit (28s rDNA) was amplified using illustra™ PuRETaq™ Ready-To-Go™ PCR beads (GE Healthcare, Buckinghamshire, United Kingdom) in a BioRad® Alpha Unit under the following temperature profile: denatured at 94° C for 2 min, annealed at 94° C for 30 sec, 55° C for 30 sec, and 72° C for 2 min (repeated for 40 cycles), then elongated at 72° C for 10 min. The forward primer ZX-1 (5'–ACCCGCTGAATTTAAGCATAT–3') (modified from van der Auwera et al. 1994) and the reverse primer 1500R (5'–GCTATCCTGAGGGAAACTTCG–3') (Olson et al. 2003, Tkach et al. 2003) were used for amplification.

PCR amplicons were loaded into a 1% molecular grade agarose gel with 1X TAE buffer. Gels were stained using SYBR® Safe DNA Gel Stain (ThermoFisher Scientific) and samples were allowed to run at 80V for ~30 min. The results of gel electrophoresis were visualized and

imaged using a KODAK Gel Logic 100 gel imaging system on an ultraviolet lamp tray. PCR amplicons were then purified using a QIAquick® PCR Purification Kit (QIAGEN Group). The kit protocol was followed with the following alterations: to bind DNA to the QIAquick column, samples were centrifuged for 60 sec at 13,000 rpm; DNA was eluted in 32 µl Buffer EB and allowed to incubate at room temperature for 5 min prior to centrifuging to increase DNA concentration. The DNA yield of purified PCR amplicons was quantified using a Nanodrop 2000 spectrophotometer and ND 2000/2000c software v. 1.4.1. DNA was sequenced by ACGT, Inc. (Wheeling, Illinois) using single pass primer extension. PCR primers and, in individual cases, the internal sequencing primer 300F (5'–CAAGTACCGTGAGGGAAAGTTG–3') (Littlewood et al. 2000) were used for sequencing. Contigs were assembled in Geneious v. 5.6.5 or 8.0.5 and aligned using MUSCLE in Geneious v. 5.6.5 or 8.0.5 using default settings. Phylogenetic trees were constructed using maximum likelihood (ML) analyses.

D1–D3 28S rDNA data for 14 specimens representing multiple species of *Anthocephalum* Linton 1891 from *H. granulata* were combined in a matrix with sequence data generated by Healy et al. (2009), Caira et al. (2014), Ruhnke et al. (2015), and Marques and Caira (2016) for 19 species in the rhinebothriidean family Anthocephalidae Ruhnke, Caira & Cox 2015: *Anthocephalum alicae* Ruhnke 1994 (KM658205); *Anthocephalum cairae* Ruhnke 1994 (KM658202); *Anthocephalum currani* Ruhnke & Seaman 2009 (KM658203); *Anthocephalum decrisantisorum* Ruhnke, Caira & Cox 2105 (KM658194); *Anthocephalum healyae* Ruhnke, Caira & Cox 2015 (KM658200); *Anthocephalum hobergi* (Zamparo, Brooks & Barriga 1999) Marques & Caira 2016 (KU295566); *Anthocephalum jensenae* Ruhnke, Caira & Cox 2015 (KM658193); *Anthocephalum mattisi* Ruhnke, Caira & Cox 2015 (FJ177059); *Anthocephalum meadowsi* Ruhnke, Caira & Cox 2015 (KM658195); *Anthocephalum michaeli* Ruhnke & Seaman 2009 (KM658204); *Anthocephalum odonnellae* Ruhnke, Caira & Cox 2015 (KM658201); *Anthocephalum papefayi* Ruhnke, Caira & Cox 2015 (KM658199); *Anthocephalum philruschi* Ruhnke, Caira & Cox 2015 (KM658196); *Anthocephalum* n. sp. 1 *sensu* Ruhnke et al. 2015 (KM658206); *Anthocephalum* n. sp. 2 *sensu* Ruhnke et al. 2015 (KM658198); *Anthocephalum*

n. sp. 3 *sensu* Ruhnke et al. 2015 (KM658192); New genus 1 n. sp. 1 *sensu* Healy et al. 2009 (FJ177107); New genus 2 cf. *sexorchidum sensu* Healy et al. 2009 (FJ177108); and New genus 4 cf. *kinabatanganensis sensu* Healy et al. 2009 (FJ177118). Taxa from the following rhinebothriidean families were used as outgroups: Rhinebothriidae Euzet 1953 (*Rhinebothrium megacanthophallus* Healy 2006 [FJ177120]), Echeneibothriidae de Beauchamp 1905 (*Pseudanthobothrium* sp. [KF685750]), and Escherbothriidae Ruhnke, Caira & Cox 2015 (*Escherbothrium* sp. [KM658197]), as well as a rhinebothriidean currently without family-level designation, New genus 11 n. sp. 1 *sensu* Healy et al. 2009 (FJ177119), and the "tetraphyllidean" *Caulobothrium opithorchis* (Riser 1955) Yamaguti 1959 (FJ177106).

Exclusion sets were generated in Gblock v. 0.91b (Castresana 2000, Talavera and Castresana 2007) accessed via the Gblock online server using default settings for the least stringent conditions, and jModelTest v. 2.1.7 (Guindon and Gascuel 2003, Darriba et al. 2012) was used to estimate the best-fitting model of evolution using Akaike Information Criterion corrections (AICc). Ten ML analyses were conducted using the desktop version of Garli v. 2.01 (Zwickl 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Doctoral dissertation, University of Texas at Austin, TX, USA) using GTR+I+Γ as the specified model of evolution. The aligned matrix consisted of 1,053 base pairs, of which 120 were excluded. Of the remaining 933 base pairs, 475 were invariable. ML bootstrap values were generated from 100 bootstrap replicates using the ML configurations. Clades with bootstrap values of 90% or greater were considered to have high nodal support. SumTrees v. 4.0.0 in DendroPy v. 4.0.3 (Sukumaran and Holder 2010) was used to display bootstrap values greater than 50% on the best tree resulting from the ten ML runs.

RESULTS

Species of Tapeworms Parasitizing *Himantura granulata*

Of the ten specimens of *Himatura granulata* examined for this study, one individual—SO-25, a small juvenile ray from the Solomon Islands—was found not to host any tapeworms. The remaining nine individuals were found to host tapeworms. For this study, more than 3,800 tapeworms were removed from spiral intestines. From these available specimens, 526 whole mounts and vouchered specimens were prepared for examination using light microscopy, 48 specimens were prepared for scanning electron microscopy, 12 scoleces and seven terminal proglottids were prepared as histological sections, and molecular sequence data were generated for 36 individuals. In total, 32 species from 13 genera across five orders were found to parasitize the nine individuals of *H. granulata* from the Solomon Islands and the two localities in northern Australia: seven lecanicephalidea species representing three genera, 12 rhinebothriidean species representing three genera, four onchoproteocephalidean species representing one genus, eight trypanorhynch species representing five genera, and one "tetraphyllidean" species (see Table 2). No species representing the Cathetocephalidea, Litobothriidea, or Phylobothriidea were encountered.

Unfortunately, it was beyond the scope of this study to positively identify all 32 species as either known species, or new to science. Instead, one new lecanicephalidean genus and its two new species are described herein, as are five new species of the rhinebothriidean genus *Anthocephalum*. Additionally, a single species representing a second new lecanicephalidean genus, a single species of the rhinebothriidean genus *Stillabothrium* Reyda & Healy 2016 (Reyda et al. 2016, in review), and a single species of the onchoproteocephalidean genus *Acanthobothrium* were confidently identified as new to science based on a combination of unique scolex morphology and/or proglottid anatomy relative to their congeners. Given the degree of host specificity typically exhibited by elasmobranch tapeworms, the remaining 14 non-trypanorhynch species are also likely new to science, but confirmation of new species status will require the examination of additional specimens and comparison to type material of congeners in

Table 2. Species of tapeworms parasitizing *Himantura granulata* from the Solomon Islands and northern Australia by host individual.

				Small Juvenile Hosts (DW less than 35 cm)						Large Mature Hosts (DW greater than 100 cm)			
				Northern Territory	Queensland	Solomon Islands				Solomon Islands			
Order	Family	Genus	Species I.D.	AU-32	CM03-74	SO-21	SO-23	SO-24	SO-25	SO-9	SO-17	SO-18	SO-19
Rhinebothriidea	Anthocephalidae	*Anthocephalum*	n. sp. 1							×	×	×	×
Rhinebothriidea	Anthocephalidae	*Anthocephalum*	n. sp. 2							×	×	×	×
Rhinebothriidea	Anthocephalidae	*Anthocephalum*	n. sp. 3		×					×	×	×	×
Rhinebothriidea	Anthocephalidae	*Anthocephalum*	n. sp. 4A/4B					×		×*	×	×	×*
Rhinebothriidea	Anthocephalidae	*Anthocephalum*	n. sp. 5							×			
Rhinebothriidea	Anthocephalidae	*Anthocephalum*	n. sp. 6	×				×		×	×	×	×
Rhinebothriidea	Rhinebothriidae	*Rhinebothrium*	sp. 1							×	×	×	×
Rhinebothriidea	Rhinebothriidae	*Rhinebothrium*	sp. 2									×	×
Rhinebothriidea	Rhinebothriidae	*Rhinebothrium*	sp. 3					×		×			
Rhinebothriidea	Rhinebothriidae	*Rhinebothrium*	sp. 4		×								
Rhinebothriidea	Rhinebothriidae	*Rhinebothrium*	sp. 5		×								
Rhinebothriidea	Escherbothriidae	*Stillabothrium*	n. sp. 1	×	×								
Onchoproteocephalidea		*Acanthobothrium*	n. sp. 1							×			×
Onchoproteocephalidea		*Acanthobothrium*	sp. 2							×			
Onchoproteocephalidea		*Acanthobothrium*	sp. 3							×		×	
Onchoproteocephalidea		*Acanthobothrium*	sp. 4	×									
Lecanicephalidea	Polypocephalidae	New Genus 12†	n. sp. 2		×	×	×	×					
Lecanicephalidea	Polypocephalidae	New Genus 12†	n. sp. 3				×			×	×	×	×
Lecanicephalidea	Polypocephalidae	"New Genus 11"†	n. sp. 3				×			×		×	×
Lecanicephalidea	Polypocephalidae	*Polypocephalus*	sp. 1							×	×	×	×
Lecanicephalidea	Polypocephalidae	*Polypocephalus*	sp. 2	×	×	×	×			×	×		×
Lecanicephalidea	Polypocephalidae	*Polypocephalus*	sp. 3		×			×			×	×	
Lecanicephalidea	Polypocephalidae	*Polypocephalus*	sp. 4							×		×	
Trypanorhyncha	Eutetrarhynchidae	*Prochristianella*	*clarkeae*		×								
Trypanorhyncha	Eutetrarhynchidae	*Prochristianella*	sp. 1									×	×
Trypanorhyncha	Eutetrarhynchidae	*Prochristianella*	sp. 2							×			
Trypanorhyncha	Eutetrarhynchidae	*Prochristianella*	sp. 3	×									
Trypanorhyncha	Eutetrarhynchidae	*Dollfusiella*	sp. 1							×			×
Trypanorhyncha	Eutetrarhynchidae	*Paroncomegas*	cf. *myliobatis*							×		×	
Trypanorhyncha	Pterobothriidae	*Pterobothrium*	cf. *australiense*							×			
Trypanorhyncha	Mixodigmatidae	*Halysiorhynchus*	sp. 1									×	
"Tetraphyllidea"		*Caulobothrium*	sp. 1	×									
			Num. Species	6	8	2	4	5	0	20	10	16	14

* presence of specimens posessing the morphological characters specific to the *Anthocephlaum* n sp. 4A/4B species complex indicated by hologenophores and molecular sequence data in addition to whole mount specimens; presence of the *Anthocephalum* n. sp. 4A/4B morphotype is conservatively counted as one species for the total number of species calculated for each host examined

† *sensu* Jensen et al. (2016)

the future. Particularly problematic from a taxonomic standpoint were the eight trypanorhynch species, because positive identification of tapeworms in this order is only possible for specimens for which the tentacles are fully everted and the tentacular armature is readily viewable; thus, all trypanorhynch species were identified to the lowest taxonomic level possible at the time of this study given the quality of available material.

Descriptions of New Taxa

New Genus 12

Diagnosis: Worms euapolytic. Scolex with scolex proper, 4 acetabula, and apical structure consisting of apical modification of scolex proper (AMSP) and apical organ. Acetabula in form of suckers. Apical modification of scolex proper cylindrical, housing apical organ; posterior portion with conspicuous hastate spinitriches, anterior rim invaginable; anterior portion devoid of hastate spinitriches, invaginable. Apical organ with external and internal components; external component retractable, non-invaginable, with central disk surrounded by 8 concave muscular, membrane-bound pads; central disk with opening to internal component; internal component glandular, heterogeneous.

Cephalic peduncle absent. Proglottids craspedote, non-laciniate; immature proglottids not laterally expanded; circumcortical longitudinal muscle bundles absent. Testes 4, arranged in single medial column, 1 layer deep in cross-section, in single field anterior to ovary. Vas deferens sinuous, extending from level posterior to ovary to posterior margin of anterior-most testis, expanded to form external seminal vesicle. Cirrus sac pyriform, angled anteriorly, containing cirrus. Cirrus armed, thin-walled. Internal seminal vesicle present. Genital pores lateral, irregularly alternating; genital atrium shallow. Ovary H-shaped in dorso-ventral view, tetralobed in cross-section, with lobate margins. Vagina medial, thin-walled, sinuous, extending from ootype region to cirrus sac, opening into genital atrium posterior to level of cirrus sac. Seminal receptacle absent. Vitellarium follicular; vitelline follicles large, in 2 lateral bands; each band consisting of 1 dorsal and 1 ventral column, extending entire length of proglottid on aporal

side, absent anterior to cirrus sac on poral side, partially interrupted by ovary. Uterus saccate, extending along median line of proglottid from near anterior margin of ovary to posterior margin of anterior-most testis. Eggs not observed. Excretory vessels in 2 lateral pairs. Parasites of *Himantura* (Myliobatiformes: Dasyatidae). Western Pacific Ocean.

Taxonomic Summary

Type species: New Genus 12 n. sp. 2.

Additional species: New Genus 12 n. sp. 3; New Genus 12 n. sp. 1 *sensu* Jensen et al. (2016).

Remarks

The phylogenetic analysis of Jensen et al. (2016) based on molecular sequence data placed New Genus 12 n. sp. 1 robustly within the family Polypocephalidae Meggitt 1924. Morphological data support this placement, including the possession of a single column of four testes, two pairs of excretory vessels, vitelline follicles largely interrupted by the ovary, and an elaborate apical structure. New Genus 12 is easily distinguished from all 24 valid lecanicephalidean genera (see Jensen et al. 2016) by its unique apical structure morphology: an extensive cylindrical apical modification of the scolex proper (AMSP) and a bipartite apical organ with an external retractable central disk surrounded by eight concave muscular, membrane-bound pads and an internal heterogeneous glandular component.

Specifically, New Genus 12 can be distinguished from the other genera in the Polypocephalidae as follows. While *Polypocephalus* Braun 1878 and *Anthemobothrium* Shipley & Hornell 1906 possess an apical organ divided into tentacles, and *Flapocephalus* Deshmukh 1979 an apical organ in the form of two muscular semi-circles, the apical organ of New Genus 12 is in the form of a central disk surrounded by eight concave muscular, membrane-bound pads. New Genus 12 differs from *Anteropora* Subhapradha 1955 (with the exception of *Anteropora comicus* [Jensen, Nikolov & Caira 2011] Jensen, Caira, Cielocha, Littlewood & Waeschenbach 2016) and *Hornellobothrium* Shipley & Hornell 1906 in possessing a scolex with acetabula in the

form of suckers rather than bothridia. However, while *A. comicus* is hyperapolytic and possess an apical modification of the scolex proper that is highly elongate, New Genus 12 is apolytic and possess an AMSP that is not highly elongate. Additionally, unlike *Hornellobothrium*, New Genus 12 does not possess laterally expanded proglottids in the anterior region of its strobila.

New Genus 12 most closely resembles *Seussapex* Jensen & Russell 2014 in its relatively large overall body size, and its possession of four acetabula in the form of suckers and a large, retractable, multipartite apical structure. However, the two genera can be distinguished from one another in that the apical organ of *Seussapex* is externally bipartite (knob-like anterior and dome-shaped posterior parts, each independently retractable) housing two glandular compartments internally, while the apical organ of New Genus 12 is externally a single unit in the form of a central disk surrounded by eight concave muscular, membrane-bound pads, housing a single heterogeneous glandular compartment internally.

Following Jensen et al. (2016), three lecanicephalidean genera (*Corrugatocephalum* Caira, Jensen & Yamane 1997; *Healyum* Jensen 2001; and *Quadcuspibothrium* Jensen, 2001) remain *incertae sedis*. Its prominent apical organ easily distinguishes New Genus 12 from *Healyum* and *Quadcuspibothrium*, both of which possess a small, internal apical organ, and from *Corrugatocephalum*, which possesses an apical organ that is sucker-like with an internal corrugated surface. New Genus 12 is further distinguished from *Quadcuspibothrium* in having acetabula in the form of suckers rather than diamond-shaped bothridia. New Genus 12 can be distinguished from *Corrugatocephalum* and *Quadcuspibothrium* in its possession of testes in a single, rather than two or more layers. While New Genus 12 possesses a cirrus armed with spinitriches, the cirrus of *Healyum* lacks spinitriches (i.e., is unarmed).

New Genus 12 n. sp. 2

(Figs. 3–5)

Description (based on whole mounts of 10 complete mature and 2 incomplete mature worms, cross-sections of 1 mature proglottid, frontal sections of 1 scolex, and 1 specimen prepared for

SEM): Worms euapolytic, 3.8–9.2 (6.8 ± 1.8; 10) mm long; maximum width at level of scolex; proglottids 47–93 (68 ± 17.3; 11) in number. Scolex (Fig. 3A) 308–527 (432 ± 74.4; 11) long by 211–295 (241 ± 32.1; 11) wide, consisting of 4 acetabula, apical modification of scolex proper, and apical organ. Acetabula in form of suckers, 47–71 (61 ± 5.9; 46; 11) in diameter. Apical modification of scolex proper (AMSP) cylindrical, housing apical organ; anterior rim invaginable; anterior portion invaginable. Apical organ with external and internal components; external component in form of central disk surrounded by 8 concave muscular, membrane-bound pads, 202–305 (253 ± 32.2; 10) long by 289–339 (307 ± 20.4; 8) wide when everted, retractable, non-invaginable; central disk with opening to internal component; muscular pads 72–124 (96 ± 18.3; 8; 19) long by 73–102 (85 ± 9.2; 9; 18) wide; internal component single heterogeneous glandular compartment.

Scolex proper with capiliform filitriches (Fig. 4F). Distal acetabular surface with hastate spinitriches and acicular filitriches (Fig. 4H). Posterior portion of AMSP with large, hastate spinitriches and capiliform filitriches (Fig. 4D); anterior portion with acicular to capiliform filitriches (Fig. 4C). External component of apical organ with acicular filitriches (Fig. 4B). Proglottids with capiliform filitriches (Fig. 4G).

Cephalic peduncle absent. Proglottids craspedote, non-laciniate. Immature proglottids 44–87 (64 ± 15.9; 11) in number, initially wider than long, becoming longer than wide with maturity; posterior-most immature proglottid 278–535 (393 ± 90.8; 12) long by 146–237 (196 ± 30.7; 12) wide. Mature proglottids 1–7 (4 ± 1.8; 11) in number, terminal proglottid 400–1,857 (911 ± 400.4; 11) long by 146–260 (198.2 ± 35.1; 11) wide. Testes 4 in number, 36–102 (60 ± 13.7; 11; 31) long by 29–157 (85 ± 30.6; 10; 27) wide, arranged in single medial column, 1 row deep in cross-section (Fig. 5C), in field from near anterior margin of proglottid to near anterior margin of ovary; may be degenerated in terminal mature proglottids. Vasa efferentia not observed. Vas deferens sinuous, extending from level posterior to ovary to posterior margin of anterior-most testis, expanded to form external seminal vesicle in terminal mature proglottids. Cirrus sac pyriform, angled slightly anteriorly, 37–114 (74 ± 22.1; 10) long by 75–124 (100 ±

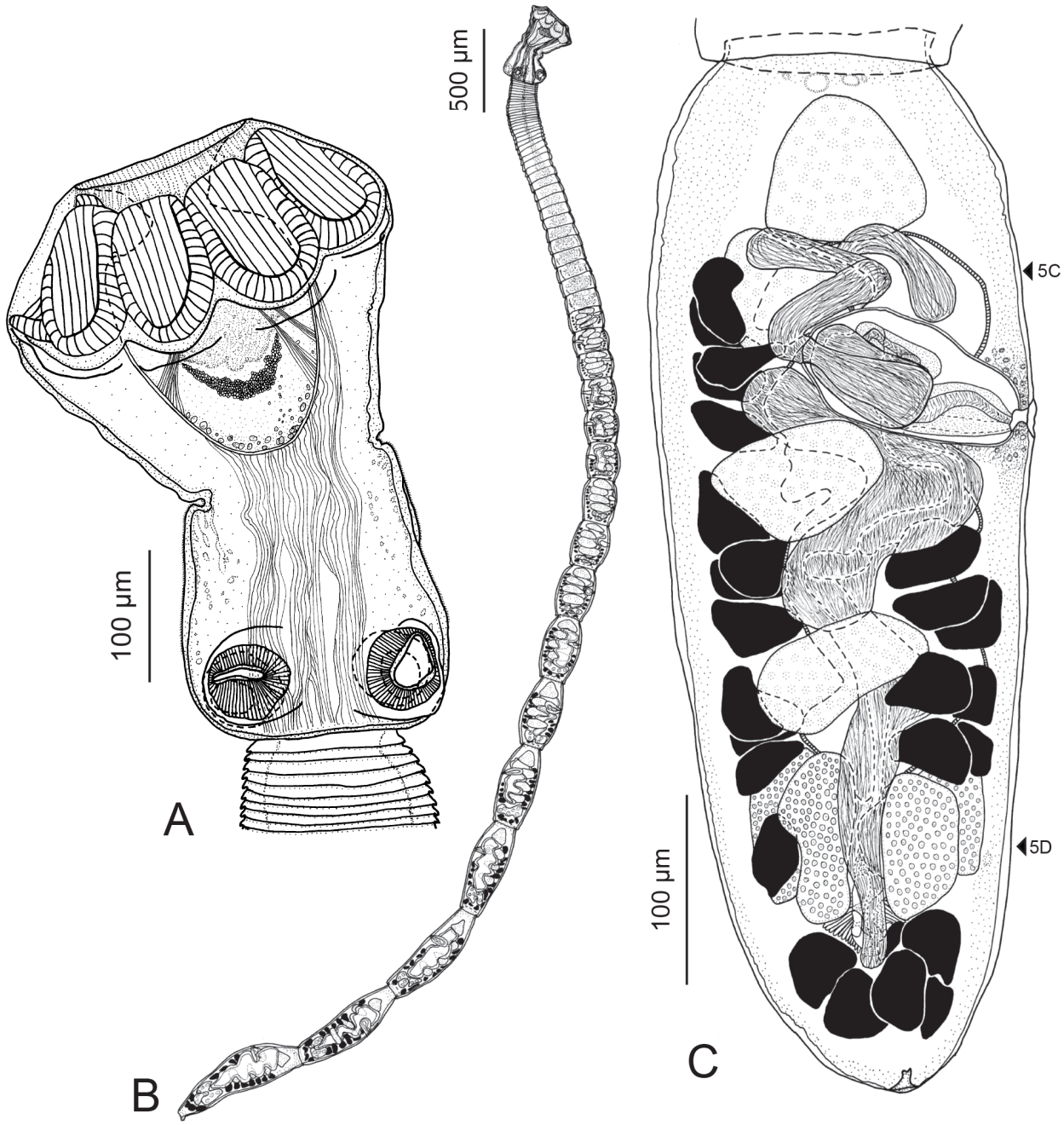

Figure 3. Line drawings of New Genus 12 n. sp. 2. (A) Scolex with apical organ everted. (B) Whole worm with apical organ everted. (C) Mature terminal proglottid. Arrows indicate levels at which cross-sections presented in Fig. 5 were taken.

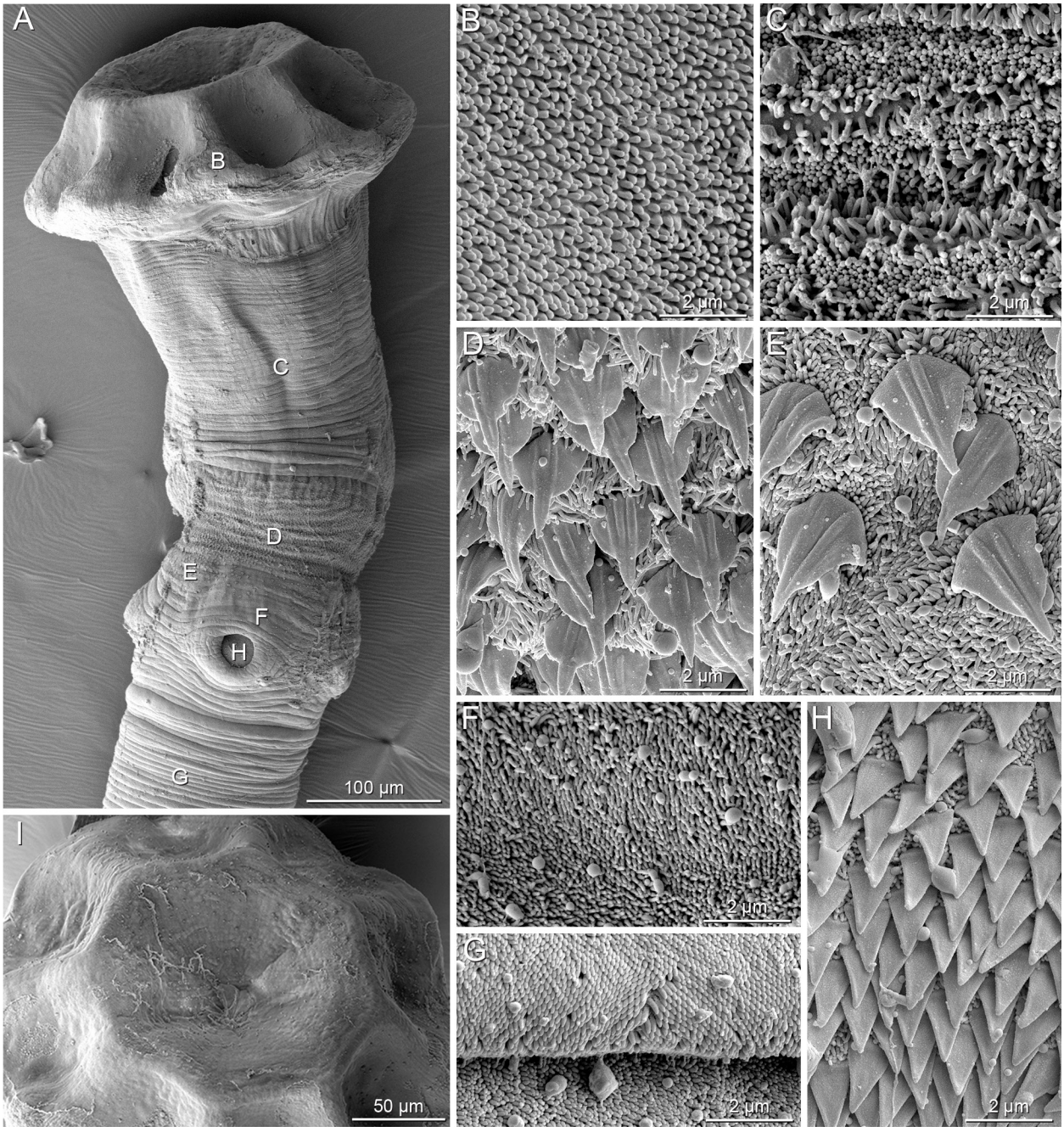

Figure 4. Scanning electron micrographs of New Genus 12 n. sp. 2. (A) Scolex with apical organ everted; small letters indicate location of details shown in Fig. 4B–H. (B) Acicular filitriches on external component of apical organ (AO). (C) Acicular to capiliform filitriches on anterior portion of apical modification of scolex proper (AMSP). (D) Large, hastate spinitriches and capiliform filitriches on posterior portion of AMSP. (E) Sparse large, hastate spinitriches on poster margin of AMSP transitioning to scolex proper (SP). (F) Capiliform filitriches on SP. (G) Capiliform filitriches on proglottid. (H) Hastate spinitriches and acicular filitriches in acetabulum. (I) Whole surface of external component of AO.

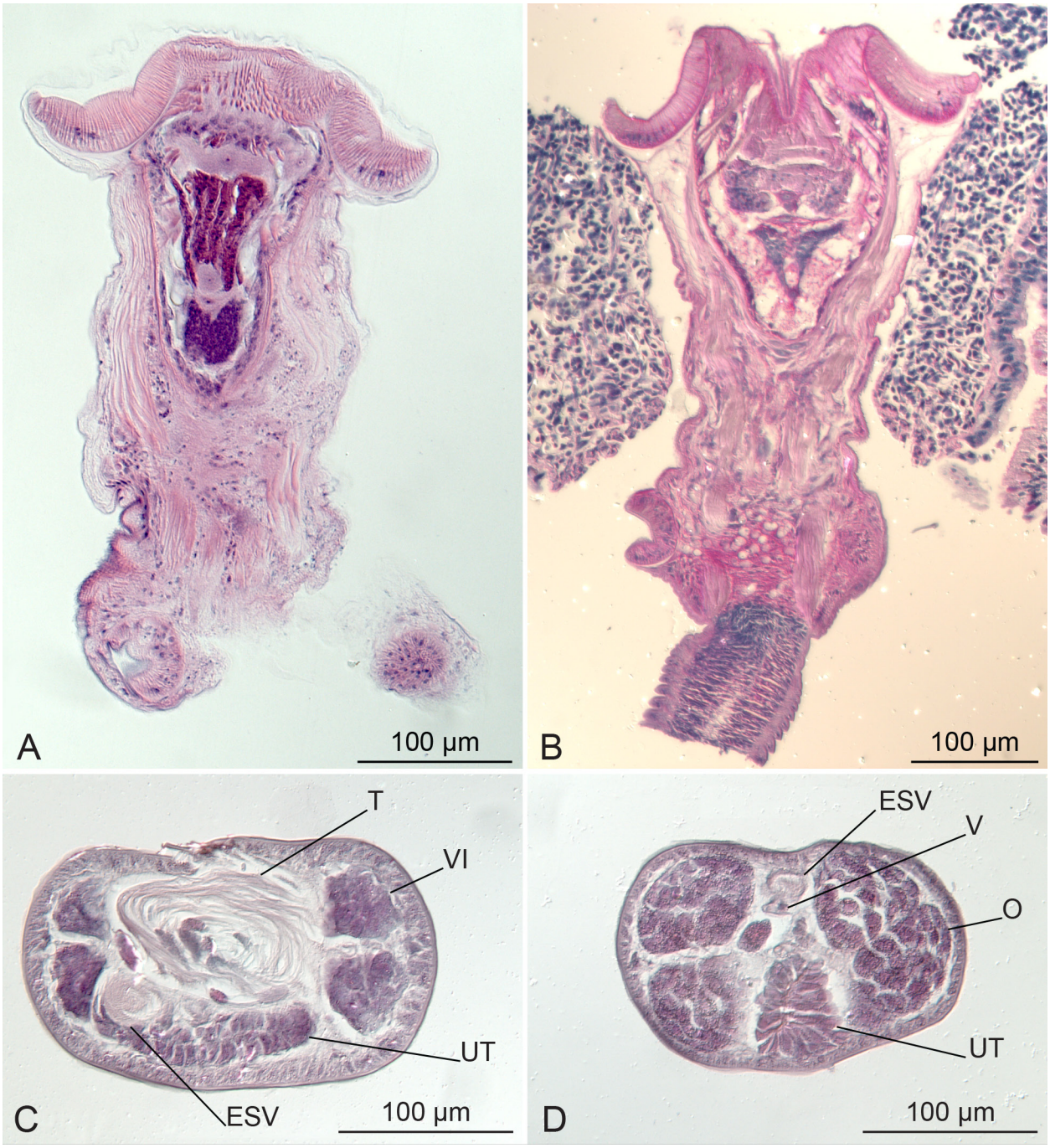

Figure 5. Photomicrographs of sections of New Genus 12 n. sp. 2. (A) Frontal section of scolex with apical organ everted stained with hematoxylin. (B) Frontal section of scolex with apical organ everted stained with PAS. (C) Cross-section through mature proglottid anterior to cirrus sac. (D) Cross-section through mature proglottid slightly posterior to ovarian bridge. *Abbreviations:* ESV, external seminal vesicle; O, ovary; T, testis; UT, uterus; V, vagina; VI, vitelline follicle.

19.7; 8) wide, containing coiled cirrus. Cirrus armed, thin-walled. Internal seminal vesicle present. Genital pores lateral, irregularly alternating, 56–74% (66 ± 5.7; 11) of proglottid length from posterior end; genital atrium shallow. Ovary H-shaped in dorso-ventral view, tetralobed in cross-section (Fig. 5D), 33–233 (139 ± 59.5; 9) long by 82–141 (116 ± 21.5; 7) wide, with lobate margins; ovarian bridge at center of ovary. Mehlis' gland near posterior margin of ovary. Vagina medial, thin-walled, sinuous, extending from ootype to genital atrium, opening into genital atrium posterior to level of cirrus sac. Seminal receptacle absent. Vitellarium follicular; vitelline follicles medullary, large, in two lateral bands; each band consisting of 1 dorsal and 1 ventral column (Fig. 5C), extending entire length of proglottid, interrupted by genital pore and largely interrupted by ovary, 14–108 (50 ± 22.3; 11; 33) long by 19–61 (39 ± 11.0; 10; 30) wide. Uterus saccate, along median line of proglottid, extending from slightly posterior to anterior margin of ovary to level of anterior-most testis, laterally displaced in mature proglottids. Eggs not observed. Excretory vessels in 2 lateral pairs.

Taxonomic Summary

Type and only host species: Himantura granulata Macleay, the mangrove whipray (Myliobatiformes: Dasyatidae).

Type locality: Near Rarumana (8°13'23.8"S; 157°0'2.4"E), Western Province, Vonavona, Solomon Islands, Solomon Sea (SO-23, SO-24).

Additional localities: Near Rarumana (8°14'13.4"S, 157°1'53.7"E), Western Province, Vonavona, Solomon Islands, Solomon Sea (SO-21); Weipa (2°35'11"S, 141°42'34"E), Queensland, Australia, Gulf of Carpentaria, Indian Ocean (CM03-74).

Site of infection: Spiral intestine.

Prevalence of infection: 40% (4 of 10 host specimens).

Type material: Holotype (QM), six paratypes (QM; four whole mounts, one proglottid cross-section series and one scolex frontal section series stained with hematoxylin); three paratypes (USNM; all whole mounts), five paratypes (LRP; three whole mounts, one

SEM voucher and one scolex frontal section series stained with PAS); one scolex prepared for SEM remains in the collection of Dr. Kirsten Jensen at the University of Kansas.

Remarks

While most lecanicephalideans, particularly polypocephalids, are small, often less than 1 mm in total length (see Caira and Jensen 2014), this new species is somewhat unusual in reaching total lengths of up to 9.1 mm. It is also of note that the apical organ of all specimens of this new species recovered was fully or mostly everted. All 15 type specimens and seven voucher specimens parasitized host specimens less than 35 cm in disk width.

New Genus 12 n. sp. 3

(Figs. 6–8)

Description (based on whole mounts of 12 complete, mature worms, cross-sections of 1 proglottid, frontal sections of 2 scoleces, and 3 specimens prepared for SEM): Worms euapolytic, 1.6–3.3 (2.1 ± 0.6; 12) mm long; maximum width 198–296 (244 ± 31.3; 13) at level of scolex; proglottids 15–30 (22 ± 4.5; 13) in number. Scolex (Fig. 6B) 385 (1) long when apical organ everted, 278–401 (313 ± 30.9; 12) long when apical organ retracted, consisting of 4 acetabula, apical modification of scolex proper, and apical organ. Acetabula in the form of suckers, 51–72 (61 ± 5.0; 14; 54) in diameter. Apical modification of scolex proper (AMSP) cylindrical, housing apical organ; anterior rim invaginable; anterior portion invaginable. Apical organ with external and internal components; external component in form of central disk surrounded by 8 concave muscular, membrane-bound pads, 229 (1) long by 249 (1) wide when everted, 192–283 (239 ± 28.8; 12) long by 160–243 (205 ± 25.8; 12) wide when retracted, non-invaginable; central disk with opening to internal component; muscular pads 51–74 (64 ± 5.3; 13; 25) long by 42–68 (60 ± 6.5; 13; 26) wide; internal component single heterogeneous glandular compartment.

Scolex proper with capiliform filitriches (Fig. 7D). Distal acetabular surface with hastate

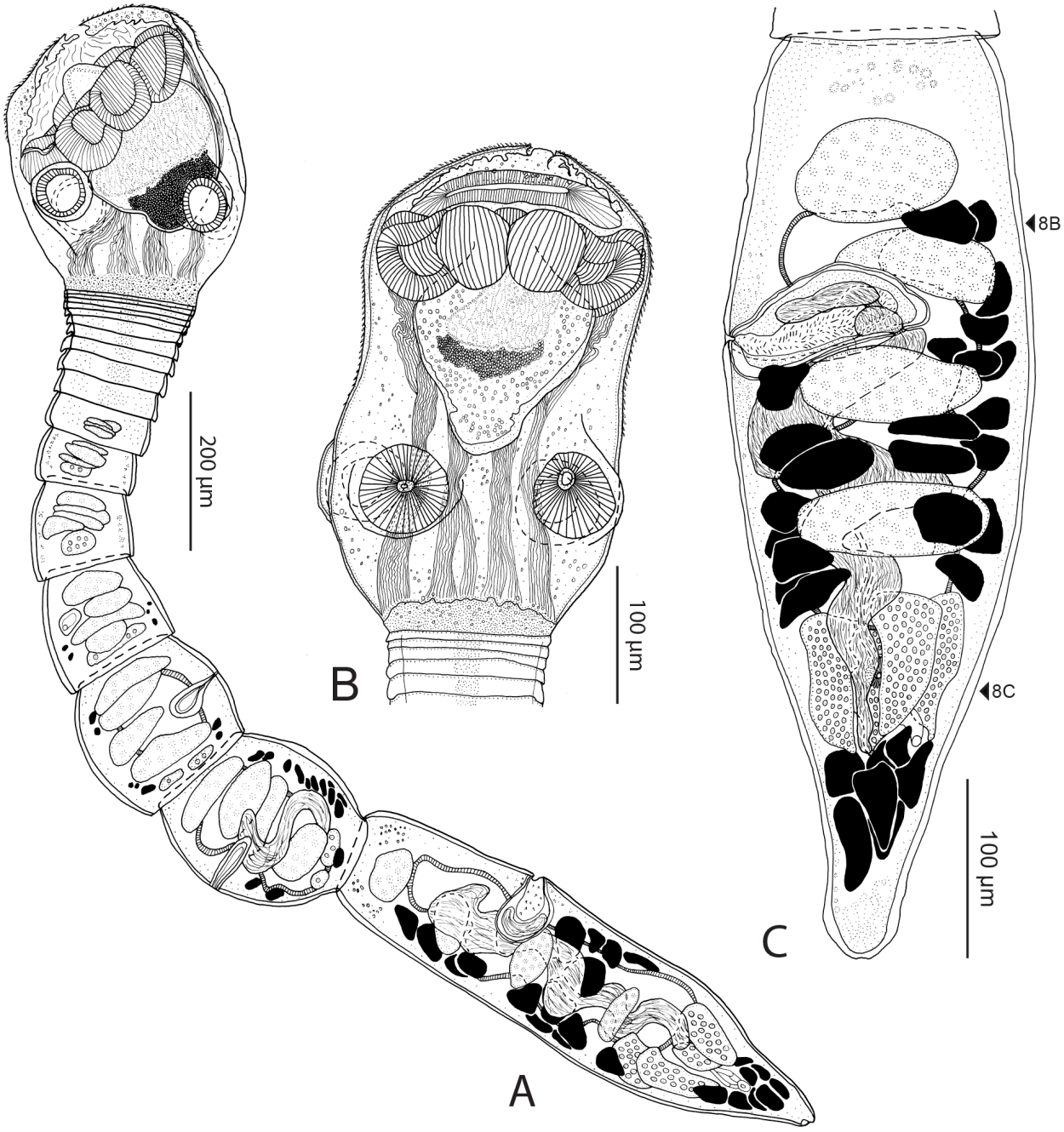

Figure 6. Line drawings of New Genus 12 n. sp. 3. (A) Whole worm with apical organ retracted. (B) Scolex with apical organ retracted. (C) Mature terminal proglottid. Arrows indicated levels at which cross-sections presented in Fig. 8 were taken.

spinitriches and acicular filitriches (Fig. 7E). Posterior portion of apical modification of scolex proper with large, hastate spinitriches and acicular to capiliform filitriches (Fig. 7B). Anterior portion of apical modification of scolex proper and apical organ microtriches not observed.

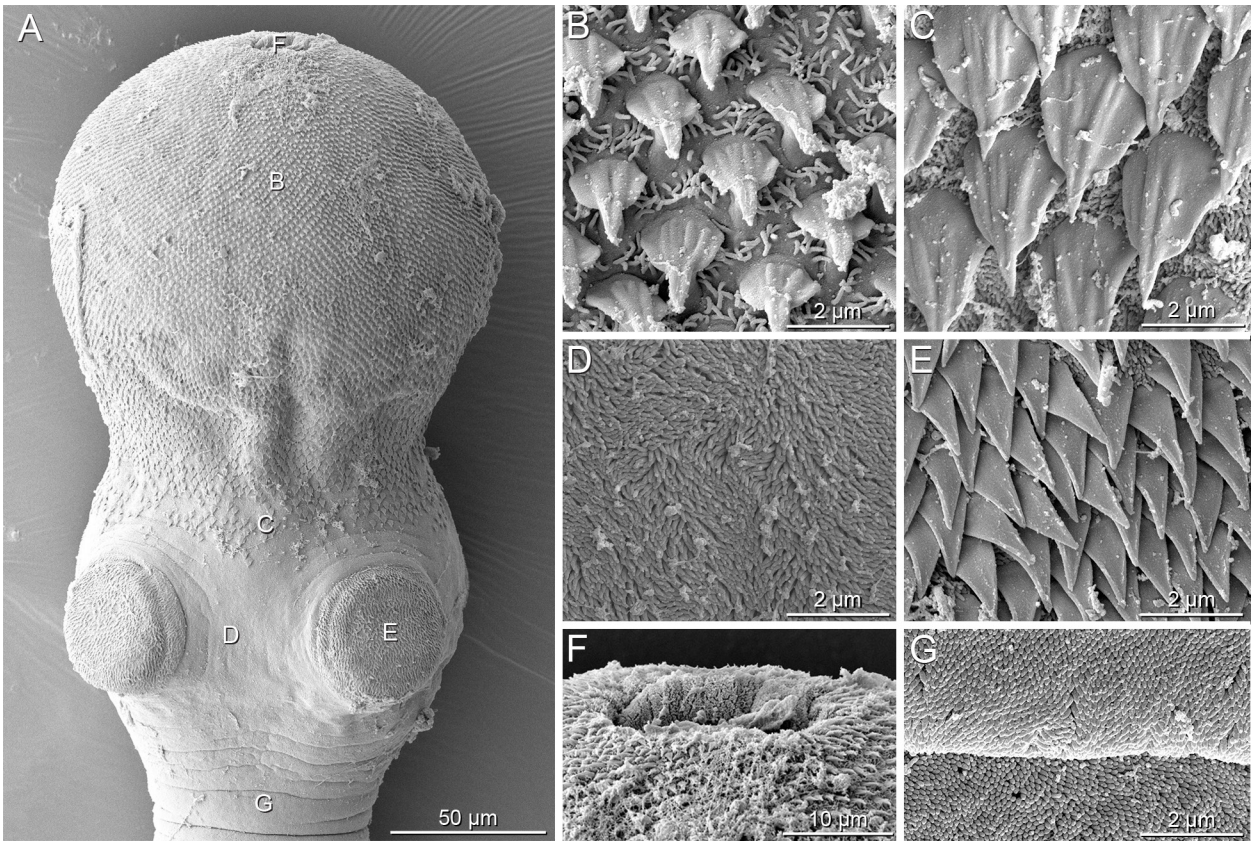

Figure 7. Scanning electron micrographs of New Genus 12 n. sp. 3. (A) Scolex with apical organ retracted; small letters indicate location of details shown in Fig. 7 B–G. (B) Large, hastate spinitriches and acicular to capiliform filitriches on posterior portion of apical modification of scolex proper (AMSP). (C) Large, hastate spinitriches on poster margin of AMSP transitioning to scolex proper (SP). (D) Capiliform filitriches on SP. (E) Hastate spinitriches and acicular filitriches in acetabulum. (F) Apex of AMSP with apical organ retracted. (G) Capiliform filitriches on proglottid.

Proglottids with capiliform filitriches (Fig. 7G).

Cephalic peduncle absent. Proglottids craspedote, non-laciniate. Immature proglottids 14–27 (20 ± 4.3; 13) in number, initially wider than long, becoming longer than wide with maturity; posterior-most immature proglottid 177–377 (246 ± 60.0; 13) long by 91–187 (138 ± 28.3; 13) wide. Mature proglottids 1–3 (2 ± 0.9; 12) in number, terminal proglottid 484–890 (612 ± 127.0; 12) long by 118–195 (148 ± 22.9; 12) wide. Testes 4 in number, 28–80 (49 ± 14.6; 12; 34) long by 32–107 (69 ± 17.0; 12; 34) wide, arranged in a single medial column, 1 row deep in cross-section, in field from anterior margin of proglottid to near anterior margin of ovary; may be degenerated in terminal mature proglottids. Vasa efferentia not observed. Vas deferens

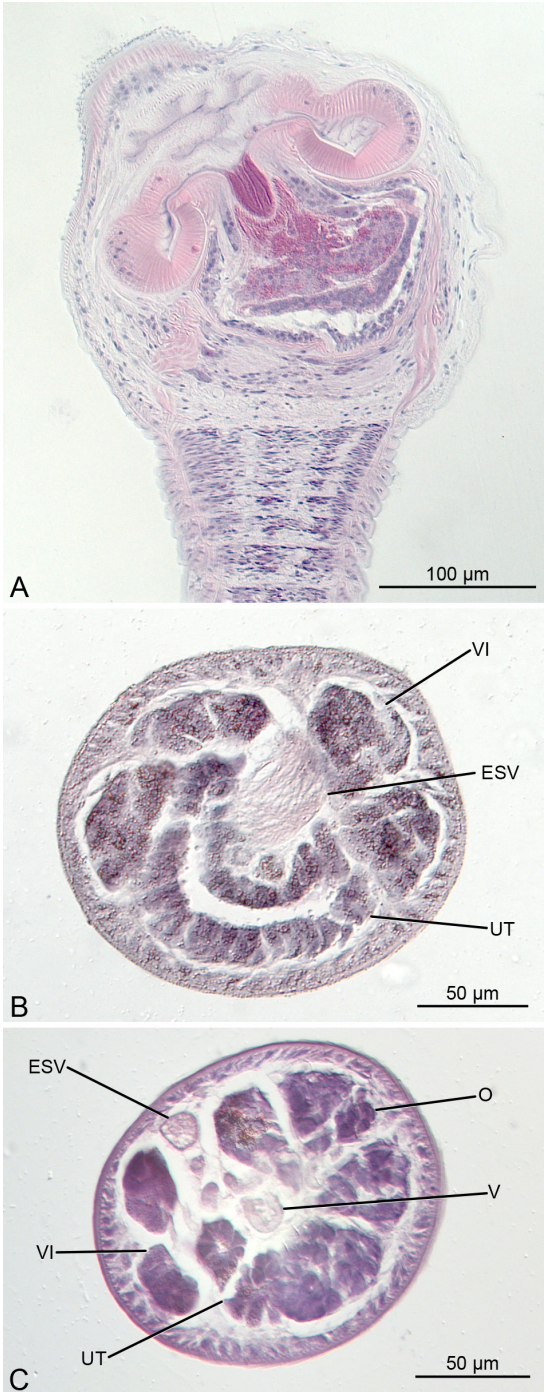

Figure 8. Photomicrographs of sections of New Genus 12 n. sp. 3. (A) Frontal section of scolex with apical organ retracted. (B) Cross-section though mature proglottid anterior to cirrus sac. (C) Cross-section through mature proglottid slightly posterior to ovarian bridge. *Abbreviations:* ESV, external seminal vesicle; O, ovary; UT, uterus; V, vagina; VI, vitelline follicle.

sinuous, extending from level posterior to ovary to posterior margin of anterior-most testis, expanded to form external seminal vesicle in terminal mature proglottids. Cirrus sac pyriform, angled slightly anteriorly, 33–86 (55 ± 17.7; 11) long by 52–150 (83 ± 28.4; 11) wide, containing coiled cirrus. Cirrus armed, thin-walled. Internal seminal vesicle present. Genital pores lateral, irregularly alternating, 61–68% (66 ± 2.3; 12) of proglottid length from posterior end; genital atrium shallow. Ovary H-shaped in dorso-ventral view, tretralobed in cross-section (Fig. 8C), 61–188 (107 ± 43.6; 11) long by 52–127 (84 ± 24.4; 12) wide, with lobate margins; ovarian bridge at center of ovary. Mehlis' gland near posterior margin of ovary. Vagina medial, thin-walled, sinuous, extending from ootype to genital atrium, opening into genital atrium posterior to level of cirrus sac. Seminal receptacle absent. Vitellarium follicular; vitelline follicles medullary, large, in two lateral bands; each band consisting of 1 dorsal and 1 ventral column (Fig, 8B), extending entire length of proglottid, interrupted by genital pore and partially interrupted by ovary, 8–59 (33.4 ± 13.2; 12; 36) long by 16–50 (26 ± 8.7; 12; 36) wide. Uterus saccate, along median line of proglottid, extending from slightly posterior to anterior margin of ovary to level of anterior-most testis, laterally displaced in mature proglottids. Eggs not observed. Excretory vessels in 2 lateral pairs

Taxonomic Summary

Type and only host species: Himantura granulata Macleay, the mangrove whipray (Myliobatiformes: Dasyatidae).

Type locality: Near Rarumana (8°13'23.8"S, 157°0'2.4"E), Western Province, Vonavona, Solomon Islands, Solomon Sea (SO-9, SO-17, SO-18, SO-19, SO-23).

Additional localities: None.

Site of infection: Spiral intestine.

Prevalence of infection: 50% (5 of 10 host specimens).

Type material: Holotype (QM), six paratypes (QM; four whole mounts, one proglottid cross-section series and one scolex frontal section series stained with hematoxylin); four

paratypes (USNM; all whole mounts), four paratypes (LRP; three whole mounts and one scolex frontal section series stained with hematoxylin); one scolex prepared for SEM and the strobila voucher of that scolex, along with two whole worms prepared for SEM, remain in the collection of Dr. Kirsten Jensen at the University of Kansas.

Remarks

This new species, though very similar in overall scolex morphology and proglottid anatomy to New Genus 12 n. sp. 2, can be distinguished from the latter species in that it possesses fewer proglottids overall (14–27 vs. 44–87, respectively) and, in general, fewer mature proglottids (on average 2 vs. 4, respectively). Consequently, the two species also differ from one another in total length. While New Genus 12 n. sp. 2 is 3.8–9.2 mm in total length, New Genus 12 n. sp. 3 only reaches a maximum total length of 3.3 mm. All but one of the individual worms of New Genus 12 n. sp. 3 examined herein presented with their apical organs fully or mostly retracted. Thus, scolex length with the apical organ everted could only be measured for a single specimen. Of the 18 type specimens and 15 voucher specimens examined in this study, 31 parasitized host specimens greater than 100 cm in disk width, while only two parasitized host specimens less than 35 cm in disk width.

Anthocephalum n. sp. 1

(Figs. 9 & 12A)

Description (based on 24 specimens: 20 whole mounts of mature worms, cross-sections of 2 mature proglottids, frontal sections of 1 scolex, and 1 scolex prepared for SEM): Worms euapolytic, 2.3−4.9 (3.1 ± 0.6; 20) mm long; maximum width 296−461 (370 ± 44.3; 20) at level of scolex; proglottids 11−21 (16 ± 2.9; 19) in number. Scolex (Fig. 9A) consisting of 4 bothridia; bothridia stalked, folded, with 63−72 (67 ± 2.5; 13) marginal loculi and oval apical sucker; apical sucker 22−55 (41 ± 6.6; 41; 19) long by 37−65 (52 ± 6.0; 32; 18) wide.

Cephalic peduncle absent. Proglottids slightly craspedote, non-laciniate. Immature

proglottids 9−18 (13 ± 2.5; 19) in number, initially wider than long, becoming longer than wide with maturity; posterior-most immature proglottid 247−430 (322 ± 46.1; 20) long by 80−130 (103 ± 14.9; 20) wide. Mature proglottids 2−5 (3 ± 0.7; 20) in number, terminal proglottids 635−1,146 (797 ± 152.1; 20) by 98−184 (130 ± 24.6; 20) wide. Testes 10−15 (12 ± 1.1; 19) in number, 23−64 (42 ± 8.1; 17; 51) long by 26−50 (39 ± 6; 16; 48) wide, arranged in 2 regular columns, 1 row deep in cross-section (Fig. 9C), in field from near anterior margin of proglottid to anterior margin of genital atrium. Vasa efferentia not observed. Vas deferens sinuous, extending from level of ovarian isthmus to posterior-most testes in very mature proglottids. Cirrus sac pyriform, recurved posteriorly, 57−132 (101 ± 16.3; 20) long by 46−109 (78 ± 17.7; 18) wide, containing coiled cirrus. Cirrus thick-walled, armed with large spinitriches (Fig. 9D). Internal seminal vesicle present. Genital pores lateral, irregularly alternating, 33−56% (44 ± 5.9; 20) of proglottid length from posterior end; genital atrium conspicuous and muscular. Ovary in posterior end of proglottid, follicular, H-shaped in dorso-ventral view, tetralobed in cross-section (Fig. 9E), symmetrical, 90−214 (125 ± 32.4; 19) long by 61−122 (94 ± 19.6; 17) wide, with lobate margins; ovarian bridge at center of ovary. Mehlis' gland near posterior margin of ovary. Ovicapt at ovarian bridge, ventral, 14−25 (18 ± 3.0; 15) in diameter. Vagina medial, thick-walled, sinuous, extending from ootype to genital atrium, opening into genital atrium anterior to cirrus sac; vastly expanded proximally. Seminal receptacle absent. Vitellarium follicular; vitelline follicles 10−38 (20 ± 6.6; 19; 57) long by 6−43 (24 ± 8.0; 18; 54) wide, medullary, in 2 lateral bands, each consisting of 1 dorsal and 1 ventral column (Fig. 9C), extending length of proglottid, interrupted by genital pore and interrupted by ovary. Uterus saccate, along median line of proglottid, ventral, extending from slightly posterior to anterior margin of ovary to anterior to field of testes. Eggs not observed. Excretory vessels in 2 lateral pairs.

Taxonomic Summary

Type and only host species: Himantura granulata Macleay, the mangrove whipray (Myliobatiformes: Dasyatidae).

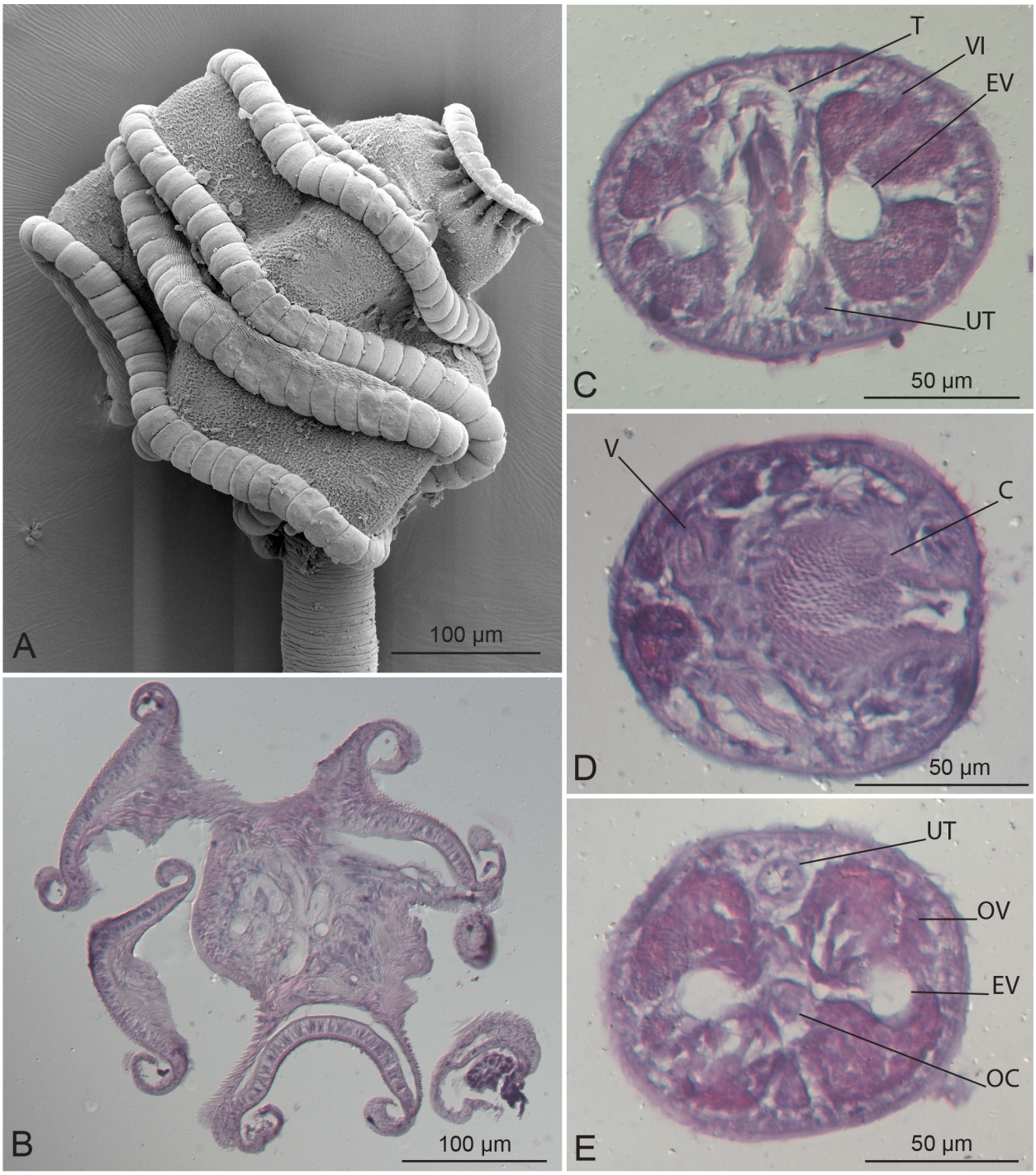

Figure 9. Scanning electron micrograph and photomicrographs of sections of *Anthocephalum* n. sp. 1. (A) Scanning electron micrograph of scolex. (B) Cross-section through scolex. (C) Cross section through mature proglottid anterior to cirrus sac. (D) Cross-section through cirrus sac illustrating large microtriches and expanded vaginal atrium. (E) Cross-section through mature proglottid slightly posterior to ovarian bridge. *Abbreviations:* C, cirrus sac; EV, excretory vessel; OV, ovary; OC, ovicapt region; T, testis; UT, uterus; V, vagina; VI, vitelline follicle.

Type locality: Near Rarumana (8°13’23.8”S, 157°0’2.4”E), Western Province, Vonavona, Solomon Islands, Solomon Sea (SO-9, SO-17, SO-18, SO-19).

Additional localities: None.

Prevalence: 4 of 10 hosts (40%).

Site of infection: Spiral intestine.

Remarks

Based on the most recent taxonomic treatment of the genus *Anthocephalum* by Ruhnke et al. (2015) and the additional species subsequently transferred to the genus by Marques and Caira (2016), 18 species of *Anthocephalum* are considered valid. *Anthocephalum* n. sp. 1 differs from all but three of these species—*Anthocephalum jensenae*, *Anthocephalum meadowsi*, and *Anthocephalum papefayi*—in having fewer testes (10–15 vs. 17 or greater). Of these three species, *A.* n. sp. 1 is most similar to *A. jensenae*, but differs from this species by its possession of a muscular genital pore, which is absent in *A. jensenae*. Additionally, *A.* n. sp. 1 has vitelline follicles arranged in a single dorsal and single ventral column on each side of the proglottid, whereas *A. jensenae* possesses vitelline follicles arranged in two to three dorsal and two to three ventral columns on each side of the proglottid. *Anthocephalum* n. sp. 1 is shorter as compared to *A. meadowsi* (2.3−4.9 mm vs. 7.9−16.8 mm) and possesses far fewer proglottids (9–18 vs. 30–40). *Anthocephalum* n. sp. 1 can be distinguished from *A. papefayi* by its possession of vitelline follicles posterior to the ovary; *A. papefayi* is currently the only described species in the genus that does not possess post-ovarian vitteline follicles. There are two species of *Anthocephalum* whose testes ranges, though not overlapping with the range of *A.* n. sp. 1, abut that of *A.* n. sp. 1 closely (i.e., may possess 17 testes); *A.* n. sp. 1 is differentiable from *Anthocephalum decrisantisorum* in terms of total length (2.3−4.9 mm vs. 6.2−15.8 mm) and total number of proglottids (9–18 vs. 20–33), and is differentiable from *Anthocephalum philruschi* in its possession of far fewer marginal loculi (63-72 in *A.* n. sp. 1 vs. 200-219 in *A. philruschi*).

Anthocephalum n. sp. 2

(Figs. 10 & 12B)

Description (based on 37 specimens: 25 whole mounts of mature worms, cross-sections of 1 mature proglottid, frontal sections of 1 scolex, and 10 scoleces prepared for SEM): Worms euapolytic, 1.8−3.5 (2.6 ± 0.4; 25) mm long; maximum width 332−521 (412 ± 47.0; 25) at level of scolex; proglottids 10−16 (13 ± 1.6; 25) in number. Scolex (Fig. 10A) consisting of 4 bothridia; bothridia stalked, folded, with 43−52 (47 ± 2.3; 24; 27) marginal loculi and oval apical sucker; apical sucker 36−59 (44 ± 5.0; 24; 52) long by 41−66 (53 ± 5.3; 25; 51) wide.

Cephalic peduncle absent. Proglottids slightly craspedote, non-laciniate. Immature proglottids 9−15 (12 ± 1.6; 25) in number, initially wider than long, becoming longer than wide with maturity; posterior-most immature proglottid 359−690 (521 ± 93.8; 25) long by 137−300 (202 ± 42.8; 25) wide. Mature proglottids 1−2 (1 ± 0.3; 25) in number, terminal proglottids 625−1,327 (949 ± 192.6; 25) by 188−338 (254 ± 43.6; 25) wide. Testes 23−38 (30 ± 3.4; 23) in number, 25−87 (40 ± 9.9; 25; 75) long by 30−84 (58 ± 11.1; 25; 75) wide, arranged in 2 regular columns, 1 row deep in cross-section (Fig. 10C), in field from near anterior margin of proglottid to anterior margin of genital atrium. Vasa efferentia not observed. Vas deferens sinuous, extending from level of ovarian isthmus to approximately third-most posterior row of testes. Cirrus sac pyriform, recurved posteriorly, 70−135 (93 ± 18.5; 23) long by 67−151 (105 ± 24.7; 23) wide, containing coiled cirrus. Cirrus thin-walled, armed; 294 (1) long by 64 (1) at base and 37 (1) at apex when fully everted. Internal seminal vesicle present. Genital pores lateral, irregularly alternating, 34−50% (40 ± 4.0; 25) of proglottid length from posterior end; genital atrium conspicuous and non-muscular. Ovary in posterior end of proglottid, follicular, H-shaped in dorso-ventral view, tetralobed in cross-section (Fig. 10D), essentially symmetrical, 128−365 (223 ± 54.5; 24) long by 101−181 (133 ± 22.3; 25) wide, with lobate margins; ovarian bridge at center of ovary. Mehlis' gland near posterior margin of ovary. Ovicapt at ovarian bridge, ventral, 22−36 (28 ± 3.6; 22) in diameter. Vagina medial, thick-walled, sinuous, extending laterally from ootype to genital atrium, opening into genital atrium anterior to level of cirrus sac. Seminal

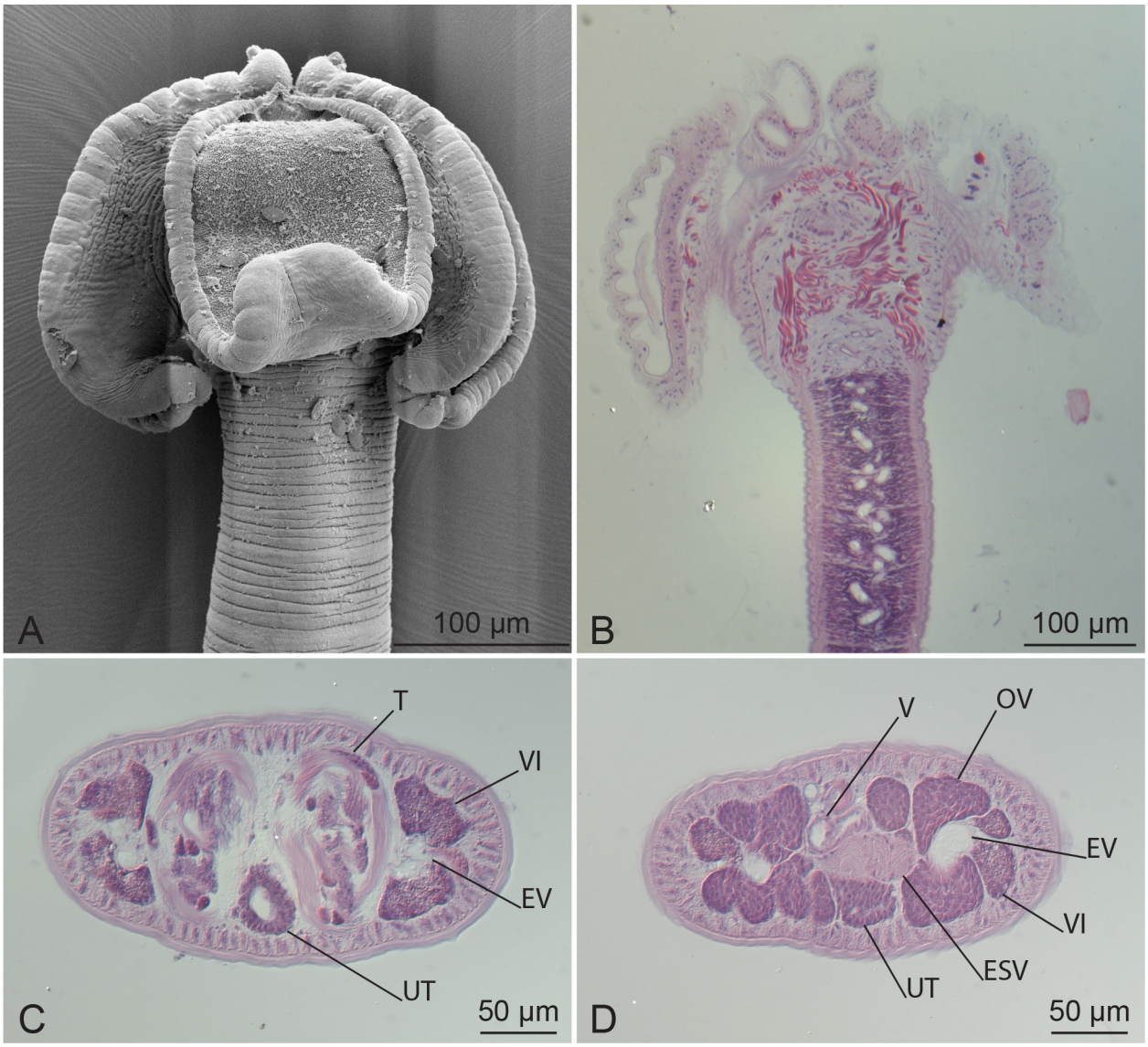

Figure 10. Scanning electron micrograph and photomicrographs of sections of *Anthocephalum* n. sp. 2. (A) Scanning electron micrograph of scolex. (B) Frontal section through scolex. (C) Cross-section through mature proglottid anterior to cirrus sac. (D) Cross-section through mature proglottid slightly anterior to ovarian bridge. *Abbreviations:* ESV, external seminal vesicle; EV, excretory vessel; OV, ovary; T, testis; UT, uterus; V, vagina; VI, vitelline follicle.

receptacle absent. Vitellarium follicular; vitelline follicles 7−37 (17 ± 6.4; 25; 75) long by 9−57 (31 ± 9.8; 25; 75) wide, medullary, in 2 lateral bands, each consisting of 1−2 dorsal and 1−2 ventral columns (Fig. 10C), extending length of proglottid, interrupted by genital pore, uninterrupted by ovary, post-poral and post-ovarian follicles present. Uterus saccate, along median line of proglottid, ventral, extending from slightly posterior to anterior margin of ovary

to posterior margin of third- or second-most anterior row of testes. Eggs not observed. Excretory vessels in 2 lateral pairs.

Taxonomic Summary

Type and only host species: Himantura granulata Macleay, the mangrove whipray (Myliobatiformes: Dasyatidae).

Type locality: Near Rarumana (8°13’23.8”S, 157°0’2.4”E), Western Province, Vonavona, Solomon Islands, Solomon Sea (SO-9, SO-17, SO-18, SO-19).

Additional localities: None.

Prevalence: 4 of 10 hosts (40%).

Site of infection: Spiral intestine.

Remarks

The short anterior extent of the uterus in *Anthocephlaum* n. sp. 2 (i.e., a uterus that does not extend anterior to the field of the testes) distinguishes this species from every described species of *Anthocephalum* to date, with the exception of *Anthocephalum wedli* (Wedl 1855) Ruhnke 2011, for which information on the anterior extent of the uterus is not reported. However, *A.* n. sp. 2 is readily distinguishable from *A. wedli* based on apolysis (*A.* n. sp. 2 is euapolytic whereas *A. wedli* is apolytic) and number of testes (23−38 in *A.* n. sp. 2 vs. 100−130 in *A. wedli*). Additionally, *A.* n. sp. 2 is the only species of *Anthocephalum* described to date possessing vitelline follicles that are not interrupted by the ovary (with the exception of *A. wedli*, for which no information on the extent of vitelline follicles is reported).

Anthocephalum n. sp. 3

(Figs. 11 & 12C)

Description (based on 31 specimens: 26 whole mounts of mature worms, cross-sections of 1 mature proglottid, facial sections of 1 scolex, and 3 scoleces prepared for SEM): Worms

euapolytic, 3.6−7.9 (5.0 ± 1.1; 26) mm long; maximum width 120−908 (523 ± 165.2; 26) at level of scolex; proglottids 17−29 (22 ± 3.4; 26) in number. Scolex (Fig. 11A) consisting of 4 bothridia; bothridia stalked, folded, with 41−57 (49 ± 3.7; 23; 39) marginal loculi and oval apical sucker; apical sucker 36−81 (51 ± 10.4; 26; 60) long by 43−91 (65 ± 11.5; 26; 58) wide.

Cephalic peduncle absent. Proglottids slightly craspedote, non-laciniate. Immature proglottids 15−25 (19 ± 2.8; 26) in number, initially wider than long, becoming longer than wide with maturity; posterior-most immature proglottid 369−832 (536 ± 105.1; 26) long by 156−318 (224 ± 40.6; 26) wide. Mature proglottids 2−4 (2 ± 0.6; 26) in number, terminal proglottids 904−1,565 (1,222 ± 165.9; 26) by 191−369 (249 ± 37.3; 26) wide. Testes 23−32 (28 ± 2.1; 26) in number, 30−73 (50 ± 8.3; 26; 78) long by 43−89 (68 ± 10.2; 26; 78) wide, arranged in 2 regular columns, 1 row deep in cross-section (Fig. 11C), in field from near anterior margin of proglottid to anterior margin of genital atrium. Vasa efferentia not observed. Vas deferens sinuous, extending from level of ovarian isthmus anteriorly to approximately halfway into field of testes. Cirrus sac slightly pyriform, not recurved, 49−89 (67 ± 8.9; 25) long by 81−134 (105 ± 13.4; 25) wide, containing coiled cirrus. Cirrus thin-walled, armed; 210 (1) long by 32 (1) wide when fully everted. Internal seminal vesicle absent. Genital pores lateral, irregularly alternating, 28−40% (35 ± 2.9; 26) of proglottid length from posterior end; genital atrium not conspicuous and non-muscular. Ovary in posterior end of proglottid, follicular, H-shaped in dorso-ventral view, tetralobed in cross-section (Fig. 11D), aporal lobes slightly longer than poral lobes, 208−455 (334 ± 64.1; 26) long by 107−238 (150 ± 29.7; 25) wide, with lobate margins; ovarian bridge at center of ovary. Mehlis' gland near posterior margin of ovary. Ovicapt at ovarian bridge, ventral, 24−39 (32 ± 4.9; 13) in diameter. Vagina medial, thick-walled, sinuous, extending laterally from ootype to genital atrium, opening into genital atrium anterior to level of cirrus sac. Seminal receptacle absent. Vitellarium follicular; vitelline follicles 8−52 (18 ± 6.7; 26; 78) long by 13−71 (38 ± 11.4; 26; 78) wide, medullary, in 2 lateral bands; each band consisting of 1−2 dorsal and 1−2 ventral columns (Fig. 11C), extending length of proglottid, uninterrupted by genital pore and ovary; post-poral and post-ovarian follicles present. Uterus saccate, along median line of

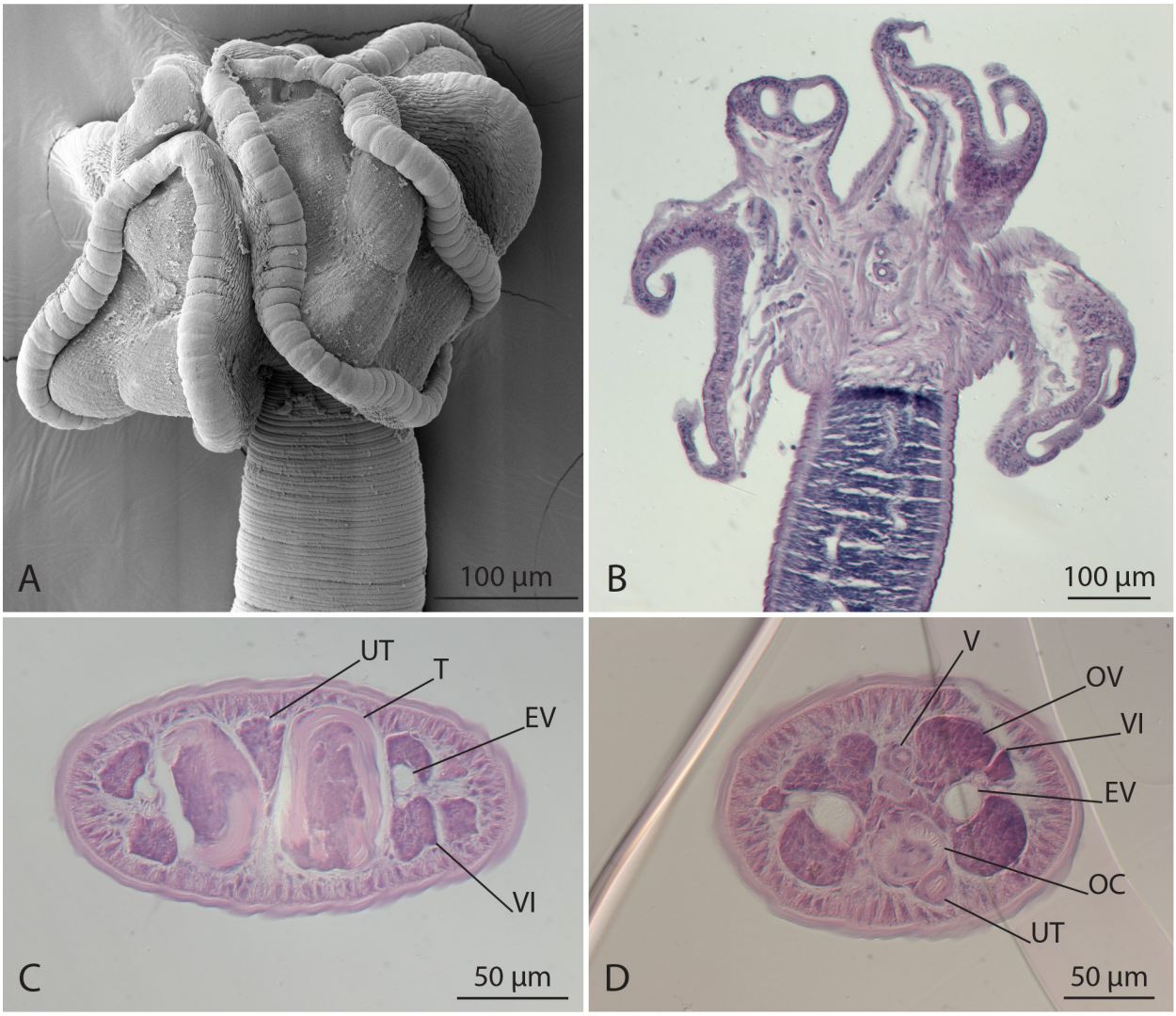

Figure 11. Scanning electron micrograph and photomicrographs of sections of *Anthocephalum* n. sp. 3. (A) Scanning electron micrograph of scolex. (B) Frontal section through scolex. (C) Cross-section through mature proglottid anterior to cirrus sac. (D) Cross-section through mature proglottid slightly anterior to ovarian bridge. *Abbreviations:* EV, excretory vessel; OC, ovicapt region; OV, ovary; T, testis; UT, uterus; V, vagina; VI, vitelline follicle.

proglottid, ventral, extending from slightly posterior to anterior margin of ovary to posterior margin of anterior-most testis. Eggs not observed. Excretory vessels in 2 lateral pairs.

Taxonomic Summary

Type and only host species: Himantura granulata Macleay, the mangrove whipray (Myliobatiformes: Dasyatidae).

Type locality: Near Rarumana (8°13’23.8”S, 157°0’2.4”E), Western Province, Vonavona, Solomon Islands, Solomon Sea (SO-9, SO-17, SO-18, SO-19).

Additional localities: Weipa (2°35’11”S, 141°42’34”E), Queensland, Australia, Gulf of Carpentaria, Indian Ocean (CM03-74).

Prevalence: 5 of 10 hosts (50%).

Site of infection: Spiral intestine.

Remarks

Anthocephalum n. sp. 3 is the second described species of *Anthocephalum* possessing vitelline follicles that are not interrupted by the ovary, the first being *A.* n. sp. 2. *Anthocephalum* n. sp. 3 is distinguishable from *A.* n. sp. 2 by more detailed characteristics of vitelline follicle extent- the vitelline follicles of *A.* n. sp. 3 are not interrupted by the genital pore, while the vitelline follicles of *A.* n. sp. 2 are interrupted by the genital pore. Additionally, *A.* n. sp. 3 possesses a uterus that extends further anteriorly than that of *A.* n. sp. 2 (posterior margin of anterior-most testis vs. posterior margin of third- or second-most anterior row of testes) and possess a vas deferens that extends halfway into the field of the testes, whereas the vas deferens extends only to approximately the third-most posterior row of testes in *A.* n. sp. 2. *Anthocephalum* n. sp. 3 is distinguished from *A. wedli*, for which none of these diagnostic characters are reported, by both apolysis (*A.* n. sp. 3 is euapolytic whereas *A. wedli* is apolytic) and number of testes (23−32 in *A.* n. sp. 3 vs. 100−130 in *A. wedli*).

***Anthocephalum* n. sp. 5**

(Fig. 12E, H)

Description (based on 5 specimens: 4 whole mounts of mature worms, and 1 scolex prepared for SEM): Worms euapolytic, 2.9−3.4 (3.1 ± 0.3; 3) mm long; maximum width 395−494 (460 ± 45.0; 4) at level of scolex; proglottids 8−10 (9 ± 0.8; 4) in number. Scolex (Fig. 12H) consisting of 4 bothridia; bothridia stalked, folded, with 81−99 (91 ± 7.1; 3; 5) marginal loculi and oval

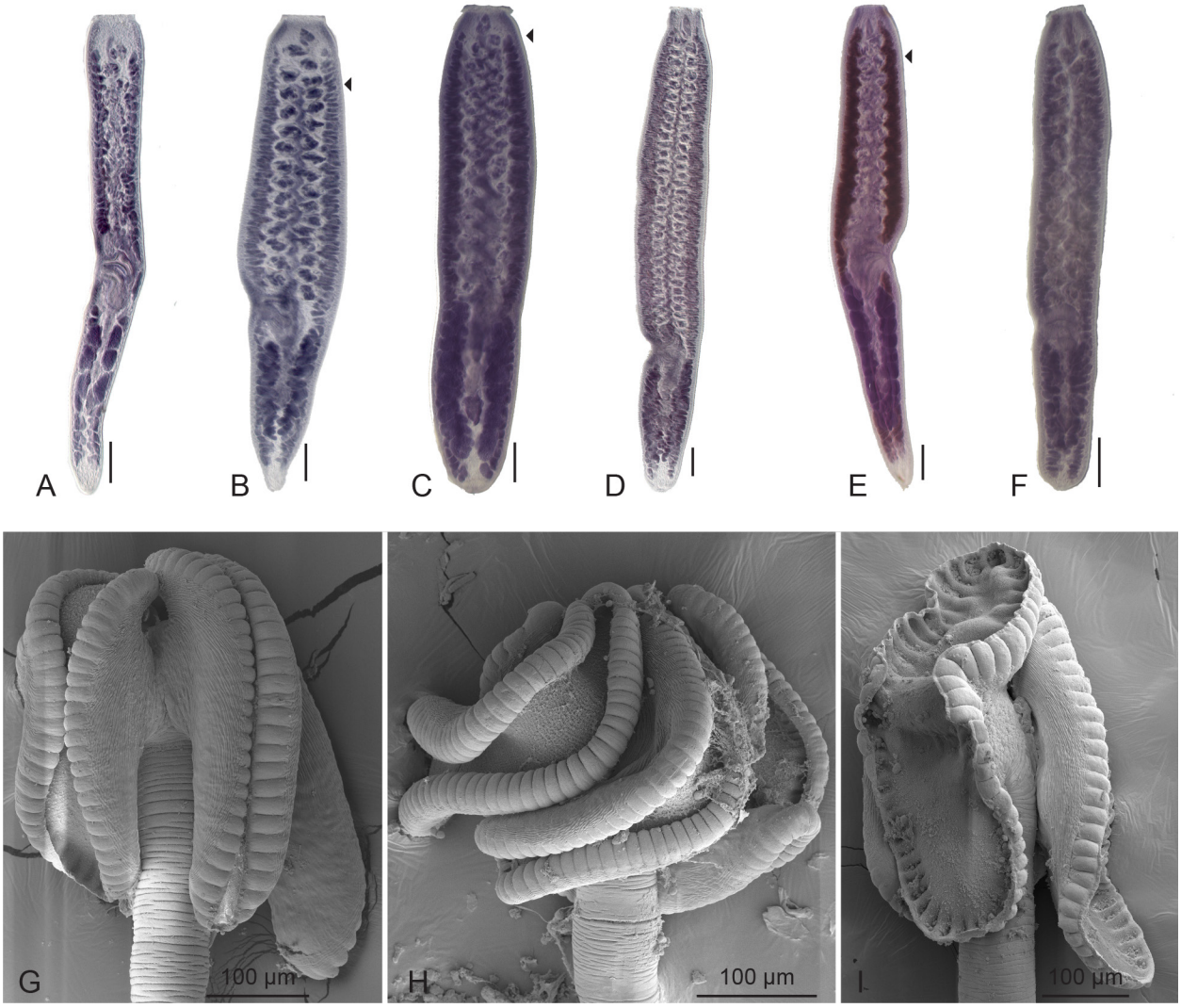

Figure 12. Photomicrographs of terminal proglottids (A–F) and scanning electron micrographs of scoleces (G–I) of *Anthocephalum* spp. (A) *Anthocephalum* n. sp. 1. (B) *Anthocephalum* n. sp. 2. (C) *Anthocephalum* n. sp. 3. (D) P *Anthocephalum* n. sp. 4A/4B morphotype. (E) *Anthocephalum* n. sp. 5. (F) *Anthocephalum* n. sp. 6. (G) *Anthocephalum* n. sp. 4A/4B morphotype. (H) *Anthocephalum* n. sp. 5. (I) *Anthocephalum* n. sp. 6. *Scale bars:* A–F, 100 µm. Arrows on B, C and E indicate anterior extent of uterus for those species for which uterus does not extend anterior to field of testes.

apical sucker; apical sucker 35−55 (43 ± 6.5; 4; 9) long by 37−59 (51 ± 8.2; 3; 7) wide.

Cephalic peduncle absent. Proglottids slightly craspedote, non-laciniate. Immature proglottids 6−8 (7 ± 1.0; 4) in number, initially wider than long, becoming longer than wide with maturity; posterior-most immature proglottid 326−622 (440 ± 127.0; 4) long by 118−146 (132 ± 14.0; 4) wide. Mature proglottids 1−2 (2 ± 0.5; 4) in number, terminal proglottids 1,314−1,571

(1,411 ± 139.6; 3) by 190−223 (203 ± 17.4; 3) wide. Testes 27−30 (29 ± 1.5; 4) in number, 38−53 (47 ± 4.1; 4; 12) long by 42−66 (52 ± 8.5; 4; 12) wide, arranged in 2 regular columns, 1 row deep in cross-section, in field from near anterior margin of proglottid to anterior margin of genital atrium. Vasa efferentia not observed. Vas deferens sinuous, extending approximately from level of ovarian isthmus anteriorly to third- or fourth-most posterior row of testes. Cirrus sac round to slightly panduriform, not recurved to recurved posteriorly, 107−132 (119 ± 11.2; 4) long by 98−134 (112 ± 15.8; 4) wide, containing coiled cirrus. Cirrus thick-walled, armed. Internal seminal vesicle present. Genital pores lateral, irregularly alternating, 41−48% (44 ± 3.4; 3) of proglottid length from posterior end; genital atrium conspicuous and non-muscular. Ovary in posterior end of proglottid, follicular, H-shaped in dorso-ventral view, tetralobed in cross-section, aporal lobes slightly longer than poral lobes, 377−453 (415 ± 38.0; 3) long by 109−132 (121 ± 16.3; 2) wide, with lobate margins; ovarian bridge at approximately center of ovary. Mehlis' gland near posterior margin of ovary. Ovicapt at ovarian bridge, ventral, 32 (1) in diameter. Vagina medial, thick-walled, sinuous, extending laterally from ootype to genital atrium, opening into genital atrium anterior to level of cirrus sac. Seminal receptacle absent. Vitellarium follicular; vitelline follicles 11−42 (21 ± 9.4; 4; 12) long by 19−35 (27 ± 4.2; 4; 12) wide, medullary, in 2 lateral bands; each band consisting of 1−2 dorsal and 1−2 ventral columns, extending from near anterior margin of proglottid to near posterior margin of proglottid, interrupted by genital pore and ovary; post-poral and post-ovarian follicles present. Uterus saccate, along median line of proglottid, ventral, extending from posterior to anterior margin of ovary to posterior margin of third- or fourth-most anterior row of testes. Eggs not observed. Excretory vessels in 2 lateral pairs.

Taxonomic Summary

Type and only host species: Himantura granulata Macleay, the mangrove whipray (Myliobatiformes: Dasyatidae).

Type locality: Near Rarumana (8°13'23.8"S, 157°0'2.4"E), Western Province, Vonavona,

Solomon Islands, Solomon Sea (SO-9).

Additional localities: None.

Prevalence: 1 of 10 hosts (10%).

Site of infection: Spiral intestine.

Remarks

Anthocephalum n. sp. 5 is the third described species of *Anthocephalum* possessing a uterus that does not extend anterior to the field of the testes, with the only other two species being *A*. n. sp. 2 and *A*. n. sp. 3. *Anthocephalum* n. sp. 5 is distinct from both of these species based on its possession of vitelline follicles that are interrupted by the ovary; vitelline follicles are continuous alongside the ovarian lobes in both *A*. n. sp. 2 and *A*. n. sp. 3. *Anthocephalum* n. sp. 5 is distinguished from *A*. *wedli*, for which extent of vitelline follicles is not reported, by both apolysis (*A*. n. sp. 5 is euapolytic whereas *A. wedli* is apolytic) and number of testes (27−30 in *A*. n. sp. 5 vs. 100–130 in *A. wedli*). It is worth noting that while all other species of *Anthocephalum* recovered from *H. granulata* are known from specimens from at least four host individuals, specimens of *A*. n. sp. 5 were recovered from only a single large mature mangrove whipray from the Solomon Islands (SO-9).

Anthocephalum n. sp. 6

(Fig. 12F, I)

Description (based on 7 specimens: 6 whole mounts of mature worms, and 1 scolex prepared for SEM): Worms euapolytic, 3.5−5.1 (4.0 ± 0.6; 6) mm long; maximum width 334−751 (474 ± 145.5; 6) at level of scolex; proglottids 11−17 (14 ± 2.6; 6) in number. Scolex (Fig. 12I) consisting of 4 bothridia; bothridia stalked, folded, with 56−88 (69 ± 14.8; 3; 6) marginal loculi and oval apical sucker; apical sucker 32−45 (36 ± 4.1; 6; 12) long by 35−47 (43 ± 3.6; 6; 11) wide.

Cephalic peduncle absent. Proglottids slightly craspedote, non-laciniate. Immature

proglottids 9−15 (12 ± 2.4; 6) in number, initially wider than long, becoming longer than wide with maturity; posterior-most immature proglottid 520−832 (623 ± 131.6; 6) long by 126−273 (179 ± 53.9; 6) wide. Mature proglottids 1−2 (2 ± 0.5; 6) in number, terminal proglottids 973−1,479 (1,165 ± 193.5; 6) by 158−273 (179 ± 53.9; 6) wide. Testes 22−46 (35 ± 9.7; 6) in number, 30−52 (39 ± 6.9; 5; 15) long by 38−62 (54 ± 7.0; 5; 15) wide, arranged in 2 regular columns, 1 row deep in cross-section, in field from near anterior margin of proglottid to anterior margin of genital atrium. Vasa efferentia not observed. Vas deferens sinuous, extending approximately from level of ovarian isthmus anteriorly to approximately one-third the total distance between anterior margin of genital atrium and anterior margin of proglottid. Cirrus sac round to slightly panduriform, not recurved to recurved slightly posteriorly, 60−93 (75 ± 12.0; 6) long by 81−104 (94 ± 8.0; 6) wide, containing coiled cirrus. Cirrus thick-walled, armed. Internal seminal vesicle absent. Genital pores lateral, irregularly alternating, 26−47% (35 ± 8.8; 6) of proglottid length from posterior end; genital atrium conspicuous and non-muscular. Ovary in posterior end of proglottid, follicular, H-shaped in dorso-ventral view, tetralobed in cross-section, aporal lobes slightly longer than poral lobes, 186−432 (280 ± 84.5; 6) long by 94−147 (119 ± 17.7; 6) wide, with lobate margins; ovarian bridge approximately at center of ovary. Mehlis' gland near posterior margin of ovary. Ovicapt at ovarian bridge, ventral, 24−28 (25 ± 2.3; 3) in diameter. Vagina medial, thick-walled, sinuous, extending laterally from ootype to genital atrium, opening into genital atrium anterior to level of cirrus sac. Seminal receptacle absent. Vitellarium follicular; vitelline follicles 5−28 (12 ± 5.3; 6; 18) long by 16−56 (32 ± 10.1; 6; 18) wide, medullary, in 2 lateral bands; each band consisting of 1 dorsal and 1 ventral column, extending from near anterior margin of proglottid to near posterior margin of proglottid, interrupted by genital pore and interrupted by middle third of ovary; post-poral and post-ovarian follicles present. Uterus saccate, along median line of proglottid, ventral, extending from posterior to anterior margin of ovary to anterior to field of testes. Eggs not observed. Excretory vessels in 2 lateral pairs.

Taxonomic Summary

Type and only host species: Himantura granulata Macleay, the mangrove whipray (Myliobatiformes: Dasyatidae).

Type locality: Near Rarumana (8°13’23.8”S, 157°0’2.4”E), Western Province, Vonavona, Solomon Islands, Solomon Sea (SO-9, SO-17, SO-18, SO-19, SO-24).

Additional localities: Darwin (12°20’11”S, 130°54’39”E), Northern Territory, Australia, Buffalo Creek, Timor Sea, Indian Ocean (AU-32).

Prevalence: 6 of 10 hosts (60%).

Site of infection: Spiral intestine.

Remarks

Anthocephalum n. sp. 6 is distinguished from *A.* n. sp. 2 and *A.* n. sp. 3 by its possession of vitelline follicles that are partially interrupted by the ovary, rather than uninterrupted, and is distinct from *A.* n. sp. 2, *A.* n. sp. 3 and *A.* n. sp. 5 in its possession of a uterus that extends anterior to the field of the testes. *Anthocephalum* n. sp. 6 can be distinguished from *Anthocephalum alicae*, *Anthocephalum currani*, *Anthocephalum duszynskii* Ruhnke 1994, *Anthocephalum gracile* Linton 1890, *Anthocephalum kingae* (Schmidt 1978) Ruhnke & Seaman 2009, and *Anthocephalum wedli* by its type of apolysis; *A.* n. sp. 6 is euapolytic whereas these six species are apolytic. *Anthocephalum* n. sp. 6 has fewer proglottids than *Anthocephalum cairae*, *Anthocephalum duszynskii*, *Anthocephalum healyae*, *Anthocephalum hobergi, Anthocephalum mattisi, Anthocephalum odonnellae* and *Anthocephalum papefayei* (9−15 vs. 80−110, 120−160, 105−133, 53–98, 34−50, 86−120 and 106−177, respectively). *Anthocephalum* n. sp. 6 also has a scolex with fewer marginal loculi than *Anthocephalum lukei* Ruhnke & Seaman 2009, *Anthocephalum meadowsi* and *Anthocephalum philruschi* (56−88 vs. 107−138, 98−134 and 200−219, respectively). *Anthocephalum* n. sp. 6 has more testes than both *Anthocephalum* n. sp. 1 and *Anthocephalum jensenae* (22–46 vs. 10–15 and 14–20, respectively). *Anthocephalum* n. sp. 6 is distinguishable from *Anthocephalum michaeli* by its possession of a uterus than extends

posteriorly beyond the anterior margin of the ovary, as the uterus in *A. michaeli* does not extend posteriorly beyond the genital pore. Overall, *A.* n. sp. 6 most closely resembles *Anthocephalum decrisantisorum*, but it can be distinguished from this species based on vitelline follicle arrangement; *A.* n. sp. 6 possesses one dorsal and one ventral column of vitelline follicles on each side of the proglottid, whereas *A. decrisantisorum* possesses vitelline follicles arranged in two to three dorsal and two to three ventral columns on each side of the proglottid.

Assessing Species Boundaries of *Anthocephalum* Using Molecular Sequence Data

For this study, sequence data were generated from the D1–D3 gene region of 28s rDNA for 19 individuals of *Anthocephalum*, 14 of which were included in the phylogenetic analysis. The morphological species boundaries of the five species of *Anthocephalum* described herein (*A.* n. sp. 1, *A.* n. sp. 2, *A.* n. sp. 3, *A.* n. sp. 5 and *A.* n. sp. 6) were corroborated by these molecular sequence data (see Fig. 13). However, these data also indicate that two additional species of *Anthocephalum* parasitizing *Himantura granulata*—heretofore referred to as *Anthocephlaum* n. sp. 4A and *Anthocephlaum* n. sp. 4B—be recognized. Five specimens from the Solomon Islands were recovered as molecularly distinct from the remaining nine sequenced specimens, and clustered as sister clades containing two and three specimens, respectively. Individuals within each clade differ from one another by 0–4 base pairs, and individuals between the two clades differ from one another by 35–38 base pairs (see Table 3). Despite distinct molecular differences, the scoleces and proglottids of the five hologenophores of these putative species were indistinguishable from one another based on any combination of quantitative and/or qualitative morphological characters. The shared morphological feature that distinguishes these two putative new species from the other five species of *Anthocephalum* parasitizing *H. granulata* is the possession of a recurved vagina. Indeed, this feature—in combination with a vastly expanded vas deferens and a genital pore opening in the posterior third of the proglottid—distinguishes these two putative new species from all species of *Anthocephalum* described to date.

In additional to the five hologenophores, 34 whole mounts of specimens possessing this

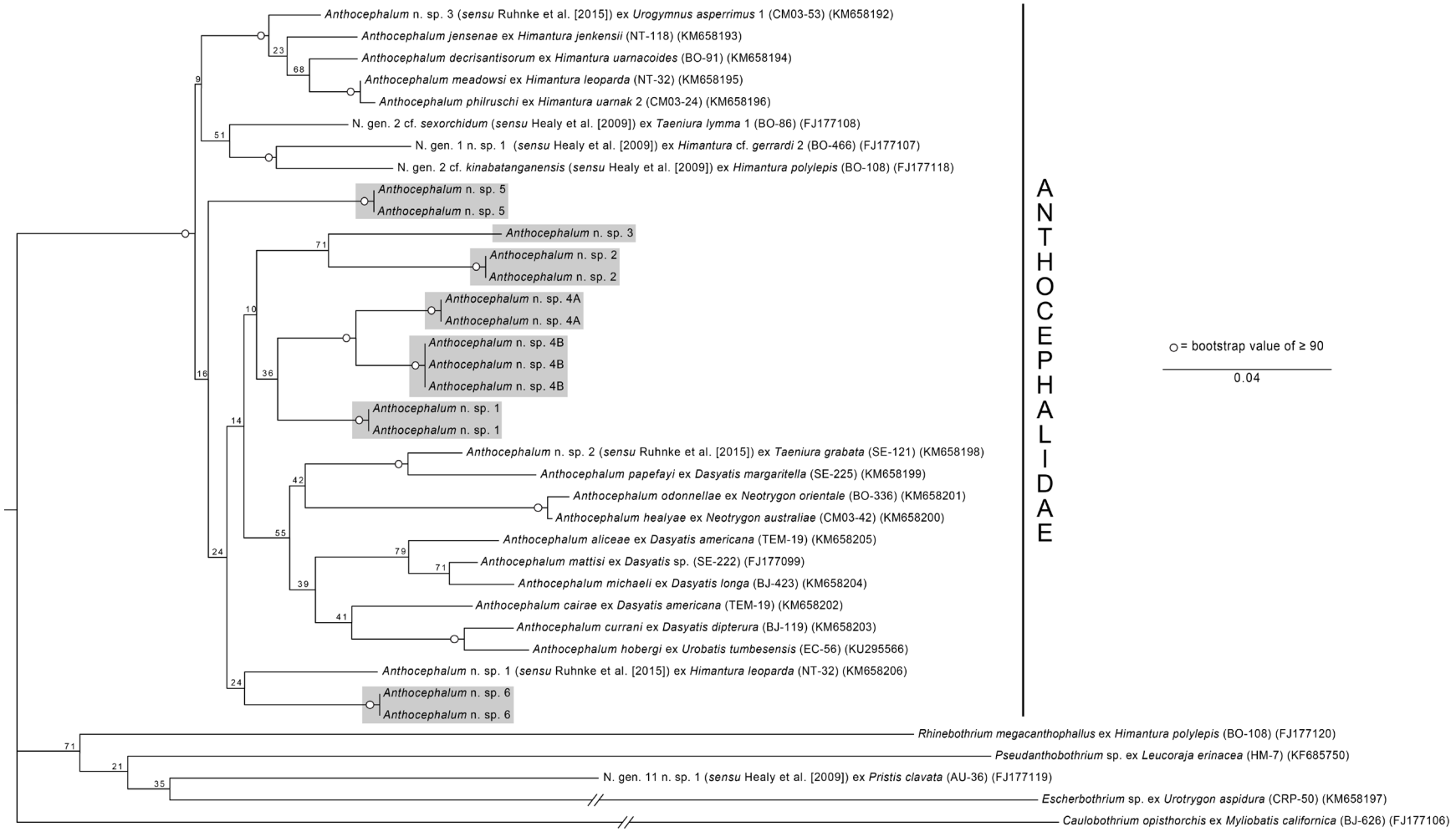

Figure 13. Phylogenetic placement of species of *Anthocephalum* from *Himantura granulata* relative to select members of the Anthocephalidae resulting from maximum liklihood analysis. Nodal support is given as bootstrap (BS) values generated from 100 BS replicates; BS values are indicated for each node, with those that are greater than or equal to 90% indicated by white dots. Specimens from *H. granulata* are highlighted in gray. Branch length scale bar indicates nucleotide substitutions per site.

Table 3. Number of base pair differences in sequences of D1–D3 28S rDNA (1,422 base pairs total) between specimens of *Anthocephalum* n. sp. 4A and *Anthocephalum* n. sp. 4B.

	A. sp. 4A - 1	*A*. sp. 4A - 2	*A*. sp. 4B - 1	*A*. sp. 4B - 2	*A*. sp. 4B - 3
A. sp. 4A - 1	—	0	38	35	35
A. sp. 4A - 2	—	—	38	35	35
A. sp. 4B - 1	—	—	—	3	4
A. sp. 4B - 2	—	—	—	—	1
A. sp. 4B - 3	—	—	—	—	—

combination of morphological characters were measured and assessed for all of the standard characters of the genus. In an effort to determine the respective species identities of these 34 specimens (i.e., whether two distinct groups of species could be recovered, each ideally containing the hologenophores of that particular species), a principal component analysis (PCA) was performed using the following measurements from each voucher specimen and hologenophore: total length, terminal proglottid length, terminal proglottid width, number of testes, cirrus sac length, cirrus sac width, distance from genital pore to posterior margin of proglottid, and ovary length. Figure 14 illustrates the first three principle components (collectively explaining 76.9% of the variance in the data) for all measured specimens and hologenophores, from which no conclusive species designations for any measured whole mount individual can be inferred. As a result, molecular sequence data suggest two species of *Anthocephalum* from *H. granulata* (*A*. n. sp. 4A and *A*. n. sp. 4B) in addition to the five species described herein, while morphological features suggest at least one additional species. In the interest of conservatism, *A*. n. sp. 4A and *A*. n. sp. 4B are together counted as one species (see Table 2).

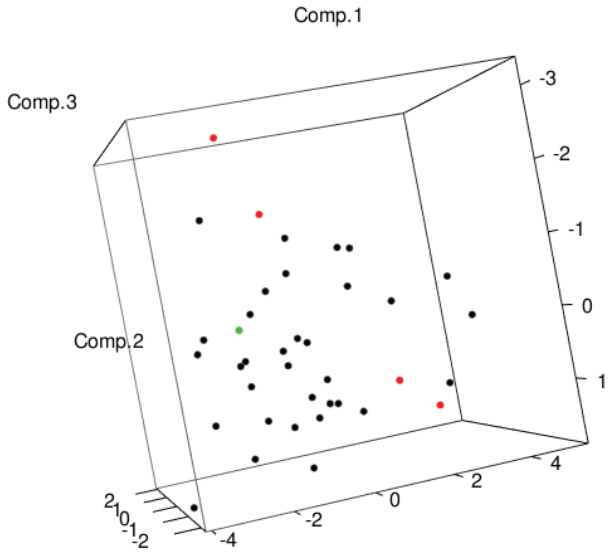

Figure 14. Point graph of first three principle components (collectively explaining 76.9% of variance in the data) from PCA of measurement data of voucher specimens and hologenophores of the *Anthocephalum* n. sp. 4A/4B species complex. Green dots represent hologenophores of *A*. sp. 4A, red dots represent hologenophores of *A*. sp. 4B, and black dots represent voucher specimens.

A Key to the Species of *Anthocephalum* from *Himantura granulata*

1. Uterus extends anterior to field of testes 2
- Uterus does not extend anterior to field of testes 3
2. Fewer than 20 testes .....*Anthocephalum* n. sp. 1
- More than 20 testes 4
3. Vitelline follicles not interrupted by ovary 5
- Vitelline follicles interrupted by ovary .....*Anthocephalum* n. sp. 5
4. Recurved vagina present ..... *Anthocephalum* n. sp. 4A /4B
- Recurved vagina absent .....*Anthocephalum* n. sp. 6
5. Vitelline follicles interrupted by genital pore .....*Anthocephalum* n. sp. 2
- Vitelline follicles not interrupted by genital pore .....*Anthocephalum* n. sp. 3

Additional Lecanicephalidean Tapeworm Species

In addition to the two new species of New Genus 12 described herein, five additional species of lecanicephalideans from two additional genera were identified: four species of *Polypocephalus* (see Fig. 15A–E), and one species that is morphologically consistent with the hologenophores of two specimens included in the phylogenetic analyses of the Lecanicephalidea by Jensen et al. (2016) and referred to therein as "New Genus 11 n. sp. 1" and "New Genus 11 n. sp. 2" (see Fig. 15F). The two species of "New Genus 11" *sensu* Jensen et al. (2016) included in their analyses were parasites of *Rhynchobatis* cf. *laevis sensu* Naylor et al. (2012b) and *Glaucostegus typus* Anonymous [Bennett], respectively (Jensen et al. 2016). Members of "New Genus 11" are united by their possession of a single row of few testes, simple tentacles, and conspicuous gladiate spinitriches on the scolex proper. Given the high degree of host specificity exhibited by the majority of lecanicephalidean species (Caira and Jensen 2014), the specimens collected from *Himantura granulata* are tentatively identified as "New Genus 11" n. sp. 3, pending comparisons of sequence data and a more detailed comparison to hologenophores. It appears from preliminary morphological assessments that "New Genus 11" n. sp. 3 from *H. granulata* possess fewer proglottids than examined specimens of "New Genus 11" n. sp. 1 from *Rhynchobatis* cf. *laevis* (~13–19 vs. ~28–41, respectively), but vouchers of "New Genus 11" n. sp. 2 were unavailable for comparison. Specimens of "New Genus 11" n. sp. 3 are known from one small juvenile ray and three large mature rays from the Solomon Islands.

For the purposes of gaining an accurate account of the total number of species parasitizing the individuals of *Himantura granulata* examined in this study, the four species of *Polypocephalus* are morphologically distinguished from one another; however, the taxonomic distinctiveness of each of these species relative to congeners remains uncertain. Members of the genus are united by their possession of a single row of few testes, simple tentacles, and a scolex proper not bearing conspicuous gladiate spinitriches. Counts given for each of the four species are based on ranges for multiple specimens. The four species from *H. granulata* are distinguished from one another as follows: *Polypocephalus* sp. 1 has 25–53 proglottids and is thus distinct

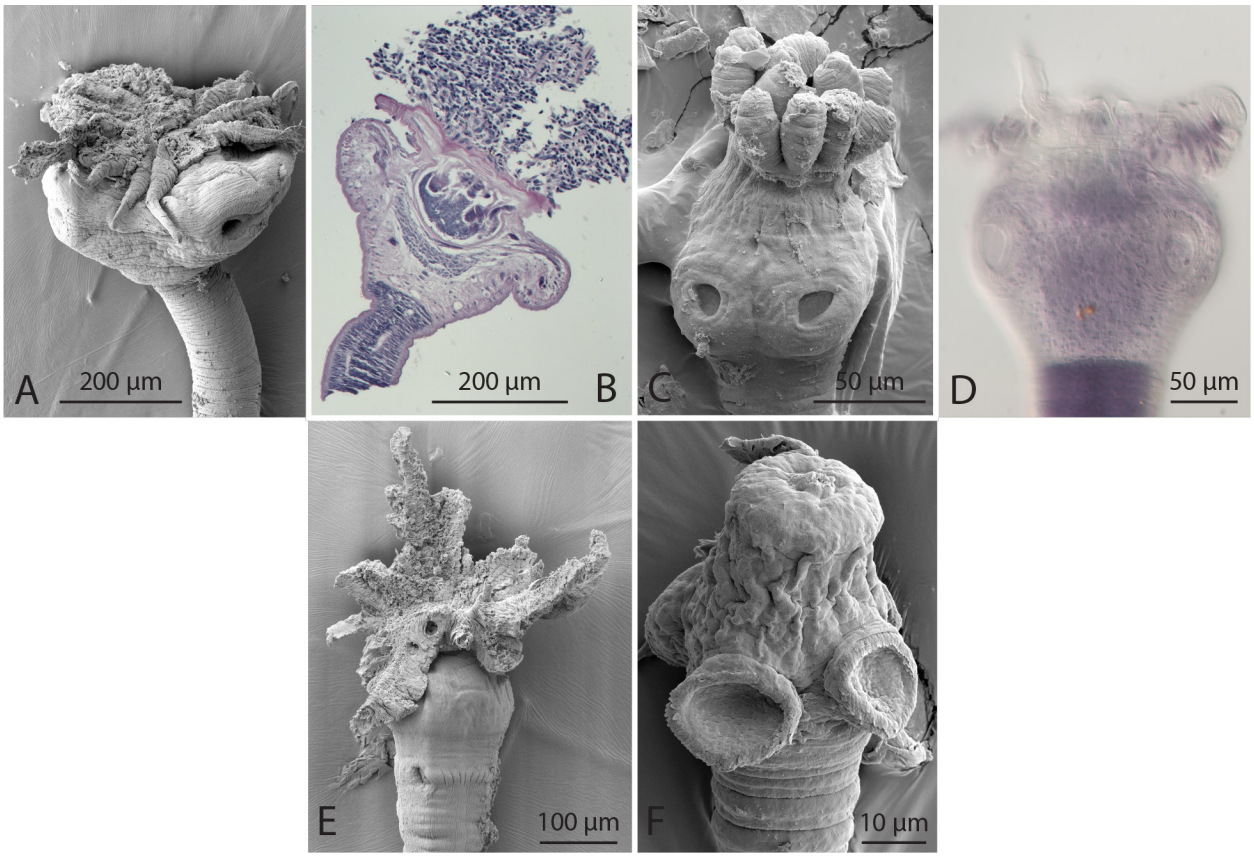

Figure 15. Scanning electron micrographs (A, C, E, F) and photomicrographs (B, D) of additional lecanicephalidean species. (A) *Polypocephalus* sp. 1. (B) Frontal section of *Polypocephalus* sp. 1. (C) *Polypocephalus* sp. 2. (D) *Polypocephalus* sp. 3. (E) *Polypocephalus* sp. 4. (F) "New Genus 11" n. sp. 3.

from *Polypocephalus* sp. 2 and *Polypocephalus* sp. 3, which have 7–18 and 9–11 proglottids, respectively. Though *P.* sp. 2 and *P.* sp. 3 possess overlapping ranges for number of proglottids, the two species can be distinguished from one another based on apolysis; *P.* sp. 2 is euapolytic, whereas *P.* sp. 3 is apolytic. *Polypocephalus* sp. 4 possesses six testes, distinguishing it from *P.* sp. 1, *P.* sp. 2 and *P.* sp. 3, all of which possess four testes. *Polypocephalus* sp. 2 is the only species of all 32 total tapeworm species identified that was found parasitizing rays of both size classes from all three host capture localities. *Poylpocephalus* sp. 1 was found only in large mature rays from the Solomon Islands, *P.* sp. 3 was found in small juvenile and large mature rays from the Solomon Islands and in the small juvenile ray from Queensland, and *P.* sp. 4 was found only in large mature rays from the Solomon Islands. Given the oioxeny of lecanicephalideans (Caira and Jensen 2014), it is likely that these four species are new to science.

Additional Rhinebothriidean Tapeworm Species

In addition to the species of *Anthocephalum* treated above, six remaining rhinebothriidean species distributed across two genera were also identified: five species of *Rhinebothrium* (see Fig. 16A–E) and one species fitting the generic diagnosis of *Stillabothrium* (Reyda et al. 2016, in review) (see Fig. 16F). Species of *Rhinebothrium* were found parasitzing hosts from the three capture localities, and the single species of *Stillabothrium* was found only parasitizing hosts from Northern Territory and Queensland (i.e., only found in northern Australia).

Following an examination of the original description and type specimens of *Rhinebothrium himanturi* from the South Australian Museum Australian Helminthological Collection (AHC) (AHC 41063 [holotype], AHC 41064 [paratype]), as well as the description and a voucher specimen of "*Rhinebothrium* sp." (AHC 41067), it can be concluded that none of the five species of *Rhinebothrium* encountered in this study are morphologically conspecific with either of these two species described or reported by Williams (1964). Members of the genus *Rhinebothrium* are united by their possession of four stalked bothridia, each subdivided into loculi by transverse septa. To date, 40 species of *Rhinebothrium* are considered valid. For the purposes of gaining an accurate account of the total number of species parasitizing the individuals of *Himantura granulata* examined for this study, the five species herein are distinguished from one another and from the two species of *Rhinebothrium* previously reported from *H. granulata* by Williams (1964); however, the taxonomic distinctiveness of these five species within the genus remains uncertain. *Rhinebothrium* is the only elasmobranch tapeworm genus outside of the Trypanorhyncha in which relaxed host specificity for multiple species has been documented. Only four of the 40 valid species of *Rhinebothrium* (i.e., *Rhinebothrium brooksi* Reyda & Marques 2011, *Rhinebothrium copianullum* [Reyda 2008] Reyda & Marques 2011, *Rhinebothrium margaritense* Mayes & Brooks 1981, and *Rhinebothrium paratrygoni* Rego & Diaz 1976) however, have been reported from more than a single host species, and three of these four species have freshwater rather than marine distributions. Given that only a single marine species of *Rhinebothrium* exhibiting relaxed host specificity has been documented, these

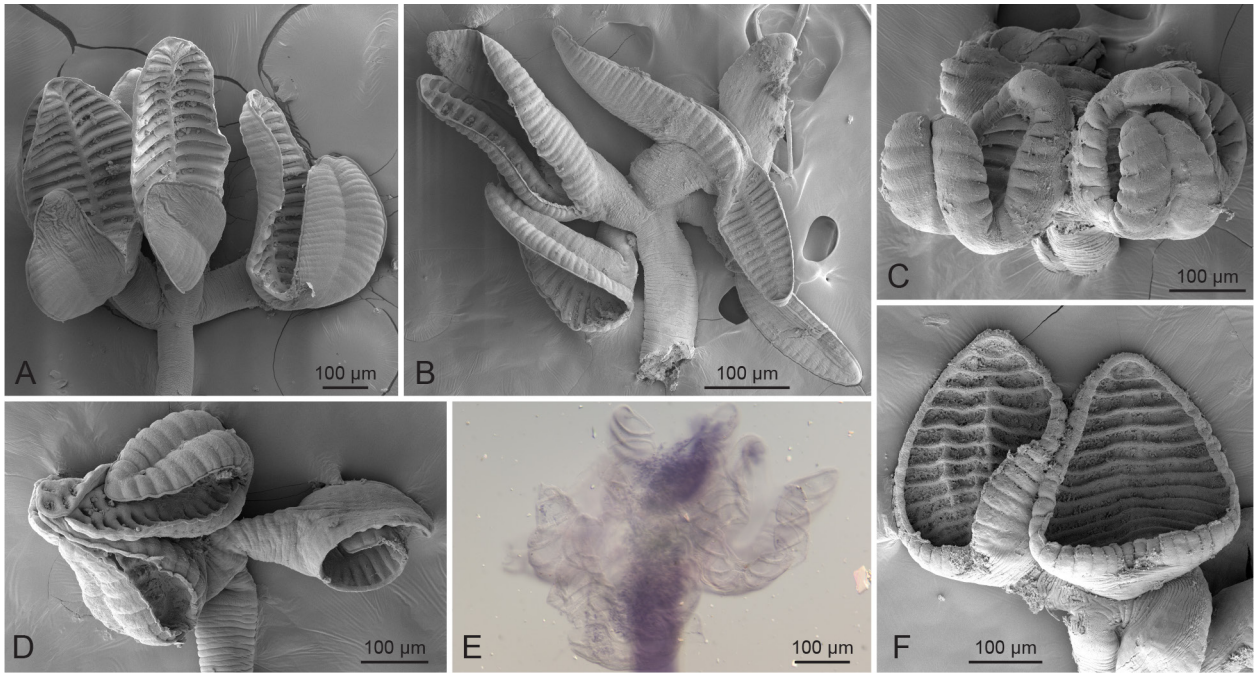

Figure 16. Scanning electron micrographs (A–D, F) and a photomicrograph (E) of scoleces of additional rhinebothriidean species. (A) *Rhinebothrium* sp. 1. (B) *Rhinebothrium* sp. 2. (C) *Rhinebothrium* sp. 3. (D) *Rhinebothrium* sp. 4. (E) *Rhinebothrium* sp. 5. (F) *Stillabothrium* n. sp. 1.

five species from *H. granulata* likely represent new species, though further investigation is warranted to test this hypothesis. Counts or measurements given for each species odentified in this study are based on ranges for multiple specimens unless otherwise indicated.

Rhinebothrium sp. 1 can be differentiated from *R. himanturi* based on a unique combination of number of loculi, number of testes, and number of proglottids (60–72 loculi, 6–10 testes and 28–38 proglottids vs. 54 loculi, 19–20 testes and 22 proglottids, respectively). Additionally, *R.* sp. 1 possesses anterior and posterior regions of the bothridia that are unequal in length and number of loculi, distinguishing it from both *R. himanturi* and "*Rhinebothrium* sp." of Williams (1964) (known only from scoleces), which both possess essentially symmetrical anterior and posterior regions of the bothridia. *Rhinebothrium* sp. 1 is known only from large mature rays from the Solomon Islands.

Rhinebothrium sp. 2 can be differentiated from both *R. himanturi* and *R.* sp. 1 based on its possession of a cirrus sac that extends posteriorly between the poral and aporal lobes of the ovary to approximately the ovarian isthmus; the posterior margin of the cirrus sac of both *R.*

himanturi and *R.* sp. 1 is anterior to the ovary. *Rhinebothrium* sp. 2 also possesses fewer testes than *R. himanturi* (9–14 vs. 19–20, respectively) and has asymmetrical anterior and posterior regions of the bothridia, distinguishing it from both *R. himanturi* and "*Rhinebothrium* sp." of Williams (1964), which possess essentially symmetrical bothridial regions. *Rhinebothrium* sp. 2 is known only from large mature rays from the Solomon Islands.

Rhinebothrium sp. 3 is distinct from *R. himanturi*, "*Rhinebothrium* sp." of Williams (1964), *R.* sp. 1 and *R.* sp. 2 based on number of loculi (28–34 vs. 54, 76, 60–72 and 60–70, respectively) and from *R. himanturi*, *R.* sp. 1 and *R.* sp. 2 based on number of testes (30–37 vs. 19–20, 6–10 and 9–14, respectively), as testes counts are not available for "*Rhinebothrium* sp." of Williams (1964). *Rhinebothrium* sp. 3 also does not possess a cirrus sac that extends posteriorly between the poral and aporal lobes of the ovary, distinguishing it from *R.* sp. 2. Additionally, preliminary measurements suggest that *R*. sp. 3 possesses a scolex that is approximately half the size of the scoleces of the four aforementioned species (~600 µm vs. ~1,050–1,400 µm maximum width). *Rhinebothrium* sp. 3 is known from both a large mature and a small juvenile ray from the Solomon Islands.

Rhinebothrium sp. 4 has fewer testes than both *R. himanturi* and *R.* sp. 3 (7–10 vs. 19–20 and 30–37, respectively) and fewer loculi than *R.* sp. 1, *R.* sp. 2 and "*Rhinebothrium* sp." of Williams (1964) (48–50 vs. 60–72, 60–70 and 76, respectively). Preliminary measurements suggest that *Rhinebothrium* sp. 4 is also approximately half the size in terms of total length as compared to *R.* sp. 1 (~3,000 µm vs. ~6,700 µm) and has fewer proglottids as compared to *R.* sp. 1 (13–22 vs. 28–38). Additionally, unlike *R.* sp. 2, *R.* sp. 4 does not possess a cirrus sac that extends posteriorly between the poral and aporal lobes of the ovary. *Rhinebothrium* sp. 4 is known only from the small juvenile ray collected from Queensland.

Rhinebothrium sp. 5 is unfortunately known only from a single specimen. However, the morphological distinctiveness of this specimen allows it to be distinguished from all of the six aforementioned species parasitzing *H. granulata*. *Rhinebothrium* sp. 5 has fewer loculi than *R. himanturi,* "*Rhinebothrium* sp." of Williams (1964), *R.* sp. 1, *R.* sp. 2, *R.* sp. 3 and *R.* sp. 4 (24

vs. 54, 76, 60–72, 60–70, 28–34 and 48–50, respectively). This species is most morphologically similar in scolex morphology to *R.* sp. 3 in that both species possess bothridia with relatively few loculi (i.e., 24 and 28–34, respectively), but *R.* sp. 5 is readily distinguished from *R.* sp. 3 based on number of testes (15–17 in *R.* sp. 5 vs. 30–37 in *R.* sp. 3).

A single rhinebothriidean species parasitizing *H. granulata* consistent in morphology with the generic diagnosis of *Stillabothrium* (see Reyda et al., in review) was also recovered. Members of this genus are united by their possession of bothridia with an anterior region divided into loculi by two or more complete or incomplete transverse septa, and a posterior region divided into an odd number of loculi by an even number of non-medial longitudinal septa. Five new species of *Stillabothrium* are described by Reyda et al. (2016) (in review) and two species are transferred to the genus from other genera. The species from *H. granulata*, *Stillabothrium* n. sp. 1, is readily distinguished from *Stillabothrium ashleyae* Willsey & Reyda 2016, *Stillabothrium davicynthiae* Daigler & Reyda 2016, and *Stillabothrium amuletum* (Butler 1987) Healy & Reyda 2016 by its lack of marginal loculi, which are present in the later three species. *Stillabothrium* n. sp. 1 differs from *Stillabothrium cadenati* (Euzet 1954) Healy & Reyda 2016 based on number of loculi in the anterior region of the bothridia (12–14 vs. 3, respectively) and number of testes (19–30 vs. 7–13, respectively). *Stillabothrium* n. sp. 1 is distinct from *Stillabothrium ashleyae*, *Stillabothrium davicynthiae*, *Stillabothrium hyphantoseptum* Herzog, Bergman & Reyda 2016, *Stillabothrium campbelli* Delgado, Dedrick & Reyda 2016 and *Stillabothrium jeanfortiae* Forti, Aprill & Reyda 2016 based on arrangement of vitelline follicles; *S.* n. sp. 1 possesses vitelline follicles that are not interrupted by the ovary, whereas the latter five species all possess vitelline follicles that are interrupted by the ovary. Based on its unique combination of these features, specimens of *Stillabothrium* n. sp. 1 from *H. granulata* can confidently be said to represent a new species. This species is known only from the two small juvenile rays from Queensland and Northern Territory.

Onchoproteocephalidean Tapeworm Species

Four species of *Acanthobothrium* (i.e., tapeworms possessing scoleces with bipronged hooks and four bothridia separated into three loculi) were identified, all but one of which were found exclusively in large mature rays from the Solomon Islands (see Fig. 17). *Acanthobothrium* is one of the most specious genera of elasmobranch tapeworms (Caira and Jensen 2014), with 185 species described to date. *Acanthobothrium* n. sp. 1 from *H. granulata* can be distinguished from the other three species of *Acanthobothrium* parasitizing this host, as well as from all but 74 of the described species of *Acanthobothrium*, by its overall large size (~3–4 cm in total length) and number of proglottids (greater than 350). This combination of features separates *A.* n. sp. 1 from all but species in categories 3–6 *sensu* Ghoshroy and Caira (2001) (i.e., species greater than 15 mm in total length and with more than 50 proglottids). However, *A.* n. sp. 1 is distinguished from these 74 species—and indeed from all known species in the genus to date—by its possession of two genital pores and two cirrus sacs, one on each side of the proglottid; a species of *Acanthobothrium* with this feature has not yet been described. *Acanthobothrium* n. sp. 1 is known only from large mature rays from the Solomon Islands.

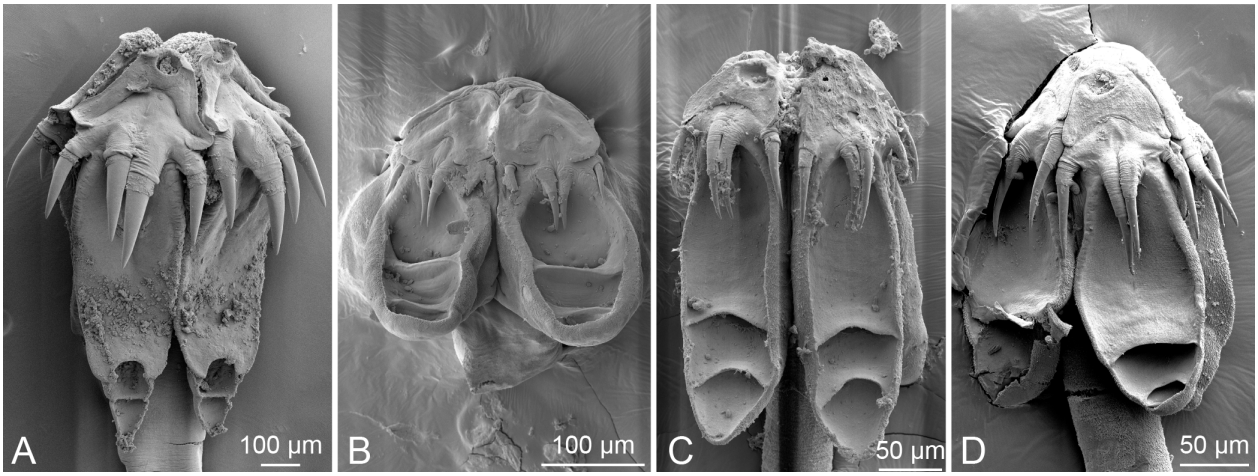

Figure 17. Scanning electron micrographs of scoleces of species of *Acanthobothrium*. (A) *Acanthobothrium* n. sp. 1. (B) *Acanthobothrium* sp. 2. (C) *Acanthobothrium* sp. 3. (D) *Acanthobothrium* sp. 4.

The remaining three species of *Acanthobothrium* from *H. granulata* can all be classified as category 1 species *sensu* Ghoshroy and Caira (2001) (i.e., species less than 15 mm in total

length with fewer than 50 proglottids, fewer than 80 testes, and poral and aporal ovarian lobes that are equal in length). In addition to their smaller size and fewer proglottids, *Acanthobothrium* sp. 2, *Acanthobothrium* sp. 3 and *Acanthobothrium* sp. 4 are all distinct from *Acanthobothrium* n. sp. 1 in that they each possess proglottids with a single genital pore and cirrus sac. These three species differ from one another in their unique combinations of number of testes (30–31 in *A*. sp. 2 vs. 14–18 in *A.* sp. 3 vs. 17–21 in *A*. sp 4) and number of proglottids (16 in *A.* sp. 2 vs. 9–11 in *A.* sp. 3 vs. 6–9 in *A*. sp. 4). Furthermore, *A.* sp. 3 possesses a vastly expanded external seminal vesicle that is not present in either *A.* sp. 2 or *A*. sp. 4, and *A.* sp. 4 is apolytic, further distinguishing it from *A.* sp. 2 and *A.* sp. 3, both of which are euapolytic. Additionally, these three species all possess distinct locular morphology (see Fig. 17). While *A.* sp. 2 and *A.* sp. 3 are both known only from large mature rays from the Solomon Islands, *A.* sp. 4 is known only from the small juvenile ray from Northern Territory.

Trypanorhynch Tapeworm Species

In total, eight species of trypanorhynchs (i.e., tapeworms with bothria and four armed tentacles) (see Fig. 18A–G) from five genera were identified from the ten host individuals examined, making the Trypanorhyncha the order with the greatest diversity at the generic level of the five tapeworm orders identified from *H. granulata*. These eight species collectively parasitized rays from all three host capture localities, and were found in both small juvenile and large mature rays. The trypanorhynchs were perhaps the most taxonomically challenging of the five orders recovered, as positive species identifications for these tapeworms are only possible based on specimens with their tentacles everted.

Three specimens of the eutetrarhynchid *Prochristianella clarkeae*—the only trypanorhynch species previously reported from *Himantura granulata*—were recovered. Two additional species of *Prochristianella* Dollfus 1946— *Prochristianella* sp. 1 and *Prochristianella* sp. 2—were also identified. Preliminary measurements suggest that individuals of *P*. sp. 1 are, on average, twice the size of the individuals of *P. clarkeae* in hand (i.e., ~5,700 µm vs. ~2,300 µm

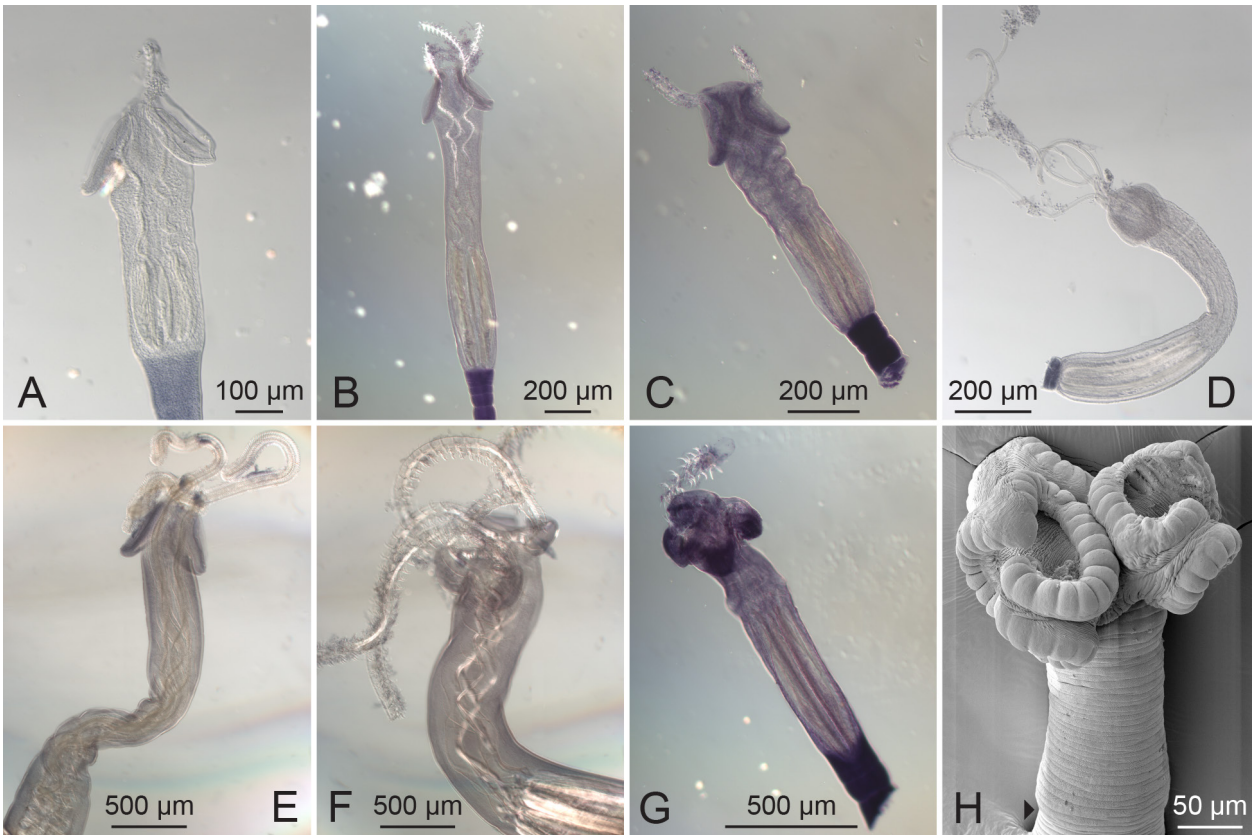

Figure 18. Photomicrographs of scoleces of species of trypanorhynchs (A–G) and scanning electron micrograph of *Caulobothrium* sp. 1. (H). (A) *Prochristianella clarkeae*. (B) *Prochristianella* sp. 1. (C) *Prochristianella* sp. 2. (D) *Dollfusiella* sp. 1. (E) *Paroncomegas* cf. *myliobatis*. (F) *Halsiorhynchus* sp. 1. (G) *Pterobothrium* cf. *australiense*. (H) *Caulobothrium* sp. 1; arrow denotes posterior margin of cephalic peduncle.

total length) and have more proglottids (i.e., more than 4 vs. 4 or fewer). Unfortunately, *P.* sp. 2 is known only from a single specimen, making its distinction from *P. clarkeae* and *P.* sp. 1 difficult; however, all three species appear to possess distinct metabasal armature that distinguishes them from one another. While specimens of *P. clarkeae* were only recovered from the small juvenile mangrove whipray from Queensland, specimens of *P.* sp. 1 and *P.* sp. 2 were both recovered from large mature mangrove whiprays from the Solomon Islands.

Two additional eutetrarhynchid species in the genera *Dollfusiella* Campbell & Beveridge 1994 and *Paroncomegas* Campbell, Marques & Ivanov 1999 were also identified. The species of *Dollfusiella*, heretofore referred to as *Dollfusiella* sp. 1, possesses two proximal rows of basal hooks that are uncinate and larger than the hooks of the metabasal armature, an unusual character

in species of *Dollfusiella*. Of the 26 species of *Dollfusiella* recognized as valid by Schaeffner and Beveridge (2013) in their taxonomic review of the genus, and the additional 27th species added by Menoret and Ivanov (2014), only seven species possess rows of enlarged proximal uncinate hooks: *Dollfusiella tenuispinis* Linton 1890; *Dollfusiella spinifer* Dollfus 1969; *Dollfusiella spinulifera* Beverdige & Jones 2000; *Dollfusiella aculeata* Beveridge, Neifar & Euzet 2004; *Dollfusiella hemispinosa* Schaeffner & Beveridge 2013; *Dollfusiella imparispinis* Schaeffner & Beveridge 2013; and *Dollfusiella spinosa* Schaeffner & Beveridge 2013. The taxonomic distinctiveness of *D.* sp. 1 from *H. granulata* is uncertain. Six of the seven species of *Dollfusiella* possessing enlarged, uncinate proximal rows of hooks are collectively reported from the eastern coast of the United States, Senegal, the Mediterranean and Malaysia; only one species—*D. spinulifera*—is reported from northern Australia. Given the low degree of host specificity in the trypanorhynchs in general (Palm 2004, Caira and Jensen 2014), and the fact that *D*. sp. 1 is known from large mature rays from the Solomon Islands, it seems likely that specimens of *D.* sp. 1 may be conspecific with *D. spinulifera*, though further taxonomic investigation is warranted.

One species of *Paroncomegas* could be identified. The morphological characteristics of this species, including its possession of tentacular swelling, very long tentacles and tentacular bulbs, metabasal armature composed of solid, falciform homeomorphous hooks, and two large macrohooks at the tentacular base, are consistent with characters reported in the description of *Paroncomegas myliobatis* Palm 2004, which is known from the purple eagle ray, *Myliobatis hamlyni* Ogilby from Indonesia (see Palm 2004). Until detailed measurements are taken to confirm the conspecificity of the specimens of *Paroncomegas* from *H. granulata* with *P. myliobatis*, this species is heretofore referred to as *Paroncomegas* cf. *myliobatis.* This species was found only from large mature mangrove whiprays from the Solomon Islands.

A single species of *Pterobothrium* Diesing 1850 was identified. This species is known only from a single specimen, but its distinctive tentacular armature allowed for the placement of this specimen within the genus *Pterobothrium*. This specimen is most morphologically similar to *Pterobothrium australiense* Campbell & Beveridge 1996 based on their shared possession of

the following morphological features: four bothria; heteroacanthous atypical, heteromorphous metabasal armature; hollow, spiniform principle hooks; short spiniform intercalary hooks; a bifid hook 5; a lack of prebulbar organs, a lack of gland cells within bulbs, and a lack of microhooks on the external surface (see Palm 2004). Until specific measurements are taken to confirm the conspecificity of the specimens from *H. granulata* with *P. australiense*, this species is heretofore referred to as *Pterobothrium* cf. *australiense.* This species was found only from a single large mature mangrove whipray from the Solomon Islands.

A single species in the genus *Halysiorhynchus* Pintner 1913 was identified. Though this species is also known only from a single specimen, its large size and possession of four bothria, six principle hooks, and characteristic basal armature consisting of a simple chainette of large solid hooks allowed for its placement within *Halysiorhynchus*. This genus is monotypic, with *Halysiorhynchus macrocephalus* (Shipley & Hornell 1906) Pintner 1913 being the only described species to date. As the species of *Halysiorhynchus* collected from *H. granulata* (heretofore referred to as *Halysiorhynchis* sp. 1) is known only from a single, incomplete specimen, confirming conspecificity of this specimen with *H. macrocephalus* is difficult; however, as *H. macrocephalus* is known from a variety of dasyatid hosts from Northern Territory, Australia (Palm 2004), it seems likely that the specimen of *H.* sp. 1 collected from a large mature mangrove whipray from the Solomon Islands is *H. macrocephalus*, though further taxonomic investigation is warranted.

“Tetraphyllidean” Tapeworm Species

Specimens representing a single “tetraphyllidean” species were encountered during this study. This species belongs to the genus *Caulobothrium* Baer 1948, and is known only from three specimens (two specimens prepared for light microscopy and one specimen prepared for SEM; Fig. 18H). This species is placed within this genus based on its possession of a large cephalic peduncle (see Fig. 18H), four loculated bothridia, and testes in a field that extends from the anterior margin of the proglottid to posterior to the genital pore. This species is known only from

the small juvenile mangrove whipray collected from Northern Territory. Currently, seven valid species of *Caulobothrium* are described; however, not enough material is available to determine whether or not *Caulobothrium* sp. 1 from *Himantura granulata* represents a new species.

Host Size and Locality Versus Tapeworm Species Assemblages

A number of differences in tapeworm species assemblages were noted for the ten specimens of *Himantura granulata* of the two size classes and from the three localities examined in this study (see Table 2). The only order known exclusively from small juvenile rays was the "Tetraphyllidea," as represented by the single species of *Caulobothrium* recovered from the small juvenile mangrove whipray from Northern Territory. The remaining four orders were present in mangrove whiprays of both size classes; however, trypanorhynchs and onchoproteocephalideans were only found in large mangrove whiprays in the Solomon Islands and small juvenile mangrove whiprays from the northern Australian localities, and were absent in small juvenile individuals from the Solomon Islands. Rhinebothriideans and lecanicephalideans were present in mangrove whiprays of both size classes from all three localities.

Species of the genus *Anthocephalum* were found in mangrove whiprays of both size classes from all three localities, but the greatest species diversity was recovered from large mature individuals from the Solomon Islands. Similarly, the majority of species of *Rhinebothrium* were recovered from large mature individuals from the Solomon Islands, though two species were found exclusively in the small juvenile mangrove whipray from Queensland. The single species of *Stillabothrium* was found only in the two small juvenile mangrove whiprays from northern Australia, and was absent from the Solomon Islands.

Three of the four species of *Acanthobothrium* were recovered exclusively from large mature individuals from the Solomon Islands, while the fourth species is known only from the single small juvenile mangrove whipray from Northern Territory; no specimens of *Acanthobothrium* were recovered from the small juvenile mangrove whiprays from the Solomon Islands or Queensland. Species of the lecanicephalidean genera New Genus 12 and "New

Genus 11" were recovered from individuals of both size classes from the Solomon Islands and Queensland, but were absent in the small juvenile mangrove whipray from Northern Territory. Species of *Polypocephalus* parasitized individuals of both size classes from all three localities. Trypanorhynchs were recovered only from large mature mangrove whiprays from the Solomon Islands and the two small juvenile mangrove whiprays from northern Australia. Species of the genus *Prochristianella* were found in individuals from all three localities, but species of the remaining four trypanorhynch genera are known exclusively form large mature individuals from the Solomon Islands. Figure 19 illustrates the relationship between host disk width and number of tapeworm species recovered, for which a significant ($p = 0.0012$) correlation was identified.

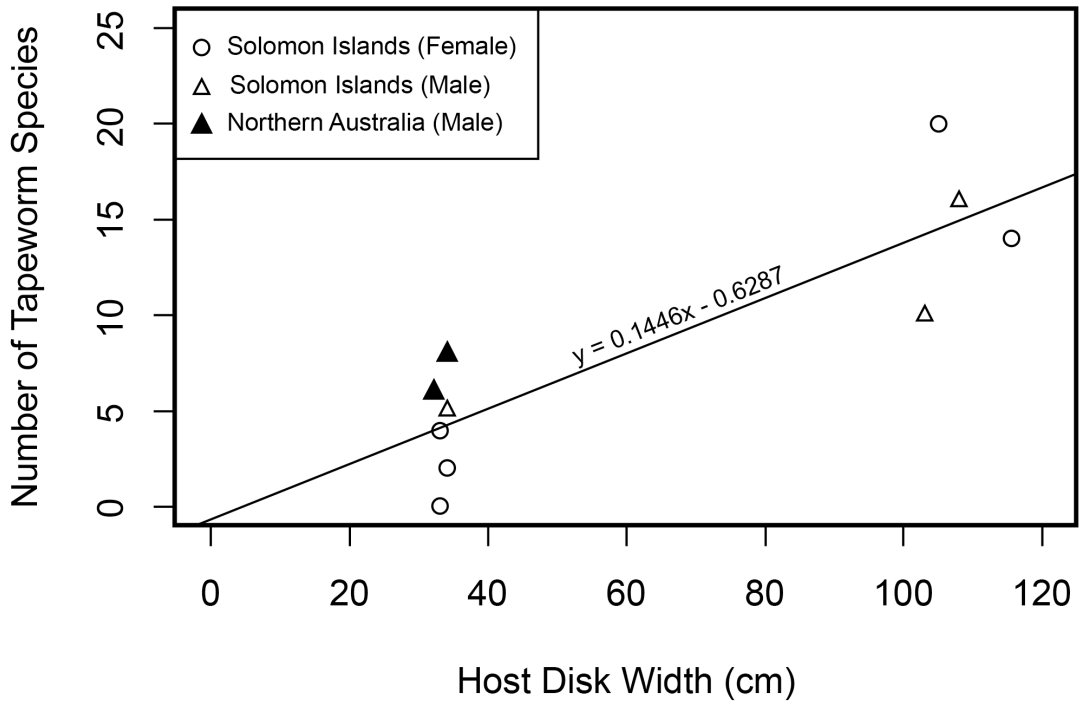

Figure 19. Relationship between host disk width and parasite species richness in the ten specimens of *Himantura granulata* examined in this study. Linear model adjusted R-squared = 0.718; $p = 0.0012$.

DISCUSSION

Summary of Tapeworm Species Parasitizing *Himantura granulata*

Prior to this study, only three species of tapeworms—one of which was not formally described—were known to parasitize *Himantura granulata*; this study has increased the total number of tapeworm species known from this host to 34 species. This count includes the two species of *Rhinebothrium* recognized by Williams (1964), *Prochristianella clarkeae* reported from *H. granulata* by Schaeffner and Beveridge (2012), and the 31 additional species identified in this study. Specimens of the two species of *Rhinebothrium* identified by Williams (1964) were not recovered in this study, but specimens of *P. clarkeae* were found parasitizing the small juvenile mangrove whipray from Queensland.

The present study is unusual in that that majority of studies of elasmobranch tapeworms do not characterize the entire fauna of a single host species, but rather tend to focus on in-depth examinations of particular groups or new taxa. Because of this, it is somewhat difficult to compare the tapeworm fauna of *H. granulata* to that of other batoids; however, available data suggest that the 34 species from 13 genera and five orders that parasitize *H. granulata* may constitute a relatively specious fauna as compared to other batoid hosts. For example, the tapeworms of the eagle ray *Aetobatus ocellatus* Kuhl have been particularly well-studied, and examinations suggest that this ray may host up to 28 species from 19 genera and five orders (see White et al. 2010, Mojica et al. 2014), an assemblage which has been touted as both diverse and complex among elasmobranchs (Caira and Jensen 2014). This level of tapeworm diversity is on par with that of *H. granulata*, but as *A. ocellatus* is a member of the Myliobatidae Bonaparte and *H. granulata* is a member of the Dasyatidae, a comparison between these two host species may not be as appropriate as an intra-familial comparison.

In-depth assessments of the tapeworms of another dasyatid, the freshwater whipray *Himantura polylepis* [Bleeker]—a close relative of *H. granulata*, according to Naylor et al. (2012a)—suggest that this large freshwater ray is known to host 18 species from 10 genera in four orders (R. Guyer, pers. comm.). This level of diversity is similar to that of *H. granulata* at

the ordinal and generic levels, yet nearly twice as many species have been identified from *H. granulata* in this study. Accumulated records from other long-studied dasyatids helps to place the tapeworm fauna of *H. granulata* in a broader context. For example, the dwarf whipray *Himantura walga* has been studied for over a century, and to date has been described as hosting 10 species of tapeworms (Shipley and Hornell 1905, 1906; Southwell 1911, 1925, 1930; Pintner 1928, Dollfus 1930, Euzet 1953, Ramadevi 1969, Muralidhar 1988, Ivanov and Campbell 2000, Twohig et al. 2008); again, fewer than half as many species as were identified from *H. granulata*. It appears that the 34 species parasitizing *H. granulata* comprise an impressively diverse fauna, though more in-depth assessments of the complete tapeworm faunas of other species of *Himantura* Müller & Henle may ultimately reveal that the high diversity of species recovered from *H. granulata* is really more typical than it appears presently.

New Genus 12: Scolex Morphology, Phylogenetic Placement, Host Associations and Geographic Distribution

The recent phylogenetic analyses of the Lecenicephalidea by Jensen et al. (2016) based on molecular sequence data placed New Genus 12, represented by New Genus 12 n. sp. 1 *sensu* Jensen et al. 2016 from *Himantura* cf. *gerrardi* 2 *sensu* Naylor et al. (2012b), robustly within the family Polypocephalidae. In fact, the proglottid anatomy of New Genus 12 (i.e., the possession of a single column of four testes, two pairs of excretory vessels, and vitteline follicles largely interrupted by the ovary) is essentially identical to that of other genera in the family (see fig. 3 of Jensen et al. 2016). While the shape of the apical organ of New Genus 12 is unique among polypocephalid genera, the complexity of the apical structure in general is most similar to that of members of the other polypocephalid genus *Seussapex*. Anterior to the scolex proper (SP) bearing four suckers, both genera possess a cylindrical apical modification of the scolex proper (AMSP) comprising distinct posterior (with hastate spinitriches) and anterior (without hastate spinitriches) regions, and an apical organ (AO). In both genera, at least the anterior rim of the AMSP is invaginable into the SP and the AO is retractable into the AMSP, and ultimately also the SP. Internal AO morphology is also quite similar in both genera; they possess one or more large

internal glandular compartment(s) associated with the external component of the AO.

The most conspicuous difference between New Genus 12 and *Seussapex* is in external AO morphology. Whereas members of *Seussapex* have a bipartite AO (i.e., posterior retractable dome-shaped part and anterior retractable and/or invaginable knob-like part) that is not conspicuously muscular, the AO of members of New Genus 12 is not bipartite and is conspicuously muscular. The eight concave muscular, membrane-bound pads of New Genus 12 are entirely unlike the components of any described lecanicephalidean scolex to date. Each muscular pad appears to be controlled by a distinct internal muscle bundle that runs the entire length of the scolex. These muscle bundles are presumably responsible for retraction of the AO into the SP. Despite the similarity of apical organ complexity, representatives of New Genus 12 and *Seussapex* included in the phylogenetic analyses by Jensen et al. (2016) were not recovered as sister taxa. Instead, the representatives of *Seussapex* were recovered as sister to the two representatives of "New Genus 11" with high support, while the representative of New Genus 12 placed—albeit with low support—as sister taxon to a clade comprising members of *Anteropora* and *Anthemobothrium* (see fig. 4 in Jensen et al. [2016]).

In addition to standard staining, one frontal scolex section series of New Genus 12 n. sp. 2 was stained using period acid-Schiff (PAS) staining. PAS-positive compounds, which stain bright magenta following PAS protocols (Bogitsh 1962), include polysaccharides, glycolipids and carbohydrate-protein complexes (i.e., tissues with high concentrations of glycogen, galactogen, and/or neutral sialomucins stain PAS-positive) (Bogitsh 1962, Bancroft and Gamble 2008). Examples of PAS-positive structures in animals include connective tissues, striated muscle, basement membranes, and some epithelial tissues (McManus 1948, Lillie 1954, Bogitsh 1962). In elasmobranch tapeworms, PAS staining has identified PAS-positive tissues in the scoleces of the trypanorhynch *Trilocularia acathiaevulgaris* Olsson 1867 (see McCullough and Fairweather 1989), the cathetocephalidean *Sanguilevator yearsleyi* Caira, Mega & Ruhnke 2005 (see Caira et al. 2005) and the lecanicephalideans *Seussapex karybares* Jensen & Russell 2014 (see Jensen and Russell 2014) and an unidentified larval species of *Polypocephalus* (see

Brockerhoff and Jones 1995). Collectively, these authors hypothesize that the presence of PAS-positive tissues in scoleces may indicate a role in adhesion or protection from host digestive enzymes and/or immune responses. In this study, PAS staining was pursued for New Genus 12 because of the morphological similarity of its internal glandular compartment to that of members of the genus *Seussapex*. The glandular compartment of *Seussapex karybares* was determined to be composed of PAS-positive tissue and was thus suggested to play a role in attachment (Jensen and Russell 2014). The tissues that appeared to stain PAS-positive in the scolex of New Genus 12 n. sp. 2 were the musclar rims of both the acetabula and the membrane-bound pads of the external AO, the central disk of the external AO, and portions of the internal glandular component of the AO (see Fig. 5B). Given that the acetabula of cyclophyllidean tapeworms have previously been shown to be composed of PAS-positive tissues (Hedrick and Daugherty 1957, Bogitsh 1963), the PAS-positive acetabula and membrane-bound pads (which resemble acetabula in their structure) of New Genus 12 n. sp. 2 are not surprising. The seemingly PAS-positive tissue of the central disk and portions of the glandular compartment of the AO may suggest, as with *Seussapex karybares*, a secretory or attachment function, but at this point, the significance of these results can only be speculated upon, and further investigation is necessary.

With the description of New Genus 12, the Lecanicephalidea now comprise 25 genera. Host associations of individual genera vary; for example, species of *Anteropora* collectively parasitze batoids of the torpediniform families Narkidae Fowler (Yamaguti 1934, Euzet 1994) and Narcinidae Gill (Subhapradha 1955, Jensen et al. 2011, Jensen et al. 2016), and the myliobatiform family Dasyatidae (Mojica et al. 2013), and sharks of orectolobiform family Hemiscylliidae Gill (Jensen 2005). In contrast, species of *Zanobatocestus* Jensen, Mojica & Caira 2014 exclusively parasitize *Zanobatus schoenleinii* Müller & Henle (Jensen et al. 2014), the sole species in the rhinopristiform family Zanobatidae Fowler. New Genus 12 is one of 10 lecanicephalidean genera reported from dasyatid stingrays. Of these 10 genera, five genera (i.e., *Anthemobothrium*, New Genus 12, *Flapocephalus*, *Seussapex*, and *Tetragonocephalum* Shipley & Hornell 1905) appear restricted to dasyatid hosts. More specifically, species of

Anthemobothrium and *Flapocephalus* parasitize species of *Pastinachus* Rüppel (see Shipley and Hornell 1906, Shinde and Deshmukh 1979); *Seussapex* is likely restricted to species of *Himantura* (see Jensen and Russell 2014); host records for *Tetragonocephalum* come from species of *Dasyatis* Rafinesque (see Yang et al. 1995, Jensen 2005), *Himantura* (see Jensen et al. 2016), *Neotrygon* Castelnau (unpublished data from Malaysian Borneo), *Pastinachus* (see Deshmukh and Shinde 1979, Shinde et al. 1985), and *Urogymnus* Müller & Henle (see Jensen et al. 2016). To date, published records for New Genus 12 are restricted to two species of *Himantura* (present study, Jensen et al. 2016). However, specimens collected as part of a survey of metazoan parasites of elasmobranchs of Borneo suggest that New Genus 12 parasitizes a diversity of species of *Himantura* as well as a species of *Neotrygon* (i.e., *N. orientale* Last, White & Séret). In fact, in addition to *H. granulata* and *H.* cf. *gerrardi* 2 *sensu* Naylor et al. (2012b) (see Jensen et al. 2016), specimens of New Genus 12 have also been recovered from *Himantura* cf. *gerrardi* 1 *sensu* Naylor et al. (2012b), *Himantura lobistoma* Manjaji-Matsumoto & Last, *Himantura polylepis*, *Himantura uarnacoides* Bleeker, and *Himantura walga*. Preliminary identifications suggest that at least six new species of New Genus 12 collectively parasitize these hosts.

Based on the locality data for the specimens described herein, the specimen included in Jensen et al. (2016), and the additional host records listed above, the geographic distribution of New Genus 12 is limited to the waters surrounding Solomon Islands, northern Australia, and Borneo (including the Kinabatangan River). While the actual geographic distribution of the genus is likely to include additional regions in the Indo-West Pacific, it is curious that despite sampling of dasyatid hosts from, for example, Viet Nam, Madagascar, the Red Sea, and northeastern India, specimens of New Genus 12 have not been recovered from rays collected from these regions. The absence from at least a subset of these regions is likely a sampling artifact.

Specimens of the two species of New Genus 12 described herein were not randomly distributed among the host individuals examined. All 22 type and voucher specimens of the large

species, New Genus 12 n. sp. 2, between 3.8 and 9.2 mm in total length, were found exclusively parasitizing small juvenile host individuals, while 31 type and voucher specimens of the small species, New Genus 12 n. sp. 3, less than 3.4 mm in total length, were found parasitizing large mature host individuals; only two specimens of New Genus 12 n. sp. 3 were found parasitizing a single individual smaller than 35 cm in disk width. Thus, despite all but two of the ten mangrove whiprays having been collected from the same locality within one to four days of one another, the two cestode congeners co-occurred in only a single, small individual (see Table 2). It seems likely that, having been collected from the same locality, these eight mangrove whiprays spent time in the same environment among the same intermediate and paratenic hosts, and so the disparate distributions of these two tapeworm species between juvenile and mature mangrove whiprays makes a random association of tapeworm species with host individuals seem unlikely.

The considerable dichotomy presented by New Genus 12 n. sp. 2—a relatively large worm—parasitizing a relatively small host individual counters the understanding of the general trend in parasitology that larger-bodied hosts tend to support larger-bodied parasites, a phenomenon referred to as Harrison's rule (Harrison 1915). This trend was originally proposed to describe the distribution of a genus of parasites among individuals of closely-related host species, and the majority of subsequent studies to have identified the trend have similarly focused on that level (e.g., Harvey and Keymer 1991, Poulin and Hamilton 1997, Johnson et al. 2005). This trend has also been described in the elasmobranch-tapeworm system, as Randhawa and Poulin (2009) noted a positive correlation between tapeworm strobila length and maximum host body size in their examination of various species of "tetraphyllidean" tapeworms and their shark and ray hosts. Unlike these previous studies, the present study examines trends at the level of a parasite genus distributed among intraspecific host individuals of different sizes. If Harrison's rule is indeed also applicable within a single host species, one would not expect the larger of the two species of New Genus 12 to exclusively parasitize small juvenile mangrove whiprays, and vice versa. That the two species of New Genus 12 do not appear to follow Harrison's rule may imply that this rule is not generally applicable within a single host species, or it may be an

artifact of relatively small and incomplete sampling of *H. granulata* across its size range (i.e., both species of New Genus 12 actually parasitize *H. granulata* of all sizes).

Anthocephalum: Host Associations and Morphological Versus Molecular Species Boundaries

Prior to this study, only three host species have been reported to be parasitized by more than a single species of *Anthocephalum*: *Dasyatis americana*, *Dasyatis longa* Garman, and *Himantura leoparda* Manjaji-Matsumoto & Last. Each of these three species has only been reported to host two species of *Anthocephalum*, making the six morphologically identified (seven molecularly diagnosed) congeners identified from *H. granulata* somewhat unusual. Given that the tapeworms of rays in the genus *Dasyatis* have been studied for over a century, it seems unlikely that the comparatively fewer species of *Anthocephalum* described from these hosts is the result of pending species descriptions or incomplete sampling. It may, however, be that hosts of *Anthocephalum* in the Indo-Pacific (i.e., other species of *Himantura* and *Neotrygon*) boast comparable levels of *Anthocephalum* species diversity to that of *H. granulata*, but have not yet been assessed as comprehensively as *H. granulata* in the present study.

This study indicates that, for *H. granulata* in the Solomon Islands and northern Australia, the majority of the species diversity of *Anthocephalum* was found in large mature mangrove whiprays. Only a single small juvenile individual from the Solomon Islands was parasitized by *Anthocephalum*, and only two individuals of *Anthocephalum* were recovered from this host individual (one individual with the morphological features of the *A.* n. sp 4A/4B species group, and one individual of *A.* n. sp. 6). Both the small mangrove whiprays from the two northern Australia localities were parasitized by *Anthocephalum*, but at similarly low intensities (two individuals of *A.* n. sp. 6 were recovered from the mangrove whipray form Northern Territory, and four individuals of *A*. n. sp. 3 were recovered from the mangrove whipray from Queensland). These intensities seem especially low given that approximately 100–500 individuals of *Anthocephalum* spp. were recovered from each large ray from the Solomon Islands. Though the

host sample size from all localities was relatively small, this striking incongruence in infection rates between small and large host individuals may indicate that *Anthocephalum* utilizes an intermediate or paratenic host that is more commonly consumed by large mature rather than small juvenile rays. Little is known about the intermediate host use of rhinebothriideans, however; work by Jensen and Bullard (2010) identified bivalves and teleosts as hosts of larval rhinebothriideans in the genera *Rhodobothrium*, *Spongiobothrium,* and *Rhinebothrium*, but nothing is known about the intermediate host use of species of *Anthocephalum*.

Five species of *Anthocephalum* parasitizing *H. granulata* were taxonomically treated herein, but two putative species—*A.* n. sp. 4A and *A.* n. sp. 4B—remain undescribed. Though molecular sequence data indicate that these two species differ from one another by 35–38 base paris (see Table 3), the hologenophores and the 32 voucher specimens studied with light microscopy form a single, seemingly homogenous morphological group, which did not allow for the morphological differentiation of *A.* n. sp. 4A from *A.* n. sp. 4B. As a PCA based on measurement data did not show a clear distinction between the two putative species (see Fig. 14), it may be that a qualitative rather than a quantitative character distinguishes them from one another; for example, the type or arrangement of microtriches on the scolex or strobila. Scolex and strobila microtriches have yet to be examined for any species of *Anthocephalum* identified in this study, and a closer examination of these characters in all seven molecularly diagnosed species is warranted.

Additionally, as one of the two specimens of *A.* n. sp 4A sequenced was immature and thus did not produce a useful hologenophore for comparison of morphological characters between the two putative species, additional sequencing of mature specimens from both species clusters may serve to generate morphologically distinct and useful hologenophores from which to derive diagnostic features in the future. It is worth noting that among the morphological characters that were helpful in distinguishing the species of *Anthocephalum* described herein from one another and from previously described species, anterior extent of the uterus and whether or not vitelline follicles were present alongside the ovary were perhaps the most

diagnostic. The three species described in this study possessing a uterus that does not extend anterior to the field of the testes (*A.* n. sp. 2, *A.* n. sp. 3 and *A.* n. sp. 5) are the first species of *Anthocephalum* reported to possess this character.

The phylogenetic tree generated for this study supports morphological species boundaries for the species of *Anthocephalum* from *H. granulata* (see Fig. 13). The object of this analysis was not to assess interrelationships within the genus, but instead to confirm the surprisingly high number of species parasitizing this host as indicated by morphology using molecular sequence data. The 14 specimens of *Anthocephalum* from *H. granulata* included in this analysis grouped into seven well-supported clades, each of which (with the exception of the *A.* n. sp. 4A and *A.* n. sp. 4B species clades) is reinforced by diagnostic morphological features. Though a monophyletic *Anthocephalum* was not recovered in this analysis, other phylogenetic assessments of the genus produced by Ruhnke et al. (2015) and Marques and Caira (2016) utilizing a combination of partial 28S and complete 18S sequence data did recover monophyly of *Anthocephalum*. The present analysis was based on only partial 28S sequence data as it was beyond the scope of this study to include 18S sequence data. Neither the two previous studies nor the present study, however, included sequence data from all described species of *Anthocephalum*- all three studies included data for only 12 or 13 of the 18 valid species, as well as three as of yet undescribed species. This study differs from that of Ruhnke et al. (2015) and Marques and Caira (2016) in its additional inclusion of the new species from *H. granulata.* None of these studies are fully complete in their assessment of the genus, however, as many undescribed species of *Anthocephalum* from additional host species likely await collection and description. It is interesting to note that in the analysis produced for this study, the species of *Anthocephalum* from *H. granulata* did not themselves form a monophyletic group.

Differences in Tapeworm Species Assemblages Between Hosts from Different Localities

Distinct differences in tapeworm community compositions were noted for hosts from the two geographically distinct regions examined in this study (i.e., the 2 capture localities

in northern Australia versus the single capture locality in the Solomon Islands). All observed differences are, however, couched in the fact that host sample sizes from all localities were relatively low, and more complete sampling may yet nullify these differences. Only a single species, *Polypocephalus* sp. 2, was found parasitzing host individuals (of both size classes) in all three capture localities, whereas *Acanthobothrium* sp. 4, *Rhinebothrium* sp. 4 and *R.* sp. 5, *Stillabothrium* n. sp. 1, *Prochristianella clarkeae* and *P.* sp. 3, and *Caulobothrium* sp. 1 were only found parasitizing mangrove whiprays from the localities in northern Australia (Queensland and Northern Territory) and were completely absent from the Solomon Islands. Additionally, in contrast to the surprisingly high diversity of species of *Anthocephalum* discovered from rays from the Solomon Islands, only two species of *Anthocephalum* were found parasitzing mangrove whiprays from northern Australia. For lecanicephalideans, New Genus 12 n. sp. 2, *Polypocephalus* sp. 2 and *P.* sp. 3 were the only species found from northern Australia; representatives of "New Genus 11" were absent from these localities despite being found in five of the eight mangrove whiprays examined from the Solomon Islands (see Table 2). It is worth noting that the only individual of *H. granulata* examined in this study whose species identification was not confirmed using NADH2 sequence data was the small juvenile mangrove whipray from Northern Territory (AU-32). Despite the lack of molecular sequence data for this individual, however, identifications based on morphological characters assessed both in the field and in the lab using detailed photographs (Caira et al. 2012b) identify the specimen confidently as *H. granulata* (J.N. Caira and K. Jensen, pers. comm.).

Despite the differences in tapeworm species assemblages of mangrove whiprays from northern Australia versus the Solomon Islands, it is difficult to tease apart the influence of host size from geography as both rays from Queensland and Northern Territory were small juveniles less than 35 cm DW and no large mature rays (i.e., greater than 100 cm DW) were captured from either of these localities to serve as a comparison to the four large mature rays collected from the Solomon Islands. This incomplete representation of *H. granulata* across its size range from northern Australian localities may mean that most differences in tapeworm species assemblages

observed between the two geographic regions are the result of a sampling artifact. Even when comparing only small juvenile rays, however, there still exists differences in tapeworm species assemblages between regions that cannot be explained as an inappropriate comparison across host age/size classes. For example, species were present in rays from northern Australia that represent genera (i.e., *Stillabothrium* n. sp. 1) and orders (i.e., *Caulobothrium* sp. 1) that were entirely absent from all individuals collected from the Solomon Islands. Additionally, species of some genera (i.e., *Acanthobothirum*) and orders (i.e., Trypanorhyncha) that parasitized the small juvenile mangrove whiprays from northern Australia were absent from small juvenile mangrove whiprays from the Solomon Islands, and were instead only found parasitizing large Solomon rays.

If the observed differences in tapeworm faunal composition between geographic regions are indeed real, and not just an artifact of small host sample size, then a multitude of potential explanations could be suggested as to why these differences exist. The most obvious explanation is that there are different intermediate and/or paratenic hosts parasitized by different tapeworm larvae available as prey in northern Australia as compared to the Solomon Islands, which would ultimately lead to the establishment of different tapeworm communities in *H. granulata* from these different regions. The elasmobranch diet literature is rich with examples of species whose diet varies across their geographic distribution (i.e., Cortés and Gruber 1990, Bethea et al. 2006, 2007; McElroy et al. 2006, Espinoza et al. 2015, Munroe et al. 2015) and thus it may not be unreasonable to suggest that the diet of *H. granulata* differs between the sampled regions. In their classification of coastal and continental shelf marine ecoregions, Spalding et al. (2007) identify each of the three localities from which the hosts examined in this study were captured as distinct ecoregions. The locality in the Solomon Islands is classified as the "Solomon Archipelago" ecoregion within the "Eastern Coral Triangle" province, the Queensland locality is classified as the "Arnhem Coast to Gulf of Carpentaria" ecoregion, and the Northern Territory locality is classified as the "Bonaparte Coast" ecoregion, both of which lie within the "Sahul Shelf" province (Spalding et al. 2007). This classification implies that each of these

three ecoregions is an "area of relatively homogenous species composition, clearly distinct from adjacent systems" (Spalding et al. 2007), lending support to the hypothesis that *H. granulata* may be exposed to and prey upon a different suite of intermediate hosts across its range, as is suggested by the regionally varying composition of its tapeworm community.

Only few studies have examined how elasmobranch tapeworm communities differ across the geographic distributions of their hosts. In their investigation of species of *Hornellobothrium* from the spotted eagle ray *Aetobatus ocellatus* in the Indo-Pacific, Mojica et al. (2014) found that rays from different regions each hosted a unique species of *Hornellobothrium*. The authors caution, however, that this may be an artifact of small host sample size. Additionally, Caira and Euzet (2001) found that while nurse sharks from opposite sides of the Atlantic Ocean shared two species of *Pedibothrium* in common, sharks from the western Atlantic hosted numerous additional species that were not found in the eastern Atlantic. Again, however, the authors raise the issue of small host sample size from both regions, which may potentially confound any comparisons. Clearly, more work is needed to elucidate how the tapeworm species assemblages may or may not vary over the geographic distribution of elasmobranch hosts.

Differences in Tapeworm Species Assemblages Between Hosts of Different Sizes from the Solomon Islands

Due to the confounding effects of potential geographic variation on the distribution of tapeworm species, the discussion of species associations as they relate to host size are relegated to comparisons between the tapeworm species assemblages of small juvenile and large mature host individuals collected from the Solomon Islands. Distinct differences in tapeworm faunas were noted between the mangrove whiprays of the two size classes collected from this region. As only four rays of each size class were sampled, however, it must be cautioned that any observed differences may simply be the result of small sample size (i.e., rays of all sizes may in fact host all species of tapeworms identified). It is also important to note that no hosts between 35 cm DW and 100 cm DW were examined, so tapeworm data is lacking for *H. granulata* across its size

range from this region.

Within the Solomon Islands, all species of trypanorhynchs and all species of *Acanthobothrium* were recovered exclusively from large mature mangrove whiprays. The majority of rhinebothriidean species from the Solomon Islands also parasitized large mangrove whiprays, with only *Anthocephalum* n. sp. 4A/4B, *A.* n. sp. 6 and *Rhinebothrium* sp. 3 found parasitzing rays of both sizes from this locality; these three species were, however, observed at lower intensities in small rays as compared to large rays (only one specimen of the *A*. n. sp. 4A/4B morphotype, one specimen of *A.* n. sp. 6, and three specimens of *R.* sp. 3 were collected from small mangrove whiprays). In terms of lecanicephalidean species assemblages, small and large rays from the Solomons appear to host more comparable faunas: *Polypocephalus* sp. 2, *P.* sp. 3, and New Genus 12 n. sp. 3 were all recovered from both small and large Solomon Island mangrove whiprays, though only two specimens of New Genus 12 n. sp. 3 were recovered from small rays, and both were recovered from a single individual. "New Genus 11" n. sp. 3 was found parasitizing three of four of the large mangrove whiprays sampled, but was found in only a single small individual in low numbers from this locality. The only species found exclusively in small rays from the Solomon Islands was New Genus 12 n. sp. 2.

Though small and large mangrove whiprays were identified upon dissection to be sexually immature and mature, respectively, a concrete age difference between individuals from these two size/maturity classes unfortunately cannot be established. A comparison of the disk width of the specimens of *H. granulata* examined in this study to the age/growth curves produced for other tropical dasyatid rays that obtain similar dimensions as adults suggests that a DW of approximately 35 cm corresponds to an age of approximately 0–1 years, and a DW of approximately 100–115 cm corresponds to an age of approximately 5–7 years (O'Shea et al. 2013, see fig.3). Last and Stevens (2009) suggest that *H. granulata* can be born as small as 14 cm DW, and measurements taken of the pups being carried by the largest ray in this study at the time of dissection indicate that pups from this host had DWs of approximately 22.5 cm. Given these data, and the relatively steep initial slopes of the growth curves for tropical dasyatid rays

produced by O'Shea et al. (2013), it seems reasonable to suggest that the four small mangrove whiprays of DW less than 35 cm examined in this study were relatively young (0–1 years) and the four mangrove whiprays of DW greater than 100 cm—in addition to being larger and sexually mature—were at least several years older. It may be that individuals of *H. granulata* with larger DWs were parasitized by more species of tapeworms due to the fact that they are older, and have had more time to consume a variety of intermediate hosts and thus to accumulate a wider diversity of tapeworms than their younger counterparts, a trend which has been noted for numerous species of bony fishes (i.e., Guégan et al. 1992, Lo et al. 1998, Gonzalez et al. 2001, Johnson et al. 2004).

There are many additional potential explanations for why larger mature rays were found to host a greater number of tapeworm species than smaller juvenile rays collected from the same area. Chief among these are differences in feeding strategies and behaviors between the two age/size classes of *H. granulata* examined, as, for predators in particular, overall body size has been shown to significantly impact prey choice (Paine 1976, Polis 1984) to the point where a predatory individual will likely share a greater proportion of its prey with a similarly-sized individual of a different species than with a conspecific of a different size (Bax 1998). All speculation on the effects of diet/feeding strategies on the tapeworm fauna of *H. granulata* must, however, be prefaced with the fact that very little is known about the intermediate or paratenic host use and degree of specificity at the level of the intermediate host for nearly all elasmobranch tapeworm species (Caira and Reyda 2005), and until more is known about these topics, these explanations must remain as strictly conjecture.

Many species of rays have been shown to be gape-limited predators, meaning the type and size of prey they are capable of consuming and the degree to which their diet is specialized are directly related to the dimensions of their mouths (Farias et al. 2006 and citations therein), which have in turn been shown to be correlated with overall body size (Dale et al. 2011). If *H. granulata*—like many cartilaginous and bony fishes—is a gape-limited predator, it would not be unreasonable to expect that a ray of DW less than 35 cm would consume different prey than

an individual three times that size. If tapeworm larvae demonstrate some degree of specificity in terms of their intermediate host preference, then consumption of different intermediate hosts by rays of different sizes would likely leaded to the establishment of different tapeworm faunas within these hosts.

A shift in diet driven by ontogenetic changes (i.e., changes as a result of aging, growth, and maturation) other than purely an increase in gape size could also be postulated for *H. granulata*; for example, it has been suggested that elasmobranchs may exhibit a shift in diet due to the fact that larger, older animals are likely stronger, faster and more experienced predators, and thus may have increased predatory effectiveness as compared to that of younger, juvenile animals (e.g., Lucifora et al. 2009, Newman et al. 2012). It is worth nothing that ontogenetic dietary shifts have been identified in elasmobranchs both as larger animals shifting their feeding strategy almost entirely from one prey species or set of prey species to another, leading to very low dietary overlap between size/age classes (i.e., Brickle et al. 2003, Hoffmayer and Parsons 2003, Bethea et al. 2006, 2007; Šantić, et al. 2013), as well as larger animals increasing or narrowing the breath of their diets as they mature (i.e., Moura et al. 2008, Dale et al. 2011, Espinoza et al. 2013, Shiffman et al. 2014). Either shift would likely affect tapeworm community composition, but as little is known about the diet and feeding strategies of *H. granulata*, it cannot be said for certain whether either is responsible for the differences in tapeworm species assemblages observed between hosts of different sizes in this study. Additionally, it may simply be—in accordance with the tenants of MacArthur and Wilson's (1967) theory of island biogeography—that larger hosts, like larger islands, can provide more available niche space in their larger spiral intestines for more tapeworm species.

There is also evidence to suggest that juvenile and mature individuals of *H. granulata* demonstrate differences in their habitat use, which—given the well-known importance of habitat use on diet (Bax 1998)—further supports small juvenile and large mature individuals of *H. granulata* pursuing different prey. In a study of the habitat use of juvenile *H. granulata* by Davy et al. (2015), it was concluded that juvenile rays associate strongly with shallow water coral reef

and intertidal mangrove habitats, and likely utilize such areas as a refuge from larger predators until they reach a size at which they are less vulnerable to predation. All individuals of *H. granulata* tagged and tracked for this behavioral study were reported as immature, and the largest individual studied had a disk width of 44.0 cm, suggesting that maturity, and thus increased safety from predators, is potentially not realized until after *H. granulata* reaches a disk width of greater than 44 cm. The study notes that no large, mature rays were captured, and only one larger individual was ever observed with the studied juvenile habitats (an individual with a disk width of approximately 100 cm), which the authors suggest is an indication of the two size classes utilizing largely non-overlapping habitats.

Collection data for the specimens assessed by Ishihara et. al (1993) for the redescription of *H. granulata* corroborate this postulation, as none of the specimens from that analysis were collected from mangrove areas, and all specimens had a disk width of 52.4 cm or greater. Additionally, the largest—presumably mature—male from that study (disk with of 97 cm) is said to have been collected by long line from a depth of 85 m, lending further support to the differential use of habitat by the two sizes classes of *H. granulata*. Though all eight juvenile and mature individuals from the Solomon Islands examined in this study were collected from the same capture locality, evidence such as that put forth by Davy et al. (2015) and Ishihara et al. (1993) suggests that mature individuals are not restricted to mangrove and intertidal habitats to the same degree that are juvenile individuals. Thus, the four large mature rays examined in this study likely enjoyed a somewhat more expanded range over which to hunt for prey, and potentially had exposure to more intermediate host species (and thus more tapeworm species) than the four small juvenile individuals examined.

Preliminary Data Investigating the Effect of Host Size in Other Elasmobranch-Tapeworm Systems

This study illuminates the need for further investigation into how tapeworm species assemblages change (or do not change) across the lifespans of different elasmobranch host

species. Unfortunately, several elements of the *H. granulata* host-parasite system made these patterns difficult to investigate in the present study, including *H. granulata*'s relatively unknown biology and life history, and its high tapeworm species diversity. Future studies would be well-served by focusing on an elasmobranch host with the following characteristics: (1) a species with known geographic distribution and life history data, (2) a species for which confident age/maturity estimations can be made based on size, (3) a species for which the diet—including any ontogenetic shifts—has been well-characterized, and (4) a species which hosts moderate tapeworm species diversity (ideally 10 or fewer tapeworm species). Initial efforts to explore the relationship between tapeworm community composition and host size in a more idealized system such as this were made using the tapeworms of the finetooth shark *Carcharhinus isodon* Valenciennes in Müller & Henle (family Carcharhinidae Jordan & Evermann).

Carcharhinus isodon is an ideal host in which to study the effect of host size on parasite diversity because it meets the four criteria postulated above, including well-understood habitat use, diet, and rates of maturation. *Carcharhinus isodon* is a migratory species of shark with a western Atlantic distribution ranging from New York to Florida and the Gulf of Mexico (Castro 1993). Juveniles and adults migrate to Bull's Bay, South Carolina each year in April and May, respectively—presumably correlated with the time at which surface waters in this area warm to temperatures above 20° C—where they remain for the summer months at shallow depths of approximately 2–7 m (Castro 1993). Sharks depart South Carolina between the months of September and October and migrate south to a wintering ground of unknown locality. Migratory patterns of *C. isodon* from the Gulf of Mexico have not been extensively studied, and the extent of separation between the coastal United States and Gulf of Mexico populations is not well understood (Castro 1993). Females are suggested to reach sexual maturity between total lengths of 125 cm and 135 cm (Castro 1993), while males mature between total lengths of approximately 119 cm and 130 cm, with both sexes maturing around 4 years of age (Carlson et al. 2003). Studies of the diet of *C. isodon* have revealed that the prey of this species consists mainly of bony fishes—particularly menhaden—under 200 mm standard length (Castro 1993,

Hoffmayer and Parsons 2003, Bethea et al. 2004), but that *C. isodon* may also opportunistically prey upon crustaceans (Castro 1993, Hoffmayer and Parsons 2003, Bethea et al. 2004) and small elasmobranchs (Castro 1993). To date, the onchoproteocephalidean *Triloculatum geeceearlensis* Caira & Jensen 2009 is the sole species of tapeworm described from *C. isodon* (see Caira and Jensen 2009). Tapeworm data from other, well-studied carcharhinids such as the blue shark *Prionace glauca* and the dusky shark *Carcharhinus obscurus* Lesueur suggest that sharks in this family tend to host on average approximately 4–7 species of tapeworms (Linton 1889, 1890; Yamaguti 1934, 1952; Joyeux and Baer 1936, Yamaguti and Miyata 1940, Robinson 1959, Ruhnke and Caira 2009, Ruhnke 2011).

Four individuals *C. isodon* were collected between 2012 and 2015 from a single locality in Bull's Bay, South Carolina: 3 immature males on June 27, 2012 less than 60 cm total length (TL) and 1 mature male on June 18, 2015 of 131 cm TL. Preliminary data suggest that the tapeworm species recovered from these four individuals are differentially distributed among them with respect to host size. While both the small juvenile sharks and the large mature shark were parasitized by tapeworms of the genera *Phoreiobothrium* and *Paraorygmatobothrium*, only the large mature shark hosted specimens of *Triloculatum geeceearlensis*, the "tetraphyllidean" genus *Anthobothrium*, and an unidentified genus in the trypanorhynch family Otobothriidae. Additionally, it appears from preliminary morphological assessments that different species of *Phoreiobothrium* and *Paraorygmatobothrium* parasitize the two host size classes. Though it must again be cautioned that host sample size is very small, these data appear to be consistent with the trend suggested by the tapeworm species assemblage data from *H. granulata* in the Solomon Islands and northern Australia (i.e., larger individuals host a greater number of tapeworm species). Since these two host species represent two very disparate taxonomic placements, morphologies, geographic distributions, and life histories within the Elasmobranchii, comparative studies between them may serve to provide context for the broader application of similar parasitological trends to elasmobranchs as a group in general.

Future Directions

As only seven new species are formally described in this study, future work on the tapeworm fauna of *H. granulata* should focus on delineating species boundaries more concretely in the remaining identified species—which were only preliminarily differentiated from one another, and not from their congeners from other hosts—and describing those taxa that are new and providing new host records for those that are not (i.e., likely most species of trypanorhynchs). At this point, *Anthocephalum* n. sp. 4A and *A.* n. sp. 4B are only distinguished from one another based on molecular sequence data, as hologenophores for these putative species are morphologically uninformative. Future sequencing efforts focused on these species would benefit from preparing the proglottids of each sequenced specimen for light microscopy and preparing the scoleces for scanning electron microscopy so as to gain as much morphological information as possible from each hologenophore. Generation of full or partial 18S sequence data for the seven species of *Anthocephalum* from *H. granulata* to be included in future phylogenetic analyses may serve to resolve interrelationships within the genus, which were not elucidated in this study using only partial 28S sequence data.

An examination of the tapeworm fauna of both additional individuals of *H. granulata* from the Solomon Islands not represented in this host size sample (i.e., between 35 cm DW and 100 cm DW), as well as additional individuals from across the species' range (i.e., from more northern Indo-Pacific regions such as Viet Nam and Cambodia) could provide fruitful for further understanding the effect of host age/size and geography, respectively, on tapeworm assemblages in this species. This additional sampling of *H. granulata* is increasingly important, as *H. granulata*—along with numerous additional elasmobranch speices—has been elevated to "near threatened" status on the IUCN Red List. This study has shown the relationship between elasmobranch host age/size and tapeworm community composition to be an avenue of research deserving of future investigation for additional elasmobranch species and their tapeworms.

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