Lecanicephalidean (Cestoda) fauna of the giant freshwater whipray, *Himantura polylepis* (Myliobatiformes: Dasyatidae), from Malaysian and Indonesian Borneo.

By

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ABSTRACT

Eight species of dasyatid stingrays (Myliobatiformes) are characterized as freshwater obligate, including the giant freshwater whipray (*Himantura polylepis*), comprising at least three separate incursions into freshwater habitats. However, a tolerance of estuarine environments has been suggested for *H. polylepis*. Eight individuals of this species were collected between 2003 and 2009 at two sites in Borneo [six from the Kinabatangan River (Malaysia) and two from Tarakan (Indonesia)]; these host specimens were examined for lecanicephalidean cestodes. Collectively, these hosts were parasitized by eight species of lecanicephalidean tapeworms: three new species of *Tetragonocephalum*, three new species of *Polypocephalus*, and one species each of two as of yet undescribed genera. This brings the total number of reported cestodes from *H. polylepis* to 18 species in nine genera of four orders. Of the nine total genera parasitizing *H. polylepis*, one is unique while seven are collectively also found parasitizing marine and euryhaline dasyatid hosts. In contrast, the South American freshwater potamotrygonids are parasitized by a total of seven genera; three of these are unique to potamotrygonids while four genera also parasitize marine stingrays. The similarity of the cestode assemblage of the giant freshwater whipray to that of its marine and euryhaline relatives might be one of several lines of evidence to support the notion that this stingray may not be restricted to freshwater.

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

Lecanicephalidea

The Lecanicephalidea, one of the nine recognized orders of elasmobranch cestodes, include more than 80 species that span 25 genera within eight families (Jensen et al., 2016). In the most recent phylogenetic treatment of the Lecanicephalidea, Jensen et al. (2016) revised four known families within the order: Tetragonocephalidae Yamaugti, 1959, Polypocephalidae Meggitt, 1924, Lecanicephalidae Braun, 1900, and Cephalobothriidae Pintner, 1928; the authors also create four new families: Aberrapecidae Jensen, Caira, Cielocha, Littlewood, and Waeschenbach, 2016; Eniochobothriidae Jensen, Caira, Cielocha, Littlewood, and Waeschenbach, 2016; Paraberrapecidae Jensen, Caira, Cielocha, Littlewood, and Waeschenbach, 2016; and Zanobatocestidae Jensen, Caira, Cielocha, Littlewood, and Waeschenbach, 2016. While no single morphological synapomorphy exists to unambiguously diagnose the group, lecanicephalideans are united in their possession of a combination of features surrounding their scolex morphology and proglottid anatomy: a scolex consisting of four bothridia or suckers, an apical modification of the scolex proper, and an apical organ; and a vagina that opens into the genital atrium posterior to the cirrus sac, as well as a vas deferens that extends from the level of the ovarian bridge to the cirrus sac often expanded to form an external seminal vesicle (Jensen, 2005; Caira and Jensen, 2014; Jensen et al., 2016).

This monophyletic order (Caira et al., 2001; Jensen, 2005; Waeschenbach et al., 2012; Caira et al., 2014; Jensen et al., 2016) primarily comprises members that parasitize batoids, though some records exist from selachid hosts (e.g., Yamaguti, 1934; Deshmukh et al., 1982; Sarada et al., 1993; Caira et al., 1997; Jensen, 2001, 2005; Pramanik and Manna, 2007). Lecanicephalideans have a circumglobal distribution (e.g., see Jensen et al., 2016) restricted to latitudes between 45°N and 35°S. The highest diversity of members of this order [67 of at least 81 valid species listed in the Global Cestode Database (http://tapewormdb.uconn.edu/index.php/parasites/species_search/)] is reported from the Indo-Pacific region (e.g., Caira and Jensen, 2014). Records of species of lecanicephalideans in the early 20th century were in association with pearl oyster fisheries operating in the Indian Ocean off India and modern-day Sri Lanka (Shipley and Hornell, 1904, 1905, 1906), with additional descriptions of a couple of species from the Atlantic Ocean (Linton, 1890) and one species from the Pacific Ocean (Shipley, 1900). Subsequent species descriptions over the next century were from across the globe, with type localities reported from the following bodies of water: Gulf of California (Jensen, 2001), Atlantic Ocean off the northeastern United States (Linton, 1916; Campbell and Williams, 1984), Caribbean Sea (e.g., Gardner and Schmidt, 1984; Ivanov and Campbell, 2000), South Atlantic Ocean (e.g., Brooks et al., 1981a), Red Sea (e.g., Hassan, 1982; Ramadan, 1986), Arabian Sea (e.g., Zaidi and Khan, 1976; Shinde and Deshmukh, 1979; Al Kawari et al., 1994), Sea of Japan (e.g., Yamaguti, 1934, 1960; Caira et al., 1997), and the Coral Sea (e.g., Butler, 1987). Targeted sampling efforts in Senegal, Southeast Asia, and Australia beginning in the late 20th century have expanded the known geographic distribution of members of this order to coastal marine environments in the South China Sea (e.g., Jensen et al., 2011; Koch et al., 2012; Mojica et al., 2013), Sulu Sea (e.g., Mojica et al., 2013), Celebes Sea (Jensen, 2006; Mojica et al., 2013), Makassar Strait (Cielocha and Jensen, 2011; Mojica et al., 2014), Timor Sea (e.g., Jensen, 2005; Cielocha et al., 2014; Jensen and Russell, 2014), Arafura Sea (e.g., Jensen, 2005; Cielocha and Jensen, 2013; Mojica et al., 2014), and the Atlantic Ocean off the southeastern United States [South Carolina, see Jensen (2005)] and Senegal (Jensen et al., 2014). Few additional records (e.g., Solomon Islands) exist from species used in molecular phylogenetic analyses e.g., Jensen et al., 2016); these await formal description.

Lecanicephalideans are known to parasitize batoids in the orders Myliobatiformes (stingrays), Torpediniformes (electric rays), and Rhinopristiformes (guitarfishes and sawfishes) (e.g., Caira and Jensen, 2014; Jensen et al., 2016) that are typically found in the coastal shelf (see Froese and Pauly, 2015). Only a single species of lecanicephalidean has been reported from a member of the Rajiformes (skates) by Yang et al. (1995) [i.e., *Lecanicephalum xiamenensis* Yang, Lui, and Lin, 1995 from *Okamejei hollandi* (Jordan and Richardson) [reported as*'Raja holandi* [sic] (Jordan and Richardson)']. Lecanicephalideans have previously only been documented from marine and euryhaline (e.g., Cielocha et al., 2014) hosts.

Overall, lecanicephalideans exhibit strict host specificity ['oioxenous' *sensu* Euzet and Combes (1980), see Caira et al. (2003); Jensen (2005)] in which each adult species parasitizes one species of definitive host. Though a complete life cycle has yet to be elucidated, lecanicephalideans are generally hypothesized to parasitize two or three intermediate hosts and an elasmobranch definitive host during their ontogenesis (Caira and Jensen, 2014). Jensen (2005) provided a summary of the larval forms of lecanicephalideans that have been reported from mollusc, crustacean, and teleost intermediate hosts. The majority of records concerning larval lecanicephalideans that have since been published are focused on economically important intermediate host species such as oysters (e.g., Zeidan et al., 2012), shrimp (e.g., Carreon et al., 2011; Carreon and Faulkes, 2014), swordfish (e.g., Garcia et al., 2010; Mattiucci et al., 2014), and queenfish (Bannai et al., 2014). Researchers who have proposed a two-host life cycle hypothesize that a single invertebrate intermediate host (i.e., a mollusc, sometimes specified as an oyster) takes in cestode eggs while filter-feeding, after which an elasmobranch definitive host preys upon the molluscan host and becomes infected (Shipley and Hornell, 1906; Jameson, 1912; Cheng, 1975; Butler, 1984). In contrast, others have suggested a three-host life cycle either consisting of (a) two invertebrate intermediate hosts and an elasmobranch definitive host, or (b) one invertebrate intermediate host, one teleost intermediate host, and an elasmobranch definitive host. Regarding the former, two different three-host hypotheses have been put forward in the literature: (1) Cake (1979) reported plerocercoid larvae of *Polypocephalus* Braun, 1878 (Polypocephalidae) from scallops in the Gulf of Mexico and suggested that an 'unknown crustacean host' (i.e., copepod) might ingest eggs, a scallop may then ingest the crustacean now infected with procercoids (i.e., larvae developed from the eggs), and an elasmobranch definitive host might then prey upon the infected scallops; and (2) Cake and Menzel (1980) proposed an alternative hypothesis pertaining to the life cycle of *Tylocephalum* Linton, 1890 (Cephalobothriidae) in which eggs are ingested by an oyster, which is consumed by a predatory gastropod (e.g., lightning whelk, apple murex, or southern oyster drill) and finally eaten by an elasmobranch definitive host.

Freshwater dasyatids

Based on the most recent estimate of extant species of elasmobranchs, approximately 5% (56 of 1,154) are known to inhabit freshwater systems (Lucifora et al., 2015). These species are restricted to the tropic and subtropic latitudes between 35°N and 35°S, with the majority of the diversity concentrated in South America and Southeast Asia (Ballantyne and Fraser, 2013). Two terms are applied herein to freshwater members based on their salinity tolerance: 'obligate freshwater' refers to species that are restricted to freshwater only, and 'euryhaline' designates species that are able to tolerate a wide salinity range inclusive of freshwater (Lucifora et al., 2015). The terms 'marginal' and 'brackish-marginal' also appear in the literature surrounding freshwater elasmobranchs (e.g., Compagno and Cook, 1995); these terms were used to refer to species that have access to freshwater but primarily inhabit brackish and estuarine waters, and are therefore not of concern in this current study. Members of Pristidae Bonaparte (sawfishes) and Carcharhinidae Jordan and Evermann (ground sharks) have been documented to enter freshwater, but they mainly occupy marine habitats. Five species of pristids are considered euryhaline [*Anoxypristis cuspidata* (Latham)*, P. pectinata* Latham*, P. pristis* (Linnaeus)*, P. clavata* Garman, and *P. zijsron* Bleeker; see Compagno and Cook, 1995; Martin, 2005; Faria et al., 2013]. Of the carcharhinids, four species are designated as euryhaline [*Carcharhinus leucas* Müller and Henle, *Glyphis gangeticus* (Müller and Henle), *G. garricki* Compagno, White, and Last, and *G. siamensis* (Steindachner) (see Compagno and Cook, 1995; Martin, 2005)], and two species are reported as obligate to freshwater [*G. glyphis* (Müller and Henle), and *G. fowlerae* Compagno, White, and Cavanagh (see Compagno and Cook, 1995; Martin, 2005)]. Freshwater stingrays within the family Potamotrygonidae Garman are the only elasmobranchs known to have radiated in freshwater (see Lovejoy, 1996); there are currently 28 species known from four genera (Eschmeyer and Fong, 2016) [23 species of *Potamotrygon* Müller and Henle; 2 species of *Heliotrygon* Carvalho and Lovejoy; 2 species of *Plesiotrygon* Rosa, Castello, and Thorson; and 1 species of *Paratrygon* Duméril]. Members of this family exclusively occupy river systems in South America.

Within the Dasyatidae, Compagno and Cook (1995) reported five euryhaline species [*Dasyatis fluviorum* Ogilby, *D. sabina* (Lesueur)*, Himantura fluviatilis* (Hamilton)*, H. uarnak* (Gmelin)*,* and *Pastinachus sephen* (Forsskål) [as '*Hypolophus sephen* (Forsskål)'], and eight obligate freshwater species [*Dasyatis garouaensis* (Stauch and Blanc)*, D. laosensis* Roberts and Karnasuta*, D. ukpam* (Smith)*, D.* sp. (from China), *Himantura polylepis* (Bleeker) [as '*H.*

chaophraya (Monkolprasit and Roberts)'], *H. krempfi* (Chabanaud)*, H. signifer* Compagno and Roberts*,* and *H.* sp. (from New Guinea)]. Martin (2005) expanded on these lists of euryhaline and obligate freshwater species (see Martin, 2005; tables 2, 4, and 5), and considered *D. ukpam* [as '*Urogymnus ukpam* (Smith)'] to be a euryhaline and not an obligate freshwater species. He also addressed taxonomic problems within freshwater and euryhaline elasmobranchs that affect the composition of species lists given by Compagno and Cook (1995): he stated that the *Dasyatis* sp. from China may be synonymous with *D. laosensis* and that *H. krempfi* may be synonymous with *H. oxyrhyncha* (Sauvage) (see Martin, 2005; table 6). Martin also seems to include *H. fluviatilis* in his expanded count of five species of *Himantura* that are obligate to freshwater because this species is included in his list of the IUCN conservation status of select elasmobranchs (see Martin, 2005; table 5), however other workers consider this species to be synonymous with *Pastinachus sephen* (see Fahmi et al., 2009; Roberts, 1998). Since Martin (2005), two additional freshwater batoids, *Himantura kittipongi* Vidthayanon and Roberts and *Himantura dalyensis* Last and Manjaji-Matsumoto, have been described. Therefore, an updated list of obligate freshwater dasyatids includes eight total species: *D. laosensis, D. garouaensis, D. ukpam, H. polylepis* (senior synonym of *H. chaophraya*), *H. dalyensis, H. signifer, H. kittipongi,* and *H. oxyrhyncha*.

Naylor et al. (2012a) conducted a phylogenetic analysis of elasmobranchs using sequence data from the mitochondrial NADH2 gene in which they included a total of 574 of approximately 1,221 described species of elasmobranchs (at the time of the study). Of the 92 valid species of dasyatids (see Ebert et al., 2013; table 1), 62 species (i.e., 67%) were included in their analysis, and 15 of 32 species (i.e., 46%) of *Himantura* were represented. Of the Myliobatiformes, three euryhaline representatives (*D. sabina, H. uarnak,* and *P. sephen*) and 11 obligate freshwater representatives (the four dasyatids *H. kittipongi, H. signifer, H. oxyrhyncha,* and *H. polylepis*; and seven potamotrygonids) were included in this phylogeny. As expected, all three euryhaline species placed within their respective genera, suggesting independently derived broad salinity tolerance for dasyatids. It is interesting to note that of the obligate freshwater species of *Himantura*, three (*H. kittipongi, H. signifer,* and *H. oxyrhyncha*) are members of a clade separate from the clade containing *H. polylepis*. Based on the analyses by Naylor et al. (2012a), a minimum of three separate incursions into freshwater can be hypothesized to have occurred within Dasyatidae: one for the clade of *H. kittipongi, H. signifer,* and *H. oxyrhyncha*; one for *H. polylepis* (including *H. dalyensis* as its presumed sister taxon; Manjaji, 2004); and, at a minimum, one for *D. laosensis, D. garouaensis*, and *D. ukpam* if these are assumed to be each other's closest relatives.

Himantura polylepis

The giant freshwater whipray, *Himantura polylepis*, is a dasyatid stingray that inhabits freshwater throughout Southeast Asia. True to its name, individuals of *H. polylepis* have been reported to reach up to two meters in disc width (Vidthayanon et al., 2013) and 600 kg in mass (Monkolprasit and Roberts, 1990; Last and Stevens, 1994; Vidthayanon et al., 2013). It is one of 32 species of *Himantura*, of which the majority of members (27 species) are found within marine waters. Of the taxa included by Naylor et al. (2012a), the closest relatives of *H. polylepis* are in fact all marine species: *H. granulata* (Macleay), *H.* sp. 1 (collected from the Arafura Sea off Northern Territory, Australia), and *Urogymnus asperrimus* (Bloch and Schneider) (see Naylor et al., 2012a; fig. 2.11). *Himantura polylepis* has been observed in rivers and estuaries from India to Indonesia: Ganges River, India (Sezaki et al., 1999; Manjaji, 2004); Mekong River spanning Laos (Glemet et al., 2013) and Thailand (Monkolprasit and Roberts, 1990); Chao Phraya River

Figure 1. Occurrence and distribution map of *Himantura polylepis* throughout its range in Southeast Asia. Blue lines indicate rivers; yellow lines indicate geographic distribution data that are not georeferenced; red dots indicate occurrence data that are georeferenced from original publications.

(Monkolprasit and Roberts, 1990; Sezaki et al., 1999; Manjaji, 2004), Tapi River (Monkolprasit and Roberts, 1990), and Bangpakong River (Monkolprasit and Roberts, 1990), Thailand; Kinabatangan River (Manjaji, 2004; Healy, 2006a) and Padas River (Manjaji, 2004), Malaysia; and the Mahakam River, Indonesia (Last and Stevens, 1994) (see Fig. 1). The occurrence map in Fig. 1 shows both collection localities for which the authors of the original publications recorded coordinates for sites where specimens of *H. polylepis* were collected (see Monkolprasit and Roberts, 1990; Sezaki et al., 1999; Manjaji, 1997, 2004; Yano et al., 2005; Glemet et al., 2013) in addition to sites for which georeferenced data was not recorded. In 2013, the International

Union for the Conservation of Nature (IUCN) listed *H. polylepis* as an endangered species due to anthropogenic threats including habitat destruction and overharvest (Vidthayanon et al., 2013). Current conservation efforts, such as the establishment of protected areas within the Kinabatangan River, Malaysia (Anderson and Jenkins, 2006), and tagging efforts in the Mekong River, Thailand (Hogan, 2009) have thus far been restricted to select rivers. *Himantura polylepis* is also among the threatened freshwater elasmobranchs that are protected under the Sabah Aquaculture and Inland Fisheries Enactment of 2003, written into the Malaysian National Plan of Action and implemented by the Department of Fisheries in 2005 (Dept. of Fisheries Malaysia, 2014).

Bleeker first described *Trygon polylepis* Bleeker from Jakarta in Java, Indonesia in 1852. Seemingly unaware of Bleeker's work, Monkolprasit and Roberts (1990) described *Himantura chaophraya* as a new species based on three specimens collected in the Chao Phraya and Mae Nam Nan rivers in Thailand. In 2008, Last and Manjaji-Matsumoto (2008) recognized *H. chaophraya* as a junior synonym of *T. polylepis*, and proposed *Himantura polylepis* as a new combination. In that same work, the authors found individuals in populations inhabiting river systems in northern Australia to be morphologically (smaller in overall body size, different squamation in tail, slightly higher vertebral count, and a much higher ratio of propterygial radials to mesopterygial radials) and molecularly (minor differences in cytochrome *b* and 16S genes) distinct from those in Southeast Asia. Based on these differences, they erected a new species, *H. dalyensis*. This action reduced the geographic distribution of *H. polylepis* to Southeast Asia.

Several researchers have suggested that the disjunct populations of *H. polylepis* across river systems in Southeast Asia may represent separate species that have diverged over time as a result of geographic separation. Sezaki et al. (1999) found differences in the cytochrome *b* gene (hereafter cyt*b*) of three specimens of *H. polylepis* (as '*H. chaophraya*') that were collected from rivers in India and Thailand. These individuals were included in a wider phylogenetic analysis of eight species of dasyatids that were collected from localities across Asia. The result of their study was that five amino acid replacements were detected in the cyt*b* gene targeted in samples from the single individual of *H. polylepis* collected from the Ganges River (India) compared to the two individuals collected from the Chao Phraya River (Thailand), which the authors interpreted to suggest that these two disjunct populations are actually part of a species complex. The authors attribute these molecular distinctions to allopatric speciation events, likely resulting from the Sunda shelf experiencing about 100 meters in sea level rise in the late Pleistocene (Ballantyne and Fraser, 2013). In her dissertation, Manjaji (2004) also included several individuals of *H. polylepis* (as '*H. chaophraya*') in her analyses that sought to investigate the relationships of species of *Himantura*; this allowed her to also speculate on their conspecificity. Morphological and molecular data (16S and cyt*b*) were generated from six specimens of '*H. chaophraya,*' two of which were collected in Australia and are therefore assumed to be members of *H. dalyensis* (subsequently described in 2008, see above) and are not further addressed here. The remaining four individuals were collected from the Ganges River in India, the Chao Phraya River in Thailand, and the Kinabatangan and Padas rivers in Malaysia. Morphological (squamation, disc shape, and overall size) and molecular distinctions indicated that these individuals represented more than one species. The analyses based on cyt*b* data consistently grouped the two specimens from Malaysia separately from those collected in India and Thailand. No taxonomic action has resulted either from the analysis of Manjaji (2004) or Sezaki et al. (1999).

Purpose of study

The goals of this study are twofold: to characterize the lecanicephalidean fauna of *H. polylepis* from individuals collected from Malaysian and Indonesian Borneo, and to evaluate the cestode fauna of *H. polylepis* compared to that of other freshwater batoids as well as its close marine relatives.

Previous reports of cestodes from *H. polylepis* include a total of 10 species across five genera within three orders. Fyler and Caira (2006) described five species of *Acanthobothrium* Blanchard, 1848 (Onchoproteocephalidea): *A. asnihae* Fyler and Caira, 2006*, A. etini* Fyler and Caira, 2006*, A. masnihae* Fyler and Caira, 2006*, A. saliki* Fyler and Caira, 2006*,* and *A. zainali* Fyler and Caira, 2006; Healy (2006a) reported three species of *Rhinebothrium* Linton, 1890 (Rhinebothriidea), including one that has since been considered a distinct, novel genus (see Healy et al., 2009; Ruhnke et al., 2015): *R. abaiensis* Healy, 2006*, R. megacanthophallus* Healy, 2006*,* and N. gen. 4 *kinabatanganensis* (as '*Rhinebothrium kinabatanganensis*' Healy, 2006); and Schaeffner and Beveridge (2014) reported two species in two genera of Trypanorhyncha: *Prochristianella clarkeae* Beveridge, 1990 and *Proemotobothrium linstowi* (Southwell, 1912) Beveridge and Campbell, 2001. The current study represents the first record of lecanicephalideans from *H. polylepis*, as well as the first account of cestodes within this order from a freshwater host.

MATERIALS AND METHODS

Host collections

A total of eight specimens of the giant freshwater whipray, *Himantura polylepis*, were examined in this study. Six individuals were collected from the Kinabatangan River off

Table 1. Collection data for individuals of *Himantura polylepis*. Question marks indicate unrecorded data.

Host ID	Sex	Disc width (cm)	Collection localities
BO-108	M	90.5	Kinabatangan River, Kampung Abai, Sabah, Malaysia (05°41'10.81"N, 118°23'08.35"E)
BO-355	?	?	Kinabatangan River, Kampung Abai, Sabah, Malaysia (05°41'10.81"N, 118°23'08.35"E)
BO-356	?	?	Kinabatangan River, Kampung Abai, Sabah, Malaysia (05°41'10.81"N, 118°23'08.35"E)
BO-358	?	?	Kinabatangan River, Kampung Abai, Sabah, Malaysia (05°41'10.81"N, 118°23'08.35"E)
BO-496	M	81.2	Kinabatangan River, Kampung Abai, Sabah, Malaysia (05°41'10.81"N, 118°23'08.35"E)
BO-497	M	45.8	Kinabatangan River, Kampung Abai, Sabah, Malaysia (05°41'10.81"N, 118°23'08.35"E)
KA-393	F	99.2	Celebes Sea, Pasar Beringin Fish Market, Tarakan, North Kalimantan, Indonesia (3°17'47.04"N, 117°34'57.26"E)
KA-397	F	127.3	Celebes Sea, Pasar Beringin Fish Market, Tarakan, North Kalimantan, Indonesia (3°17'47.04"N, 117°34'57.26"E)

Kampung Abai (5°41'10.81'N, 118°23'8.35'E) in Sabah, Malaysia, during three trips in April 2003, May 2004, and July 2008: Jensen and colleagues collected three males (260, 238.4, and 145.4 cm in total length [TL]) via long line from 2003–2004 (host specimen nos. BO-108, BO-496, and BO-497); Malaysian collaborators collected three specimens (host specimen nos. BO-355, BO-356, and BO-358) in June 2009, though their sex and measurements were not recorded (see Table 1). Two female specimens (220.1 cm and 50.2 cm in TL, respectively) were purchased from local fishermen at the Pasar Beringin fish market in Tarakan (3°17'47.04'N, 117°34'57.26'E), North Kalimantan, Indonesia (host specimen nos. KA-393 and KA-397, respectively). Additional host information is available in the Global Cestode Database (http://elasmobranchs.tapewormdb.uconn.edu) and can be retrieved by searching elasmobranch host specimen Collection Codes (e.g., 'BO') and Collection Numbers (e.g., '108').

Each individual was dissected in the field by mid-ventral incision to remove the spiral intestine. The intestine was then opened with a longitudinal incision and examined for cestodes. A selection of the worms found in the field were fixed in 95% ethanol for generation of molecular sequence data. The remainder of the cestodes as well as the spiral intestine were fixed in 10% formalin buffered with water (1:9 ratio) and transferred to 70% ethanol in the laboratory for long-term storage. Host identifications of four of the whiprays from the Kinabatangan River (BO-355, BO-356, BO-496, and BO-497) and the two individuals from Tarakan (KA-393 and KA-397) were verified using NADH2 sequence data (Naylor et al., 2012b).

Morphological methods

Cestodes were sorted to order under a Leica MZ16 dissecting scope. Lecanicephalidean worms were then sorted to genus and prepared as whole mounts for light microscopy (LM) as follows. They were hydrated in a graded ethanol series, stained with Delafield's haematoxylin, dehydrated in a graded ethanol series, cleared with methyl salicylate, and mounted in Canada balsam on glass slides. Scoleces mounted for scanning electron microscopy (SEM) were hydrated in a graded ethanol series, transferred to 1% osmium tetroxide for no less than 6 hours and up to overnight at 4°C, dehydrated in a graded ethanol series, and placed in hexamethyldisilizane (HMDS, Electron Microscopy Sciences, Hatfield, PA) for 15 minutes. They were allowed to air-dry before being mounted on an aluminum stub with double-sided adhesive carbon tape. Specimens were sputter-coated with ~30–35 nm of gold and examined with a FEI Versa 3D Dual Beam field emission scanning electron microscope at the Microscopy and Analytical Imaging Laboratory, University of Kansas, Lawrence, Kansas. Strobila vouchers of scoleces examined with SEM were mounted using the aforementioned protocol for whole-mounted specimens. Specimens reserved for histological sectioning (longitudinal or cross-sections) were dehydrated in a graded ethanol series, stained with Fast Green, and cleared in xylene prior to embedding in paraffin wax in plastic block holders. Serial sections of scoleces and proglottids in paraffin were cut at 7 µm intervals using an Olympus Cut 4060 retracting rotary microtome (Triangle Biomedical Sciences, Durham, North Carolina); sections were floated on glass slides using 2.5% sodium silicate solution and dried on a slide warmer at 40°C for several hours. Sections were stained with Delafield's haematoxylin, counter-stained with eosin, differentiated in Scott's solution, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Additional proglottid and scoleces prepared for histological sectioning in plastic were dehydrated in a graded ethanol series according to the EMS Catalog #14653 with the following modifications: dehydration for 30 minute intervals each in 70, 95 [stained with Fast Green for last 30 seconds], and 100% ethanol, and a final dehydration in 100% ethanol for one hour and up to overnight at room temperature; infiltration in a 1:1 solution of 100% ethanol and infiltration solution for 2 hours at 4°C; and infiltration in 100% infiltration solution at 4°C overnight). Specimens were embedded in a ratio of 100 ml of Technovit H7100 Glycol Methacrylate (GMA) (Kluzer, Wehrheim, Germany) to 1 g of benzoyl peroxide in plastic block holders, cut with glass knives using the same microtome at 4-6 µm intervals, floated on single droplets of distilled water on Superfrost (Fisherbrand; Fisher, Pittsburg, Pennsylvania) microscope slides (10-15 per slide) and allowed to air-dry. Sections were stained with Delafield's haematoxylin, counter-stained with eosin, differentiated in Scott's solution, dehydrated in a graded ethanol series, dried in a 60°C oven, and mounted in Canada balsam.

Specimens prepared as histological sections were imaged using a Lecia DFC 480 or Lumenera Infinity 3 attached to a Zeiss Axioskop 2 Plus compound microscope (Zeiss, Thornwood, New York). Line drawings were made using a camera lucida attached to the aforementioned compound microscope.

Measurements are given in micrometers unless otherwise stated and are reported as ranges followed in parentheses by the mean, standard deviation, number of individual specimens examined, and the total number of measurements if more than one measurement was taken per worm. Microthrix terminology follows Chervy (2009).

Museum abbreviations for collections in which type specimens will be deposited are as follows: LRP, Lawrence R. Penner Parasitology Collection, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA; MZUM(P), Muzium Zoologi, Universiti Malaya, Kuala Lumpur, Malaysia; MZB, Museum Zoologicum Bogoriense, Center for Biology, Indonesian Institute of Science, Cibinong, Jakarta-Bogor, Java, Indonesia; and USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.

RESULTS

Summary of lecanicephalideans parasitizing *Himantura polylepis*

Over one thousand individual lecanicephalideans were recovered from six of the eight individuals of *H. polylepis* examined in this study. In total, these lecanicephalideans represent eight new species in four genera: three new species of *Tetragonocephalum*, three new species of *Polypocephalus*, and one new species each representing two as of yet undescribed genera (i.e. N. gen. 12 and N. gen. 11 *sensu* Jensen et al., 2016). All lecanicephalideans are species that are new

Table 2. Presence of species of lecanicephalideans in specimens of *Himantura polylepis*. Question mark indicates the identification of a single specimen of *Polypocephalus* that was not identified to species.

Cestode species	Specimen No.							
	BO-108	BO-355	BO-356	BO-358	BO-496	BO-497	KA-393	KA-397
Tetragonocephalum n. sp. 1	X			no lecanicephalideans				no lecanicephalideans
Tetragonocephalum n. sp. 2							X	
Tetragonocephalum n. sp. 3	X							
Polypocephalus n. sp. 1	X	?			X	X		
Polypocephalus n. sp. 2	X				X		X	
Polypocephalus n. sp. 3	X				X			
N. sp. of n. gen. 12	X							
N. sp. of n. gen. 11	X		X		X	X	X	

to science and are reported for the first time. One host individual (BO-108) displayed the highest species richness by far as it was parasitized by seven of the eight new taxa. A range of 1–4 (2.5 average) species of lecanicephalideans were recovered from four of the host individuals (i.e. BO-356, BO-496, BO-497, and KA-393). Only a single representative of *Polypocephalus* was removed from the intestine of the BO-355 host individual, however the condition of this cestode was too poor to identify it to species. No lecanicephalideans were recovered from either the BO-358 or KA-397 host individuals.

The number of lecanicephalidean specimens available for examination varied greatly among species recovered. As a result, species for which many specimens were available were treated much more comprehensively than those that had limited material. Two of the three new species of *Tetragonocephalum* (*T.* n. sp. 1 and *T.* n. sp. 2) had sufficient material for description using a combination of light and scanning-electron microscopy, and histological sectioning. Unfortunately, only a single individual of *T.* n. sp. 3 was recovered; this worm was prepared for light microscopy and was found to be unique from its congeners, justifying designation as a new species. The three species are formally described and distinguished from their congeners herein.

Treatment of nominal taxa of *Tetragonocephalum* and descriptions of new species

To unambiguously determine new species status, species parasitizing *H. polylepis* should be compared to all other valid species of *Tetragonocephalum*. What follows is a summary of and justifications for determinations of taxonomic status of nominal species of *Tetragonocephalum.* Historically, a total of 27 nominal taxa have been attributed to *Tetragonocephalum*; the validity or identity of most of these is doubtful. Based on examination of the original literature, the taxonomic status of these 27 names/taxa was determined to be either unavailable, *nomina nuda*, *incertae sedis*, *species inquirendae*, or valid. What follows are detailed explanations in support of our determinations (see also Jensen, 2005):

(1) Four names, *T. govindi* Khamkar and Shinde, 2012, *T. panjiensis* Khamkar, 2011, *T. pulensis* Kankale, 2014, and *T. sepheni* Lanka, Hippargi, and Patil, 2013, published after 1999, are unavailable for nomenclatural purposes. The authors (Khamkar, 2011; Khamkar and Shinde, 2012; Lanka et al., 2013; Kankale, 2014) neither explicitly fixed a holotype or syntypes, nor included a statement of intent that types will be (or have been) deposited in a collection thus violating Article 16.4 of the International Code of Zoological Nomenclature (ICZN; 1999).

(2) Seven names, *T. janardane* Wankhede, 1990, '*Tetragonicephalum* [sic] *karachiensis* Bilqees and Fatima, 1982', '*Tetragonicephalum* [sic] *stegostomai* Bilqees and Fatima, 1982,' '*Tetragonicephalum* [sic] *varium* Bilqees and Fatima, 1982,' *T. meenae*, '*T. shindei* Shipley et Hornell, 1906,' and '*T. marnrle*, Shipley et Hornell, 1906,' published after 1930 but before 1999, are considered *nomina nuda* because they violate Article 13 of the ICZN (1999) since they do not appear to have been published with accompanying descriptions. Specifically, the name *T. janardane* was listed in an abstract by Wankhede (1990), which does not constitute a published work (see Article 9; ICZN; Ride et al., 1999). The names *Tetragonicephalum* [sic] *karachiensis,*

Tetragonicephalum [sic] *stegostomai,* and *Tetragonicephalum* [sic] *varium* were used in a list of cestode parasites from marine fishes in Pakistan by Bilqees (1995). 'Bilqees and Fatima (1982)' (see Bilqees, 1995; pg. 75) was given as the authority, however the citation was incomplete [i.e., 'Bilqees, F.M. and Fatima, H. 1980c. Three new speceis [sic] of genus *Tetragonocephalum* Shipley and Hornell, 1905 (Cestoda: Lecanicephalidae Braun, 1900)'; pg. 86] and suggested a publication date of 1980. Reference to these names could not be found in any other publication or database. 'Bilqees and Fatima (1982)' could not be located. In addition, given the citation, the spelling of *Tetragonocephalum* as '*Tetragonicephalum*' in the list appears to have been in error. The names *T. meenae* and *T. shindei* appear in Mohekar et al. (2002) in a list of species collected from the west coast of Maharashtra, India. The former was given without authority or citation and the original description (if one exists) could not be located and the latter was erroneously attributed to 'Shipley et Hornell, 1906' (Mohekar et al., 2002; pg. 98); a publication describing this taxon could not be located. The name *T. marnrle* was included in a list of species of *Tetragoncephalum* in Lanka et al. (2013), who attribute the name to 'Shipley et Hornell (1906)' (see Lanka et al., 2013; pg. 11); again, a publication describing this taxon could not be located.

(3) Two species, *Tetragonocephalum aetiobatidis* Shipley and Hornell, 1905 and *T. akajeinensis* Yang, Liu, and Lin, 1995, are most likely members of genera other than *Tetragonocephalum* and are therefore considered *incertae sedi*s. The original description of *T. aetiobatidis* is inconsistent with the current diagnosis of *Tetragonocephalum* in that the species was described to possess craspedote proglottids and suckers directed anteriorly and to lack a bisaccate uterus (Shipley and Hornell, 1905); it is thus more consistent with the diagnosis of *Adelobothrium* Shipley, 1900. *Tetragonocephalum akajeinensis* is most likely a member of *Tylocephalum* Linton, 1890 due to its craspedote strobila and lobed ovary (see Yang et al.,

1995). I refrain from creating new combinations until the new generic placement can be unambiguously confirmed.

(4) Nine species are considered to be *species inquirendae*. They were reported from only two species of stingrays, all from off the coast of India. *Tetragonocephalum aurangabadensis* Shinde and Jadhav, 1990*, T. madhulatae* (Andhare and Shinde, 1994) Jensen, 2005*, T. madrasensis* (Andhare and Shinde, 1994) Jensen, 2005*, T. raoi* Deshmukh and Shinde, 1979*,* and *T. ratnagiriensis* Shinde and Jadhav, 1990 were described from *Dasyatis zugei* (as '*Trygon zugei*'; see Deshmukh and Shinde, 1979; Shinde and Jadhav, 1990; Andhare and Shinde, 1994). *Tetragonocephalum alii* Deshmukh and Shinde, 1979*, T. bhagawatii* Shinde, Mohekar, and Jadhav, 1985*, T. sephenis* Deshmukh and Shinde, 1979*,* and *T. shipleyi* Shinde, Mohekar, and Jadhav, 1985 were described from *Pastinachus sephen* (as '*Trygon sephen*'; see Deshmukh and Shinde, 1979; Shinde et al., 1985). Poor or incomplete descriptions, doubtful or uncertain host identifications, differentiation from what appears to be a random assortment of taxa rather than 'related or similar taxa' (Recommendation 13A, ICZN; Ride et al., 1999), incomplete reference to relevant taxonomic literature, and the lack of access to types render these species unrecognizable and their identity doubtful; they are thus considered to be *species inquirendae*. Consequently, the new species of *Tetragonocephalum* described below were not compared to these *species inquirendae*.

(5) Only five species are recognized as valid in this study and are differentiated from the three new species described herein. These are *T. passeyi, T. simile* (Pintner, 1928) Ivanov and Campbell, 2000*, T. trygonis* Shipley and Hornell, 1905*, T. uarnak* (Shipley and Hornell, 1906) Pintner, 1928*,* and *T. yamagutii* Muralidhar, 1988. Detailed comparisons are presented below

between the three new species of *Tetragonocephalum* and the five species considered valid herein.

It should finally be noted that the, albeit substandard, illustrations in the original description of six species of *Tylocephalum* (i.e., *Tylocephalum alii* Andhare and Shinde, 1994; *Tylocephalum chiralensis* Vijayalakshmi and Sarada, 1995; *Tylocephalum choudhurai* Pramanik and Manna, 2007; *Tylocephalum girindrai* Pramanik and Manna, 2007; *Tylocephalum haldari* Pramanik and Manna, 2007; and *Tylocephalum krisnai* Pramanik and Manna, 2007) suggest these to be more consistent with the diagnosis of *Tetragonocephalum* than that of *Tylocephalum* (Andhare and Shinde, 1994; Vijayalakshmi and Sarada, 1995; Pramanik and Manna, 2007). All of these species were described from India within the last 25 years. For the same reasons as stated above, all are considered to be *species inquirendae*. Given their status, new combinations are not formally created here and the species are not treated further herein.

***Tetragonocephalum* n. sp. 1**

(Figs. 2, 3, 5A-E)

Description [based on 28 specimens: 21 whole-mounted specimens (11 immature, 10 mature); 1 cross-section series of mature proglottids; 5 specimens prepared for SEM; 1 specimen sequenced; and their 7 whole-mounted vouchers].

Worms 3.7–10.5 mm (6.1 ± 2.0; 9) long; maximum width at level of scolex; 22–39 (31 ± 5.2; 10) proglottids, euapolytic. Scolex 313–460 (371 ± 42.0; 19) long by 331–473 (400 ± 36.6; 21) wide, consisting of scolex proper, apical modification of scolex proper, and apical organ. Scolex proper 120–337 (213 ± 46.1; 19) long by 331–473 (400 ± 36.6; 21) wide, bearing 4 acetabula. Acetabula sucker-like in form, round, 60–90 (73 ± 7.5; 23; 92) in diameter. Apical modification of scolex proper cylindrical, narrower than scolex proper, bearing apical organ. Apical organ large, globular in form, muscular, non-invaginable, non-retractable, with glandular surface, 149–285 (196 ± 38.5; 18) long by 248–395 (321 ± 39.7; 22) wide, 111–202 (145 ± 26.7; 16) high at apex.

Scolex proper (Fig. 5E) and rims of acetabula (Fig. 5B) covered with acicular filitriches. Surface of apical organ (Fig. 5D) and strobila (Fig. 5C) covered with capilliform filitriches.

Cephalic peduncle absent. Proglottids acraspedote, non-laciniate. Immature proglottids 20–38 (28 ± 5.2; 10) in number, initially wider than long, becoming longer than wide (1–18 [6 ± 4.1; 24] immature proglottids longer than wide); posterior-most immature proglottid 340–1,206 (595 ± 221.5; 22) long by 193–354 (271 ± 41.5; 21) wide. Mature proglottids 1–5 (3 ± 1.2; 10) in number, terminal mature proglottid 430–1,397 (864 ± 243.7; 12) long by 204–355 (286 ± 38.5; 12) wide. Gravid proglottids absent. Testes 27–45 (34 ± 5.6; 24) in number, 12–27 (20 ± 3.7; 13; 39) long by 29–57 (44 ± 8.8; 13; 39) wide, extending from anterior margin of proglottid to anterior margin of cirrus sac, arranged in 2 irregular columns in dorso-ventral view, essentially 2 layers deep in cross-section (Fig. 3A). Vas deferens minimal, sinuous, entering cirrus sac at its proximal end, visible from anterior margin of ovary to cirrus sac. External seminal vesicle absent. Internal seminal vesicle not observed. Cirrus sac pyriform, oriented anteriorly, 57–165 (127 ± 33.4; 12) long by 64–149 (122 ± 23.6; 12) wide, containing coiled cirrus. Cirrus armed with spinitriches. Genital pores lateral, irregularly alternating, 40–54% (47 ± 3.6; 12) of proglottid length from posterior end. Genital atrium expansive, conspicuous. Ovary essentially oval in dorso-ventral view, incomplete ring-shaped in cross-section (Fig. 3B), 88–212 (126 ± 34.8; 11) long by 102–184 (134 ± 25.7; 11) wide; ovarian bridge at center of ovary. Mehlis' gland posterior to ovarian bridge. Vagina thick-walled, extending along median line from ootype to anterior margin of ovary, entering genital atrium at proximal end; vaginal sphincter not observed. Uterus bisaccate, constricted at level of genital atrium, medial in proglottid, extending from posterior margin of ovary to anterior region of testis field; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles 6–28 (13 ± 5.9; 12; 35) long by 19–83 (37 ± 17.4; 12; 35) wide, in 3 fields: 1 field anterior to cirrus sac arranged in 2 irregular columns in dorso-ventral view (1 or more layers deep in cross-section), 1 field between genital atrium and anterior margin of ovary, and 1 field posterior to ovary. Excretory ducts in two lateral pairs. Eggs not observed.

Taxonomic summary

Type host: Himantura polylepis (Bleeker, 1852), giant freshwater whipray (Myliobatiformes: Dasyatidae).

Additional hosts: None.

Site of infection: Spiral intestine.

Type locality: Kampung Abai (05°41’N, 118°23’E), Kinabatangan River, Sabah, Malaysia (host specimen no. BO-108).

Additional localities: None.

Type specimens deposited: Holotype (whole mount of mature worm; MZUM[P]) and 3 paratypes (whole mounts of 1 mature and 2 immature worms; MZUM [P]); 10 paratypes (9 whole mounts of 5 mature and 4 immature worms, and 1 cross sections series of mature worm and its whole-mounted scolex voucher; USNM); 8 paratypes (whole mounts of 3 mature and 5 immature worms; LRP); 1 scolex (with its whole-mounted strobila voucher) prepared for SEM retained in the personal collection of K. J. at the University of Kansas.

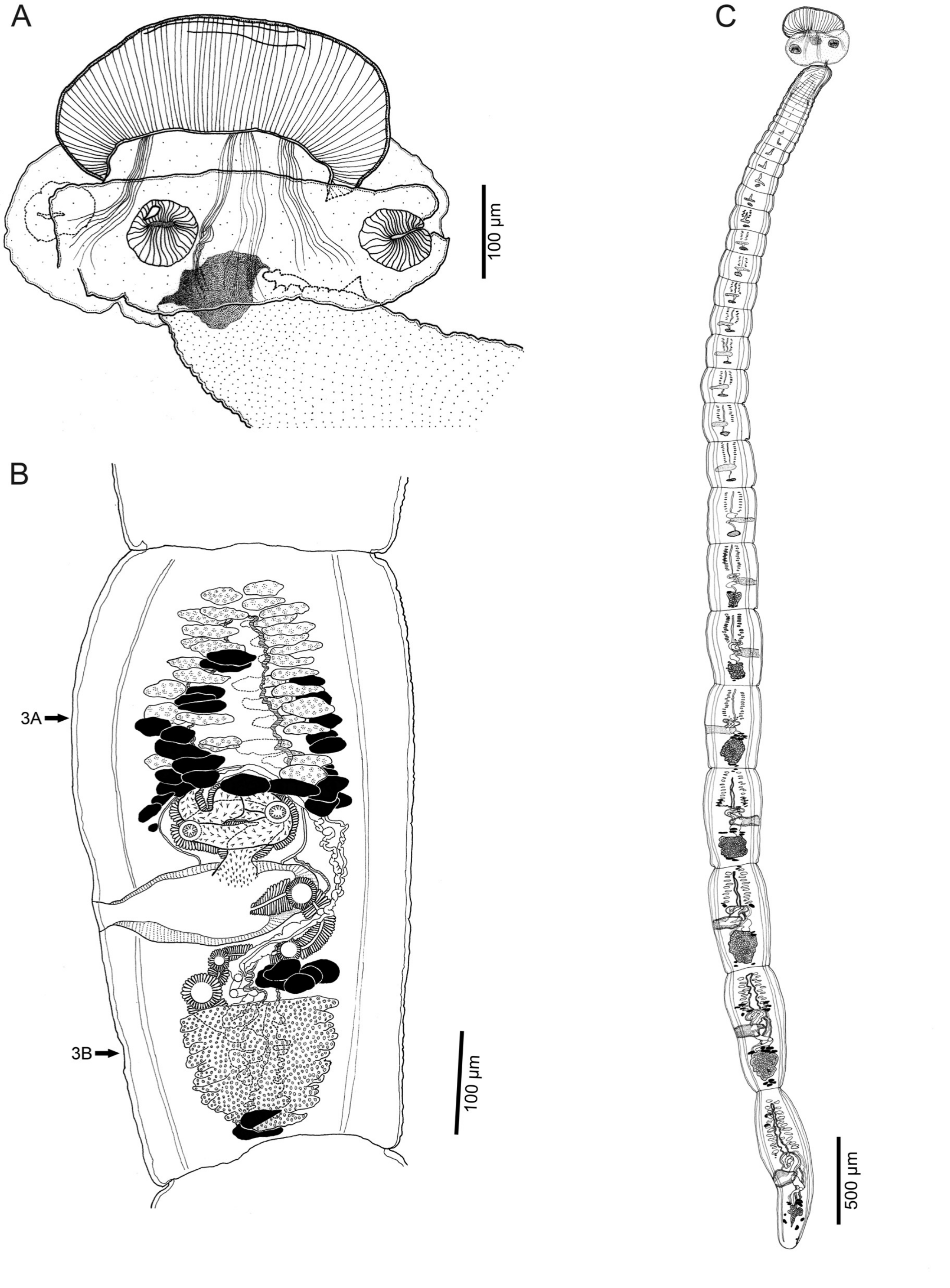

Figure 2. Line drawings of *Tetragonocephalum* n. sp. 1. (**A**) Scolex. (**B**) Mature subterminal proglottid; arrows indicate level at which sections in Fig. 3 were taken. (**C**) Whole worm.

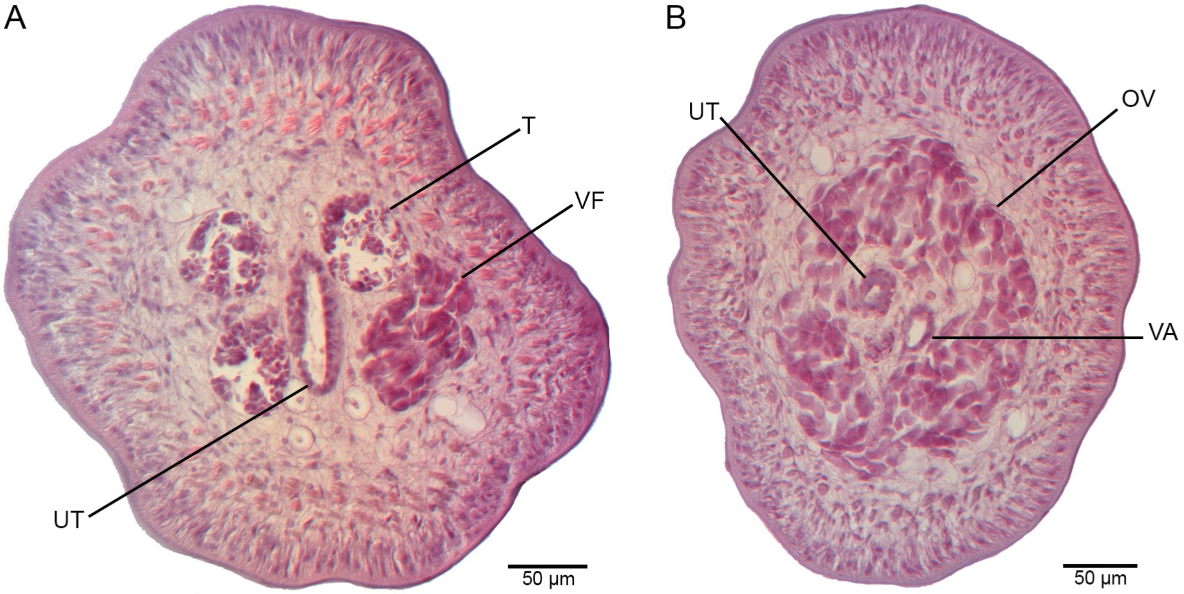

Fig. 3. Light micrographs of cross-sections through mature proglottids of *Tetragonocephalum* n. sp. 1. (**A**) At level anterior to cirrus sac showing testes and expanded uterus. (**B**) At level of ovary. Abbreviations: OV, ovary; T, testis; UT, uterus; VA, vagina; VF, vitelline follicle.

Remarks

Tetragonocephalum n. sp. 1 is a member of *Tetragonocephalum* due to its possession of a large muscular, non-retractable apical organ, extensive genital atrium, and bisaccate uterus. The possession of a euapolytic (i.e., absence of gravid proglottids on the strobila), rather than an apolytic strobila differentiates *T.* n. sp. 1 from all five species currently considered valid. In addition, *T.* n. sp. 1 is shorter in total length than *T. passeyi, T. trygonis,* and *T. uarnak,* (3.7–10.5 mm vs. 11.6–28.7 mm, 20–40 mm, 35 mm, respectively), and has fewer proglottids than *T. trygonis* and *T. simile* (22–39 vs. >60 [based on fig. 3 of Shipley and Hornell 1905, p. 54] and 'approximately 75' [Pintner, 1928, p. 103], respectively). A fewer number of testes distinguishes *T.* n. sp. 1 from *T. passeyi* and *T. yamagutii* (27–45 vs. 54–73 and 54–56, respectively). *Tetragonocephalum* n. sp. 1 can be further differentiated from *T. yamagutii* in possessing a much shorter ovary (88–159 long vs. 400–520 long). *Tetragonocephalum* n. sp. 1 is additionally distinguished from *T. simile* in possessing a shorter scolex (313–460 vs. 494), and from *T. uarnak* in possessing a narrower strobila (205–357 at terminal or subterminal proglottids vs. 700 'in the middle [of the strobila]' [Shipley and Hornell, 1906, p. 76]).

***Tetragonocephalum* n. sp. 2**

(Figs. 4, 5F)

Description [based on 17 specimens: 13 whole-mounted (8 immature, 5 mature); 2 specimens prepared for SEM; 2 specimens sequenced; and their 4 whole-mounted vouchers].

Worms 5.7–16.4 mm (9.6 ± 4.4; 5) long; maximum width at level of scolex; 17–30 (23 ± 5.4; 4) proglottids, euapolytic. Scolex 542–752 (616 ± 56.1; 13) long by 413–633 (515 ± 69.1; 15) wide, consisting of scolex proper, apical modification of scolex proper, and apical organ. Scolex proper 355–531 (428 ±49.3; 13) long by 413–633 (515 ± 69.1; 15) wide, bearing 4 acetabula. Acetabula sucker-like in form, round, 102–130 (115 ± 8.0; 14; 60) in diameter. Apical modification of scolex proper cylindrical, narrower than scolex proper, bearing apical organ. Apical organ large, globular in form, muscular, non-invaginable, non-retractable, 216–365 (289 ± 40.0; 13) long by 361–515 (457 ± 47.3; 15) wide, 144–267 (189 ± 34.0; 13) high at apex.

Microtriches on apical organ, scolex proper, rims of acetabula, or strobila not observed.

Cephalic peduncle absent. Proglottids acraspedote, non-laciniate. Immature proglottids 16–27 (21 ± 4.6; 4) in number, initially wider than long, becoming longer than wide; posterior-most immature proglottid 318–1,607 (1,062 ± 367.7; 4) long by 186–261 (211 ± 35.0; 4) wide. Mature proglottids 1–3 (2 ± 1.0; 5) in number, terminal or subterminal mature proglottid 825–1,891 (1,280 ± 421.7; 5) long by 178–238 (199 ± 25.6; 5) wide. Gravid proglottids absent. Testes 17–37 (24 ± 7.7; 5) in number, 16–43 (26 ± 8.6; 5; 15) long by 18–42 (33 ± 8.0; 5; 15) wide, extending from anterior margin of proglottid to anterior margin of cirrus sac, arranged in 2 irregular columns in dorso-ventral view, essentially 2 layers deep. Vas deferens minimal, essentially straight, visible from anterior margin of ovary to cirrus sac, entering cirrus sac at proximal end. External seminal vesicle absent. Internal seminal vesicle not observed. Cirrus sac pyriform, oriented anteriorly, 91–164 (133 ± 28.4; 5) long by 66–107 (86 ± 20.5; 3) wide, containing cirrus. Cirrus armed with spinitriches. Genital pores lateral, irregularly alternating, 34–54% (45 ± 8.4; 4) of proglottid length from posterior end. Genital atrium expansive. Ovary essentially oval in dorso-ventral view, 135–455 (233 ± 129.0; 5) long by 70–90 (81 ± 8.9; 5) wide; ovarian bridge toward posterior end of ovary. Mehlis' gland posterior to ovarian bridge. Vagina thick-walled, medial, straight, extending from ootype to genital pore, opening into genital atrium posterior to cirrus sac; vaginal sphincter not observed. Uterus bisaccate, constricted at level of genital atrium, medial in proglottid, extending from posterior margin of ovary to anterior region of proglottid; uterine duct opening into uterus at level of anterior margin of ovary; uterine pore absent. Vitellarium follicular; vitelline follicles 8–28 (15 ± 6.7; 5; 15) long by 13–36 (18 ± 5.9; 5; 15) wide, in 3 fields: 1 field anterior to cirrus sac arranged in 2 irregular columns in dorso-ventral view, 1 field between genital atrium and anterior margin of ovary, and 1 field posterior to ovary. Excretory ducts in 2 lateral pairs. Eggs not observed.

Taxonomic summary

Type host: Himantura polylepis (Bleeker, 1852), giant freshwater whipray (Dasyatidae, Myliobatiformes).

Additional hosts: None.

Site of infection: Spiral intestine.

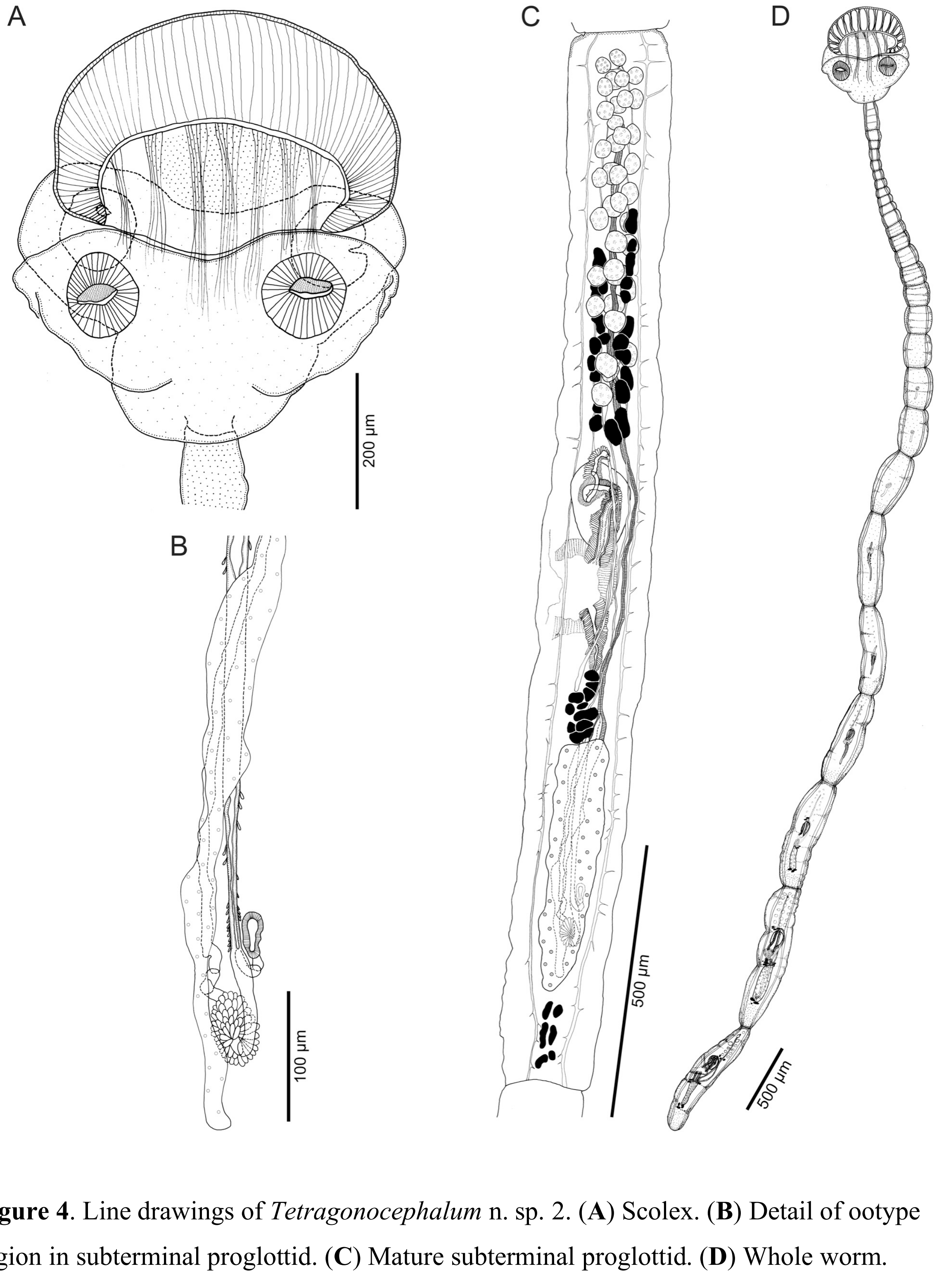

Figure 4. Line drawings of *Tetragonocephalum* n. sp. 2. (**A**) Scolex. (**B**) Detail of ootype region in subterminal proglottid. (**C**) Mature subterminal proglottid. (**D**) Whole worm.

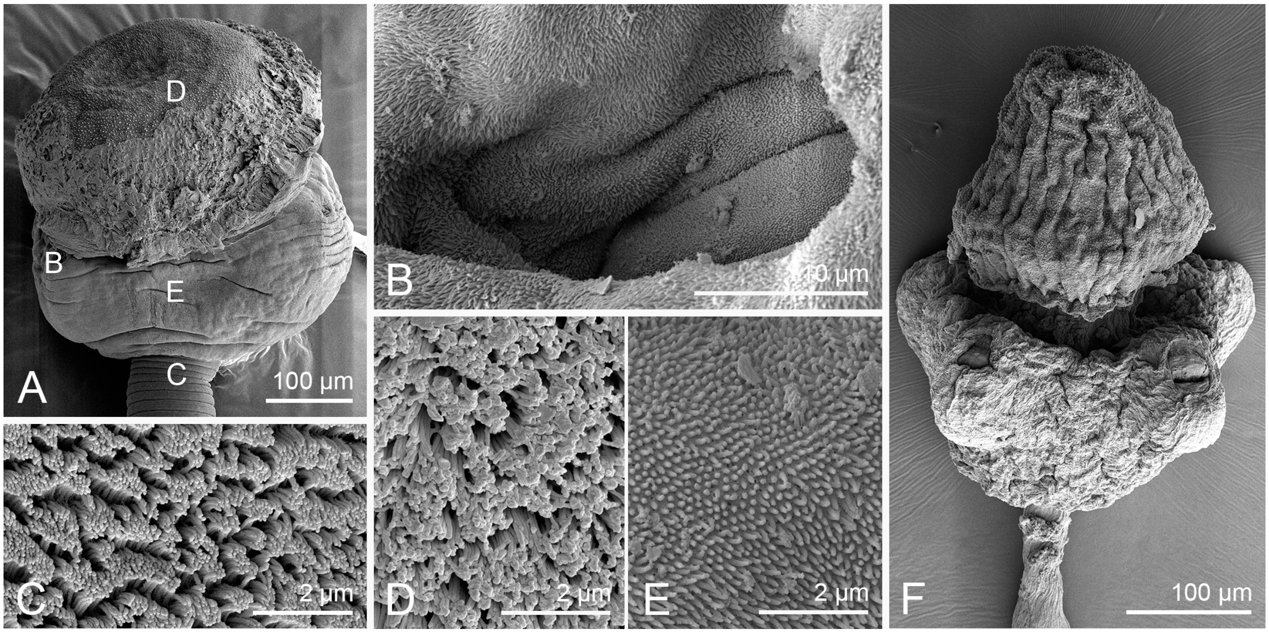

Figure 5. Scanning electron micrographs of *Tetragonocephalum* n. sp. 1 and *T.* n. sp. 2. (**A-E**), *T.* n. sp. 1. (**A**) Scolex; small letters indicate location of details shown in B-E. (**B**) Acetabulum with acicular filitriches. (**C**) Proglottid surface with capilliform filitriches. (**D**) Apical organ with capilliform filitriches. (**E**) Scolex proper with acicular filitriches. (**F**) Scanning electron micrograph of scolex of *T.* n. sp. 2.

Type locality: Pasar Beringin (03°17’47.04’N, 117°34’57.26’E), Celebes Sea, Tarakan, North Kalimantan, Indonesia (host specimen no. KA-393).

Additional localities: None.

Type specimens deposited: Holotype (whole mount of mature worm; MZB) and 1 paratype (whole mount of immature worm; MZB); 5 paratypes (whole mounts of 2 mature and 3 immature worms; LRP); 5 paratypes (whole mounts of 3 mature and 2 immature worms; USNM); 1 scolex (with its whole-mounted strobila voucher) prepared for SEM retained in the personal collection of K. J. at the University of Kansas.

Remarks

The presence of a large muscular, non-retractable apical organ, an extensive genital atrium, and a bisaccate uterus clearly identifies *Tetragonocephalum* n. sp. 2 as a member of *Tetragonocephalum*. This new species differs from five of the six valid species (*T. passeyi*, *T. simile*, *T. trygonis, T. uarnak*, and *T. yamagutii*) in possessing a euapolytic rather than apolytic strobila. In addition, *T.* n. sp. 2 is shorter in total length than *T. trygonis* and *T. uarnak* (5.7–16.4 mm vs. 20–40 mm and 35 mm, respectively), and has fewer proglottids than *T. trygonis* and *T. simile* (17–30 vs. >60 [fig. 3, Shipley and Hornell 1905, p. 54] and ‘approximately 75’ [Pintner 1928, p. 103], respectively). *Tetragonocephalum* n. sp. 2 has a longer scolex than *T. simile* (542–752 vs. 494 [Pintner, 1928, fig. 38, p. 92]); as well as a longer apical organ (216–235 vs. 120–160), longer scolex proper (355–531 vs. 180–210), and larger acetabula (diameter of 102–130 vs. 30–50) than that of *T. yamagutii*. Additionally, *T.* n. sp. 2 possesses a narrower maximum width of the strobila than *T. uarnak* (234–276 [at posterior-most proglottids] vs. 700 [‘in the middle’] [Shipley and Hornell, 1906, p. 76]). The new species is further differentiated from *T. passeyi* and *T. yamagutii* in having fewer testes (17–37 vs. 54–73 and 54–56, respectively) and possessing a narrower ovary (70–90 vs. 135–370 and 270–310, respectively). *Tetragonocephalum* n. sp. 2 is distinguished from *T.* n. sp. 1, the only other euapolytic species, in its possession of a longer scolex (542–752 vs. 313–460), larger acetabula (diameter of 102–130 vs. 60–90), and a narrower ovary (70–90 vs. 102–184). The new species also possesses a less conspicuous genital atrium and genital pore, in addition to a slighter strobila than that of *T.* n. sp. 1.

***Tetragonocephalum* n. sp. 3**

(Fig. 6)

Description (based on 1 whole-mounted gravid specimen).

Worm 6.9 mm long; maximum width at middle of strobila; 34 proglottids, apolytic. Scolex consisting of scolex proper, apical modification of scolex proper, and apical organ. Scolex proper 399 wide, bearing 4 acetabula. Acetabula sucker-like in form, essentially round, 61–71 (67 ± 4.2; 1; 4) in diameter. Apical modification of scolex proper cylindrical, narrower than scolex proper, bearing apical organ. Apical organ large, globular in form, muscular, non-invaginable, non-retractable, 412 wide.

Cephalic peduncle absent. Proglottids acraspedote, non-laciniate. Immature proglottids 21 in number, wider than long; posterior-most immature proglottid 91 long by 284 wide. Mature proglottids 2 in number; posterior-most mature proglottid 186 long by 326 wide. Gravid proglottids 11 in number; terminal proglottid 1,029 long by 280 wide. Testes 17 in number, 22–31 (25 ± 5.0; 1; 3) long by 32–45 (39 ± 6.5; 1; 3) wide, extending from anterior margin of proglottid to anterior margin of cirrus sac, arranged in 2 irregular columns in dorso-ventral view, essentially 2 layers deep. Vas deferens minimal, sinuous, visible from anterior margin of ovary to cirrus sac, entering cirrus sac at proximal end. External seminal vesicle absent. Internal seminal vesicle not observed. Cirrus sac pyriform, oriented anteriorly, 111 long by 91 wide, containing coiled cirrus. Cirrus armed with spinitriches. Genital pores lateral, irregularly alternating, 39% of proglottid length from posterior end. Genital atrium expansive, conspicuous. Ovary oval in dorso-ventral view, 54 long by 204 wide, symmetrical; ovarian bridge at center of ovary. Mehlis' gland near posterior margin of ovary. Vagina thick-walled, medial, sinuous, extending from ootype to genital pore, opening into genital atrium posterior to cirrus sac;

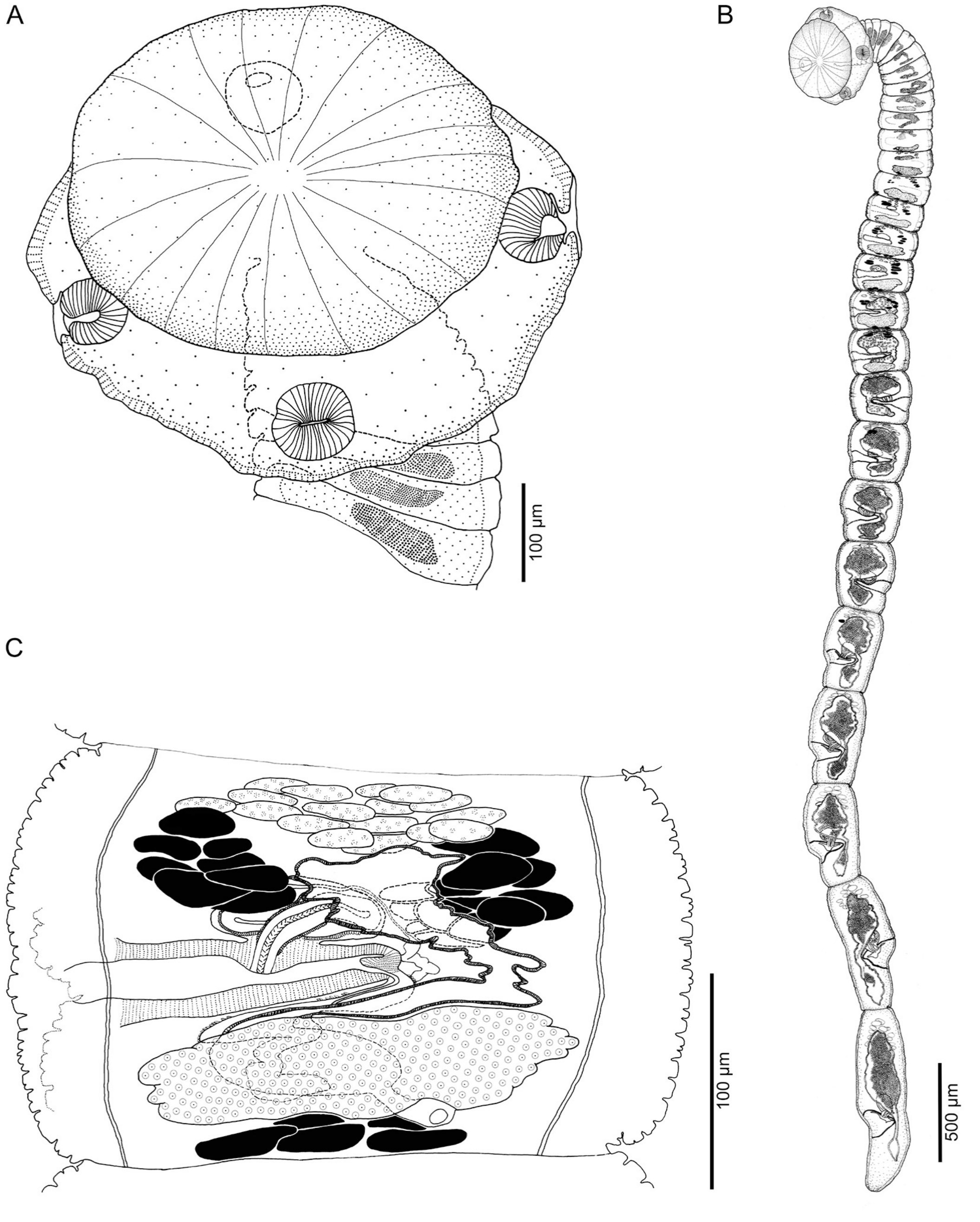

Figure 6. Line drawings of *Tetragonocephalum* n. sp. 3. (**A**) Scolex. (**B**) Mature proglottid. (**C**) Whole worm.

vaginal sphincter not observed. Uterus bisaccate, constricted at level of genital atrium, medial in proglottid, extending from near posterior margin of ovary to posterior extent of testes; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles 8–14 (10 ± 3.2; 1; 3) long by 37–65 (49 ± 14.6; 1; 3) wide, in 2 fields: 1 field anterior to cirrus sac arranged in two irregular columns in dorso-ventral view, and 1 field posterior to ovary. Excretory ducts in two lateral pairs. Eggs not observed.

Taxonomic summary

Type host: Himantura polylepis (Bleeker, 1852), giant freshwater whipray (Dasyatidae, Myliobatiformes).

Additional hosts: None.

Site of infection: Spiral intestine.

Type locality: Kampung Abai (05°41'N, 118°23'E), Kinabatangan River, Sabah, Malaysia (host specimen no. BO-108).

Additional localities: None.

Type specimen deposited: Holotype (whole mount of gravid worm; MZUM[P]).

Remarks

As seen in the two previous new species, *Tetragonocephalum* n. sp. 3 is readily placed within *Tetragonocephalum* due to its possession of a large muscular, non-retractable apical organ, an extensive genital atrium, and bisaccate uterus. The new species differs from *T. passeyi, T. trygonis*, and *T. uarnak* in its shorter total length (6.9 mm vs. 11.7–28.7 mm, 20–40 mm, and 35 mm, respectively). Moreover, *Tetragonocephalum* n. sp. 3 has approximately half the number of proglottids as *T. trygonis* and *T. simile* (34 vs. >60 [Shipley and Hornell 1905, Fig. 3, p. 54] and 'approximately 75' [Pintner 1928, p. 103], respectively). Additionally, *T.* n. sp. 3 has fewer testes than *T. passeyi* and *T. yamagutii* (17 vs. 54–73 and 54–56, respectively), and is further distinguished from *T. yamagutii* in possessing a much wider scolex proper (473 vs. 90–120). *Tetragonocephalum* n. sp. 3 differs from *T.* n. sp. 1 and *T.* n. sp. 2 in being apolytic rather than euapolytic, possessing fewer testes (17 vs. 27–45 and 20–37, respectively) and having a conspicuously shorter ovary (54 vs. 88–212 and 135–455, respectively). Only a single specimen of this new species was available for this study, but it is clearly distinct from its congeners, including the two new species of *Tetragonocephalum*, based on the aforementioned features.

Treatment of nominal taxa of *Polypocephalus* and descriptions of new species

A total of 41 nominal taxa have been associated with *Polypocephalus* to date. An examination of the associated original descriptions revealed that the validity of the majority of these species is questionable. The taxonomic treatment of each nominal species is addressed below (see also Jensen, 2005).

(1) Five names published after 1999, without explicit fixation of holotypes or syntypes or deposition in a collection, a violation of Article 16.4 of the International Code of Zoological Nomenclature (ICZN; Riede et al., 1999), are considered unavailable for nomenclatural purposes: *P. harnesis* Lanka, 2006, *P. kuhlii* Vankara, Vijayalakshmi, and Vijayalakshmi, 2006, *P. budhadebae* Jadhav, 2007, *P. mirkarwarensis* Dandwate and Jadhav, 2009, and *P. waltairensis* Mote, 2012 (see Lanka, 2006; Vankara et al., 2006; Jadhav, 2007; Dandwate and Jadhav, 2009; Mote, 2012). Additionally, the hosts from which *P. harnesis* and *P. waltairensis* are described, a perciform fish and requiem shark, respectively (Lanka, 2006; Mote, 2012), are highly suspect.

(2) Seven names have appeared in the literature without accompanying descriptions or definitions that are ‘purported to differentiate the taxon,’ a violation of Article 13 of the ICZN (Ride et al., 1999). These names are thus considered *nomina nuda* and are nomenclaturally unavailable. ‘*Polypocephalus braunii*’ was mentioned in the remarks section of new species descriptions by Jadhav et al. (1986), Jadhav and Shinde (1989), and Shinde et al. (1991), all of whom cite ‘Shinde (1981)’ as the authority; however, this publication could not be found and appears not to have been published. The name ‘*P. testicularis*’ was included in a publication by Jadhav and Shinde (1989) when they differentiate their new species from other species of *Polypocephalus*, though no authority is given and no associated description can be found. The name ‘*P. trygoni*’ was mentioned in the remarks section and distinguished from new species of the genus by Jadhav and Shinde (1989), Mote (2012), and Jadhav (2007), with only the latter author including the authority ‘Jadhav William Thretfull [sic], 1986’ (Jadhav, 2007, p. 170); an extensive literature search for this source yielded no associated publication. The names ‘*P. pandei*’ and ‘*P. sakriensis*’ are mentioned in Jadhav (2007) without authorities listed or the original publications cited; their associated publications, if they exist, could not be located. The name ‘*P. rakhamalii*’ is used in the figure caption of a description of a new species in the genus by Dandwate and Jadhav (2009, fig. 1, p. 420); no authority is listed and this is presumed to be a mistake. The name ‘*p.* [sic] *rhynchobatis*’ is used by Mote (2012) to differentiate a new species of the genus; no authority is given and no associated description can be located, and it may therefore be that the comparison was actually meant to be to *P. rhinobatidis* Subhapradha, 1951.

(3) Fifteen species are considered *species inquirendae* due to doubtful host associations, descriptions based on morphological features known not to be useful in distinguishing species in this genus, or incomplete species descriptions. In addition, ten of these are suspect in their host

associations, and those that are described from the same host species are not distinguished sufficiently from one another so that they could be recognized if recollected: five species (*P. alii* Shinde and Jadhav, 1981, *P. katpurensis* Shinde and Jadhav, 1981, *P. singhii* Shinde and Jadhav, 1981, *P. djeddensis* Jadhav and Shinde, 1989, *P. himanshui* Pramanik and Manna, 2006) were described from the same host species, *Rhynchobatus djiddensis* (Forsskål) (see Shinde and Jadhav, 1981; Jadhav and Shinde, 1989; Pramanik and Manna, 2006), which is an unusually high number of congeners reported from the same host species. Similarly, three species are described from *Pastinachus sephen* (reported as '*Trygon sephen*' by Shinde and Jadhav, 1981; and Deshmukh et al., 1982): *P. thapari* Shinde and Jadhav, 1981, *P. pratibhai* Deshmukh, Jadhav, and Shinde, 1982, and *P. maharashtra* Deshmukh, Jadhav, and Shinde, 1982. Additionally, suspect morphological features were used to describe the latter species (e.g., *P. maharashtra* is reported to possess 12 testes, when members of *Polypocephalus* have been described to possess 4 or 6 testes). *Polypocephalus visakhapatnamensis* Vankara, Vijaya, and Lakshmi, 2007 was described based on specimens collected from two different host species, *Himantura uarnak* (Gmelin) and *Dasyatis zugei* (Müller and Henle) (see Vankara et al., 2007) without specification of the type host; if our assumption of host specificity of *Polypocephalus* is correct, this constitutes a mixed type series. *Polypocephalus indicus* Deshmukh, Jadhav, and Shinde, 1982, was described from a requiem shark (Deshmukh et al., 1982); though a few records of lecanicephalideans observed in sharks exist (e.g., Yamaguti, 1934; Sarada et al., 1993; Caira et al., 1997; Jensen, 2001; Jensen, 2005; Pramanik and Manna, 2007), this phenomenon has not been observed to occur in members of *Polypocephalus* and this record is highly suspect. Descriptions of four species, *P. karbharii* Deshmukh, Jadhav, and Shinde, 1982, *P. ratnagiriensis* Jadhav, Shinde, and Sawrade, 1986, *P. digholensis* Deshmukh, Jadhav, and

Shinde, 1982, and *P. bombayensis* Shinde, Dhule, and Jadhav, 1991, lacked comprehensive morphological diagnoses and were based on either very few or an undisclosed number of specimens (see Deshmukh et al., 1982; Jadhav et al., 1986; Shinde et al., 1991). These reasons in combination with a lack of access to type specimens render these species indistinguishable and unrecognizable from other members within the genus.

(4) The remaining 14 species, *P. radiatus* Braun, 1878, *P. medusia* (Linton, 1890) Southwell, 1925, *P. elongatus* (Southwell, 1912) Southwell, 1925, *P. affinis* Subhapradha, 1951, *P. coronatus* Subhapradha, 1951, *P. lintoni* Subhapradha, 1951, *P. rhinobatidis*, *P. vitellaris* Subhapradha, 1951, *P. rhynchobatidis* Subhapradha, 1951, *P. vesicularis* Yamaguti, 1960, *P. saoudi* Hassan, 1982, *P. caribbensis* (Gardner and Schmidt, 1984) Jensen, 2005, *P. moretonensis* Butler, 1987, and *P. helmuti* Jensen, 2005, are considered valid herein.

Sufficient specimens of two of the three new species of *Polypocephalus* (*P.* n. sp. 1 and *P.* n. sp. 2) were available for characterization with light and scanning-electron microscopy and were prepared as histological sections. Only six individuals of *P.* n. sp. 3 were found within two host individuals; these were mounted for light microscopy. All three new species of *Polypocephalus* are formally described and distinguished from their congeners here.

***Polypocephalus* n. sp. 1**

(Figs. 7, 8, 10A-G)

Description [based on 21 whole-mounted specimens: 18 whole-mounted gravid specimens; 2 cross-section series (1 of scolex and 1 of mature proglottids); and 1 specimen prepped for SEM; and their associated vouchers].

Worms 1.0–2.8 mm (1.9 ± 0.6; 18) long; maximum width at level of strobila; 5–9 (8 ± 1.4; 18) proglottids, apolytic. Scolex 122–167 (148 ± 15.6; 15) long by 155–196 (174 ± 12.6; 15) wide, bearing 4 acetabula. Acetabula sucker-like in form (Fig. 7A, B), round, 37–72 (53 ± 6.5; 17; 68) in diameter. Apical modification of scolex proper with expandable aperture at apex, housing apical organ. Apical organ external, primarily glandular, divided into 16 tentacles (Fig. 8A); tentacles 77–145 (111± 28.3; 3; 6) long by 13–34 (20 ± 5.3; 7; 18) wide, completely invaginable. Tentacular pouch 105–137 (116 ± 11.8; 10) long by 95–129 (108 ± 12.4; 10) wide.

Scolex proper covered with acicular filitriches (Fig. 10C). Rims of acetabula and apical modification of the scolex proper covered with hastate spinitriches and acicular filitriches (Fig. 10F). Distal acetabular surface covered with acicular filitriches (Fig. 10G). Strobila covered with capilliform filitriches (Fig. 10E).

Cephalic peduncle absent. Proglottids acraspedote, non-laciniate. Immature proglottids 3–6 (4 ± 0.9; 18) in number, wider than long becoming longer than wide, 84–223 (146 ± 40.2; 18) long by 83–140 (108 ± 18.4; 18) wide. Mature proglottids 1–2 (1 ± 0.5; 18) in number, longer than wide, 124–374 (256 ± 63.1; 18) long by 95–152 (125 ± 15.6; 18) wide. Gravid proglottids 1–4 (2 ± 1.0; 18) in number, 396–863 (659 ± 140.5; 18) long by 118–169 (139 ± 16.2; 18) wide. Testes 4 in number, 19–66 (42 ± 11.0; 18; 54) long by 34–97 (64 ± 15.4; 17; 51) wide, extending from anterior margin of proglottid to anterior margin of ovary, arranged in single medial column in dorso-ventral view (Fig. 8B), displaced to lateral margin of proglottid by gravid uterus. Vas deferens sinuous, extending from level of ootype to cirrus sac. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac pyriform, angled anteriorly, 39–78 (60 ± 9.6; 13) long by 21–42 (32 ± 5.9; 12) wide, containing coiled cirrus. Cirrus thin-walled, armed with spinitriches. Genital pores lateral, irregularly alternating, 39–54% (46 ± 3.8;

18) of proglottid length from posterior end; genital atrium shallow. Vagina thin-walled, medial, sinuous, opening into genital atrium posterior to cirrus sac; vaginal sphincter absent; seminal receptacle not observed. Uterus thick-walled, sub-lateral in mature proglottids, extending from level of ovary to posterior of anterior-most testis. Ovary H-shaped in dorso-ventral view, symmetrical, tetralobed in cross-section (Fig. 8C), 35–92 (65 ± 15.1; 18) long by 48–111 (82 ± 16.9; 14) wide. Vitellarium follicular; vitelline follicles arranged in 2 lateral bands, extending from posterior extent of proglottid to posterior of cirrus sac on poral side and level of second anterior-most testis on aporal side, 9–50 (21 ± 7.2; 18; 54) long by 9–36 (20 ± 5.2; 16; 48). Excretory ducts in 2 lateral pairs. Eggs in terminal proglottids clumped together in fibrous matrix (Fig. 8D).

Taxonomic summary

Type host: Himantura polylepis (Bleeker, 1852), giant freshwater whipray (Dasyatidae, Myliobatiformes).

Additional hosts: None.

Site of infection: Spiral intestine.

Type locality: Kampung Abai (05°41’N, 118°23’E), Kinabatangan River, Sabah, Malaysia (host specimen nos. BO-108, BO-496, and BO-497).

Additional localities: None.

Type specimens deposited: Holotype (whole mount) and 3 paratypes (whole mounts; MZUM[P]); 10 paratypes (9 whole mounts and 1 scolex cross-section series; USNM); 6 paratypes (5 whole mounts and 1 proglottid cross-section series; LRP); 3 scoleces (with their associated whole-mounted strobila vouchers) prepared for SEM retained in the personal collection of K. J. at the University of Kansas.

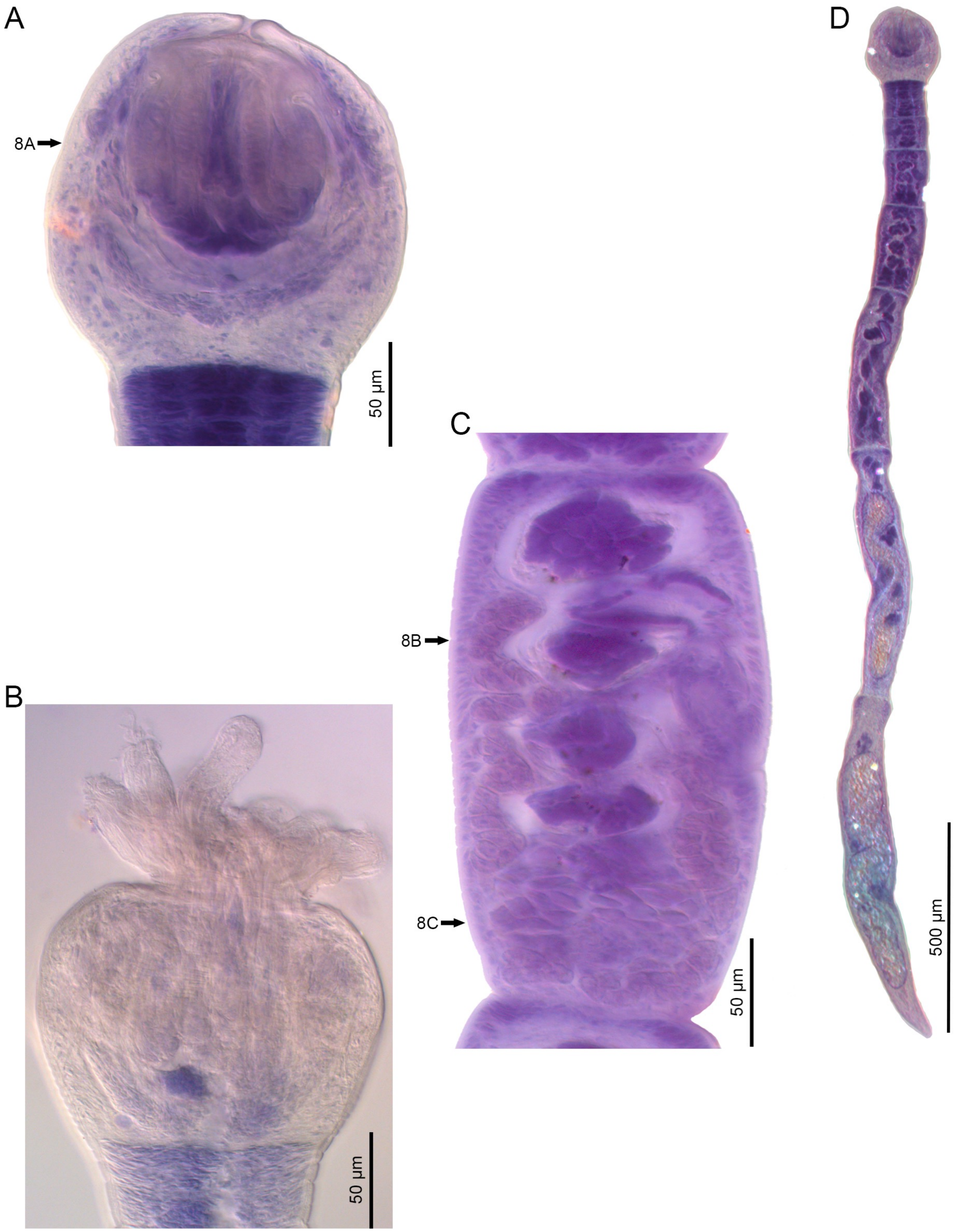

Figure 7. Light micrographs of *Polypocephalus* n. sp. 1. (**A**) Scolex with tentacles invaginated; arrow indicates location of section in Fig. 8A. (**B**) Scolex with tentacles everted. (**C**) Mature proglottid; arrows indicate location of sections in Figs. 8B and 8C. (**D**) Whole worm.

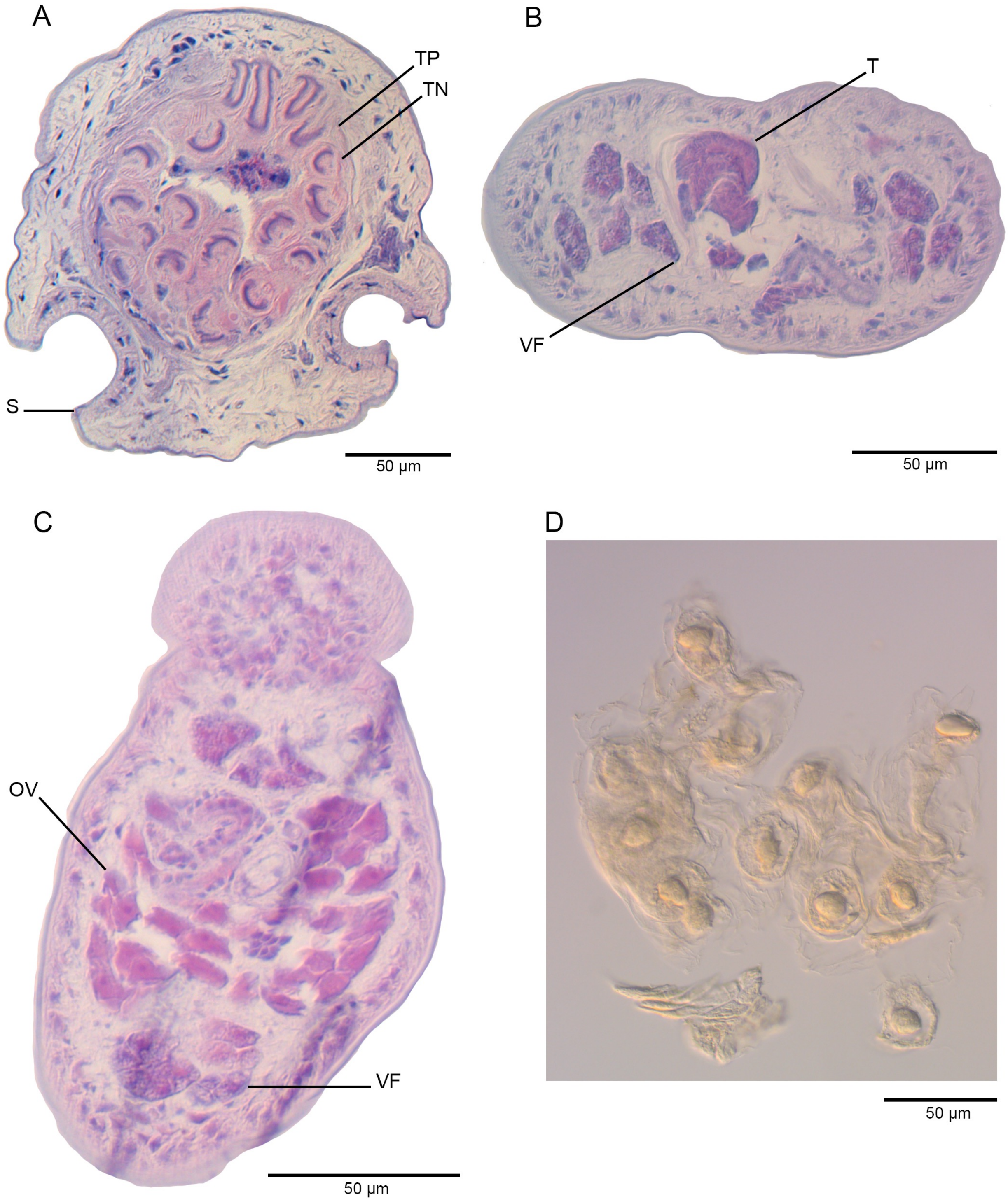

Figure 8. Light micrographs of egg mount and cross-sections through scolex and mature proglottids of *Polypocephalus* n. sp. 1. (**A**) Cross-section through scolex showing tentacles in tentacular pouch. (**B-C**) Cross-sections through mature proglottids. (**B**) At level of testis. (**C**) At level of ovary. (**D**) Whole mount of eggs. Abbreviations: OV, ovary; S, sucker; T, testis; TN, tentacle; TP, tentacular pouch; VF, vitelline follicle.

Remarks

Polypocephalus n. sp. 1 is a member of *Polypocephalus* due to a combination of features: an apical organ divided into tentacles, few testes arranged in a single medial column, and an H-shaped ovary that is tetralobed in cross-section. Acraspedote proglottids distinguish *P.* n. sp. 1 from six of its congeners which possess craspedote proglottids: *P. vesicularis, P. coronatus, P. lintoni, P. vitellaris, P. saoudi,* and *P. caribbensis*. Possession of 4 rather than 6 testes differentiates *P.* n. sp. 1 from *P. affinis, P. helmuti, P. radiatus,* and *P. rhinobatidis*. A relatively short total length distinguishes *P.* n. sp. 1 to *P. elongatus, P. vesicularis,* and *P. vitellaris* (1.0–2.8 mm vs. 39–50 mm, 7.8 mm, and 11 mm, respectively). *Polypocephalus* n. sp. 1 has fewer proglottids than *P. moretonensis, P. coronatus, P. lintoni, P. vesicularis,* and *P. vitellaris* [5–9 vs. 120, 40–48, 'over 50' (Subhapradha, 1951; p. 220), 'about 100' (Yamaguti, 1960; p. 46), and 40, respectively). A shorter scolex differentiates *P.* n. sp. 1 from *P. vesicularis, P. medusia,* and *P. elongatus* (122–167 vs. 280, 480, and 500, respectively). *Polypocephalus* n. sp. 1 has a shorter cirrus sac than *P. vescisularis, P. lintoni, P. vitellaris,* and *P. saoudi* (39–78 vs. 150–200, 135, 135, and 110–135, respectively). *Polypocephalus* n. sp. 1 has fewer tentacles than *P. moretonensis* (16 vs. 40) and more tentacles than *P. saoudi* and *P. vesicularis* (10 and 14, respectively). A shorter ovary length distinguishes *P.* n. sp. 1 from *P. saoudi* (35–92 vs. 110–135). The cirrus sac of *P.* n. sp. 1 is anteriorly oriented rather than that of *P. rhynchobatidis*, which is posteriorly oriented. The absence of an external seminal vesicle differentiates *P.* n. sp. 1 from *P. moretonensis*.

***Polypocephalus* n. sp. 2**

(Figs. 9, 10H-N)

Description (based on 16 specimens: 15 whole-mounted gravid specimens, and 1 specimen prepped for SEM and its associated whole-mounted voucher).

Worms 1.7–5.2 mm (2.8 ± 8.3; 15) long; maximum width at level of scolex; 13–30 (20 ± 5.6; 15) proglottids, apolytic. Scolex 272–399 (337 ± 39.3; 13) long by 280–382 (319 ± 34.2; 11) wide, bearing 4 acetabula. Acetabula sucker-like in form (Fig. 10K), round, 60–100 (78 ± 9.5; 15; 60) in diameter. Apical modification of scolex proper with expandable aperture at apex, housing apical organ. Apical organ external, primarily glandular, divided into at least 10–14, likely 16 total tentacles; tentacles 320–400 (364± 40.5; 1; 3) long by 25–32 (28 ± 2.6; 4; 8) wide, completely invaginable. Tentacular pouch with invaginated tentacles 205–342 (261 ± 45.8; 9) long by 186–272 (233 ± 32.4; 7) wide.

Scolex proper covered with acicular filitriches (Fig. 10J). Rims of acetabula covered with gladiate spinitriches and acicular filitriches (Fig. 10M). Strobila covered with capilliform filitriches (Fig. 10L).

Cephalic peduncle absent. Proglottids acraspedote, non-laciniate. Immature proglottids 9–25 (15 ± 4.8; 15) in number, wider than long, becoming longer than wide, 97–221 (156 ± 38.9; 15) long by 85–170 (114 ± 22.8; 15) wide. Mature proglottids 1–5 (3 ± 1.2; 15) in number, longer than wide, 120–391 (260 ± 75; 15) long by 93–178 (124 ± 24.9; 15) wide. Gravid proglottids 1–6 (3 ± 1.7; 15) in number, 345–791 (547 ± 154.5; 15) long by 102–249 (146 ± 42.2; 15) wide. Testes 4 in number, 15–72 (34 ± 13.2; 15; 45) long by 24–65 (38 ± 8.7; 12; 36) wide, extending from anterior margin of proglottid to anterior margin of ovary, arranged in single medial column in dorso-ventral view, displaced to lateral margin of proglottid by gravid uterus. Vas deferens medial, sinuous, extending from level of ootype to cirrus sac, entering cirrus sac at distal end. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac pyriform, angled anteriorly, 44–93 (62 ± 20.5; 6) long by 31–50 (42 ± 7.4; 9) wide, containing coiled cirrus. Cirrus thin-walled, armed with spinitriches. Genital pores lateral, irregularly alternating, 44–69% (51 ± 6; 15) of proglottid length from posterior end; genital atrium shallow. Vagina thin-walled, medial, straight, opening into genital atrium posterior to cirrus sac; vaginal sphincter absent; seminal receptacle not observed. Uterus thick-walled, medial in mature proglottids, extending from level of ovary to posterior of anterior-most testis. Ovary H-shaped in dorso-ventral view, symmetrical, tetralobed, 46–92 (65 ± 11.5; 15) long by 60–106 (76 ± 16.3; 10) wide. Vitellarium follicular; vitelline follicles arranged in 2 lateral bands, extending from posterior extent of proglottid to posterior of cirrus sac on poral side and level of second anterior-most testis on aporal side, 9–47 (23 ± 7.8; 15; 45) long by 11–38 (19 ± 5.8; 13; 39). Excretory ducts in 2 lateral pairs. Eggs not observed.

Taxonomic summary

Type host: Himantura polylepis (Bleeker, 1852), giant freshwater whipray (Dasyatidae, Myliobatiformes).

Additional hosts: None.

Site of infection: Spiral intestine.

Type locality: Kampung Abai (05°41’N, 118°23’E), Kinabatangan River, Sabah, Malaysia (host specimen nos. BO-108 and BO-496).

Additional localities: Pasar Beringin (03°17’47.04’N, 117°34’57.26’E), Celebes Sea, Tarakan, North Kalimantan, Indonesia (KA-393).

Type specimens deposited: Holotype (whole mount of gravid worm; MZB) and 2 paratypes

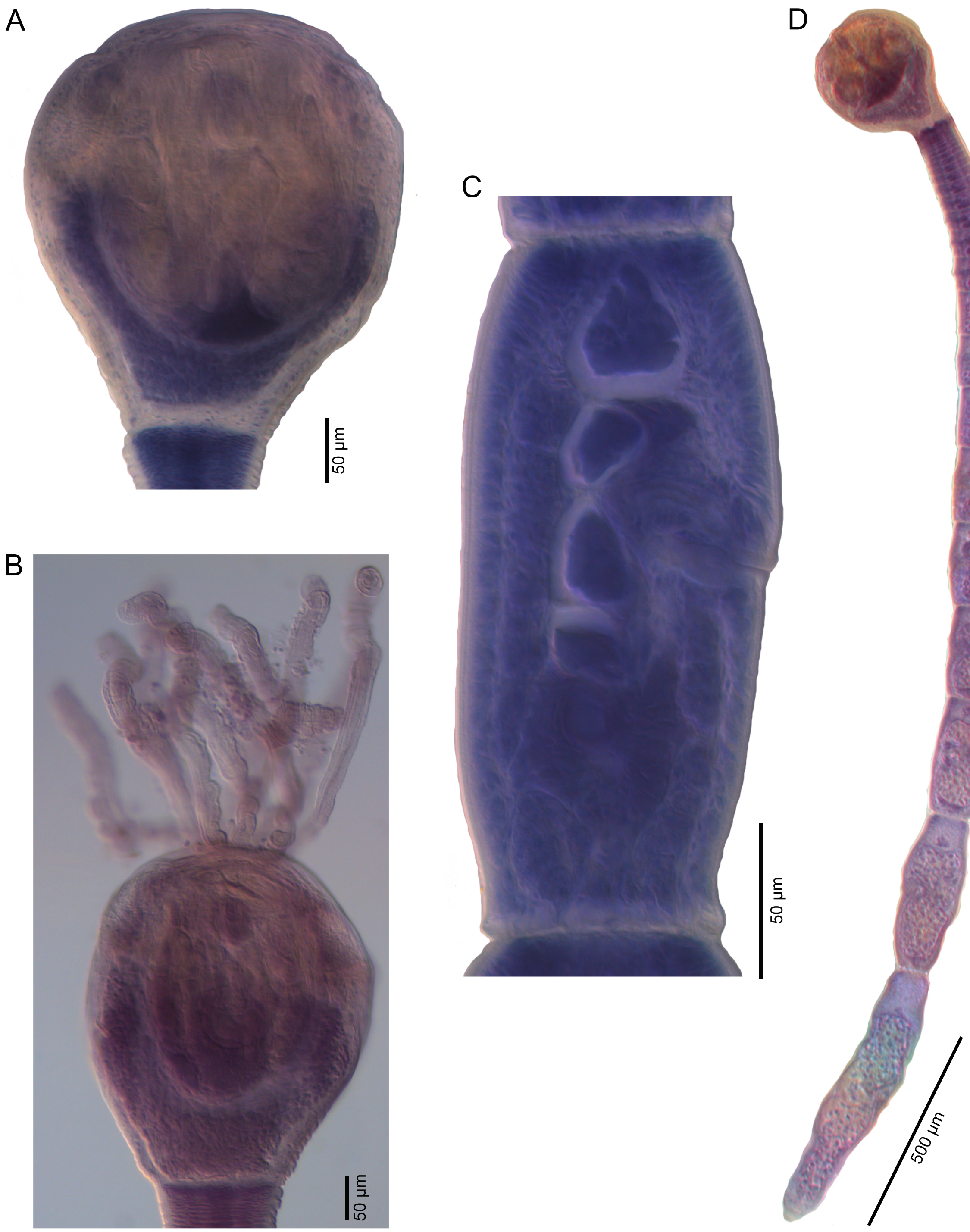

Figure 9. Light micrographs of *Polypocephalus* n. sp. 2. (**A**) Scolex with tentacles invaginated. (**B**) Scolex with tentacles everted. (**C**) Mature proglottid. (**D**) Whole worm.

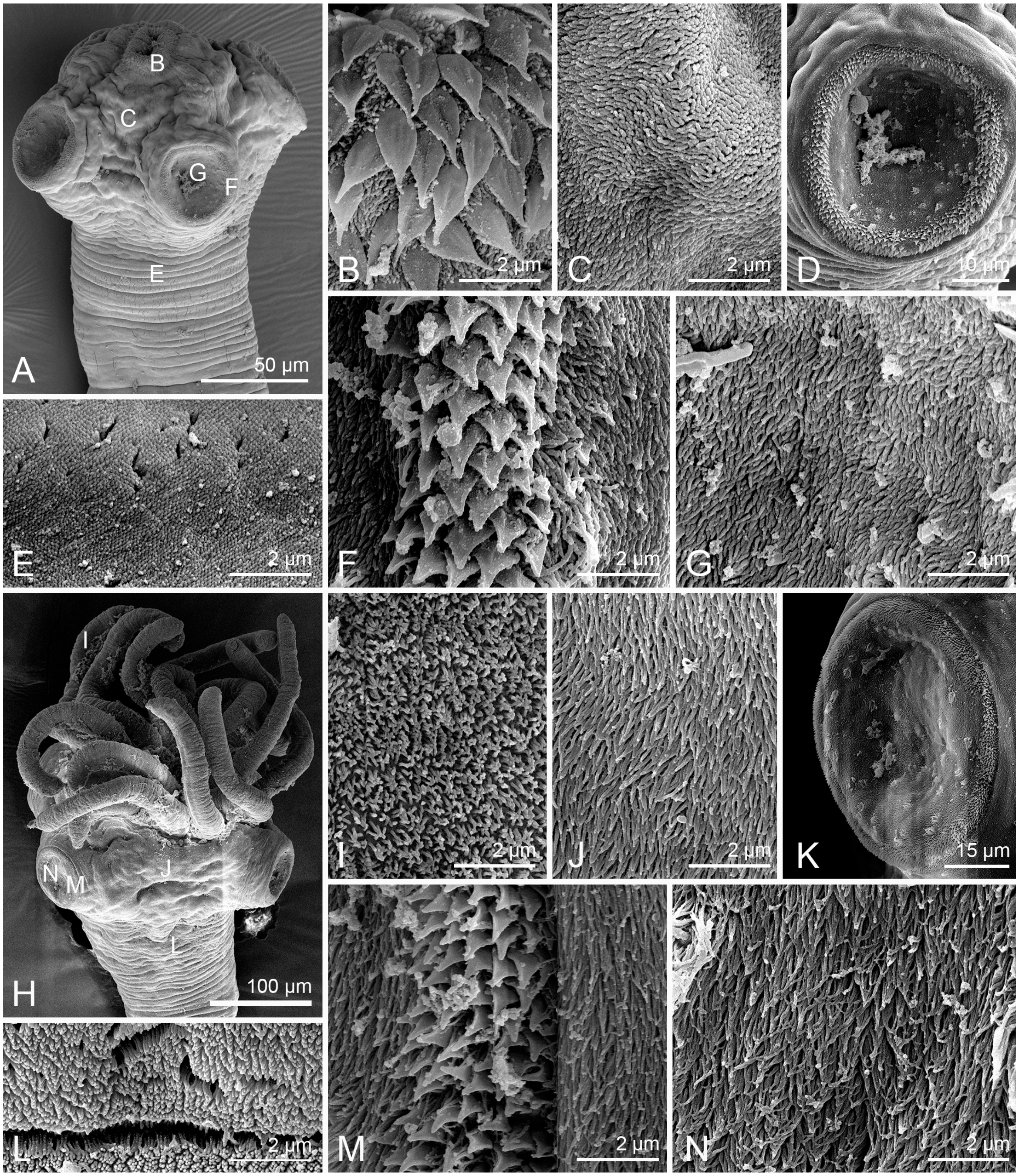

Figure 10. Scanning electron micrographs of *Polypocephalus* n. sp. 1 and *P.* n. sp. 2. (**A-G**) *Polypocephalus* n. sp. 1. (**A**) Scolex with tentacles invaginated; small letters indicate location of details shown in B-G. (**B**) Apical modification of the scolex proper. (**C**) Scolex proper. (**D**) Acetabulum. (**E**) Proglottid surface. (**F**) Proximal acetabular surface. (**G**) Distal acetabular surface. (**H-N**) *Polypocephalus* n. sp. 2. (**H**) Scolex with tentacles fully everted; small letters indicate location of details shown in I-N. (**I**) Tentacular surface. (**J**) Scolex proper. (**K**) Acetabulum. (**L**) Proglottid surface. (**M**) Proximal acetabular surface. (**N**) Distal acetabular surface.

(whole mounts; MZB); 6 paratypes (whole mounts; USNM); 6 paratypes (whole mounts; LRP); 1 scolex (with its associated whole-mounted strobila voucher) prepared for SEM retained in the personal collection of K. J. at the University of Kansas.

Remarks

An apical organ divided into invaginable tentacles, few testes arranged in a single medial column, and an H-shaped ovary that is tetralobed place *P.* n. sp. 2 within the genus *Polypocephalus*. Of the 15 species of *Polypocephalus* that are recognized in this study as valid, *P.* n. sp. 2 can easily be distinguished from *P. vesicularis, P. coronatus, P. lintoni, P. vitellaris, P. saoudi,* and *P. caribbensis* in its possession of acraspedote, rather than craspedote, proglottids. *Polypocephalus* n. sp. 2 has four rather than six testes, differentiating it from *P. affinis, P. helmuti, P. radiatus,* and *P. rhinobatidis*. A shorter total length (1.7–5.2 mm vs. 18 mm, 72 mm, 39–50 mm, 7.8 mm, and 11 mm, respectively) distinguishes *P.* n. sp. 2 from *P. radiatus, P. rhinobatidis*, *P. elongatus*, *P. vesicularis*, and *P. vitellaris*. *Polypocephalus* n. sp. 2 has fewer proglottids (i.e., 13–30) than *P. radiatus, P. rhinobatidis, P. elongatus, P. vesicularis, P. vitellaris, P. moretonensis,* and *P. lintoni*, all of which possess 40 or more proglottids. A shorter scolex differentiates *P.* n. sp. 2 from *P. medusia* and *P. elongatus* (272–399 vs. 480 and 500, respectively). *Polypocephalus* n. sp. 2 has fewer tentacles than *P. morentonensis* (likely 16 vs. 40). *Polypocephalus* n. sp. 2 has a shorter ovary than that of *P. saoudi* (46–92 vs. 350–440). An anteriorly oriented cirrus sac distinguishes *P.* n. sp. 2 from *P. rhynchobatidis*. The absence of an external seminal vesicle further differentiates *P.* n. sp. 2 from *P. moretonensis*. *Polypocephalus* n. sp. 2 is distinct from *P.* sp. 1 in its higher proglottid count (13–30 vs. 5–9), a longer scolex (272–399 vs. 122–167), longer tentacles (300–393 vs. 77–145), and a longer (205–342 vs. 105-137) and wider (186-272 vs. 95–129) tentacular pouch.

The number of tentacles is readily determined by examination of a scolex with fully everted tentacles through light or scanning electron microscopy, or by examination of cross-sections through the scolex with invaginated tentacles. However, tentacles of only two of the individuals of this species mounted for light microscopy were everted. Though 10 and 14 tentacles were determined for these specimens, it is likely that not all tentacles were everted and that this species, like *P.* n. sp. 1, possesses 16 tentacles.

***Polypocephalus* n. sp. 3**

(Figs. 11, 12)

Description (based on 4 whole-mounted gravid specimens).

Worms 5.1–7.4 mm (6.2 ± 1.1; 3) long; maximum width at level of scolex; 23–36 (30 ± 6.2; 4) proglottids, apolytic. Scolex 210–391 (270 ± 82.7; 4) long by 547–628 (568 ± 39.6; 4) wide, bearing 4 acetabula. Acetabula sucker-like in form (Fig. 11A), round, 108–133 (119 ± 7.4; 4; 14) in diameter. Apical modification of scolex proper with expandable aperture at apex, housing apical organ. Apical organ external, primarily glandular, divided into at least 14–15, likely 16 total tentacles; tentacles 113–171 (137± 17.0; 4; 12) long by 19–62 (47 ± 14.5; 4; 12) wide, not extending above apex of scolex, completely invaginable. Tentacular pouch observed for one individual with tentacles inverted, 160 long by 271 wide.

Cephalic peduncle absent. Proglottids acraspedote, non-laciniate. Immature proglottids 15–23 (18 ± 3.4; 4) in number, wider than long, becoming longer than wide, 171–208 (187 ± 15.3; 4) long by 176–239 (201 ± 29.6; 4) wide. Mature proglottids 2–7 (5 ± 2.1; 4) in number, longer than wide, 196–382 (293 ± 79.9; 4) long by 164–228 (192 ± 30.6; 4) wide. Gravid proglottids 2–11 (7 ± 4.7; 4) in number, 429–1,149 (687 ± 317.9; 4) long by 174–270 (237 ±

42.6; 4) wide. Testes 4 in number, 41–70 (52 ± 7.2; 4; 12) long by 65–102 (86 ± 15.0; 2; 6) wide, extending from anterior margin of proglottid to anterior margin of ovary, arranged in single medial column in dorso-ventral view (Fig. 12A). Vas deferens extensive, sinuous, medial, extending from level of ootype to poster of anterior-most testis, entering cirrus sac at distal end. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac pyriform, angled anteriorly, 47–81 (66 ± 17.5; 3) long by 56–75 (63 ± 10.3; 3) wide, containing coiled cirrus. Cirrus thin-walled, armed with spinitriches. Genital pores lateral, irregularly alternating, 58–66% (61 ± 3.5; 4) of proglottid length from posterior end; genital atrium shallow. Vagina thin-walled, medial, sinuous, opening into genital atrium posterior to cirrus sac; vaginal sphincter absent; seminal receptacle not observed. Uterus thick-walled, medial in mature proglottids, extending from level of ovary to posterior of anterior-most testis. Ovary H-shaped in dorso-ventral view, symmetrical, tetralobed in cross-section (Fig. 12B), 39–120 (75 ± 38.7; 4) long by 103–130 (114 ± 12.7; 4) wide. Vitellarium follicular; vitelline follicles arranged in 2 lateral bands, extending from posterior extent of proglottid to posterior of cirrus sac on poral side and level of second anterior-most testis on aporal side, 15–44 (26 ± 10.0; 4; 12) long by 28–35 (32 ± 3.1; 2; 6). Excretory ducts in 2 lateral pairs. Eggs not observed.

Taxonomic summary

Type host: Himantura polylepis (Bleeker, 1852), giant freshwater whipray (Dasyatidae, Myliobatiformes).

Additional hosts: None.

Site of infection: Spiral intestine.

Type locality: Kampung Abai (05°41’N, 118°23’E), Kinabatangan River, Sabah, Malaysia (host specimen nos. BO-108 and BO-496).

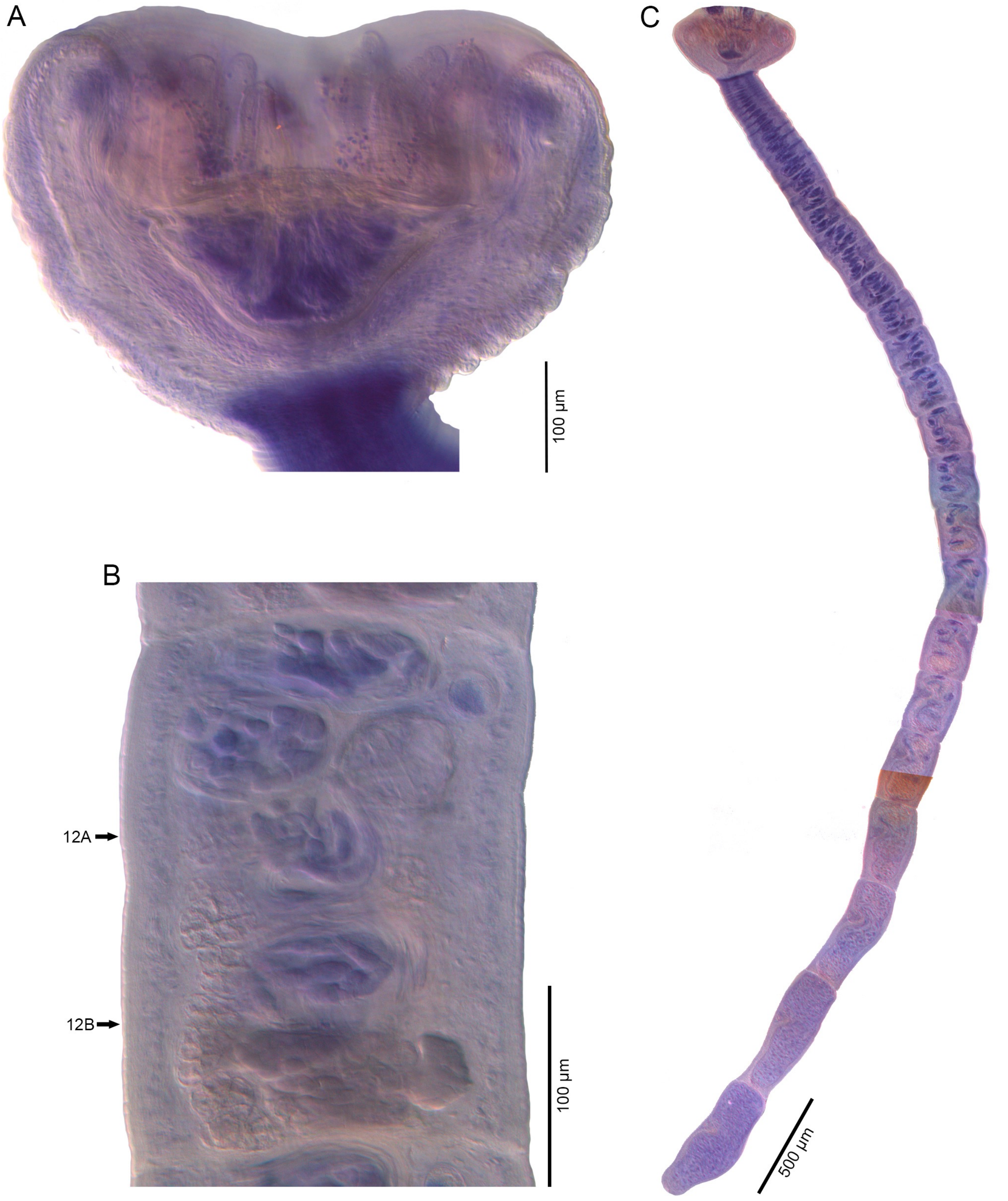

Figure 11. Light micrographs of *Polypocephalus* n. sp. 3. (**A**) Scolex. (**B**) Mature proglottid; arrows indicate level at which sections in Fig. 12 were taken. (**C**) Whole worm.

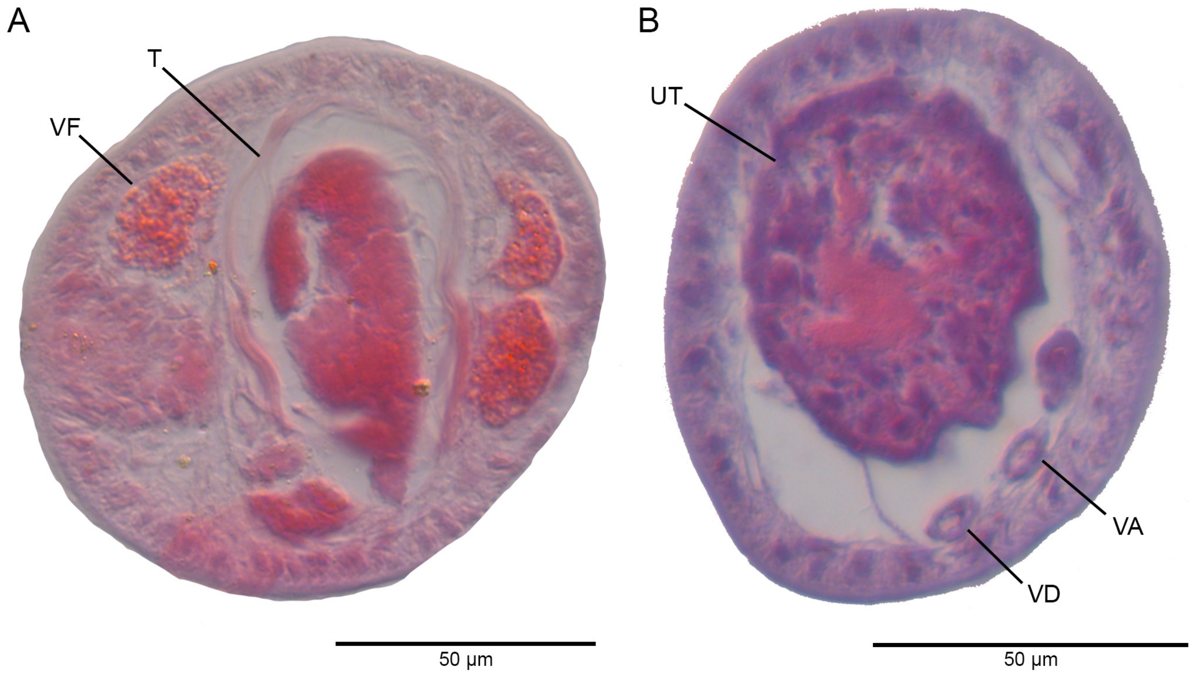

Figure 12. Light micrographs of cross-sections through mature proglottids of *Polypocephalus* n. sp. 3. (**A**) At level posterior to cirrus sac showing testes and vitellaria. (**B**) At level of uterus anterior to ovary. Abbreviations: T, testis; UT, uterus; VA, vagina; VD, vas deferens; VF, vitelline follicle.

Additional localities: None.

Type specimens deposited: Holotype (whole mount of gravid worm; MZUM[P]); 2 paratypes (whole mounts of 2 gravid worms; USNM); 1 paratype (whole mount of gravid worm; LRP).

Remarks

The combination of an apical organ divided into invaginal tentacles, few testes arranged in a single medial column, and an H-shaped ovary that is tetralobed in cross-section places *Polypocephalus* n. sp. 3 in *Polypocephalus*. The possession of acraspedote proglottids distinguishes *P.* n. sp. 3 from *P. vesicularis, P. coronatus, P. lintoni, P. vitellaris, P. saoudi,* and *P. caribbensis*. *Polypocephalus* n. sp. 3 has fewer testes (four vs. six, respectively) than *P. affinis, P. helmuti, P. radiatus,* and *P. rhinobatidis.* A longer total length differentiates *P.* n. sp. 3 from *P. vitellaris* and *P. elongatus* (5.1–7.4 mm vs. 11 mm and 39–50 mm, respectively). Fewer proglottids distinguish *P.* n. sp. 3 from *P. moretonensis, P. lintoni,* and *P. vesicularis* [23–36 vs. 120, 'over 50' (Subhapradha, 1951; p. 220), and 'about 100' (Yamaguti, 1960; p. 46), respectively]. *Polypocephalus* n. sp. 3 has shorter scolex than that of *P. medusia* and *P. elongatus* (210–391 vs. 480 and 500, respectively), and a longer (210–391 vs. 75–100) and wider (547–628 vs. 125–163) scolex than that of *P. rhynchobatidis*. A shorter cirrus sac differentiates *P.* n. sp. 3 from *P. vesicularis, P. lintoni, P. vitellaris,* and *P. saoudi* (47–81 vs. 150–200, 135, 135, and 110–135, respectively). *Polypocephalus* n. sp. 3 has fewer tentacles (likely 16 vs. 40) than *P. moretonensis*. A shorter ovary (39–120 vs. 350–440) distinguishes *P.* n. sp. 3 from *P. saoudi*. An anteriorly, and not posteriorly, oriented cirrus sac differentiates *P.* n. sp. 3 from *P. rhynchobatidis*. *Polypocephalus* n. sp. 3 is further differentiated from *P. moretonensis* in its absence of an external seminal vesicle. Larger acetabula distinguish *P.* n. sp. 3 from *P. medusia* and *P. rhynchobatidis* (108–133 vs. 60–80 and 35, respectively). *Polypocephalus* n. sp. 3 has shorter terminal proglottids than *P. medusia* (429–1,149 vs. 2,000). The new species is readily distinguished from *P.* n. sp. 1 and *P.* n. sp. 2 due to its wider scolex (210–391 vs. 122–167 and 272–399, respectively) and larger acetabula (108–133 vs. 37–72 and 60–100, respectively).

Material for this species was limited to only four intact specimens, which were prepared for light microscopy. Everted tentacles were observed only for three of the four individuals. Although 14 and 15 tentacles were determined for these specimens, it is likely that not all tentacles were everted and that this species, like *P.* n. sp. 1 and *P.* n. sp. 2, possesses 16 tentacles.

Treatment of new species of lecanicephalidean genera not formally described

New species of N. gen. 12 (*sensu* Jensen et al., 2016)

(Fig. 13)

A single representative belonging to N. gen. 12 (*sensu* Jensen et al., 2016) was recovered from a single host individual and mounted for light microscopy for morphological investigation. Measurements were taken in an attempt to distinguish it from two of its congeners found in *H. granulata* collected in the Solomon Islands for which a manuscript is now in preparation (K. Herzog, pers. comm.), however a formal description is not included herein due to lack of sufficient material. This worm most closely resembles members of N. gen. 12 (*sensu* Jensen et al., 2016) due to its possession of an apical organ with eight invaginable muscular pads, an apical organ with muscular and glandular components, extensive external seminal vesicle, and conspicuous microtriches on the apical modification of scolex proper.

Descriptive measurements and features (based on 1 whole-mounted worm).

Worm 1,771 long; maximum width at level of scolex; 15 proglottids. Scolex 187 long by 205 wide, consisting of 4 acetabula, apical modification of scolex proper, and apical organ. Acetabula sucker-like in form, round, 55–67 (62 ± 5.5; 1; 4) in diameter. Apical modification of scolex proper cylindrical, housing apical organ, invaginable. Muscular pads 46–55 (51 ± 6.4; 1; 2) long by 59–61 (60 ± 1.4; 1; 2) wide when retracted.

Microtriches on apical modification of scolex proper conspicuous (i.e., visible with light microscopy).

Cephalic peduncle absent. Proglottids craspedote, non-laciniate. Immature proglottids 12 in number, initially wider than long, becoming longer than wide; posterior-most immature proglottid 127 long by 132 wide. Mature proglottids 3 in number, terminal mature proglottid 676 long by 139 wide. Gravid proglottids absent. Testes 4 in number, 33–46 (41 ± 7.0; 1; 3) long by 50–88 (68 ± 19.0; 1; 3) wide, extending from anterior margin of proglottid to anterior margin of ovary, arranged in a single medial column in dorso-ventral view, 1 row deep. Vas deferens extensive, sinuous, visible from level of ootype to posterior margin of anterior-most testis, expanded to form external seminal vesicle in mature proglottids. Internal seminal vesicle present. Cirrus sac pyriform, oriented anteriorly, 34 long by 23 wide, containing coiled cirrus. Cirrus thin-walled, armed with spinitriches. Genital pores lateral, irregularly alternating, 78% of proglottid length from posterior end; genital atrium shallow. Ovary H-shaped in dorso-ventral view, 96 long by 58 wide; ovarian bridge at center of ovary. Mehlis' gland posterior to ovarian bridge. Vagina thin-walled, sinuous, extending along median line of proglottid from ootype to genital atrium, entering genital atrium posterior to cirrus sac; vaginal sphincter not observed. Uterus saccate, medial, extending from anterior margin of ovary to posterior region of anterior-most testis, laterally displaced in mature proglottids; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles large, in 2 lateral bands 2 rows deep, extending from posterior of anterior-most testis to posterior extent of proglottid, interrupted by genital pore and ovary, 41–79 (59 ± 19.0; 1; 3) long by 14–19 (16 ± 2.5; 1; 3) wide. Excretory vessels in 2 lateral pairs. Eggs not observed.

Taxonomic summary

Host: Himantura polylepis (Bleeker, 1852), giant freshwater whipray (Dasyatidae, Myliobatiformes).

Site of infection: Spiral intestine.

Locality: Kampung Abai (05°41'N, 118°23'E), Kinabatangan River, Sabah, Malaysia (host specimen no. BO-108).

Remarks

This individual parasitizing *H. polylepis* is most like the smaller of its congeners in both total length and proglottid count; it can be differentiated from it by its shorter scolex (187 vs.

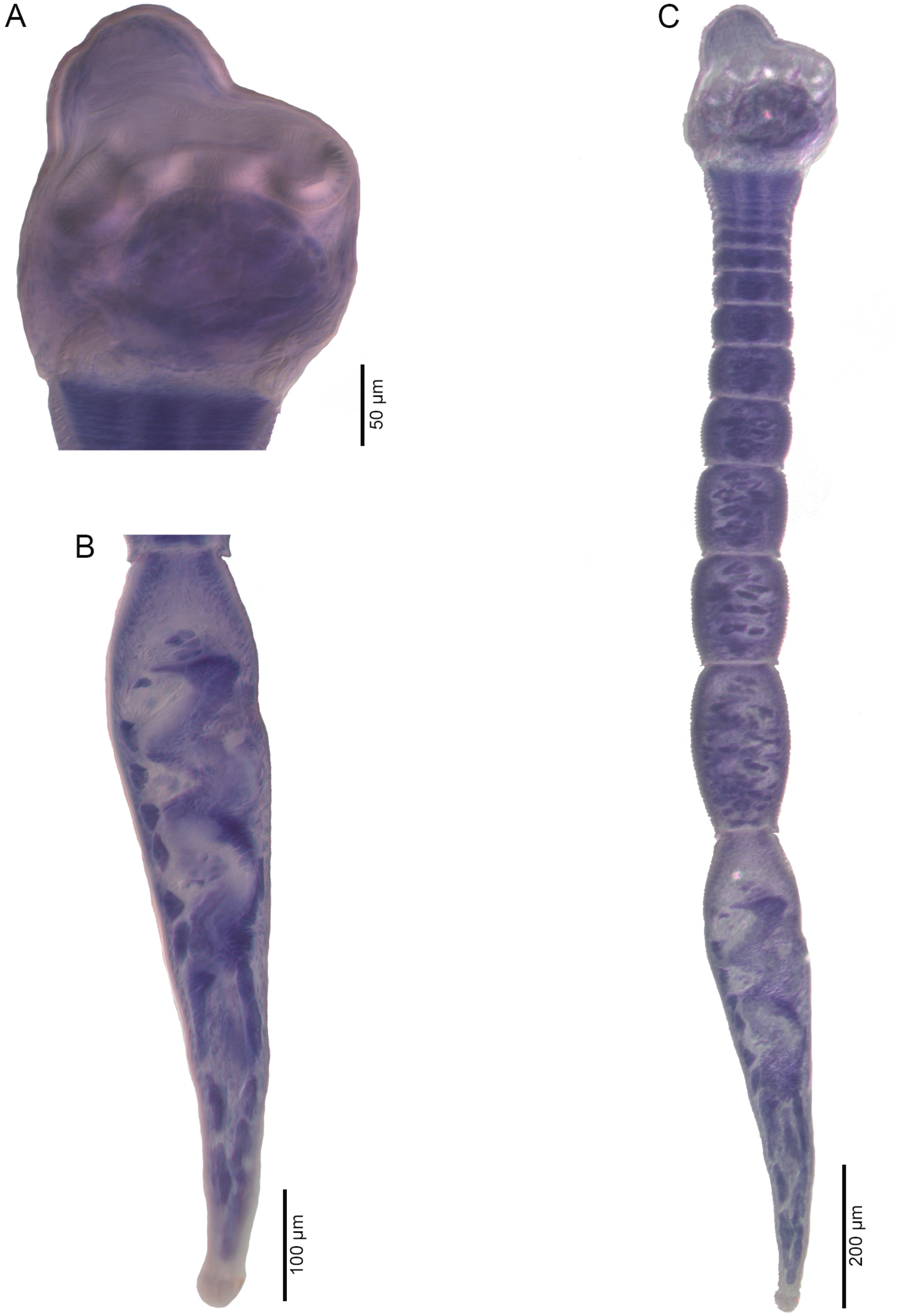

Figure 13. Light micrographs of new species of N. gen. 12 (*sensu* Jensen et al., 2016). (**A**) Scolex. (**B**) Mature terminal proglottid. (**C**) Whole worm.

278–401, retracted), narrower cirrus sac (23 vs. 52–150), and a genital pore positioned more anteriorly in the proglottid (78% vs. 61–68%) (K. Herzog, pers. comm.).

New species of N. gen. 11 (*sensu* Jensen et al., 2016)

(Figs. 14, 15)

Individuals consistent in morphology with N. gen. 11 (*sensu* Jensen et al., 2016) were mounted for light and scanning-electron microscopy. However, the small size of these worms (~300 µm in total length) precluded histological sectioning. Due to these limitations, every attempt was made to characterize this species based on the information available, but a formal description is not provided.

Members of this new species were found in five of the eight host individuals examined (host specimen nos. BO-108, BO-356, BO-496, BO-497, and KA-393). These cestodes resemble the two individuals of N. gen. 11 from *Rhynchobatus* cf. *laevis* (host specimen no. NT-49) and *Glaucostegus typus* (host specimen no. AU-62) that were included in the lecanicephalidean phylogeny by Jensen et al. (2016) in their minute size (not exceeding approximately 300 µm in total length), possession of short tentacles, a narrow scolex, craspedote proglottids, and overall slender body form. They closely resembled the scolex of the representative of N. gen. 11 from *Glaucostegus typus* (host specimen no. AU-62) included in the scanning electron micrograph plate of lecanicephalidean genera (see fig. 1 in Jensen et al., 2016). However, this species differs from these individuals most conspicuously in its possession of six rather than four testes [as defined for the species by Jensen et al. (2016) based on an image of the hologenophore (*sensu* Pleijel et al., 2008)], and the lack of gladiate spinitriches on its scolex proper (see Fig. 15).

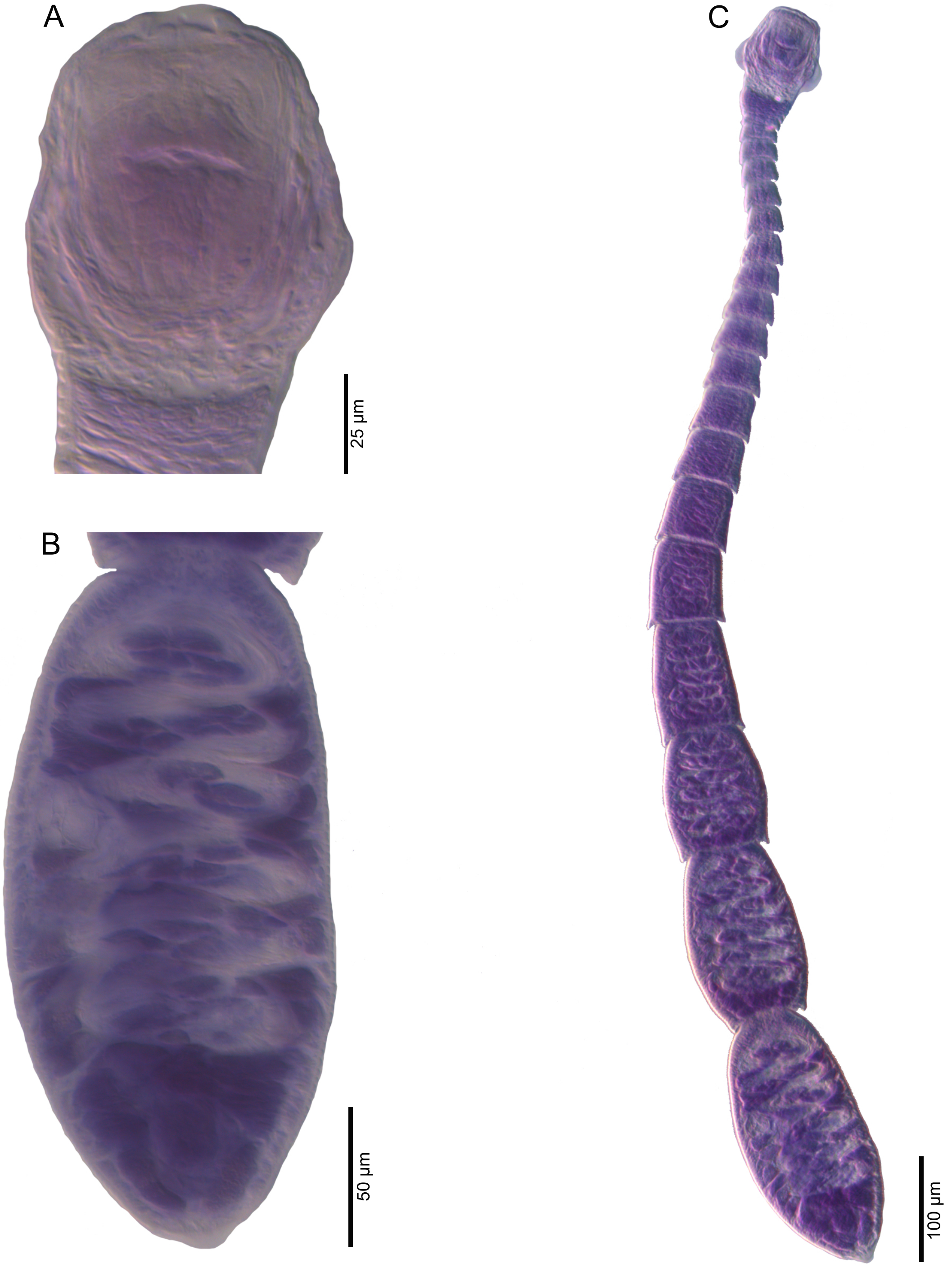

Figure 14. Light micrographs of new species of N. gen. 11 (*sensu* Jensen et al., 2016). (**A**) Scolex. (**B**) Mature terminal proglottid. (**C**) Whole worm.

Figure 15. Scanning electron micrograph of scolex of new species of N. gen. 11 (*sensu* Jensen et al., 2016) with tentacles invaginated.

DISCUSSION

A total of eight species of lecanicephalideans in four genera [i.e., *Tetragonocephalum, Polypocephalus*, N. gen. 11 (*sensu* Jensen et al, 2016), and N. gen. 12 (*sensu* Jensen et al, 2016)] were recovered from *H. polylepis* in this study. This number of lecanicephalideans reported from a single host species is not unusual. For comparison, a total of 20 species in nine genera have been documented from the myliobatid *Aetobatus ocellatus* (Kuhl) (see Shipley, 1900; Shipley and Hornell, 1905, 1906; Southwell, 1911, 1925; MacCallum, 1917; Butler, 1987; Jadhav and Shinde, 1987; Jensen, 2005; Pramanik and Manna, 2007; White et al., 2010; Mojica et al., 2014; Jensen and Russell, 2014). Neither is this number of lecanicephalidean species particularly low. For example, five lecanicephalidean species in three genera have been reported from the rhinopristid *Glaucostegus granulatus* (Cuvier) (see Braun, 1878; Subhapradha, 1951, 1955; Deshmukh, 1980; Jensen, 2005; Cielocha et al., 2014). The lecanicephalidean diversity reported from *H. polylepis* is most comparable to that of its marine congener, *Himantura granulata*, which is parasitized by seven lecanicephalidean species in three genera [i.e., *Polypocephalus*, N. gen. 11 (*sensu* Jensen et al, 2016), and N. gen. 12 (*sensu* Jensen et al, 2016)] (K. Herzog, pers. comm.).

Most of the over 80 known lecanicephalideans have been reported from marine hosts (e.g., Caira and Jensen, 2014). Records from euryhaline host species do exist and are extensive. For example, these records include those from the euryhaline dasyatids *H. uarnak* (see Shipley and Hornell, 1906; Pintner, 1928; Jensen and Russell, 2014)*, D. fluviorum* (see Butler, 1987)*,* and *P. sephen* (see Shipley and Hornell, 1906; Deshmukh and Shinde, 1979; Shinde and Deshmukh, 1979), as well as from pristids *A. cuspidata* (see Southwell, 1911; Cielocha et al., 2014), *P. pristis* (see Cielocha et al., 2014)*,* and *P. clavata* (see Cielocha et al., 2014]. It appears from the literature that cestodes are opportunistically described from hosts or host groups occasionally as single species (e.g., *Aberrapex manjajiae* Jensen, 2006 from *Taeniura lymma*; Jensen, 2006), and more commonly as multiple species in a single genus (e.g., species of *Hornellobothrium* from *Aetobatus ocellatus*, Mojica et al., 2014; species of *Anteropora* Subhapradha, 1955 from dasyatids, Mojica et al., 2013); reports of entire cestode faunas (e.g., cestodes from *Himantura walga*, Twohig et al., 2008) or cestodes of a particular order (e.g. Schaeffner and Beveridge, 2014) are rare. Therefore, the absence of data for lecanicephalideans from a particular host does not necessarily mean that they are not present, rather that the host specimen(s) may not have been examined for those specific cestodes or that these cestodes are present but have not been described. This is the first record of lecanicephalideans from what has been characterized as an obligate freshwater host (see Martin, 2005).

This study broadens our understanding of the diversity and distribution of members of *Tetragonocephalum* and *Polypocephalus*. For the former genus, the number of valid species is increased from five to eight. It is not unusual for three congeners of *Tetragonocephalum* to be reported from a single host species, as is the case in *H. polylepis*. In fact, *H. walga* has been reported to host *T. simile* (see Pintner, 1928; Ivanov and Campbell, 2000) and *T. trygonis* (see Shipley and Hornell, 1905) off the coast of Sri Lanka, as well as *T. yamagutii* (see Muralidhar, 1988) off the coast of India. This is the first record of species of *Tetragonocephalum* from Borneo. Additionally, the three new species of *Polypocephalus* reported from *H. polylepis* increases the number of valid species within the genus from 14 to 17. This number of species of *Polypocephalus* reported from a single host species, again, is not unusual within this genus. Three other batoids have each been reported to host at least three species of *Polypocephalus*: three valid species are known from *Glaucostegus granulatus* [*P. affinis* and *P. rhinobatidis* reported by Subhapradha (1951), and *P. radiatus* reported by Braun (1878)]; four valid species are documented from *Rhynchobatus djiddensis* [*P. coronatus*, *P. lintoni, P. rhynchobatidis*, and *P. vitellaris* reported by Subhapradha (1951)]; five additional species, considered here to be *species inquirendae*, are also purported to parasitize *R. djiddensis* [*P. alii, P. katpurensis,* and *P. singhii* reported by Shinde and Jadhav (1981); *P. djeddensis* reported by Jadhav and Shinde (1989); and *P. himanshui* reported by Pramanik and Manna (2006)], however these records are questionable; and three species of *Polypocephalus* are described from *Pastinachus sephen* [*P. maharashtra* and *P. pratibhai* were reported by Deshmukh et al. (1982); and *P. thapari* was reported by Shinde and Jadhav (1981)], though these records are also suspect and require verification. This is also the first record of species of *Polypocephalus* from Borneo.

The parasite fauna of other species of freshwater dasyatids remains poorly known. As part of this study, spiral intestines of four specimens likely to represent *H. dalyensis*, the freshwater whipray, collected from Cairns, Queensland (Australia) in 2002 (host specimen nos. CMJ-1 through CMJ-4) were examined for cestodes. Preliminary investigation shows that two of the four host specimens (host specimen nos. CMJ-3 and CMJ-4) were collectively infected with members of *Acanthobothrium, Rhinebothrium*, and *Polypocephalus*, all three genera of which are also known from *H. polylepis*. Information on the lecanicephalidean fauna, or cestode fauna in general, of the other six obligate freshwater dasyatids (i.e., *H. kittipongi, H. oxyrhyncha, H. signifer, D. garouaensis, D. laosensis,* or *D. ukpam*) is entirely lacking. While the spiral intestines of 17 individuals of *H. signifer* and 20 individuals of *H. oxyrhyncha* were collected in Indonesian Borneo between 2007–2008 (see GCD; Caira et al., 2016), none have been examined for cestodes to date. It could be worthwhile to examine these 37 specimens for lecanicephalideans and as well as target collection of *H. signifer* and *H. oxyrhyncha* in other sites within their respective geographic ranges (Indonesia, Malaysia, and Thailand for *H. signifer*; and Cambodia and Thailand for *H. oxyrhyncha*) in order to compare their cestode assemblages to that of *H. polylepis*. Investigation of *H. kittipongi* and *D. laosensis* in the Chao Phraya and Mekong Rivers in Thailand and Laos, and *D. garouaensis* and *D. ukpam* in the river systems of western Africa would additionally aid in the understanding of the cestode fauna of freshwater dasyatids overall. However, these four latter species are either listed as endangered by the IUCN (i.e., *H. kittipongi*) or they are themselves poorly known, for example with respect to their exact geographic ranges (i.e., *D. laosensis, D. garouaensis,* and *D. ukpam*); the combination of these factors would make future collection of these species difficult.

In contrast, the parasite fauna of the potamotrygonid river stingrays of South America has been the focus of much taxonomic effort (e.g., Brooks et al., 1981b; Marques, 2000; Reyda, 2007; Reyda and Marques, 2011). A total of 28 species of cestodes within seven genera among four orders (Onchoproteocephalidea, Phyllobothriidea, Rhinebothriidea, and Trypanorhyncha) are reported from six species [*Potamotrygon circularis* Garman, *P. hystrix* (Müller and Henle), *P. magdalenae* (Duméril), *P. motoro* (Müller and Henle), *P. orbignyi* (Castelnau), and *Paratrygon aiereba* (Müller and Henle)] in this family of South American freshwater rays (see Lopez-Neyra and Diaz-Ungria, 1958; Brooks and Thorson, 1976; Rego and Dias, 1976; Mayes et al., 1978; Rego, 1979; Mayes et al., 1981; Brooks et al., 1981b; Marques et al., 2001; Marques and Brooks, 2003; Ivanov, 2004, 2005; Luchetti et al., 2008; Reyda, 2008; Menoret and Ivanov, 2011; Reyda and Marques, 2011; Marques and Reyda, 2015). Three of the seven genera appear unique to potamotrygonids; these are the onchoproteocephalidean *Potamotrygonocestus* Brooks and Thorson, 1976; the phyllobothriidean *Nandocestus* Reyda, 2008; and the rhinebothriidean *Rhinebothroides* Mayes, Brooks, and Thorson, 1981. However, given that members of *Rhinebothroides* have nested within *Rhinebothrium* in previous phylogenetic analyses (see Reyda and Marques, 2011; Marques and Caira, 2016), the former genus may in the future be synonymized with the latter. The remaining four genera (the onchoproteocephalidean *Acanthobothrium*; the rhinebothriideans *Rhinebothrium* and *Anindobothrium* Marques, Brooks, and Lasso, 2001; and the trypanorhynch *Paroncomegas* Campbell, Marques, and Ivanov, 1999) also parasitize batoids exclusively from marine waters (e.g., Campbell and Beveridge, 2002; Campbell, 1975; Brooks, 1977; Palm, 2004). It is hypothesized that the potamotrygonids radiated into river systems within South America during the late Oligocene to early Miocene (Lovejoy et al., 1998; Marques, 2000); this relatively long period of evolutionary time could account for the

Table 3. Comprehensive list of cestodes reported from *Himantura polylepis*.

Order	Species Reported	Citations
Onchoproteocephalidea	*Acanthobothrium asnihae*	Fyler and Caira (2006)
	Acanthobothrium etini	Fyler and Caira (2006)
	Acanthobothrium masnihae	Fyler and Caira (2006)
	Acanthobothrium saliki	Fyler and Caira (2006)
	Acanthobothrium zainali	Fyler and Caira (2006)
Rhinebothriidea	*Rhinebothrium abaiensis*	Healy (2006)
	Rhinebothrium megacanthophallus	Healy (2006)
	New genus 4 *kinabatanganensis*	Healy (2006); Healy et al. (2009)
Trypanorhyncha	*Prochristianella clarkeae*	Schaeffner and Beveridge (2014)
	Proemotobothrium linstowi	Schaeffner and Beveridge (2014)
Lecanicephalidea	New species of New genus 12	(This study)
	New species of New genus 11	(This study)
	Polypocephalus n. sp. 1	(This study)
	Polypocephalus n. sp. 2	(This study)
	Polypocephalus n. sp. 3	(This study)
	Tetragonocephalum n. sp. 1	(This study)
	Tetragonocephalum n. sp. 2	(This study)
	Tetragonocephalum n. sp. 3	(This study)

number of unique genera in this family of rays, having evolved in concert with their hosts, while other cestode fauna have not. Further support of a cestode transition to freshwater in the South American system was provided by Caira and Orringer (1995), who proposed a freshwater life cycle for the onchoproteocephalidean *Potamotrygonocestus magdalenensis* Brooks and Thorson, 1976 parasitizing *Potamotrygon magdalenae*. The authors suggested that *P. magdalenensis* may utilize mayfly nymphs as an intermediate host in the Ciénega Grande River, Colombia.

The main question remains: is the cestode fauna of *H. polylepis*, an obligate freshwater host (*sensu* Martin, 2005), unique compared that of its marine relatives. Of the 18 species of cestodes now known to parasitize *H. polylepis* (see Table 3), only the trypanorhynchs *Prochristianella clarkeae* and *Proemotobothrium linstowi* have been reported from a diversity of other hosts. For example, according to Palm (2004), *P. clarkeae* parasitizes 11 species of batoid hosts within eight genera [i.e., the myliobatiform genera *Neotrygon* Castelnau, *Dasyatis*

Rafinesque, *Himantura* (Müller and Henle), and *Urolophus* (Müller and Henle); the rajiform genus *Dipturus* (Rafinesque); the rhinopristiform genera *Rhynchobatus* (Müller and Henle) and *Aptychotrema* Norman; and the chimaeriform genus *Callorhinchus* Lacepéde]. *Proemotobothrium linstowi* is reported from three species of hosts in three genera: the myliobatiform genus *Aetobatus* Blainville, the rhinoprostid genus *Rhynchobatus,* and the carcharhinid shark genus *Carcharhinus* Blainville. This observation is expected as members of the Trypanorhyncha are much more relaxed in their host associations than members of the other elasmobranch-hosted orders; the trypanorhynchs are therefore less useful in assessing the uniqueness of the cestode assemblage within *H. polylepis*. The oioxenous specificity (i.e., a species of parasite restricted to a single species of host) exhibited by the additional 16 species that are reported from *H. polylepis* (see Table 3) is well-established in the onchoproteocephalidean, rhinebothriidean, and lecanicephalidean tapeworms (see Caira et al., 2001; Caira et al., 2003; Jensen, 2005; Caira and Jensen, 2015). A species level comparison of the cestode fauna of marine and freshwater dasyatid hosts is therefore not useful given that each stingray species has its unique set of cestode species. Rather, comparison of the respective cestode fauna of *H. polylepis* and its marine relatives at the generic level might provide insight into whether any of the cestodes reported from *H. polylepis* might be associated with a freshwater environment. Table 4 shows all nine genera found in *H. polylepis* and their presence or absence in a broad sampling of marine and euryhaline dasyatids based on published and unpublished data.

Of the seven non-trypanorhynchan genera parasitizing *H. polylepis*, members of *Acanthobothrium* and *Rhinebothrium* are the genera found most ubiquitous in other dasyatid hosts (see Table 4). The majority of species of *Acanthobothrium* are described from members of

Myliobatiformes (e.g., Baer and Euzet, 1962; Campbell, 1969; Ghoshroy and Caira, 2001), with some records from Torpediniformes (electric rays) (e.g., Caira and Burge, 2001), Rajiformes (skates) (e.g., Williams, 1968; Carvajal and Goldstein, 1969), Rhinopristiformes (guitarfishes) (e.g., Appy and Dailey, 1973; Campbell and Beveridge, 2002), Heterodontiformes (horn sharks) (e.g., Yamaguti, 1934; Drummond, 1937; Appy and Dailey, 1973), Carcharhiniformes (ground sharks) (e.g., Euzet, 1959; Vardo-Zalik and Campbell, 2011), and Orectolobiformes (carpet sharks) (e.g., Williams, 1962; Campbell and Beveridge, 2002). On the whole, members of *Acanthobothrium* are ubiquitous in elasmobranchs. Of the 184 known species of *Acanthobothrium* currently considered valid, 173 parasitize marine elasmobranchs, six are reported from potamotrygonids, and five are described from *H. polylepis*. Species of *Rhinebothrium* are mostly reported from dasyatids (e.g., Linton, 1890; Baer, 1948; Euzet, 1959; Williams, 1964; Campbell, 1975), with additional records from myliobatid (e.g., Euzet, 1955), potamotrygonid (e.g., Reyda, 2008; Reyda and Marques, 2011; Menoret and Ivanov, 2011), and rhinobatid (e.g., Butler, 1987; Golestaninasab and Malek, 2015) hosts, and a single rajiform (Euzet and Carvajal, 1973) host. Forty species are currently recognized as valid: six species have been reported from potamotrygonids, two species from *H. polylepis*, and 32 species from marine batoids.

The 14 valid species *Polypocephalus* recognized prior to this study are mostly known from dasyatids (e.g., Linton, 1890; Southwell, 1912; Butler, 1987) and rhinopristiforms (e.g., Braun, 1878; Subhapradha, 1951); additional records exist from myliobatids (Gardner and Schmidt, 1984; Jensen, 2005). All species were described from marine batoids. The five valid species of *Tetragonocephalum* recognized prior to this study have been described from species of *Himantura* (Shipley and Hornell, 1905, 1906; Pintner, 1928; Muralidhar, 1988; Ivanov and

Campbell, 2000; Jensen, 2005). Jensen et al. (2016) reported an undescribed species from the porcupine ray, *Urogymnus asperrimus* (Bloch and Schneider), and suggested that another species also parasitizes the red stingray, *Dasyatis akajei* (Müller and Henle) (see Table 4). All previous records of *Tetragonocephalum* come from marine dasyatids. Preliminary data indicates that representatives of N. gen. 11 (*sensu* Jensen et al., 2016) parasitize at least 12 species of batoids in five genera (i.e., the myliobatiform genera *Himantura, Dasyatis,* and *Pastinachus*; and the rhinopristiform genera *Glaucostegus* Bonaparte*,* and *Rhynchobatus*). Members of N. gen. 12 (*sensu* Jensen et al., 2016) are further restricted to at least six species of batoids in two myliobatiform genera (i.e., *Himantura* and *Neotrygon*) (K. Herzog, pers. comm.). Species diversity of N. gen. 11 and N. gen. 12 remain to be determined. To date, all unpublished records are from marine batoids.

Of the nine genera that are reported from *H. polylepis*, only N. gen. 4 is unique to this host species. Despite recent extensive collecting efforts of tapeworms of elasmobranchs broadly in Southeast Asia (see e.g., Healy, 2006b; Fyler and Caira, 2006; Jensen and Caira, 2006; Jensen et al., 2011; Koch et al., 2012; Mojica et al., 2013, 2014; Schaeffner and Beveridge, 2014), N. gen. 4 has not been seen in any other elasmobranch species from that region or elsewhere. It is possible that members of this genus parasitize other species of *Himantura*, albeit at possibly low prevalence and intensity. As it stands, among the more than 1,200 tapeworm individuals recovered from *H. polylepis*, this genus is currently only known from the eight specimens used for the original description (Healy, 2006a), one specimen voucher sequenced (see Healy et al., 2009), and a single additional specimen (K. Jensen, pers. comm.). Examination of additional specimens of *H. dalyensis*, or specimens of *H. signifer* or *H. oxyrhyncha*, might be the most likely candidate host for other species in this genus. As outlined above, the other eight genera

Table 4. Presence or absence of cestode genera parasitizing *Himantura polylepis* in selected marine and euryhaline dasyatid hosts.

Host Species	*Acanthobothrium*	*Rhinebothrium*	N. gen. 4	*Polypocephalus*	*Tetragonocephalum*	N. gen. 12	N. gen. 11	*Proemotobothrium* §	*Prochristianella*	Citations
Himantura polylepis	X	X	X	(this study)	(this study)	(this study)	(this study)	X	X	Fyler and Caira (2006); Healy (2006); Healy et al. (2009); Schaeffner and Beveridge (2014)
Himantura sp. 1 †	X									Fyler et al. (2009)
Himantura dalyensis*	x	x		x						
Himantura cf. *gerrardi* 2				x	x	X	x		X	Jensen et al. (2016); Schaeffner and Beveridge (2014)
Himantura granulata †	x	X		X		x	x		x	Williams (1964); Healy (2006); Schaeffner and Beveridge (2014); Jensen et al. (2016); K. Herzog (pers. comm.)
Himantura kittipongi					no records					
Himantura leoparda				x	X					Jensen (2005)
Himantura lobistoma		X		x		x	x		X	Healy (2006); Schaeffner and Beveridge (2014)
Himantura oxyrhyncha					no records					
Himantura signifer					no records					
Himantura uarnacoides	X	X		x	x	x	x		X	Healy (2006); Reyda and Caira (2006); Healy et al. (2009); Schaeffner and Beveridge (2014)
Himantura uarnak ‡	X			x	X		x		X	Shipley and Hornell (1906); Pintner (1928); Maheswari et al. (1987); Campbell and Beveridge (2002); Palm (2004); Schaeffner and Beveridge (2014)
Himantura walga	X	X			X	x	x		X	Shipley and Hornell (1905, 1906); Pintner (1928); Euzet (1953); Muralidhar (1988); Ivanov and Campbell (2000); Healy (2006); Twohig et al. (2008); Schaeffner and Beveridge (2014)
Dasyatis akajei	X	X		x	x					Healy (2006); Jensen et al. (2016)
Dasyatis americana	X	X		x			x		X	Campbell (1969, 1970, 1975); Palm (2004)
Dasyatis dipterura	X	X								Ghoshroy and Caira (2001); Healy (2006)
Dasyatis centroura	X	X		X					X	Linton (1890, 1924); Southwell (1925); Baer (1948); Palm (2004); Vardo-Zalik and Campbell (2011)
Dasyatis fluviorum ‡*				X					X	Butler (1987); Palm (2004)
Dasyatis garouaensis*					no records					
Dasyatis laosensis*					no records					
Dasyatis longa	X									Marques et al. (1995); Monks et al. (1996); Marques et al. (1997)
*Dasyatis pastinaca**	X									MacCallum (1921); Wardle and McLeod (1952)
Dasyatis sabina ‡		X		x					X	Palm (2004); Healy (2006)
Dasyatis say	X	X		x					X	Linton (1908); Palm (2004); Healy (2006)
Dasyatis ukpam*					no records					
Dasyatis zugei	X	X							X	Yamaguti (1952); Wang and Yang (2001); Schaeffner and Beveridge (2014)
Pastinachus astrus ‡	X	X		X						Verma (1928); Baer and Euzet (1962); Hassan (1983); Campbell and Beveridge (2002); Healy (2006)
Neotrygon cf. *kuhlii*	X			X		x			X	Southwell (1912, 1925); Baer and Euzet (1962); Wang (1984); Palm (2004)
Urogymnus asperrimus †	X	X		x	X				X	Hornell (1912); Southwell (1925); Brooks and Deardorff (1988); Healy (2006); Schaeffner and Beveridge (2014); Jensen et al. (2016)
Taeniura lymma		X		X						Hassan (1982); Ramadan (1984); Healy (2006)

X = reported in literature

x = preliminary observations (described genera from the Global Cestode Database located at http://tapewormdb.uconn.edu/index.php/parasites/species_search/

* Species not included in Naylor et al. (2012a)

† Closest relatives of *H. polylepis* according to Naylor et al. (2012a)

‡ Euryhaline species

§ records also from rhynchobatid and myliobatid batoids and carcharhinid sharks (see Palm, 2004; Schaeffner and Beveridge, 2014)

Bolded species are designated as freshwater obligate reported from *H. polylepis* are primarily found within marine and euryhaline dasyatids, myliobatids, and rhinopristids, although an individual record of *Proemotobothrium* from the myliobatid *Aetobatus narinari* exists at the generic level (see Palm, 2004). Therefore, the cestode fauna reported from *H. polylepis* is overall not unique to this host species with the exception of N. gen. 4, but rather is similar to that of its congeners. This degree of overlap of cestode genera parasitizing freshwater elasmobranchs compared to their marine counterparts is dissimilar to that of potamotrygonids, where three of seven genera (i.e., 42%) are unique to this family of rays versus only one of nine unique genera (i.e., 11%) in *H. polylepis*.

The lack of a distinct cestode assemblage at the generic level in *H. polylepis* suggests that this host species may not be restricted to freshwater systems. In fact, there has been disagreement in the literature amongst researchers regarding the salinity tolerance and habitat range of *H. polylepis*. Some refer to *H. polylepis* as a ‘freshwater’ (e.g. Commission, 1997) or an ‘obligate freshwater’ species (e.g. Martin, 2005), and others suggest that it is instead a euryhaline species (Monkolprasit and Roberts, 1990; Last and Manjaji-Matsumoto, 2008; Last et al., 2010). The parasite fauna suggests that *H. polylepis* might be able to tolerate a broader salinity range, entering estuaries and preying on some of the same types of intermediate hosts as its marine dasyatid relatives.

A potential method to better estimate the amount of time that *H. polylepis* spends in freshwater would be to conduct a blood plasma ion concentration analysis. Previous studies have utilized this test to determine the physiological effects of salinity on the body chemistry of fishes (Edwards and Marshall, 2013; table 1.1). Ion concentration can therefore be used as an indicator of time spent in freshwater versus saltwater by using it to gauge decreased osmoregulatory capability in freshwater species lost over generations. Piermarini and Evans (1998) found differences in sodium, chloride, and urea ion concentration contributing to total osmolality of blood plasma between individuals of *Dasyatis sabina* collected from the St. Johns River, Florida and the Atlantic Ocean off Florida; saltwater specimens had higher levels of all three types of ions. Furthermore, Griffith et al. (1973) found even lower ionic levels of these same compounds within potamotrygonids. This family of rays was found to have an extremely low concentration of urea in their blood plasma compared to the individuals of *D. sabina* examined by Piermarini and Evans (1998). Testing the blood plasma ionic composition of *H. polylepis* would contribute to determining if this species is truly obligate to freshwater riverine systems. It is expected that the blood plasma ions of *H. polylepis* would instead be similar to euryhaline or marine species.

The results of this study generate more questions than answers, and there are several components that require additional investigation. Bearing in mind that *H. polylepis* is an endangered species and that new collection of specimens is unlikely now or in the future, hypothetical targeting of this species for collection throughout its observed range in both river systems and estuary zones from India to Indonesia would allow for a more accurate understanding of its overall cestode fauna. New collections would also provide an opportunity to target the cestode species for which molecular material was not available in order to incorporate them into the existing lecanicephalidean phylogeny (see Jensen et al., 2016) to test if the multiple species of *Tetragonocephalum* and *Polypocephalus* parasitizing *H. polylepis* are each other's closest relatives, or if they are more closely related to members of these two genera from marine hosts.

Examination of the cestode fauna of the other seven freshwater dasyatids would allow for comparison to *H. polylepis* to (1) verify if the cestode assemblages of freshwater dasyatids are more similar to freshwater or marine dasyatids overall, (2) determine if their cestodes are each other's closest relatives by incorporating them into the most recent lecanicephalidean phylogeny (Jensen et al., 2016), and (3) possibly give further insight into the salinity tolerance of these seven freshwater dasyatids. Though the sample size of the two infected specimens of *H. dalyensis* from Cairns is low, they did confirm the presence of three cestode genera that are also present in *H. polylepis* as well as other dasyatids. Collection of additional specimens of *H. dalyensis* from rivers within their range (i.e. Northern Australia and Papua New Guinea) would give the opportunity to identify the representatives of *Acanthobothrium, Rhinebothrium,* and *Polypocephalus* to the species level, as well as any other cestodes that might parasitize this host. Additionally, it might be interesting to determine if the same species of cestodes are found in the Australia and the Papua New Guinea populations of *H. dalyensis*, thereby suggesting that members of these populations may forage in the same areas or even breed with one another.

LITURATURE CITED

Al Kawari, K. S., M. F. Saoud, and M. Q. Wanas. 1994. Helminth parasites of fishes from the Arabian Gulf: On *Eniochobothrium qatarense* sp. nov. (Cestoda: Lecanicephalidea) and the affinities of *Eniochobothrium* Shipley and Hornell, 1906, *Litobothrium* Dailey, 1969 and *Renyxa* Kurochkin and Slankis, 1973. *Japanese Journal of Parasitology* 43: 97–104.

Anderson, A. B. and C. N. Jenkins. 2006. Applying Nature's Design. *Columbia University Press*. New York, New York. 157–169.

Andhare, V. V. and G. B. Shinde. 1994. Three new species of the genus *Tylocephalum* (Cestoda: Lecanicephalidae) at Madras, M. S., India. *Indian Journal of Parasitology* 18(1): 83–89.

Appy, R. G. and M. D. Dailey. 1973. Two new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from elasmobranchs of the eastern Pacific. *Journal of Parasitology* 59: 817–820.

Baer, J. G. 1948. Contributions a l'étude des cestodes des sélaciens. I-IV. *Bulletin de la Société Neuchâteloise des Sciences Naturelles* 71: 63–122.

Baer, J. G. and L. Euzet. 1962. Revision critique des cestodes tétraphyllides décrits par T. Southwell (1re partie). *Bulletin de la Société Neuchâteloise des Sciences Naturelles* 85: 143–172.

Ballantyne, J. S. and D. I. Fraser. 2013. Euryhaline elasmobranchs. *Fish Physiology*. Academic Press. 32: 126–198.

Bannai, M. A., S. A. Al-Daraji, and E. T. Muhammad. 2014. Lecanicephalidea cestode larvae parasite in *Scomberoides cammmersoniaus* fish, Arabian Gulf. *International Journal of Marine Science* 4: 68 pp.

Bilqees, F. M. 1995. Teaching and research in marine parasitology - problems and solution. *Proceedings of Parasitology* 20: 63–89.

Bleeker, P. 1852. Bijdrage tot de kennis der Plagiostomen van den Indischen Archipel. *Lange* 24: 73.

Braun, M. 1878. Zwei neue Bandwürmer. *Arbeiten aus dem Zoologisch-Zootomischen Institut in Würzburg, Neue Folge* 4: 297–302.

Brooks, D. R. 1977. Six new species of tetraphyllidean cestodes, including a new genus, from a marine stingray *Himantura schmardae* (Werner, 1904) from Colombia. *Proceedings of the Helminthological Society of Washington* 44: 51–59.

Brooks, D. R. and T. B. Thorson. 1976. Two tetraphyllidean cestodes from the freshwater stingray *Potamotrygon magdalenae* Dumeril 1852 (Chondrichthyes: Potamotrygonidae) from Colombia. *Journal of Parasitology* 62: 943–947.

Brooks, D. R. and T. L. Deardorff. 1988. *Rhinebothrium devaneyi* n. sp. (Eucestoda: Tetraphyllidea) and *Echinocephalus overstreeti* Deardorff and Ko, 1983 (Nematoda: Gnathostomatidae) in a thorny back ray, *Urogymnus asperrimus*, from Enewetak Atoll, with phylogenetic analysis of both species groups. *Journal of Parasitology* 74: 459–465.

Brooks, D. R., M. A. Mayes and T. B. Thorson. 1981a. Cestode parasites in *Myliobatis goodei* Garman (Myliobatiformes: Myliobatidae) from Río de la Plata, Uruguay, with a summary of cestodes collected from South American elasmobranchs during 1975-1979. *Proceedings of the Biological Society of Washington* 93: 1239–1252.

Brooks, D. R., M. A. Mayes and T. B. Thorson. 1981b. Systematic review of cestodes infecting freshwater stingrays (Chondrichthyes: Potamotrygonidae) including four new species from Venezuela. *Proceedings of the Helminthological Society of Washington* 48: 43–64.

Butler, S. A. 1984. Taxonomy and biology of some tetraphyllidean cestodes from Moreton Bay. *Department of Parasitology*. *University of Queensland*. St. Lucia, Queensland, Australia. 1–187. Ph.D. Thesis.

Butler, S. A. 1987. The taxonomic history of the family Lecanicephalidae Braun, 1900, a little known group of marine cestodes. *Systematic Parasitology* 10: 105–115.

Caira, J. N. and A. N. Burge. 2001. Three new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from the ocellate electric ray, *Diplobatis ommata*, in the Gulf of California, Mexico. *Comparative Parasitology* 68: 52–65.

Caira, J. N. and K. Jensen. 2014. A digest of elasmobranch tapeworms. *Journal of Parasitology* 100(4): 373–391.

Caira, J. N. and K. Jensen. 2015. Insights on the identities of sharks of the *Rhizoprionodon acutus* (Elasmobranchii: Carcharhiniformes) species complex based on three new species of *Phoreiobothrium* (Cestoda: Onchoproteocephalidea). *Zootaxa* 4059: 335–350.

Caira, J. N., K. Jensen, E. Barbeau. Editors. 2016. Global Cestode Database. World Wide Web electronic publication. www.tapewormdb.uconn.edu. Accessed May 11, 2016.

Caira, J. N., K. Jensen, and C. J. Healy. 2001. Interrelationships among tetraphyllidean and lecanicephalidean cestodes. In *Interrelationships of the Platyhelminthes*, D. T. Littlewood and R. A. Bray (editors). Taylor & Francis, London. 135–158.

Caira, J. N., K. Jensen, and K. E. Holsinger. 2003. On a new index of host specificity. *Taxonomy, ecology and evolution of metazoan parasites*, C. Combes and J. Jourdane (Editors). Presses Universitaires de Perpignan. Perpignan. 161–201.

Caira, J. N., K. Jensen, A. Waeschenbach, P. D. Olson, and D. T. Littlewood. 2014. Orders out of chaos - molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships. *International Journal for Parasitology* 44(1): 55–73.

Caira, J. N., K. Jensen, Y. Yamane, A. Isobe, and K. Nagasawa. 1997. On the tapeworms of *Megachasma pelagios*: Description of a new genus and species of lecanicephalidean and additional information on the trypanorhynch *Mixodigma leptaleum*. *Biology of the megamouth shark* 16: 181–191.

Caira, J. N. and D. J. Orringer. 1995. Additional information on the morphology of *Potamotrygonocestus magdalenensis* (Tetraphyllidea: Onchobothriidae) from the freshwater sintgray *Potamotrygon magdalenae* in Colombia. *Journal of the Helminthological Society of Washington* 62: 22–26.

Cake, E. W., Jr. 1979. *Polypocephalus* sp. (Cestoda; Lecanicephalidae): A description of tentaculo-plerocercoids from bay scallops of the northeastern Gulf of Mexico. *Proceedings of the Helminthological Society of Washington* 46: 165–170.

Cake, E. W., Jr. and R. W. Menzel. 1980. Infections of *Tylocephalum metacestodes* in commercial oysters and three predaceous gastropods of the eastern Gulf of Mexico. *Proceedings of the National Shellfisheries Association* 70: 94–104.

Campbell, R. A. 1969. New species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from Chesapeake Bay, Virginia. *Journal of Parasitology* 55: 559–570.

Campbell, R. A. 1970. Notes on tetraphyllidean cestodes from the Atlantic Coast of North America, with descriptions of two new species. *Journal of Parasitology* 56: 498–508.

Campbell, R. A. 1975. Tetraphyllidean cestodes from western North Atlantic selachians with descriptions of two new species. *Journal of Parasitology* 61: 265–270.

Campbell, R. A. and I. Beveridge. 2002. The genus *Acanthobothrium* (Cestoda: Tetraphyllidea: Onchobothriidae) parasitic in Australian elasmobranch fishes. *Invertebrate Systematics* 16: 237–344.

Campbell, R. A. and A. D. Williams. 1984. *Tylocephalum* Linton, 1890 (Cestoda: Lecanicephalidea) from the cownose ray, *Rhinoptera bonasus* (Mitchill, 1815) with a discussion of its validity and systematic relationships. *Proceedings of the Helminthological Society of Washington* 51: 121–134.

Carreon, N. and Z. Faulkes. 2014. Position of larval tapeworms, *Polypocephalus* sp., in the ganglia of shrimp, *Litopenaeus setiferus*. *Integrative and Comparative Biology*. 54(2): 143–148.

Carreon, N., Z. Faulkes, and B. L. Fredensborg. 2011. *Polypocephalus* sp. infects the nervous system and increases activity of commercially harvested white shrimp (*Litopenaeus setiferus*). *Journal of Parasitology* 97: 755–759.

Carvajal, J. G. and R. J. Goldstein. 1969. *Acanthobothrium psammobati* sp. n. (Cestoda: Tetraphyllidea: Onchobothriidae) from the skate, *Psammobatis scobina* (Chondrichthyes: Rajidae) from Chile. *Zoologischer Anzeiger* 182: 432–435.

Cheng, T. C. 1975. New geographic records for *Tylocephalum* metacestodes. *Journal of Invertebrate Pathology* 26: 395–396.

Chervy, L. 2009. Unified terminology for cestode microtriches: a proposal from the International Workshops on Cestode Systematics in 2002–2008. *Folia Parasitologica* 56(3): 199–230.

Cielocha, J. J. and K. Jensen. 2011. A revision of *Hexacanalis* Perrenoud, 1931 (Cestoda: Lecanicephalidea) and description of *H. folifer* n. sp. from the zonetail butterfly ray *Gymnura zonura* (Bleeker) (Rajiformes: Gymnuridae). *Systematic Parasitology* 79: 1–16.

Cielocha, J. J. and K. Jensen. 2013. *Stoibocephalum* n. gen. (Cestoda: Lecanicephalidea) from the sharkray, *Rhina ancylostoma* Bloch & Schneider (Elasmobranchii: Rhinopristiformes), from northern Australia. *Zootaxa* 3626(4): 558–568.

Cielocha, J. J., K. Jensen, and J. N. Caira. 2014. *Floriparicapitus*, a new genus of lecanicephalidean tapeworm (Cestoda) from sawfishes (Pristidae) and guitarfishes (Rhinobatidae) in the Indo-West Pacific. *Journal of Parasitology* 100(4): 485–499.

Commission, I. S. S. 1997. Elasmobranch Biodiversity, Conservation and Management. S. L. Fowler, T. M. Reed, and F. A. Dipper (Editors). International Union for Conservation of Nature and Natural Resources, Sabah, Malaysia. *International Seminar and Workshop* 25: 1–258.

Compagno, L. J. and S. F. Cook. 1995. The exploitation of freshwater elasmobranchs: status of taxa and prospects for the future. *Journal of Aquariculture and Aquatic Sciences* 7: 62–90.

Dandwate, R. R. and B. V. Jadhav. 2009. A new lecanicephalidean cestode endoparasite on *Trygon sephen* from Ratnagiri, India. *Uttar Pradesh Journal of Zoology* 29(3): 419–421.

Department of Fisheries Malaysia. 2014. Malaysia National Plan of Action for the Conservation and Management of Shark (Plan 2). *Ministry of Agriculture and Agro-based Industry Malaysia*. Putrajaya, Malaysia. 1–51.

Deshmukh, R. A. 1980. On a new cestode *Spinocephalum rhinobatii* gen. et sp. nov. (Cestoda: Lecanicephalidae) from a marine fish from west coast of India. *Rivista di Parassitologia* 41: 27–32.

Deshmukh, R. K., B. V. Jadhav, and G. B. Shinde. 1982. Five new species of the genus *Polypocephalus* Braun, 1878. (Cestoda: Lecanicephalidea) India. *Marathwada University Journal of Science (Biological Sciences)* 21: 79–86.

Deshmukh, R. A. and G. B. Shinde. 1979. Three new species of *Tetragonocephalum* Shipley and Hornell, 1905 (Cestoda: Tetragonocephalidae) from marine fishes of west coast of India. *Bioresearch* 3: 19–23.

Drummond, F. H. 1937. Reports of the expedition of the McCoy Society for Field Investigations and Research. *Proceedings of the Royal Society of Victoria* 49: 401–404.

Ebert, D. A., H. Ho, W. T. White, and M. R. de Carvalho. 2013. Introduction to the systematics and biodiversity of sharks, rays, and chimaeras (Chondrichthyes) of Taiwain. *Zootaxa* 3752(1): 5–19.

Edwards, S. L. and W. S. Marshall. 2013. Principles and patterns of osmoregulation and euryhalinity in fishes. In Euryhaline Fishes. *Fish Physiology* 32: 1–44.

Eschmeyer, W. N. and J. D. Fong. 2016. Online Catalog of Fishes. *California Academy of Sciences.* http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp#Potamotrygonidae. Accessed on 01 April 2016.

Euzet, L. 1953. Cestodes tétraphyllides nouveaux ou peu connus de *Dasyatis pastinaca* (L.). *Annales de Parasitologie Humaine et Comparée* 28: 339–351.

Euzet, L. 1955. Quelques cestodes de *Myliobatis aquila* L. Recueil des Travaux des Laboratoires de Botanique, Géologie et Zoologie de la Faculté des Sciences de l´Université de Montpellier. *Série Zoologique* 1: 18–27.

Euzet, L. 1959. Recherches sur les cestodes tétraphyllides des sélaciens des cotes de France. Doctoral Dissertation. *Université de Montpellier*. Montpellier, France.

Euzet, L. and J. Carvajal-Garay. 1973. *Rhinebothrium* (Cestoda, Tetraphyllidea) parasites de raies du genre Psammobatis au Chili. *Bulletin du Muséum National d´Histoire Naturelle, 3e Série Zoologie* 101: 779–787.

Euzet, L. and C. Combes. 1980. Les problémes de l'espéce chez les animaux parasites: Les Problémes de l'Espéce dans le Régne Animal. Tome 3. C. J. Génermont, J. and M. Lamotte (editors). *Mémoires de la Société Zologique de France* 239–285.

Fahmi, W. White, and B. M. Manjaji. 2009. *Pastinachus sephen*. The IUCN Red List of Threatened Species 2009: e.T161332A5400078. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161332A5400078.en. Accessed on 03 April 2016.

Faria, V. V., M. T. McDavitt, P. Charvet, T. R. Wiley, C. A. Simpfendorfer, and G. P. Naylor. 2013. Species delineation and global population structure of critically endangered sawfishes (Pristidae). *Zoological Journal of the Linnean Society* 167(1): 136–164.

Froese, R. and D. Pauly. Editors. 2015. FishBase. World Wide Web electronic publication. www.fishbase.org. Accessed on 01 April 2016.

Fyler, C. and J. N. Caira. 2006. Five new species of *Acanthobothrium* (Tetraphyllidea: Onchobothriidae) from the freshwater stingray *Himantura chaophraya* (Batoidea: Dasyatidae) in Malaysian Borneo. *Journal of Parasitology* 92(1): 105–125.

Fyler, C. A., J. N. Caira, and K. Jensen. 2009. Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from an unusual species of *Himantura* (Rajiformes: Dasyatidae) from northern Australia. *Folia Parasitologica* 56(2): 107–128.

Garcia, A., S. Mattiucci, S. Damiano, M. N. Santos, and G. Nascetti. 2010. Metazoan parasites of swordfish, *Xiphias gladius* (Pisces: Xiphiidae) from the Atlantic Ocean: implications for host stock identification. *ICES Journal of Marine Science* 68: 175–182.

Gardner, S. L. and G. D. Schmidt. 1984. *Discobothrium caribbensis* sp. n., a lecanicephalidean cestode from a yellow-spotted stingray, *Urolophus jamaicensis*, in Jamaica. *Journal of Parasitology* 70: 303–304.

Ghoshroy, S. and J. N. Caira. 2001. Four new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from the whiptail stingray *Dasyatis brevis* from the Gulf of California, Mexico. *Journal of Parasitology* 87: 354–372.

Glemet, R., M. R. Bezuijen, S. C. Leslie, B. Stuart, R. J. Timmins, J. W. Duckworth, J. Maxwell, S. Seateun, N. Sivongxay, C. Vongsombath, S. Viravong, D. Singhanouvong, and O. Ongkeo. 2013. Ecological Survey of the Mekong River between Louangphabang and Vientiane Cities. *International Union for Conservation of Nature* 1–241.

Golestaninasab, M. and M. Malek. 2015. Two new species of *Rhinebothrium* (Cestoda: Rhinebothriidea) from granulated guitarfish *Glaucostegus granulatus* in the Gulf of Oman. *Journal of Helminthology* 1–14.

Griffith, R. W., P. K. Pang, A. K. Srivastava, and G. E. Pickford. 1973. Serum composition of freshwater stingrays (Potamotrygonidae) adapted to fresh and dilute seawater. *Biological Bulletin* 144(2): 304–320.

Hassan, S. H. 1982. *Polypocephalus saoudi* n. sp. lecanicephalidean cestode from *Taeniura lymma* in the Red Sea. *Journal of the Egyptian Society of Parasitology* 12: 395–401.

Hassan, S. H. 1983. *Acanthobothrium manteri* sp. n. a tetraphyllidean cestode (Oncobothriidae) from *Dasyatis sephen*. *Journal of the Egyptian Society of Parasitology* 13: 75–80.

Healy, C.J. 2006a. Three new species of *Rhinebothrium* (Cestoda Tetraphyllidea) from the freshwater whipray, *Himantura chaophraya*, in Malaysian Borneo. *Journal of Parasitology* 92(2): 364–374.

Healy, C. J. 2006b. A revision of selected Tetraphyllidea (Cestoda): *Caulobothrium, Rhabdobothrium, Rhinebothrium, Scalithrium,* and *Spongiobothrium*. *Unoversity of Connecticut*. Storrs, Connecticut, USA. Ph. D. dissertation.

Healy, C. J., J. N. Caira, K. Jensen, B. L. Webster, and D. T. Littlewood. 2009. Proposal for a new tapeworm order, Rhinebothriidea. *International Journal for Parasitology* 39: 497–511.

Hogan, Z. 2009. The Megafishes Project. *The National Geographic*. Retrieved 24 April, 2014. http://environment.nationageographic.com/environment/freshwater/about-megafishes-project/.

Hornell, J. 1912. New cestodes from Indian fishes. *Records of the Indian Museum* 7: 197–204.

Ivanov, V. A. 2004. A new species of *Rhinebothroides* Mayes, Brooks & Thorson, 1981 (Cestoda: Tetraphyllidea) from the ocellate river stingray in Argentina, with amended descriptions of two other species of the genus. *Systematic Parasitology* 58: 159–174.

Ivanov, V. A. 2005. A new species of *Acanthobothrium* (Cestoda: Tetraphyllidea: Onchobothriidae) from the ocellate river stingray, *Potamotrygon motoro* (Chondrichthyes: Potamotrygonidae), in Argentina. *Journal of Parasitology* 91(2): 390–396.

Ivanov, V. A. and R. A. Campbell. 2000. Emendation of the generic diagnosis of *Tylocephalum* (Cestoda: Lecanicephalidea: Tetragonocephalidae), and description of *Tylocephalum brooksi* n. sp. *Journal of Parasitology* 86: 1085–1092.

Jadhav, B. V. 2007. Bioinformatics. *APH Publishing Corporation*, New Delhi, India. 155–183.

Jadhav, B. V. and G. B. Shinde. 1987. *Tylocephalum aurangabadensis* sp. nov. (Cestoda: Lecanicephalidae) from a marine fish *Aetobatis narinari* [sic] from Arabian Sea. *Indian Journal of Helminthology* 39: 88–91.

Jadhav, D. H. and G. B. Shinde. 1989. A new species of the genus *Polypocephalus* (Cestoda: Lecanicephalidea) at Ratnagiri, M. S., India. *Rivista di Parassitologia* 6: 189–191.

Jadhav, B. V., G. B. Shinde, and D. V. Sarwade. 1986. *Polypocephalus ratnagiriensis* sp. nov. (Cestoda: Lecanicephalidae) from *Trygon zugei*, India. *Indian Journal of Helminthology* 38: 88–92.

Jameson, H. L. 1912. Studies on pearl-oysters and pearls—The structure of the shell and pearls of the Ceylon pearl-oyster (*Margaritifera vulgaris* Schumacher): with an examination of the cestode theory of pearl production. *Proceedings of the Zoological Society of London* 2: 260–358.

Jensen, K. 2001. Four new genera and five new species of lecanicephalideans (Cestoda: Lecanicephalidea) from elasmobranchs in the Gulf of California, Mexico. *Journal of Parasitology* 87: 845–861.

Jensen, K. 2005. Tapeworms of elasmobranchs (Part I): a monograph on the Lecanicephalidea (Platyhelminthes, Cestoda). *Bulletin of the University of Nebraska State Museum* 18: 1–241.

Jensen, K. 2006. A new species of *Aberrapex* Jensen, 2001 (Cestoda: Lecanicephalidea) from *Taeniura lymma* (Forsskål) (Myliobatiformes: Dasyatidae) from off Sabah, Malaysia. *Systematic Parasitology* 64: 117–123.

Jensen, K. and J. N. Caira. 2006. The status of *Rhoptrobothrium* Shipley et Hornell, 1906 (Cestoda: Tetraphyllidea), with redescription of the type species, *R. myliobatidis*, and description of three new species from two species of *Aetomylaeus* (Myliobatiformes: Myliobatidae) from Malaysian Borneo. *Folia Parasitologica* 53: 189–207.

Jensen, K., J. N. Caira, J. J. Cielocha, D. T. Littlewood, and A. Waeschenbach. 2016. When proglottids and scoleces conflict: phylogenetic relationships and a family-level classification of the Lecanicephalidea (Platyhelminthes: Cestoda). *International Journal for Parasitology* 46(5): 281–310.

Jensen, K., K. R. Mojica, and J. N. Caira. 2014. A new genus and two new species of lecanicephalidean tapeworms from the striped panray, *Zanobatus schoenleinii* (Rhinopristiformes: Zanobatidae), off Senegal. *Folia Parasitologica* 61(5): 432–440.

Jensen, K., P. Nikolov, and J. N. Caira. 2011. A new genus and two new species of Anteroporidae (Cestoda: Lecanicephalidea) from the darkspotted numbfish, *Narcine maculata* (Torpediniformes: Narcinidae), off Malaysian Borneo. *Folia Parasitologica* 58(2): 95–107.

Jensen, K and S. L. Russell. 2014. *Seussapex*, a new genus of lecanicephalidean tapeworm (Platyhelminthes: Cestoda) from the stingray genus *Himantura* (Myliobatiformes: Dasyatidae) in the Indo-West Pacific with investigation of mode of attachment. *Folia Parasitologica* 61(3): 231–241.

Kankale, N. M. 2014. A new species of genus—*Tetragonocephalum pulensis* from a marine fish *Trygon zugei*. *Indian Journal of Applied Research* 4(4): 568–569.

Khamkar, D. D. 2011. A new species of *Tetragonocephalum panjiensis* N. Sp. (Eucestoda: Lecanicephalidae) from *Trygon zugei* at Panji, GoA, India. *Journal of Experimental Sciences* 2(4): 13–14.

Khamkar, D. D. and G. B. Shinde. 2012. A new species *Tetragonocephalum govindi* n.sp. (Eucestoda: Lecanicephalidea) from *Trygon zugei* at Panji, Goa, India. *Trends in Parasitology Research* 1(2): 22–24.

Koch, K. R., K. Jensen, and J. N. Caira. 2012. Three new genera and six new species of lecanicephalideans (Cestoda) from eagle rays of the genus *Aetomylaeus* (Myliobatiformes: Myliobatidae) from Northern Australia and Borneo. *Journal of Parasitology* 98(1): 175–198.

Lanka, L. P. 2006. A new lecanicephalidean cestode *Polypocephalus harnesis*, n. sp. from *Scomberomorus guttatus* at Ratnagiri (M.S.) India. *Rivista di Parassitologia* 22(3): 199–204.

Lanka, L., R. Hippargi, and S. R. Patil. 2013. A new *Tetragonocephalum sepheni* (Cestoda: Lecanicephalidae) from *Trygon sephen* at Ratnagiri in Maharashtra, India. *Journal of Entomology and Zoology Studies* 1(3): 11–14.

Last, P. R. and B. M. Manjaji-Matsumoto. 2008. *Himantura dalyensis* sp. nov., a new estuarine whipray (Myliobatoidei: Dasyatidae) from northern Australia. *CSIRO Marine and Atmospheric Research* 22: 283–292.

Last, P. R. and J. D. Stevens. 1994. Sharks and rays of Australia. *CSIRO*, Melbourne. 1st edition.

Linton, E. 1890. Notes on Entozoa of marine fishes of New England, with descriptions of several new species. Part II. United States Commission of Fish and Fisheries. Part XV. *Report of the Commissioner for 1887* 15: 718–899.

Linton, E. 1908. IX. Helminth fauna of the Dry Tortugas. I. Cestodes. *Papers from the Tortugas Laboratory of the Carnegie Institution of Washington* 1(102): 157–190.

Linton, E. 1916. Notes on two cestodes from the spotted sting-ray. *Journal of Parasitology* 3: 34–37.

Linton, E. 1924. Notes on cestode parasites of sharks and skates. *Proceedings of the United States National Museum* 64: 1–114.

López-Neyra, C. R. and C. Diaz-Ungria. 1958. Cestodes de Venezuela. V. Cestodes de vertebrados Venezolanos [segunda nota]. Novedades Cientificas Contribucciones ocasionales del Museo de Historia Natural La Salle, Caracas, Venezuela. *Serie Zoológica 2*3: 1–41.

Lovejoy, N. R. 1996. Systematics of myliobatid elasmobranchs: with emphasis on the phylogeny and historical biogeography of Neotropical freshwater stingrays (Potamotrygonidae: Rajiformes). *Zoological Journal of the Linnean Society* 117(3): 207–257.

Lovejoy, N. R., E. Bermingham, and A. P. Martin. 1998. Marine incursion into South America. *Nature* 396: 421–422.

Luchetti, N. M., F. P. Marques, and P. Charvet-Almeida. 2008. A new species of *Potamotrygonocestus* Brooks & Thorson, 1976 (Eucestoda: Tetraphyllidea) from *Plesiotrygon iwamae* Rosa, Castello & Thorson (Mylliobatoidea: Potamotrygonidae) and a redescription of *Potamotrygonocestus chaoi* Marques, Brooks & Araujo, 2003. *Systematic Parasitology* 70: 131–145.

Lucifora, L. O., M. R. Carvalho, P. M. Kyne, and W. T. White. 2015. Freshwater sharks and rays. *Current Biology Magazine*. Elsevier Ltd. 25: 3 pp.

MacCallum, G. A. 1917. Some new forms of parasitic worms. *Zoopathologica* 1: 43–75.

Maheswari, J. U., C. V. Lakshmi, and K. H. Rao. 1985. Studies on a new species of *Acanthobothrium* from *Dasyatis uarnak* (Forskal) from Waltair Coast. *Rivista di Parassitologia* 2: 39–44.

Maheswari, J. U., S. Sanaka, C. V. Lakshmi, and K. H. Rao. 1987. *Acanthobothrium waltairensis* n. sp. (Cestoda: Tetraphyllidea) parasite of *Dasyatis uarnak* (Pisces: Condrichtehyes [sic]) from India. *Revista Ibérica de Parasitología* 47: 33–36.

Manjaji, B. M. 1997. Elasmobranchs recorded from rivers and estuaries in Sabah. *Elasmobranch biodiversity, conservation and management: Proceedings of the International Seminar and Workshop, Sabah, Malasia*. 168–180.

Manjaji, B.M. 2004. Taxonomic and phylogenetic systematics of the Indo-Pacific whip-tailed stingray genus *Himantura* Müller & Henele 1837 [Chondrichthyes: Myliobatiformes: Dasyatidae]. *University of Tasmania*. Tasmania, Australia. Ph. D. dissertation.

Marques, F. P. 2000. Evolution of neotropical freshwater stingrays and their parasites: taking into account space and time. *University of Toronto*. Ph. D. dissertation.

Marques, F. P. and D. R. Brooks. 2003. Taxonomic Revision of *Rhinebothroides* (Eucestoda: Tetraphyllidea: Phyllobothriidae), Parasites of Neotropical Freshwater Stingrays (Rajiformes: Myliobatoidei: Potamotrygonidae). *Journal of Parasitology* 89(5): 994–1017.

Marques, F. P., D. R. Brooks, and R. Barriga. 1997. Six species of *Acanthobothrium* (Eucestoda: Tetraphyllidea) in stingrays (Chondrichthyes: Rajiformes: Myliobatoidei) from Ecuador. *Journal of Parasitology* 83(3): 475–484.

Marques, F. P., D. R. Brooks, and C. A. Lasso. 2001. *Anindobothrium* n. gen. (Eucestoda: Tetraphyllidea) inhabiting marine and freshwater potamotrygonid stingrays. *Journal of Parasitology* 87: 666–672.

Marques, F. P., D. R. Brooks, and S. Monks. 1995. Five new species of *Acanthobothrium* van Beneden, 1849 (Eucestoda: Tetraphyllidea: Onchobothriidae) in stingrays from the Gulf of Nicoya, Costa Rica. *Journal of Parasitology* 81(6): 942–951.

Marques, F. P. and J. N. Caira. 2016. *Pararhinebothroides*—neither the sister-taxon of *Rhinebothroides* nor a valid genus. *Journal of Parasitology* 102(2) 249–259.

Marques, F. P. and F. B. Reyda. 2015. *Rhinebothrium jaimei* sp. n. (Eucestoda: Rhinebothriidea: Rhinebothriidae): a new species from Neotropical freshwater stingrays (Potamotrygonidae). *Folia Parasitologica* 62: 1–9.

Martin, R. A. 2005. Conservation of freshwater and euryhaline elasmobranchs: a review. *Journal of the Marine Biological Association of the United Kingdom* 85: 1049–1073.

Mattiucci, S., A. Garcia, P. Cipriani, M. N. Santos, G. Nascetti, and R. Cimmaruta. 2014. Metazoan parasite infection in the scordfish, *Xiphias gladius*, from the Mediterranean Sea and comparison with Atlantic populations: implications for its stock characterization. *Parasite* 21: 1–13.

Mayes, M. A., D. R. Brooks, and T. B. Thorson. 1978. Two new species of *Acanthobothrium* van Beneden 1849 (Cestoidea: Tetraphyllidea) from freshwater stingrays in South America. *Journal of Parasitology* 64: 838–841.

Mayes, M. A., D. R. Brooks, and T. B. Thorson. 1981. Two new tetraphyllidean cestodes from *Potamotrygon circularis* Garman (Chondrichthyes: Potamotrygonidae) in the Itacuaí River, Brazil. *Proceedings of the Helminthological Society of Washington* 48(1): 38–42.

Menoret, A. and V. A. Ivanov. 2011. Descriptions of two new freshwater Neotropical species of *Rhinebothrium* (Cestoda: Rhinebothriidea) from *Potomotrygon motoro* (Chondrichthyes: Potamotrygonidae). *Folia Parasitologica* 58(3): 178–186.

Mohekar, A. D., H. K. Bhagwan, and C. J. Hiware. 2002. Biodiversity of cestode parasites parasitizing piscian host from Elasmobranchii collected along west coast of Maharashtra. *Uttar Pradesh Journal of Zoology* 22(1): 97–98.

Mojica, K. R., K. Jensen, and J. N. Caira. 2013. Revision of *Anteropora* (Cestoda: Lecanicephalidea) and descriptions of five new species from stingrays (Myliobatiformes: Dasyatidae) in Borneo. *Raffles Bulletin of Zoology* 61(2): 491–506.

Mojica, K. R., K. Jensen, and J. N. Caira. 2014. The ocellated eagle ray, *Aetobatus ocellatus* (Myliobatiformes: Myliobatidae), from Borneo and Northern Australia as host of four new species of *Hornellobothrium* (Cestoda: Lecanicephalidea). *Journal of Parasitology* 100(4): 504–515.

Monkolprasit, S. and T. R. Roberts. 1990. *Himantura chaophraya*, a new freshwater stingray from Thailand. *Japanese Journal of Ichthyology* 37(3): 5 pp.

Monks, S., D. R. Brooks, and G. P. Ponce de León. 1996. A new species of *Acanthobothrium* van Beneden, 1849 (Eucestoda: Tetraphyllidea: Onchobothriidae) in *Dasyatis longus* Garman (Chondrichthyes: Myliobatiformes: Dasyatididae) from Chamela Bay, Jalisco, Mexico. *Journal of Parasitology* 82(3): 484–488.

Mote, A. 2012. On new species of the genus *Polypocephlus* [sic], Braun, 1878 with its new species. *Journal of Experimental Sciences* 3(5): 35–37.

Muralidhar, A. 1988. A new cestode *Tetragonocephalum yamagutii* sp. n. (Cestoda: Tetragonocephalidae) from a marine fish, *Trygon walga* at Madras (east coast of India). *Rivista di Parassitologia* 5: 257–260.

Naylor, G. J., J. N. Caira, K. Jensen, K. A. Rosana, N. Straube, and C. Lakner. 2012a. Elasmobranch phylogeny: a mitochondrial estimate based on 595 species. J. C. Carrier, J. A. Musak, and M. R. Heithaus (Editors), The biology of sharks and their relatives. *CRC Press.* Boca Raton, FL. 31–57.

Naylor, G. J., J. N. Caira, K. Jensen, K. A. Rosana, W. T. White, and P. R. Last. 2012b. A DNA sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History* 367: 1–262.

Palm, H. W. 2004. The Trypanorhyncha Diesing, 1863. *PKSPL-IPB PRESS*, Bogor. 710 pp.

Piermarini, P. M. and D. H. Evans. 1998. Osmoregulation of the Atlantic Stingray (*Dasyatis sabina*) from the Freshwater Lake Jesup of the St. Johns River, Florida. *Physiological Zoology* 71(5): 7pp.

Pintner, T. 1928. Die sogenannte Gambothriidae, Linton, 1899. (partial translation by I. Schwink). *Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere* 50: 55–116.

Pleijel, F., U. Jondelius, E. Norlinder, A. Nygren, B. Oxelman, C. Schander, P. Sundberg, and M. Thollesson. Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution* 48: 369–371.

Pramanik, P. B. and B. Manna. 2006. *Polypocephalus himanshui* n.sp. (Cestoda: Lecanicephalidae) from *Rhynchobatus djeddensis* Forsskal, 1775 From Bay of Bengal at Digha Coast, West Bengal, India. *Indian Journal of Animal Health* 45(2): 125–138.

Pramanik, P. B. and B. Manna. 2007. Six new and two known species of the genus *Tylocephalum* Linton, 1890 (Cestoida: Lecanicephalidae) in cartilageous fishes from Bay of Bengal at Digha coastal waters, West Bengal, India. *Journal of Natural History* 3(2): 12–22.

Ramadan, M. M. 1984. A review of the cestode genus *Rhinebothrium* Linton, 1889 (Tetraphyllidae), with a description of two new species of the sting ray *Taeniura lymma* from the Red Sea. *Journal of the Egyptian Society of Parasitology* 14: 85–94.

Ramadan, M. M. 1986. Cestodes of the genus *Cephalobothrium* Shipley and Hornel, 1906 (Lecanicephaliidae), with description of *C. ghardagense* n. sp. and *C. taeniurai* n. sp. from the Red Sea fishes. *Japanese Journal of Parasitology* 35: 11–15.

Rego, A. A. 1979. Contribuição ao conhecimento dos helmintos de raias fluviais Paratrygonidae. *Revista Brasileira de Biologia* 39(4): 879–890.

Rego, A. A. and A. P. Dias. 1976. Estudos de cestóides de peixes do Brasil. 3.a Nota: Cestóides de raias fluviais Paratrygonidae. *Revista Brasileira de Biologia* 36: 941–956.

Reyda, F. B. 2007. Patterns of diversity and host specificity in the cestodes of Neotropical freshwater stingrays. *University of Connecticut*. Storrs, Connecticut, USA. Ph. D. dissertation.

Reyda, F. B. 2008. Intestinal helminths of freshwater stingrays in southeastern Peru, and a new genus and two new species of cestode. *Journal of Parasitology* 94(3): 684–699.

Reyda, F. B. and J. N. Caira. 2006. Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from *Himantura uarnacoides* (Myliobatiformes: Dasyatidae) in Malaysian Borneo. *Comparative Parasitology* 73(1): 49–71.

Reyda, F. B. and F. P. Marques. 2011. Diversification and species boundaries of *Rhinebothrium* (Cestoda; Rhinebothriidea) in South American freshwater stingrays (Batoidea; Potamotrygonidae). *PLOS One* 6(8): 1–26.

Ride, W. D., H. G. Cogger, C. Dupuis, O. Kraus, A. Minella, F. C. Thompson, and P. K. Tubbs. 1999. International Code of Zoological Nomenclature. 4th edition. The International Trust for Zoological Nomenclature. *The Natural History Museum.* London, England. 306 pp.

Roberts, T. R. 1998. Francis Hamilton and the freshwater stingrays described in his Gangetic fishes (1822). *Archives of Natural History* 25(2): 267–279.

Ruhnke, T. R., J. N. Caira, and A. Cox. 2015. The cestode order Rhinebothriidea no longer family-less: a molecular phylogenetic investigation with erection of two new families and description of eight new species of *Anthocephalum*. *Zootaxa* 3904(1): 051–081.

Sarada, S., C. V. Lakshmi, and K. H. Rao. 1993. Studies on a new species of *Cephalobothrium stegostomi* (Cestoda: Lecanicephalidea) from *Stegostoma fasciatum* from Waltair coast. *Indian Journal of Parasitology* 17: 1–4.

Schaeffner, B.C. and I. Beveridge. 2014. The trypanorhynch cestode fauna of Borneo. *Zootaxa* 3900(1): 21–49.

Sezaki, K., R. A. Begum, P. Wongrat, M. P. Srivastava, S. SriKantha, K. Kikuchi, H. Ishihara, S. Tanaka, T. Taniuchi, and S. Watabe. 1999. Molecular phylogeny of Asian freshwater and marine stingrays based on the DNA nucleotide and deduced amino acid sequences of the cytochrome *b* gene. *Fisheries Science* 65(4): 563–570.

Shinde, G. B. and R. A. Deshmukh. 1979. On a new species of *Flapocephalus* Deshmukh R. A., 1977 (Cestoda: Lecanicephalidae Braun, 1900) from a marine fish at Veraval, west coast of India. *Rivista di Parassitologia* 40: 295–298.

Shinde, G. B., B. B. Dhule, and D. H. Jadhav. 1991. *Polypocephalus bombayensis* sp. nov. (Cestoda: Lecanicephalidae) from a marine fish *Aetobatus flagellum* at Kakinada (A. P.), India. *Indian Journal of Helminthology* 43: 77–79.

Shinde, G. B. and B. V. Jadhav. 1981. Four new species of the genus *Polypocephalus* Braun, 1878 (Cestoda: Lecanicephalidae) from the marine fishes of India. *Indian Journal of Parasitology* 5: 1–7.

Shinde, G. B. and D. H. Jadhav. 1990. Two species of the genus *Tetragonocephalum* (Cestoda: Tetragonocephalidae) at Ratnagiri, (M. S.), India. *Indian Journal of Parasitology* 14(1): 9–12.

Shinde, G. B., A. D. Mohekar, and B. V. Jadhav. 1985. Two new species of the genus *Tetragonocephalum* Shipley and Hornell, 1905 (Cestoda: Lecanicephalidea) from west coast of India. *Indian Journal of Parasitology* 9: 79–82.

Shipley, A. E. 1900. A description of the Entozoa collected by Dr. Willey during his sojourn in the Western Pacific. *Zoological Results based on material from New Britain, New Guinea, Loyalty Islands and elsewhere, collected during the years 1895, 1896, and 1897* 5: 531–568.

Shipley, A. E. and J. Hornell. 1904. The parasites of the pearl oyster. *Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar (Herdman), Part 2.* 11: 77–106.

Shipley, A. E. and J. Hornell. 1905. Further report on parasites found in connection with the pearl oyster fishery at Ceylon. *Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar (Herdman), Part 3*. 49–56.

Shipley, A. E. and J. Hornell. 1906. Report on the cestode and nematode parasites from the marine fishes of Ceylon. *Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar (Herdman), Part 5*. 43–96.

Southwell, T. 1911. Description of nine new species of cestode parasites, including two new genera from marine fishes of Ceylon. *Ceylon Marine Biological Reports* 1: 216–225.

Southwell, T. 1912. A description of ten new species of cestode parasites from marine fishes of Ceylon, with notes on other cestodes from the same region. *Ceylon Marine Biological Report* 1: 259–278.

Southwell, T. 1925. A monograph on the Tetraphyllidea with notes on related cestodes. *Memoirs of the Liverpool School of Tropical Medicine (New Series)* 2: 1–368.

Subhapradha, C. K. 1951. On the genus *Polypocephalus* Braun, 1878 (Cestoda), together with descriptions of six new species from Madras. *Proceedings of the Zoological Society of London* 121: 205–235.

Subhapradha, C. K. 1955. Cestode parasites of fishes of Madras Coast. *Indian Journal of Helminthology* 7(2): 41–132.

Twohig, M. E., J. N. Caira, and C. A. Fyler. 2008. Two new cestode species from the dwarf whipray, *Himantura walga* (Batoidea: Dasyatidae), from Borneo, with comments on site and mode of attachment. *Journal of Parasitology* 94(5): 1118–1127.

Vankara, A. P., C. V. Lakshmi, and J. V. Lakshmi. 2007. *Polypocephalus viskhapatnamensis* sp. nov. (Lecanicephalidea: Polypocephalidae) from *Himantura uarnak* (Forsskål) and *Dasyatis* (Amphotistius) *zugei* (Müller & Henle) from Visakhapatnam coast. *Journal of Parasitic Diseases* 31(2): 152–154.

Vardo-Zalik, A. M. and R. A. Campbell. 2011. Five new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Tetraphyllidea) in elasmobranchs from the northwest Atlantic and Gulf of Mexico with first records from smooth-hound sharks and guitarfish. *Zootaxa* 2838: 41–64.

Verma, S. C. 1928. Some cestodes from Indian fishes, including four new species of Tetraphyllidea and revised keys to the genera *Acanthobothrium* and Gangesia. *Allahabad University Studies* 4: 119–176.

Vidthayanon, C., I. Baird, and Z. Hogan. 2013. *Himantura polylepis*. The IUCN Red List of Threatened Species 2013: e.T195320A8956611. http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T195320A8956611.en. Accessed on 29 March 2016.

Vijayalakshmi, C. and S. Sarada. 1995. Studies on the new species *Tylocephalum chiralensis*, parasite from *Dasyatis* (Himantura) *uarnak* (Förskal) from Chirala coast, Andhra Pradesh, India. *Boletín Chileno de Parasitología* 50: 73–75.

Waeschenbach, A., B. L. Webster, and D. T. Littlewood. 2012. Adding resolution to ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with large fragments of mtDNA. *Molecular Phylogenetics and Evolution* 63(3): 834–847.

Wang, P. Q. 1984. Notes on some cestodes of fishes in Fujian Province, with a list of fish cestodes recorded from China. *Wuyi Science Journal* 4: 71–84.

Wang, Y. and W. Yang. 2001. *Rhinebothrium xiamenesis* n. sp. (Eucestoda: Tetraphyllidea) in *Dasyatis zugei* from the coast of Xiamen, China. *Journal of Parasitology* 87(1): 185–187.

Wankhede, H. J. 1990. *Tetragonocephalum janardane* n. sp. (Cestoda: Tetragonocephalidae) from *Trygon zugei*. *Proceedings of the Seventy-seventh annual session of the Indian Science Congress Association Part III*. Abstract.

Wardle, R. A. and J. A. McLeod. 1952. The Zoology of Tapeworms. *University of Minnesota Press*. Minneapolis, Minnesota. 780 pp.

White, W. T., P. R. Last, G. J. Naylor, K. Jensen, and J. N. Caira. 2010. Clarification of *Aetobatus ocellatus* (Kuhl, 1823) as a valid species, and a comparison with *Aetobatus narinari* (Euphrasen, 1790) (Rajiformes: Myliobatidae). *CSIRO Marine & Atmospheric Research Paper* 32: 141–164.

Williams, H. H. 1962. *Acanthobothrium* sp. nov. (Cestoda: Tetraphyllidea) and a comment on the order Biporophyllaeidea. *Parasitology* 52: 67–76.

Williams, H. H. 1964. Some new and little known cestodes from Australian elasmobranchs with a brief discussion on their possible use in problems of host taxonomy. *Parasitology* 54: 737–748.

Williams, H. H. 1968. *Acanthobothrium quadripartitum* sp. nov. (Cestoda: Tetraphyllidea) from *Raja naevus* in the North Sea and English Channel. *Parasitology* 58: 105–110.

Yamaguti, S. 1934. Studies on the helminth fauna of Japan. Part 4. Cestodes of fishes. *Japanese Journal of Zoology* 6: 1–112.

Yamaguti, S. 1952. Studies on the helminth fauna of Japan. Part 49. Cestodes of fishes, II. *Acta Medicinae Okayama* 8: 1–76.

Yamaguti, S. 1960. Studies on the helminth fauna of Japan: Part 56. Cestodes of fishes, III. *Publications of the Seto Marine Biological Laboratory* 8: 41–50.

Yang, W., G. Lui, and Y. Lin. 1995. Two new species of cestode (Lecanicephalidea: Lecanicephalidae) from marine fishes in Xiamen, South Fujian, P. R. of China. (in Chinese, English abstract). *Journal of Xiamen University (Natural Science)* 34: 109–112.

Yano, K., A. Ahmad, A. C. Gambang, A. H. Idris, A. R. Solahuddin, and A. Aznan. 2005. Sharks and Rays of Malaysia and Brunei Darussalam. *Southeast Asian Fisheries Development Center*. Kuala Terengganu. 241 pp.

Zaidi, D. A. and D. Khan. 1976. Cestodes of fishes from Pakistan. *Biologia* 22: 157–179.

Zeidan, G. C., M. D. Luz, and G. Boehs. 2012. Parasites of economically important bivalves from the southern coast of Bahia State, Brazil. *Revista Brasileira de Parasitologia Veterinária* 21(4): 391–398.