



Phylogenetic analysis and diversity of peculiar new lecanicephalidean tapeworms (Eniochobothriidae) from cownose rays across the globe

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ABSTRACT

The three members of the lecanicephalidean tapeworm family Eniochobothriidae are unusual among tapeworms in that they lack a vagina and possess a series of expanded proglottids forming a trough at the anterior end of their body. They exclusively parasitise cownose rays of the genus *Rhinoptera* (Myliobatiformes: Rhinopteridae). New collections from six of the nine known species of cownose rays from the waters off Australia, Mexico, Mozambique, Senegal, Taiwan and the United States (off Mississippi, Louisiana and South Carolina) yielded eight new species and a new genus of eniochobothriids. Here we erect *Amiculucestus*, gen. nov. and describe six of the eight new species – four in the new genus and two in *Eniochobothrium* – expanding the number of genera in the family to two and the number of described species in the family to nine. Morphological work was based on light and scanning electron microscopy. The tree resulting from a maximum likelihood analysis of sequence data for the D1–D3 region of the 28S rDNA gene for 11 species of eniochobothriids supports the reciprocal monophyly of both genera. The mode of attachment to the mucosal surface of the spiral intestine of the host was investigated using histological sections of worms *in situ*. These cestodes appear to use the anterior trough-like portion of their body, which consists of an unusual series of barren proglottids, rather than their scolex, to attach to the mucosal surface. Based on our new collections, we estimate that the total number of eniochobothriids across the globe does not exceed 27 species.

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Introduction

Our knowledge of cestodes parasitising cownose rays of the genus *Rhinoptera* Cuvier (Rhinopteridae Jordan & Evermann) has expanded considerably with the recent works of [Stephan and Caira \(2022\)](#) on the ‘tetraphyllidean’ genus *Duplicibothrium* Williams & Campbell, 1978, and [Herzog and Jensen \(2022\)](#) on the trypanorhynch genus *Rhinoptericola* Carvajal & Campbell, 1975. Beyond describing five new cestode species, these studies have also provided clarity on the identities of specimens of *Rhinoptera* collected globally as part of parasitologically focused survey work over the past two decades. Eight species of *Rhinoptera* are currently recognised as valid ([Last et al. 2016](#)). The tree resulting from a neighbour-joining analysis of sequence data for the *NADH2* gene by [Naylor et al. \(2012\)](#) provided evidence of the possible existence of a ninth species among specimens of *Rhinoptera jayakari* Boulenger based on the existence of two subgroups among specimens originally identified as this species. This possibility was echoed by [Last et al. \(2016\)](#). Sequence data generated here for this same gene place the specimens of *Rhinoptera* we collected from Mozambique in one of these subgroups and the specimen we collected from Taiwan in the other. Given the type locality of *R. jayakari* is the Gulf of Oman, and one of the subgroups includes a specimen from

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the Persian Gulf, we refer to host specimens in that subgroup, which includes the specimens from Mozambique, as *R. jayakari* and refer to those in the other subgroup, which includes the specimen from Taiwan, as *R. cf. jayakari*.

With the identities of species of *Rhinoptera* resolved, the initial goal of this study was to describe six morphologically distinctive species of cestodes in the order Lecanicephalidea that we found parasitising six of the nine species of *Rhinoptera* collected from the waters off Australia, Mexico, Mozambique, Senegal, Taiwan and the United States (off Mississippi, Louisiana and South Carolina) from 1997 to 2016. We initially considered these small cestodes all to belong to *Eniochobothrium* Shipley & Hornell, 1906, the only genus in the family Eniochobothriidae Jensen, Caira, Cielocha, Littlewood & Waeschenbach (see Jensen *et al.* 2016), currently comprising three species. Somewhat unexpectedly, morphological evidence and the results of a phylogenetic analysis of sequence data for the D1–D3 region of the 28S rRNA gene (hereafter referred to as 28S) suggest that a subset of the species belong to a second related, but distinct, genus. Two new species of *Eniochobothrium* are described. *Eniochobothrium qatarse* Al Kawari, Saoud & Wanas, 1994 is redescribed based on new material from *R. jayakari* and *R. cf. jayakari* collected off Mozambique and Taiwan respectively. The generic diagnosis of *Eniochobothrium* of Jensen (2005) is revised to accommodate the new species. A new genus is established to house the four other new species, each of which is also described below.

Materials and methods

Host collections

Worms used in this study were collected from the following 12 specimens of cownose rays (Rhinopteridae) representing six species: one female specimen of *Rhinoptera bonasus* (Mitchell) (host accession number CH-18; 91-cm disc width, DW) collected 17 June 2013 in Charleston Harbor, SC, USA (32°45'2.53"N, 79°53'48.28"W); four specimens of *Rhinoptera brasiliensis* Müller, consisting of one male (host accession number MS05-49; 92 cm DW) collected 19 June 2005 in the Gulf of Mexico off the south side of East Ship Island, MS, USA (30°14'24.54"N, 88°52'25.25"W), one female (host accession number MS05-298; 97 cm DW) collected 25 April 2006 in the Gulf of Mexico off the west tip of Horn Island, MS, USA (30°14'37.70"N, 88°46'37.62"W), one of unknown sex and size (host accession number MS05-375) collected 27 August 2006 in the Gulf of Mexico off the west of south tip of Chandeleur Islands, LA, USA (29°57'9.54"N, 88°50'38.98"W), and one female (host accession number MX-73; 101 cm DW) collected 26 October 2016 in the Gulf of Mexico off Campeche, Mexico; two female specimens of *Rhinoptera jayakari* (host accession numbers MZ-1 and MZ-4; 85 and 92 cm DW respectively) collected 23 June

2016 in the Mozambique Channel off Tofo, Inhambane, Mozambique (23°47'33.02"S, 35°31'16.38"E); one female specimen of *Rhinoptera cf. jayakari* (host accession number TW-17; 67 cm DW) collected 12 May 2005 in the Taiwan Strait off Magong, Penghu Island, Taiwan (23°33'49"N, 119°34'31"E); two male specimens of *Rhinoptera marginata* (Geoffroy Saint-Hilaire), one (host accession number SE-80; 72 cm DW) collected 12 January 2003 in the Atlantic Ocean off Saint Louis, Senegal (16°01'28"N, 16°30'33"W), the other (host accession number SE-231; 78.8 cm DW) collected 10 January 2004 in the Atlantic Ocean off Joal, Senegal (14°10'30"N, 16°51'12"W); and two specimens *Rhinoptera neglecta* Ogilby, one male and one female (host accession numbers AU-85 and AU-87; 138 and 129 cm DW respectively), collected 11 August 1997 in Fog Bay, Timor Sea, off Dundee Beach, NT, Australia (12°45'33"S, 130°21'7"E). Additional details about host specimens and their collecting localities are available by searching on host accession numbers (e.g. collection code 'CH' and collection number '18' for CH-18) in the Global Cestode Database (www.elasmobranchs.tapewormdb.uconn.edu). Host identifications were verified using *NADH2* sequence data following Naylor *et al.* (2012). Host taxonomy follows Last *et al.* (2016).

In each case, the spiral intestine was removed through a mid-ventral incision of the body, opened with a longitudinal incision and rinsed with sea water. A subset of the worms encountered was fixed in 95% ethanol for molecular work and the remaining gut contents were fixed in 10% seawater-buffered formalin. In most cases, the spiral intestine was subsequently fixed in 10% seawater-buffered formalin. All formalin-fixed material was later transferred to 70% ethanol for storage.

Morphological methods and terminology

Cestode specimens prepared as whole mounts were hydrated in distilled water, stained with Delafield's hematoxylin, differentiated in tap water, destained in 70% acid ethanol, neutralised in 70% basic ethanol, dehydrated in a graded series of ethanol concentrations, cleared with methyl salicylate and mounted in Canada balsam on glass slides.

Cestode specimens prepared for histology were stained in Fast Green, dehydrated in a graded series of ethanol concentrations, cleared in xylene and embedded in paraffin. Serial sections were cut using an Olympus Cut 4060 retracting rotary microtome (Triangle Biomedical Sciences, Durham, NC, USA) at a thickness of 5–7 µm, floated on a 2.5% sodium silicate solution on glass slides and allowed to dry on a slide warmer set to 40°C. Sections were subsequently deparaffinised in xylene, hydrated in a graded series of ethanol concentrations, stained with Delafield's hematoxylin, differentiated in Scott's solution, counter-stained with eosin, dehydrated in a graded series of ethanol concentrations, cleared in xylene and mounted in Canada balsam. Pieces of spiral intestine with cestode specimens *in situ* were prepared following the same protocol.

One specimen of *Eniochobothrium qatarense* from *Rhinoptera* cf. *jayakari* from Taiwan was embedded in glycol methacrylate (GMA) for histology as follows. The specimen was dehydrated in a graded series of ethanol concentrations, transferred to a 1:1 solution of 100% ethanol and Technovit H7100 infiltrating resin (Heraeus Kluser GmbH, Wehrheim, Germany) for 2 h, moved to pure infiltrating resin and refrigerated at 4°C overnight. The specimen was subsequently embedded in Technovit H7100 in plastic block holders. Serial sections were cut using the Olympus Cut 4060 retracting rotary microtome at a thickness of 2–4 µm floated on drops of distilled water on Fisherbrand Superfrost Plus charged microscope slides (Fisher Scientific, Pittsburgh, PA, USA) and allowed to air-dry. Sections were stained with Delafield's hematoxylin, differentiated in Scott's solution, rinsed with distilled water, dried for ~2 min in a 60°C oven, counter-stained in eosin, dehydrated in a graded series of ethanol concentrations, dried for ~2 min in a 60°C oven and then mounted in Canada balsam.

For the preparation of semi-permanent mounts of eggs, whole worms were placed in lactophenol for 4 h. Worms were subsequently placed in a drop of lactophenol on a glass slide, the terminal proglottid broken open with insect pins to release the eggs and the eggs were mounted in lactophenol under a cover slip. To seal, cover slips were ringed twice with nail polish.

For examination with scanning electron microscopy (SEM), cestode specimens were hydrated in distilled water, transferred to 1% osmium tetroxide and left overnight at 4°C, dehydrated in a graded series of ethanol concentrations and placed in hexamethyldisilazane (HMDS, Electron Microscopy Sciences, Hatfield, PA, USA) for 30 min. Following removal of the excess HMDS, specimens were allowed to air-dry, mounted on aluminum stubs with double-sided adhesive carbon dots (PELCO tabs, Ted Pella, Inc., Redding, CA, USA), sputter-coated with 30–35 nm of gold and examined with a Hitachi S4700 field emission scanning electron microscope (Hitachi, Schaumburg, IL, USA) at the Microscopy and Analytical Imaging Research Resource Laboratory, University of Kansas, Lawrence, KS, USA. If only a portion of a worm was prepared as histological sections or for examination with SEM, the remainder of the worm was prepared as a whole-mounted voucher as described above.

Measurements were made using the image analysis software Infinity Analyze (ver. 7.0.2.920, Teledyne Lumenera, Ottawa, ON, Canada) with an Infinity 3-6URC USB camera (Teledyne Lumenera) attached to a Zeiss Axioskop 2 plus compound microscope (Zeiss, Thornwood, NY, USA). Unless noted otherwise, measurements are given in micrometres. In the text, measurements and counts are given as the range. In Tables 1 and 2, measurements and counts are given as the range (mean ± s.d.; number of worms measured; number of measurements, if more than one measurement was taken per worm). Measurements of reproductive organs were taken from mature proglottids unless noted otherwise. Photomicrographs

of histological sections and eggs were taken using the same compound microscope and camera. The photomicrograph of the worm attached was taken using an Infinity 5-5C USB camera (Teledyne Lumenera) attached to a Leica MZ16 stereo-microscope (Leica Microsystems Inc., Buffalo Grove, IL, USA).

The unusual morphology of eniochobothriids requires some explanation if their descriptions are to be understood in the context of those of other elasmobranch tapeworms. The body of eniochobothriids consists of a scolex and strobila. The tiny scolex is typically only tenuously attached to the strobila and thus it is common for worms to lack a scolex. The anterior region of the strobila bears a series of laterally expanded proglottids that appear to function solely in attachment; there is no evidence that these proglottids ever develop reproductive organs. The region of the strobila bearing these laterally expanded barren proglottids is concave, forming a trough. The trough is followed by a series of typical reproductive proglottids, with the most immature located on the portion of the strobila immediately posterior to the trough and the most developed located at the terminal end of the strobila. At any single point in time, worms typically bear only a small number of reproductive proglottids in which the reproductive organs are visible; development of the reproductive organs appears to be rapid, rather than gradual. Some species are highly protandrous, which can lead to the false impression that some worms are male and others female. If meaningful comparisons are to be made among species, the measurements presented need to reflect all of these aspects of the morphology and biology of these worms. As a consequence, worm length is reported both as total length (i.e. with the scolex) and strobilar length (i.e. without the scolex). Proglottid numbers are presented as total number of proglottids, number of barren proglottids comprising the trough, and number of reproductive proglottids. Measurements of proglottid features are presented separately for mature terminal proglottids and gravid terminal proglottids. In the case of species in the new genus, measurements are also presented separately for proglottids in which the male reproductive system is mature and for those in which the female reproductive system is mature.

Terminology for microtriches follows Chervy (2009). Museum abbreviations used throughout are as follows: LRP, Lawrence R. Penner Parasitology Collection, University of Connecticut, Storrs, CT, USA; QM, Queensland Museum, Invertebrate Collection, Worms & Echinoderms Department, Brisbane, Qld, Australia; NMNS, National Museum of Natural Science, Zoology Department, Taichung, Taiwan; USNM, National Museum of Natural History, Smithsonian Institution, Department of Invertebrate Zoology, Washington, DC, USA.

Molecular methods

Sequence data for 28S were generated for a total of 15 specimens of eniochobothriids using Sanger sequencing. Genomic DNA was extracted from a portion of the strobila

Table 1. Measurements for *Eniochobothrium qatarense* and new species of *Eniochobothrium*.

Cestode species	<i>Eniochobothrium qatarense</i> Al Kawari, Saoud & Wanas, 1994	<i>Eniochobothrium overstreeti</i> , sp. nov.	<i>Eniochobothrium vegrande</i> , sp. nov.
Host species	<i>Rhinoptera jayakari</i> (TH); <i>Rhinoptera</i> cf. <i>jayakari</i>	<i>Rhinoptera brasiliensis</i>	<i>Rhinoptera</i> cf. <i>jayakari</i>
Locality	Mozambique, Mozambique Channel; Penghu Island, Taiwan, Taiwan Strait	Mississippi, USA, Gulf of Mexico; Campeche, Mexico, Gulf of Mexico	Penghu Island, Taiwan, Taiwan Strait
Mature worm length (with scolex)	2886–5234 (3694 ± 105.9; 4)	961–1215 (1072 ± 130.1; 3)	1304–1610 (<i>n</i> = 2)
Mature strobila length	2821–5084 (3619 ± 1012.6; 4)	863–1148 (1019 ± 142; 4)	1130–1480 (1296 ± 150.8; 5)
Number of proglottids (in mature worms)	39–50 (44 ± 5.1; 4)	27–29 (28 ± 1.0; 4)	22–26 (24 ± 1.7; 5)
Gravid worm length (with scolex)	3706–7480 (4973 ± 947.7; 15)	1230–1798 (1505 ± 219.2; 7)	1286–1840 (1597 ± 257.5; 4)
Gravid strobila length	3623–7458 (4857 ± 1001.6; 15)	1144–1692 (1403 ± 215.4; 7)	1147–1708 (1470 ± 273.9; 4)
Number of proglottids (in gravid worms)	27–45 (37 ± 5.8; 14)	23–33 (29 ± 3.6; 7)	21–23 (22 ± 1.2; 3)
Scolex length	68–169 (116 ± 37.7; 14)	82–105 (95 ± 8.2; 9)	136–184 (151 ± 17.8; 6)
Scolex width	73–115 (98 ± 14.1; 19)	71–89 (79 ± 5.8; 9)	68–101 (90 ± 12.6; 6)
Acetabula length	42–54 (47 ± 3.0; 8; 35)	33–47 (39 ± 4; 10; 20)	41–54 (48 ± 4.7; 6; 12)
Acetabula width	28–45 (39 ± 4.4; 18; 32)	27–39 (31 ± 3.4; 10; 18)	28–41 (34 ± 4.1; 6; 11)
Apical organ length	24–50 (35 ± 6.2; 17)	31–47 (37 ± 5.7; 10)	48–60 (55 ± 5.1; 6)
Apical organ width	17–30 (23 ± 3.4; 17)	24–29 (26 ± 1.6; 10)	25–30 (28 ± 1.9; 6)
Number of barren proglottids forming trough	16–24 (19 ± 2.3; 19)	15–21 (17 ± 1.6; 11)	11–15 (14 ± 1.6; 8)
Trough length	613–888 (754 ± 79.9; 19)	244–420 (307 ± 47.6; 11)	272–469 (363 ± 88.6; 8)
Trough width	456–852 (655 ± 87.4; 19)	131–296 (242 ± 48.6; 10)	118–197 (161 ± 26.9; 8)
Number of reproductive proglottids in mature worms	21–28 (25 ± 3.0; 4)	9–11 (11 ± 1.0; 4)	9–12 (10 ± 1.1; 5)
Number of reproductive proglottids in gravid worms	6–26 (17 ± 6.5; 15)	8–15 (11 ± 2.1; 7)	8–11 (10 ± 1.3; 4)
Number of immat. proglottids	3–25 (16 ± 6.7; 19)	8–14 (10 ± 1.5; 11)	6–10 (8 ± 1.2; 9)
Posterior-most immat. proglottid length	168–460 (281 ± 80.3; 19)	108–375 (196 ± 93.0; 11)	58–137 (90 ± 26.3; 9)
Posterior-most immat. proglottid width	313–499 (384 ± 53.0; 19)	188–230 (208 ± 12.9; 11)	129–187 (152 ± 21.1; 9)
Number of mature proglottids	1–3 (2 ± 0.5; 19)	1 (<i>n</i> = 4) (in mature worms); 0 (<i>n</i> = 7) (in gravid worms)	1–2 (1 ± 0.5; 9)
Terminal mature proglottid length	1073–1525 (1327 ± 215.3; 4)	482–647 (570 ± 83.5; 4)	448–617 (559 ± 69.6; 5)
Terminal mature proglottid width	395–472 (436 ± 35.9; 4)	204–223 (215 ± 8.1; 4)	172–215 (186 ± 18.1; 5)
Posterior-most mature proglottid length in gravid worms	776–1493 (1051 ± 204.3; 15)	–	171–294 (234 ± 50.2; 4)
Posterior-most mature proglottid width in gravid worms	399–733 (524 ± 83.0; 15)	–	144–176 (162 ± 14.1; 4)
Number of gravid proglottids	1–2 (1 ± 0.3; 14)	1 (<i>n</i> = 7)	1 (<i>n</i> = 4)
Terminal gravid proglottid length	1543–3393 (2181 ± 471.5; 15)	645–881 (771 ± 87.1; 7)	632–838 (744 ± 102.9; 4)
Terminal gravid proglottid width	411–665 (531 ± 62.7; 15)	222–316 (267 ± 36.5; 7)	186–216 (206 ± 13.9; 4)
Number of testes	29–50 (41 ± 5.9; 19; 26)	20–29 (23 ± 2.6; 10)	14–17 (16 ± 1.3; 8)
Testes length	21–67 (33 ± 9.9; 19; 33)	16–40 (27 ± 6.5; 11; 33)	13–25 (18 ± 3.4; 5; 15)
Testes width	11–53 (24 ± 8.6; 19; 33)	17–51 (33 ± 9.1; 11; 33)	11–32 (17 ± 5.4; 5; 15)

(Continued on next page)

Table 1. (Continued)

Cestode species	<i>Eniochobothrium qatarense</i> Al Kawari, Saoud & Wanas, 1994	<i>Eniochobothrium overstreeti</i> , sp. nov.	<i>Eniochobothrium vegrande</i> , sp. nov.
Host species	<i>Rhinoptera jayakari</i> (TH); <i>Rhinoptera cf. jayakari</i>	<i>Rhinoptera brasiliensis</i>	<i>Rhinoptera cf. jayakari</i>
Locality	Mozambique, Mozambique Channel; Penghu Island, Taiwan, Taiwan Strait	Mississippi, USA, Gulf of Mexico; Campeche, Mexico, Gulf of Mexico	Penghu Island, Taiwan, Taiwan Strait
Number of aporal testes	22–37 (30 ± 3.9; 19; 26)	14–23 (17 ± 2.5; 11)	10–14 (12 ± 1.2; 8)
Number of preaporal testes	0 (n = 19)	0 (n = 11)	0 (n = 8)
Number of postaporal testes	7–18 (12 ± 3.4; 19; 26)	5–7 (6 ± 0.7; 10)	4–6 (5 ± 1.1; 8)
Cirrus sac length	603–843 (755 ± 64.5; 18)	307–331 (316 ± 10.7; 4)	305–422 (346 ± 45.6; 5)
Cirrus sac width	100–148 (118 ± 12.5; 18)	47–55 (53 ± 3.7; 4)	45–60 (52 ± 5.8; 5)
Ovary width	195–328 (256 ± 41.8; 18)	101–139 (117 ± 16; 4)	77–143 (105 ± 27.6; 5)
Ovarian lobe length	126–504 (305 ± 110.7; 17; 33)	129–232 (181 ± 47; 4; 8)	92–214 (156 ± 41.9; 5; 10)
Genital pore position in posterior-most mature proglottid (%)	71–84 (78 ± 3.1; 19)	75–82 (78 ± 3.1; 4)	55–79 (70 ± 9.2; 9)
Aporal field of vitelline follicles length	172–597 (377 ± 105.0; 17)	104–184 (145 ± 35.5; 4)	133–176 (150 ± 18.2; 5)
Aporal field of vitelline follicles width	78–184 (128 ± 28.6; 17)	45–64 (56 ± 8; 4)	36–69 (45 ± 13.6; 5)
Poral field of vitelline follicles length	188–536 (328 ± 85.7; 16)	92–139 (116 ± 25.2; 4)	98–138 (118 ± 16.5; 5)
Poral field of vitelline follicles width	97–182 (136 ± 34.7; 16)	42–62 (54 ± 9.7; 4)	42–67 (53 ± 11; 5)
Oncosphere length	14–21 (17 ± 1.4; 3; 28)	11–17 (13 ± 1.6; 2; 17)	–
Oncosphere width	9–15 (12 ± 1.5; 3; 28)	9–14 (11 ± 1.5; 2; 17)	–
Cocoon length	93–120 (106 ± 8.0; 3; 19)	70–92 (81 ± 8.2; 2; 9)	–
Cocoon width	57–100 (76 ± 9.8; 3; 19)	54–71 (61 ± 5.4; 2; 9)	–
Number of oncospheres per cocoon	45–74 (60 ± 7.6; 3; 16)	21–33 (26 ± 3.3; 2; 9)	–

TH, type host.

using a MasterPure Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) and eluted in 60 µL of TE buffer. 28S was amplified using illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare, Chicago, IL, USA) and the primer pair ZX-1 (5′-ACCCGCTGAATTTAA GCATAT-3′) (modified from van der Auwera *et al.* 1994) and 1500R (5′-GCTATCCTGAGGGAACTTCG-3′) (Olson *et al.* 2003; Tkach *et al.* 2003) with a thermocycler temperature profile as follows: 2 min of denaturation at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 55°C and 2 min at 72°C, and 10 min or elongation at 72°C. Both strands were cycle sequenced on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using ABI Big Dye dioxo terminators (ver. 3.1) at the University of Connecticut or by ACGT, Inc. (Wheeling, IL, USA) using a single pass primer extension. Contigs were assembled using Geneious Prime 2019 (Biomatters, Inc., San Diego, CA, USA).

Phylogenetic methods

In addition to the 28S sequence data for the 15 specimens of eniochobothriids generated *de novo* in this study, the

phylogenetic analysis included 28S sequence data downloaded from GenBank for seven specimens originally identified as species of *Eniochobothrium* (KU249056, KF685859, AF286928, KF685860, GQ470202, GQ470201 and KU249055) from Olson *et al.* (2001), Jensen and Bullard (2010), Caira *et al.* (2014) and Jensen *et al.* (2016). Also included was a lecanicephalid (*Collicocephalus baggioi* Koch, Jensen & Caira, 2012; KU249072) and a polypocephalid (*Anteropora japonica* (Yamaguti, 1934) Euzet, 1994; MN701004) as outgroups. Sequences were preliminarily assembled in Geneious Prime 2019 (Biomatters, Inc., San Diego, CA, USA) and aligned using webPRANK (see <https://www.ebi.ac.uk/goldman-srv/webprank/>; Löytynoja and Goldman 2010) with default settings, but with the '+ F flag' removed. JModelTest (ver. 2.1.7, see <https://github.com/ddarriba/jmodeltest2>; Guindon and Gascuel 2003; Darriba *et al.* 2012) was run on the resulting multiple sequence alignment and TIM3+I+G was determined to best fit the data based on corrected Akaike Information Criterion (AICc) values. A maximum likelihood (ML) analysis with 1000 iterations and a ML bootstrap analysis with 1000 bootstrap replicates were conducted using Garli (ver. 2.01, see <https://code.google.com/archive/p/garli/>;

Table 2. Measurements for new species of *Amiclucestus*.

Cestode species	<i>Amiclucestus calli</i>, sp. nov.	<i>Amiclucestus australiensis</i>, sp. nov.	<i>Amiclucestus herzogae</i>, sp. nov.	<i>Amiclucestus penghuensis</i>, sp. nov.
Host species	<i>Rhinoptera brasiliensis</i>	<i>Rhinoptera neglecta</i>	<i>Rhinoptera jayakari</i>	<i>Rhinoptera cf. jayakari</i>
Locality	Mississippi and Louisiana, USA, Gulf of Mexico	Fog Bay, NT, Australia, Timor Sea	Mozambique, Mozambique Channel	Penghu Island, Taiwan, Taiwan Strait
Number of proglottids (all maturities)	32–45 (38 ± 5; 10)	21–33 (31 ± 3.2; 14)	32–38 (35 ± 2.2; 16)	35–47 (n = 2)
Male mature worm length (with scolex)	1578–1928 (n = 2)	1068–1201 (1135 ± 54.3; 4)	–	1249 (n = 1)
Male mature strobila length	1360–2190 (1695 ± 267.7; 7)	1018–1138 (1082 ± 53.2; 5)	1324–1809 (1591 ± 191.4; 8)	1206 (n = 1)
Female mature worm length (with scolex)	2321–2349 (n = 2)	1598–1777 (1660 ± 101.7; 3)	–	–
Female mature strobila length	1856–2392 (2136 ± 218.1; 5)	1512–2023 (1694 ± 209.6; 5)	1816–2075 (1929 ± 111.1; 5)	1951 (n = 1)
Gravid worm length (with scolex)	3226 (n = 1)	1422 (n = 1)	1972 (n = 1)	2506 (n = 1)
Gravid strobila length	3126 (n = 1)	1342–1970 (1756 ± 296; 4)	1872–1988 (1920 ± 60.4; 3)	2398 (n = 1)
Scolex length	94–136 (116 ± 15.3; 7)	85–110 (95 ± 8.7; 8)	100–121 (n = 2)	108–195 (156 ± 33.7; 5)
Scolex width	90–107 (99 ± 5.4; 7)	61–83 (75 ± 7.9; 7)	87–100 (n = 2)	93–145 (123 ± 19.0; 5)
Acetabula length	43–53 (49 ± 2.5; 7; 13)	36–46 (41 ± 3.1; 8; 16)	43–45 (44 ± 0.8; 2; 5)	44–77 (63 ± 11.8; 5; 10)
Acetabula width	32–43 (38 ± 3.6; 6; 11)	24–34 (28 ± 3.2; 6; 10)	26–35 (30 ± 3.6; 2; 5)	34–54 (42 ± 7.4; 5; 10)
Apical organ length	36–51 (44 ± 5.4; 7)	30–37 (34 ± 2.6; 8)	32–34 (n = 2)	55–78 (69 ± 8.9; 5)
Apical organ width	23–30 (26 ± 2.2; 7)	17–23 (20 ± 2.1; 8)	23–24 (n = 2)	23–45 (34 ± 8.4; 5)
Number of barren proglottids forming trough	17–22 (19 ± 1.7; 15)	18–23 (21 ± 1.5; 14)	17–21 (19 ± 1.1; 17)	14–18 (16 ± 1.3; 7)
Trough length	299–613 (434 ± 91.2; 15)	357–589 (483 ± 90.6; 14)	333–444 (386 ± 37.3; 17)	282–403 (349 ± 48.3; 7)
Trough width	226–318 (256 ± 25.3; 14)	177–244 (200 ± 21.8; 10)	154–232 (197 ± 21.7; 15)	92–215 (163 ± 37.7; 7)
Number of reproductive proglottids in mature & gravid worms	14–23 (19 ± 3.4; 13)	9–12 (11 ± 1.1; 13)	12–19 (16 ± 1.9; 16)	21–29 (24 ± 4.2; 3)
Number of immature proglottids	13–22 (18 ± 3.4; 13)	8–11 (10 ± 1.1; 13)	11–18 (15 ± 1.9; 15)	20–28 (23 ± 4.2; 3)
Posterior-most immat. proglottid length	77–413 (172 ± 83.5; 13)	44–281 (102 ± 63; 14)	90–214 (140 ± 39.7; 12)	61–197 (134 ± 68.5; 3)
Posterior-most immat. proglottid width	165–281 (214 ± 37.1; 12)	71–129 (99 ± 17.9; 14)	120–156 (140 ± 11.6; 12)	139–153 (144 ± 7.6; 3)
Number of mature proglottids in mature worms	1 (n = 12)	1 (n = 10)	1 (n = 13)	1 (n = 2)
Number of mature proglottids in gravid worms	0 (n = 1)	0 (n = 4)	0 (n = 3)	0 (n = 1)
Terminal male-mature proglottid length	580–1228 (838 ± 207.9; 7)	467–653 (541 ± 71.2; 5)	595–1024 (814 ± 141.5; 8)	579 (n = 1)
Terminal male-mature proglottid width	251–293 (270 ± 13.7; 7)	123–149 (137 ± 10.9; 5)	167–205 (186 ± 14.5; 8)	180 (n = 1)
Terminal female-mature proglottid length	944–1223 (1060 ± 107.9; 5)	801–1172 (925 ± 155.4; 5)	1013–1259 (1085 ± 103; 5)	1139 (n = 1)

(Continued on next page)

Table 2. (Continued)

Cestode species	<i>Amiculucestus calli</i> , sp. nov.	<i>Amiculucestus</i> <i>australiensis</i> , sp. nov.	<i>Amiculucestus herzogae</i> , sp. nov.	<i>Amiculucestus</i> <i>penguensis</i> , sp. nov.
Host species	<i>Rhinoptera brasiliensis</i>	<i>Rhinoptera neglecta</i>	<i>Rhinoptera jayakari</i>	<i>Rhinoptera</i> cf. <i>jayakari</i>
Locality	Mississippi and Louisiana, USA, Gulf of Mexico	Fog Bay, NT, Australia, Timor Sea	Mozambique, Mozambique Channel	Penghu Island, Taiwan, Taiwan Strait
Terminal female-mature proglottid width	293–350 (324 ± 25.3; 5)	166–245 (202 ± 29.4; 5)	200–233 (219 ± 14; 4)	210 (n = 1)
Number of gravid proglottids	1 (n = 1)	1 (n = 4)	1 (n = 3)	1 (n = 1)
Gravid proglottid length	1383 (n = 1)	627–1067 (849 ± 181.2; 4)	951–1128 (1046 ± 89.2; 3)	1326 (n = 1)
Gravid proglottid width	333 (n = 1)	184–282 (231 ± 40.4; 4)	251–282 (267 ± 15.6; 3)	326 (n = 1)
Number of testes	35–58 (47 ± 8.8; 10)	11–16 (14 ± 1.4; 9)	19–29 (23 ± 2.9; 10)	24–31 (27 ± 2.7; 7)
Testes length	10–41 (23 ± 7.5; 3; 24)	17–32 (24 ± 4.2; 7; 21)	11–25 (15 ± 3.1; 8; 24)	13–21 (17 ± 4.1; 1; 3)
Testes width	28–76 (47 ± 12.6; 3; 24)	18–31 (24 ± 4.3; 7; 21)	22–45 (33 ± 7.1; 8; 24)	33–37 (34 ± 2.0; 1; 3)
Number of aporal testes	17–37 (28 ± 6; 12)	8–11 (9 ± 0.9; 9)	11–16 (13 ± 1.8; 10)	13–16 (15 ± 1.2; 7)
Number of preporal testes	8–20 (15 ± 4.3; 12)	3–6 (5 ± 0.9; 9)	7–13 (10 ± 1.7; 10)	10–15 (12 ± 1.9; 6)
Number of postporal testes	0–4 (2 ± 1.3; 10)	0 (n = 9)	0 (n = 10)	0 (n = 7)
Cirrus sac length	211–304 (260 ± 30.4; 3; 11)	175–255 (214 ± 28.2; 10)	228–339 (301 ± 29.3; 13)	301–349 (323 ± 24.2; 3)
Cirrus sac width	63–97 (80 ± 12.7; 3; 10)	51–64 (58 ± 4.9; 10)	71–98 (89 ± 8.3; 13)	70–89 (79 ± 9.5; 3)
Cirrus length	at least 406 (n = 9)	–	at least 88 (n = 3)	–
Cirrus width	14–16 (15 ± 0.6; 3; 6)	–	18 (n = 1)	–
Cirrus width at base	23–36 (30 ± 4.3; 3; 9)	–	24–26 (25 ± 1.0; 3)	–
Ovary width	150–263 (217 ± 52.7; 5)	98–161 (131 ± 28.2; 5)	123–190 (152 ± 25.9; 5)	151 (n = 1)
Ovarian lobe length	384–644 (489 ± 97.0; 5; 9)	198–383 (310 ± 67.5; 5; 10)	328–490 (416 ± 53.9; 5; 10)	441–450 (n = 1)
Genital pore position in posterior-most mature proglottid (%)	61–74 (68 ± 4.1; 13)	56–72 (64 ± 5.6; 10)	48–60 (56 ± 3.1; 12)	61 (n = 1)
Vitelline follicle length	11–17 (15 ± 2; 2; 12)	11–16 (13 ± 1.9; 6)	7–15 (11 ± 2.5; 5; 15)	16–22 (18 ± 3.2; 1; 3)
Vitelline follicle width	33–79 (50 ± 13.2; 2; 12)	16–27 (21 ± 4; 6)	31–62 (49 ± 10.7; 5; 15)	37–65 (53 ± 14.3; 1; 3)

Zwickl 2006) on the University of Kansas Center for Research Computing Shared Community Cluster. Default Garli configurations were used with the following alterations: streefname=random, attachmentspertaxon=48 (i.e. twice the number of terminal taxa) and output-phyliptree=1. For the ML analysis, the following search settings were used: searchreps=1000, genthreshfortopterm=100000 and significanttopochange=0.00001. And for the ML bootstrap analysis, the following search settings were used: searchreps=1, bootstrapreps=1000, genthreshfortopterm=10000 and significanttopochange=0.01. Clades with bootstrap values of 95% or greater were considered to have high nodal support. Bootstrap values were mapped on the best tree resulting from the ML analysis using SumTrees (ver. 4.5.2) in DendroPy (ver. 4.5.2, see <https://github.com/jeetsukumaran/DendroPy>; Sukumaran and Holder 2010).

Results

Phylogenetic analysis

The 15 specimens of eniochobothriids for which sequence data were generated in this study represent 10 species. GenBank and voucher info for the 12 of the 15 specimens of the eight described species included the analysis are given in the taxonomic summaries below. The remaining three specimens represent two undescribed species. For these latter specimens, GenBank and voucher information is as follows: *Eniochobothrium* sp. nov. 4 (GenBank number ON040620, hologenophore (photo voucher) LRP number 10821, KW83; and GenBank number ON04061, hologenophore LRP number 10822, KW117) and *Amiculucestus* sp. nov. 5 (GenBank number ON040633, hologenophore LRP number 10834, KW268).

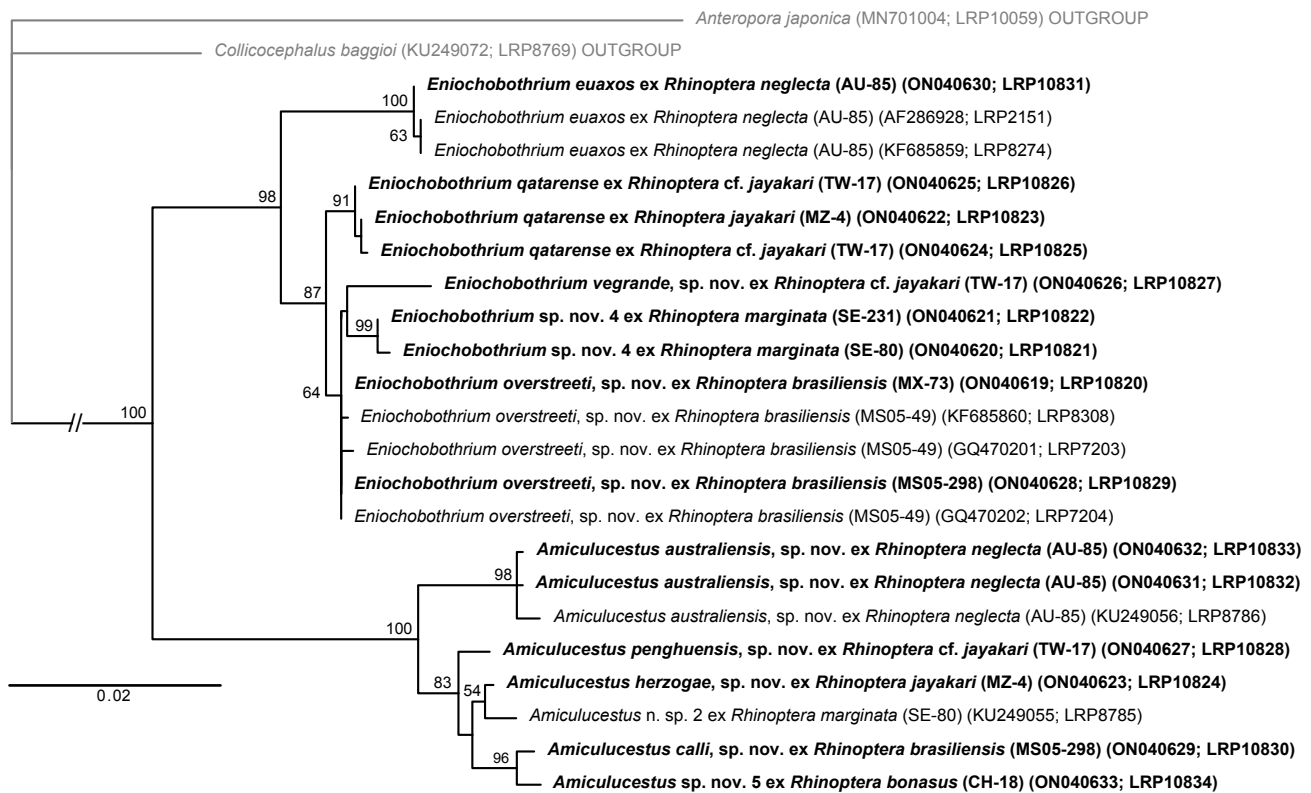


Fig. 1. Phylogenetic tree of the interrelationships of eniochobothriids resulting from a maximum likelihood analysis of sequence data for the D1–D3 region of the 28S rRNA gene. Branch leading to ingroup is shown at reduced length indicated by front slashes (//). Nodal support is indicated by bootstrap (BS) support values; only BS support values ≥ 50 are provided. Sequences generated as part of this study are indicated in bold. Taxon labels are given as cestode species name, host species name with host accession number in parentheses, followed by GenBank accession number and hologenophore specimen number, also in parentheses. Scale bar indicates nucleotide substitutions per site.

The tree resulting from the maximum likelihood phylogenetic analysis is shown in Fig. 1. The topology suggests the existence of two distinct subclades of eniochobothriids. Morphologically, the species in the two subclades differ conspicuously from one another. Based on these molecular and morphological differences we are erecting a new genus for one of the subclades. The subclade that contains species that are consistent morphologically with the diagnosis of *Eniochobothrium* based on its type species, *Eniochobothrium gracile* Shipley & Hornell, 1906, as provided by Shipley and Hornell (1906), retains the generic name *Eniochobothrium*. The new genus *Amiculucestus*, gen. nov. is erected to house the species in the other subclade.

Taxonomic treatments

Genus *Eniochobothrium* Shipley & Hornell, 1906, amended

Diagnosis

Family Eniochobothriidae Jensen, Caira, Cielocha, Littlewood & Waeschenbach, 2016. Worms apolytic. Scolex with four

acetabula and apical modification of scolex proper housing apical organ, weakly connected to strobila; acetabula in form of suckers; distal and proximal surfaces of acetabula covered with both spathulate and small gladiate spinitriches; apical modification of scolex proper cone shaped, with terminal aperture; apical organ small, glandular, non-eversible, non-protrusible. Cephalic peduncle absent. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough, and posterior region of reproductive proglottids; circumcortical longitudinal muscle bundles absent. Proglottids craspedote, non-lacinate. Testes numerous, 1–2 layers deep, arranged in aporal and postporal fields, occasionally also in preporal field, overlapping fields of vitelline follicles. Vas deferens conspicuous, thick walled, extending from level of ovary to near anterior margin of distal end of cirrus sac. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac U-shaped, thick walled, highly muscular. Cirrus armed. Ovary H-shaped in dorso-ventral view, bilobed in cross-section, with smooth or lobulated margins. Vagina absent; seminal receptacle present. Genital pores lateral, irregularly or regularly alternating; genital atrium conspicuous. Uterus saccate, medial, stopping short of level of genital pore. Vitellarium follicular;

vitelline follicles medullary, in two lateral fields of multiple follicles, restricted to region between cirrus sac and ovary. Excretory vessels four, arranged in one dorsal and one ventral pair. Oncospheres grouped in cocoons. Parasites of batoids of the genus *Rhinoptera* (Rhinopteridae). Circumtropical.

Type species: Eniochobothrium gracile Shipley & Hornell, 1906.

Additional species

Eniochobothrium euaxos Jensen, 2005; *Eniochobothrium overstreeti*, sp. nov.; *Eniochobothrium qatarense* Al Kawari, Saoud & Wanas, 1994; *Eniochobothrium vegrande*, sp. nov.

Remarks

This generic diagnosis is an amended version of the most recent generic diagnosis of *Eniochobothrium* of Jensen (2005), informed by the familial diagnosis of Jensen *et al.* (2016). It reflects the updated terminology for the different strobilar regions, the arrangement of the testes, the shape of the cirrus sac and the vitelline follicles. The genital atrium for the genus was reassessed and is herein described as conspicuous rather than shallow.

Eniochobothrium qatarense Al Kawari, Saoud & Wanas, 1994 redescription

(Fig. 2–4a, 5–7)

Based on four mature and fifteen gravid worms, cross-sections of one worm, cross-sections of one worm *in situ*, longitudinal section of one worm *in situ*, three semi-permanent preparations of eggs and two specimens prepared for SEM.

Worms apolytic; mature worms 2886–5234 long, mature strobilae 2821–5084 long, with 39–50 proglottids; gravid worms 3706–7480 long, gravid strobilae 3623–7458 long, with 27–45 proglottids. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough and posterior region of reproductive proglottids (Fig. 2a); circumcortical longitudinal muscle bundles absent.

Scolex 68–169 long, 73–115 wide, bearing four acetabula, apical modification of scolex proper and apical organ (Fig. 2b). Acetabula in form of suckers, sessile, 42–54 long, 28–45 wide. Apical modification of scolex proper in form of small cone with terminal aperture, housing apical organ. Apical organ small, internal, non-protrusible, non-eversible, primarily glandular, 24–50 long, 17–30 wide. Cephalic peduncle absent.

Distal surfaces (Fig. 3d) and rims of acetabula (Fig. 3e), and region of scolex proper posterior to acetabula (Fig. 3f) covered with spathulate spinitriches and small gladiate spinitriches. Apical modification of scolex proper and scolex

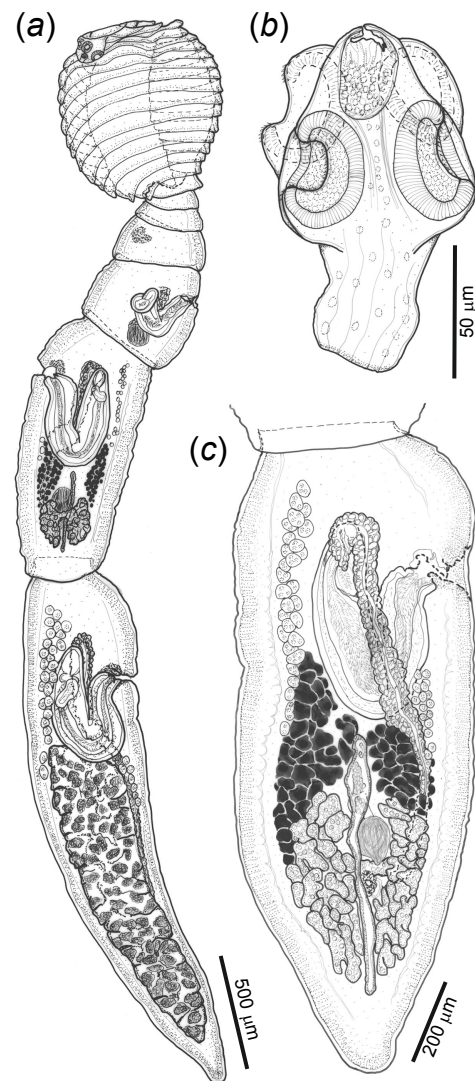


Fig. 2. Line drawings of *Eniochobothrium qatarense* Al Kawari, Saoud & Wanas, 1994 (a) Gravid whole worm (voucher, USNM number 1672273). (b) Scolex (voucher, USNM number 1672274). (c) Terminal mature proglottid (voucher, USNM number 1672274).

proper anterior to acetabula (Fig. 3c) covered with small gladiate spinitriches. Barren proglottids of trough covered with scolopate spinitriches on both concave (Fig. 3g) and convex (Fig. 3h) surfaces. Reproductive proglottids covered with capilliform filitriches away from posterior proglottid margins (Fig. 3j) and with scolopate spinitriches near or at posterior proglottid margins (Fig. 3i).

Proglottids craspedote, non-laciniate. Trough with 16–24 proglottids, 613–888 long, 456–852 wide. Reproductive proglottids in mature worms 21–28 in number, in gravid worms 6–26 in number; immature proglottids 3–25 in number, posterior-most immature proglottid 168–460 long, 313–499 wide; mature proglottids 1–3 in number; terminal mature proglottid 1073–1525 long, 395–472 wide; posterior-most mature proglottid in gravid worms 776–1493 long, 399–733

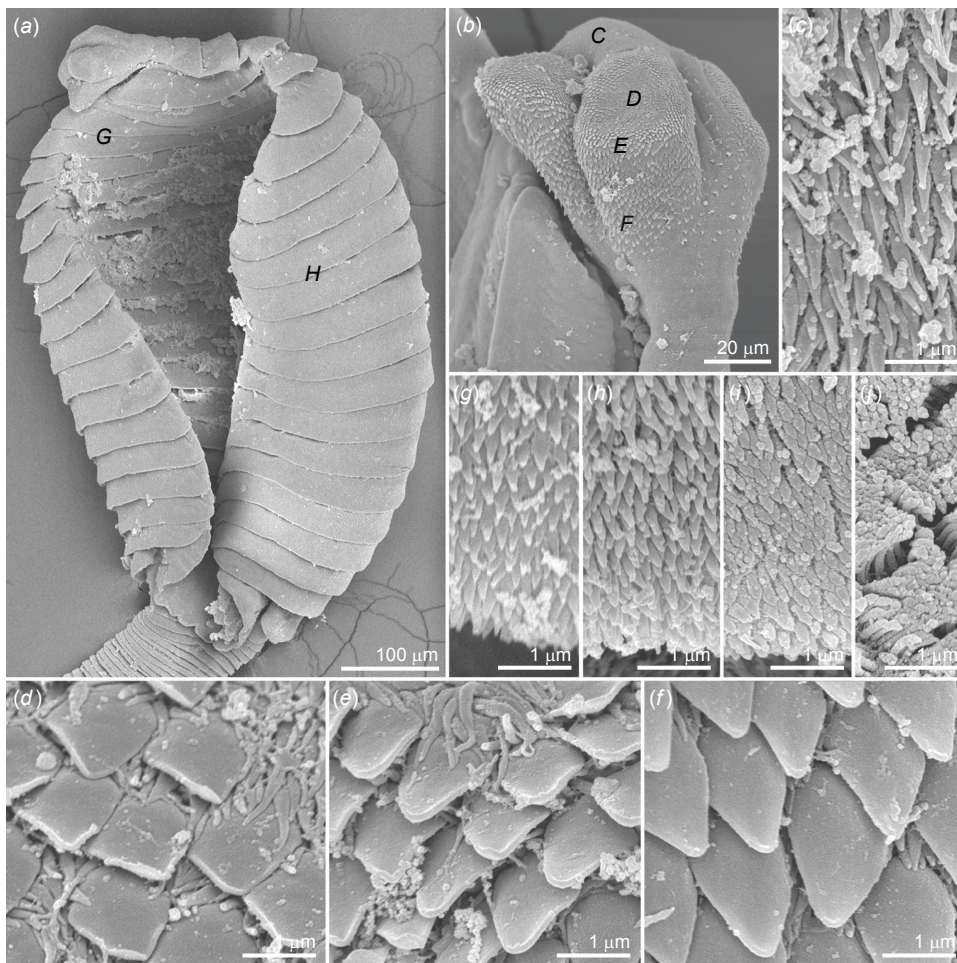


Fig. 3. Scanning electron micrographs of *Eniochobothrium qatarense* Al Kawari, Saoud & Wanas, 1994. (a) Scolex and anterior region of strobila comprising barren, laterally expanded proglottids forming trough; uppercase italic letters indicate location of details shown in (g) and (h). (b) Scolex; uppercase italic letters indicate location of details shown in (c–f). (c) Surface of scolex proper anterior to acetabula. (d) Distal acetabular surface. (e) Surface of acetabular rim. (f) Surface of scolex proper posterior to acetabula. (g) Concave surface of trough. (h) Convex surface of trough. (i) Surface of posterior margin of reproductive proglottid. (j) Surface of reproductive proglottid away from margin.

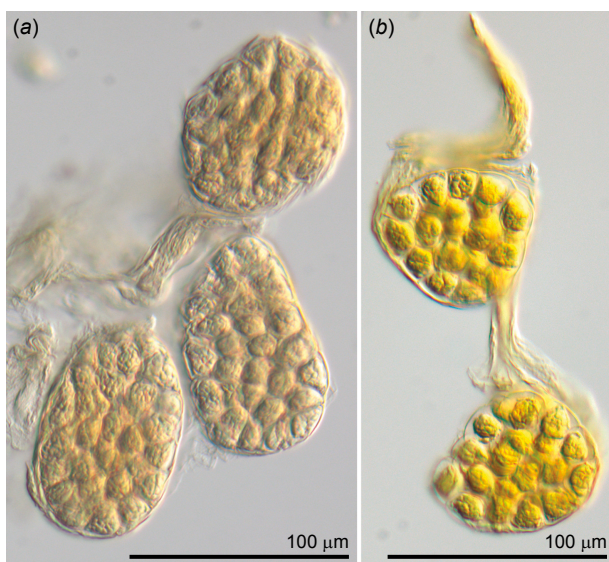


Fig. 4. Photomicrographs of developing oncospheres grouped in cocoons. (a) *Eniochobothrium qatarense* Al Kawari, Saoud & Wanas, 1994. (b) *Eniochobothrium overstreeti*, sp. nov.

wide; gravid proglottids 1–2 in number, 1543–3393 long, 411–665 wide.

Testes 29–50 in total number, 21–67 long, 11–53 wide, 1–2 layers deep in cross-section, extending from near anterior margin of proglottid to midlevel of vitelline field on aporal side, restricted to region posterior to pore between midlevel of cirrus sac and midlevel of vitelline field on poral side (Fig. 2c); aporal testes 22–37 in number; preporal testes absent; postporal testes 7–18 in number. Vas deferens conspicuous, thick walled, extending from level of ootype anteriorly along lateral margin of proglottid, then diagonally towards midline of proglottid to enter cirrus sac at its anteromedial margin. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac U-shaped (Fig. 2c), thick walled, highly muscular, 603–843 long, 100–148 wide, containing cirrus. Cirrus armed. Ovary H-shaped in dorso-ventral view (Fig. 2c), bilobed in cross-section (Fig. 5d), with strongly lobulated margins, symmetrical, 195–328 wide; ovarian lobes 126–504 long. Vagina absent; seminal receptacle conspicuous, located immediately anterior to ovarian isthmus. Genital pores lateral, regularly alternating, 71–84% of proglottid length from posterior end in mature proglottids; genital atrium conspicuous. Uterus saccate,

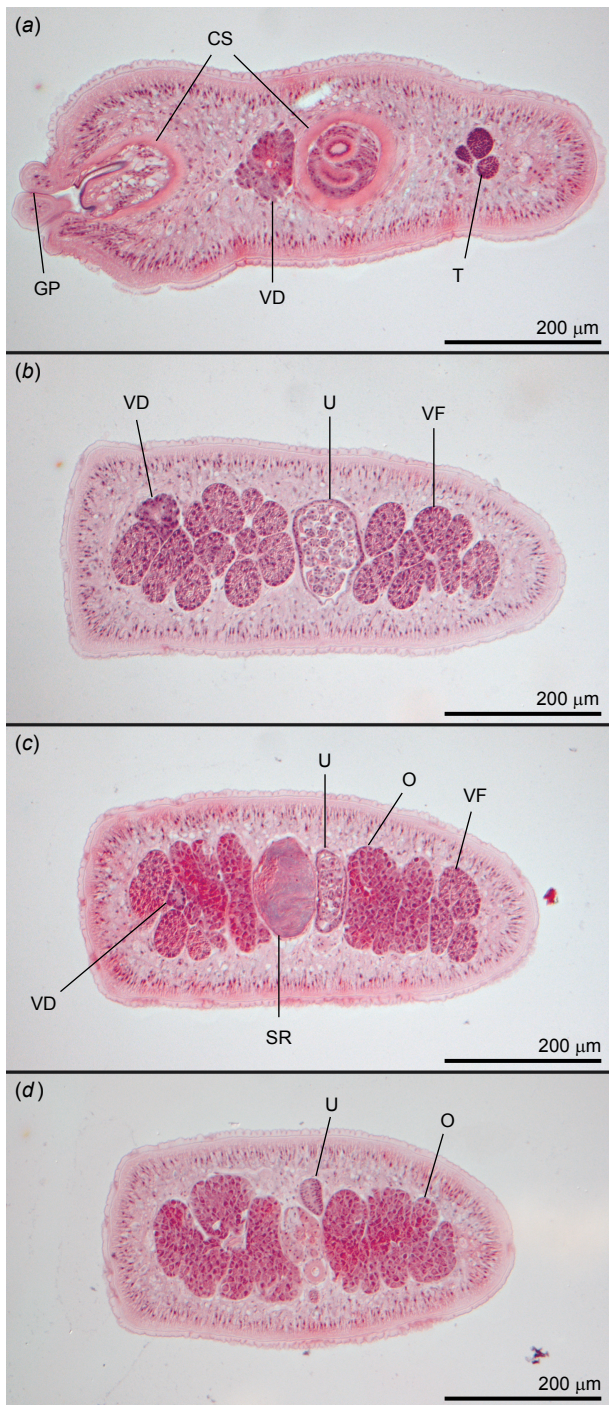


Fig. 5. Photomicrographs *Eniochobothrium qatarense* Al Kawari, Saoud & Wanas, 1994. (a) Cross-section through early gravid proglottid at level of genital pore. (b) Cross-section through early gravid proglottid at level of vitelline fields. (c) Cross-section through early gravid proglottid at level of seminal receptacle anterior to ovarian isthmus. (d) Cross-section through early gravid proglottid slightly posterior to ovarian isthmus. Abbreviations: CS, cirrus sac; GP, genital pore; O, ovary; SR, seminal receptacle; T, testes; U, uterus; VD, vas deferens; VF, vitelline follicle.



Fig. 6. Photomicrographs *Eniochobothrium qatarense* Al Kawari, Saoud & Wanas, 1994. (a) Worm attached to surface of spiral intestine. (b) Histological section perpendicular to surface of spiral intestine showing worm *in situ*. (c) Histological section parallel to surface of spiral intestine showing worm *in situ*. Abbreviations: S, scolex.

medial, extending from posterior margin of ovary to near posterior margin of cirrus sac; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles medullary, arranged in two lateral fields (Fig. 2c); aporal

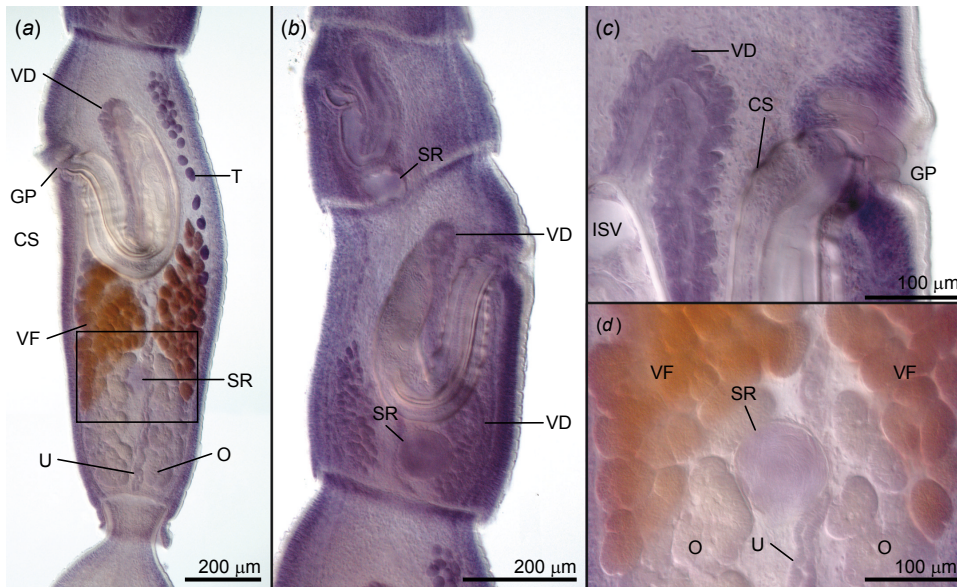


Fig. 7. Photomicrographs of *Eniochobothrium qatarse* Al Kawari, Saoud & Wanas, 1994. (a) Mature proglottid; rectangle indicates region shown in (d). (b) Immature proglottids (same specimen as in a) showing empty (anterior) and sperm-filled (posterior) seminal receptacle. (c) Close-up of region of genital pore and vas deferens in mature proglottid. (d) Detail of region with seminal receptacle in mature proglottid. Abbreviations: CS, cirrus sac; GP, genital pore; ISV, internal seminal vesicle; O, ovary; SR, seminal receptacle; VD, vas deferens; T, testes; U, uterus; VF, vitelline follicle.

field extending from midlevel of ovary to midlevel of cirrus sac, 172–597 long, 78–184 wide; poral field extending from anterior margin of ovary to posterior margin of cirrus sac, 188–536 long, 97–182 wide. Excretory vessels four, arranged in one dorsal and one ventral pair. Developing oncospheres subspherical, thin-walled, 14–21 long, 9–15 wide, grouped in cocoons; cocoons oblong, 93–120 long, 57–100 wide, containing 45–74 oncospheres (Fig. 4a).

Type host

Rhinoptera jayakari Boulenger, Oman cownose ray (Myliobatiformes: Rhinopteridae).

Additional hosts

Rhinoptera cf. *jayakari* (Myliobatiformes: Rhinopteridae).

Type locality

Persian Gulf off Qatar.

Additional localities

Tofo, Inhambane, Mozambique, Mozambique Channel (23°47'33.02"S, 35°31'16.38"E); Magong, Penghu Island, Taiwan, Taiwan Strait (23°33'49"N, 119°34'31"E).

Site of infection

Spiral intestine.

Type specimens

Holotype (HCUQ number 6111.87) and paratypes (HCUQ number 6112.87), Helminth Collection, Department of

Zoology, University of Qatar (HCUQ) (now Biology Museum, Qatar University, Doha, Qatar).

Voucher specimens

Two whole mounts (gravid worms) (NMNS numbers 8637-004–8637-005); twelve whole mounts (two mature and eight gravid worms), cross-sections of one worm and one worm *in situ*, longitudinal sections of one worm *in situ*, and two semi-permanent preparation of eggs (USNM numbers 1672269–1672283); seven whole mounts (two mature and five gravid worms), one semi-permanent preparation of eggs, and two SEM vouchers (LRP numbers 10932–10941). Specimens prepared for SEM retained in the collection of K. Jensen.

Sequence data

GenBank number ON040622 (hologenophore LRP number 10823, KW227); GenBank number ON040624 (hologenophore LRP number 10825, KW232); GenBank number ON040625 (hologenophore LRP number 10826, KW234).

Remarks

To confirm the conspecificity of our specimens from Mozambique and Taiwan with those of *E. qatarse* described from the Persian Gulf, colleagues in the Department of Biological and Environmental Sciences, Qatar University, kindly provided us with images of specimens on two slides (one containing a single specimen, and one with three specimens and a detached proglottid), all identified as *E. qatarse* by the original authors, and deposited in the Biology Museum at Qatar University. Although we believe these to be type specimens, there appeared to be no museum numbers on the slides or numbers were not visible. It was, thus, impossible to unambiguously confirm their identity as type material.

The description of Al Kawari *et al.* (1994) and the specimens from Qatar University suggest that the measurements provided in the original description were a combination of measurements of features from both mature and gravid worms. Since *E. qatarense* was described, our understanding of the unusual morphology of *Eniochobothrium* has advanced (see Jensen 2005; Jensen *et al.* 2016) and so too has our recognition of the importance of distinguishing between mature and gravid worms. Moreover, this distinction is needed if measurements are to be informative for distinguishing among species. Thus, the redescription of *E. qatarense* presented here is based solely on newly collected material.

Our redescription is generally consistent with that of Al Kawari *et al.* (1994). One notable exception is that, although Al Kawari *et al.* (1994) considered a vagina to be present albeit 'hardly traced in whole-mounted preparations' (p. 101), no evidence of a vagina was seen in the specimens examined here. In fact, our work on multiple species of *Eniochobothrium* has shown that members of this genus actually lack a vagina (see also Jensen 2005 and Jensen *et al.* 2016). Based on molecular and morphological data, the host associations of *E. qatarense* are expanded from *Rhinoptera jayakari* to also include *Rhinoptera* cf. *jayakari*. This report also greatly expands the geographic distribution of the species beyond the Persian Gulf to include the western Indian Ocean and the western Pacific Ocean.

Eniochobothrium overstreeti, sp. nov.

(Fig. 4b, 8a–c, 9)

ZooBank urn:lsid:zoobank.org:act:32E27DEE-A4D3-49B3-9891-DF64F46AC1AF

Based on three mature worms, one mature strobila, seven gravid worms, cross-sections of two worms, two semi-permanent preparation of eggs and three worms prepared for SEM.

Worms apolytic; mature worms 961–1215 long, mature strobilae 863–1148 long, with 27–29 proglottids; gravid worms 1230–1798 long, gravid strobilae 1144–1692 long, with 23–33 proglottids. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough and posterior region of reproductive proglottids (Fig. 8a); circumcortical longitudinal muscle bundles absent.

Scolex 82–105 long, 71–89 wide, bearing four acetabula, apical modification of scolex proper and apical organ (Fig. 8b). Acetabula in form of suckers, sessile, 33–47 long, 27–39 wide. Apical modification of scolex proper in form of small cone with terminal aperture, housing apical organ. Apical organ small, internal, non-protrusible, non-eversible, primarily glandular, 31–47 long, 24–29 wide. Cephalic peduncle absent.

Distal surfaces (Fig. 9e) and rims of acetabula (Fig. 9f), and region of scolex proper posterior to acetabula (Fig. 9g) covered with spatulate spiniriches and small gladiate spiniriches.

Apical modification of scolex proper and scolex proper anterior to acetabula (Fig. 9c) covered with small gladiate spiniriches; gladiate spiniriches on margins of terminal aperture more densely arranged (Fig. 9d). Barren proglottids of trough covered with scolopate spiniriches on both concave (Fig. 9h, i) and convex (Fig. 9j) surfaces. Reproductive proglottids covered with capilliform filitriches away from posterior proglottid margins (Fig. 9l) and with scolopate spiniriches near or at posterior proglottid margins (Fig. 9k).

Proglottids craspedote, non-lacinate. Trough with 15–21 proglottids, 244–420 long, 131–296 wide. Reproductive proglottids in mature worms 9–11 in number, in gravid worms 8–15 in number; immature proglottids 8–14 in number, posterior-most immature proglottid 108–375 long, 188–230 wide; mature proglottids in mature worms 1 ($n = 4$) in number, in gravid worms 0 ($n = 7$) in number; terminal mature proglottid 482–647 long, 204–223 wide; gravid proglottids 1 ($n = 7$) in number, 645–881 long, 222–316 wide.

Testes 20–29 in total number, 16–40 long, 17–51 wide, 1–2 layers deep in cross-section, extending from near anterior margin of proglottid to midlevel of vitelline field on aporal side, restricted to region posterior to pore between midlevel of cirrus sac and anterior margin of vitelline field on poral side (Fig. 8c); aporal testes 14–23 in number; preporal testes absent; postporal testes 5–7 in number. Vas deferens conspicuous, thick walled, extending from level of ootype anteriorly along lateral margin of proglottid, then diagonally towards midline of proglottid to enter cirrus sac at its anteromedial margin. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac U-shaped, thick walled, highly muscular, 307–331 long, 47–55 wide, containing cirrus. Cirrus armed. Ovary H-shaped in dorsoventral view (Fig. 8c), bilobed in cross-section, with weakly lobulated margins, symmetrical, 101–139 wide; ovarian lobes 129–232 long. Vagina absent; seminal receptacle conspicuous, located immediately anterior to ovarian isthmus. Genital pores lateral, regularly alternating, 75–82% of proglottid length from posterior end in mature proglottids; genital atrium conspicuous. Uterus saccate, medial, extending from posterior margin of ovary to near posterior margin of cirrus sac; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles medullary, arranged in two lateral fields (Fig. 8c); aporal field extending from midlevel of ovary to midlevel of cirrus sac, 104–184 long, 45–64 wide; poral field extending from midlevel of ovary to posterior margin of cirrus sac, 92–139 long, 42–62 wide. Excretory vessels four, arranged in one dorsal and one ventral pair. Developing oncospheres subspherical, thin-walled, 11–17 long, 9–14 wide, grouped in cocoons; cocoon oblong, 70–92 long, 54–71 wide, containing 21–33 oncospheres (Fig. 4b).

Type and only known host

Rhinoptera brasiliensis Müller, Brazilian cownose ray (Myliobatiformes: Rhinopteridae).

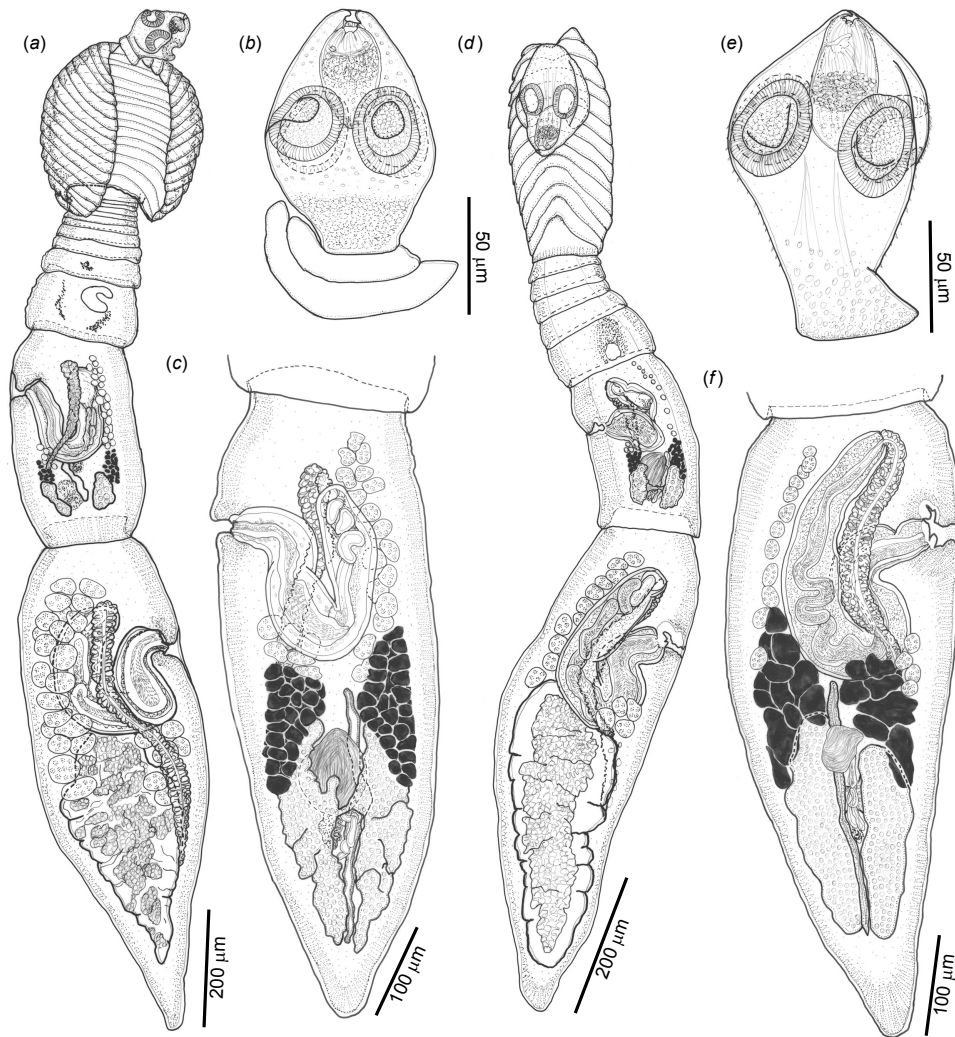


Fig. 8. Line drawings of *Eniochobothrium overstreeti*, sp. nov. (a–c) and *Eniochobothrium vegrande*, sp. nov. (d–f). (a) Gravid whole worm of *E. overstreeti*, sp. nov. (holotype, USNM number 1672266). (b) Scolex of *E. overstreeti*, sp. nov. (paratype, USNM number 1672264). (c) Terminal mature proglottid of *E. overstreeti*, sp. nov. (paratype, USNM number 1672262). (d) Gravid whole worm of *E. vegrande*, sp. nov. (holotype, NMNS number 8637-006). (e) Scolex of *E. vegrande*, sp. nov. (paratype, USNM number 1672287). (f) Terminal mature proglottid of *E. vegrande*, sp. nov. (paratype, USNM number 1672285).

Type locality

West tip of Horn Island, MS, USA, Gulf of Mexico (30°14'37.70"N, 88°46'37.62"W).

Additional locality

South side of East Ship Island, MS, USA, Gulf of Mexico (30°14'24.54"N, 88°52'25.25"W); Campeche, Mexico, Gulf of Mexico.

Site of infection

Spiral intestine.

Type specimens deposited

Holotype (USNM number 1672266), and six paratypes, cross-sections of one worm, and one semi-permanent preparation of eggs (USNM numbers 1672260–1672265, 1672267–1672268); four paratypes, cross-sections of one worm, and one semi-permanent preparation of eggs (LRP numbers 10923–10931). Specimens prepared for SEM retained in the collection of K. Jensen.

Sequence data

GenBank number KF685860 (hologenophore LRP number 8308, PBI907, TE-91) (as *Eniochobothrium* sp. nov. 1;

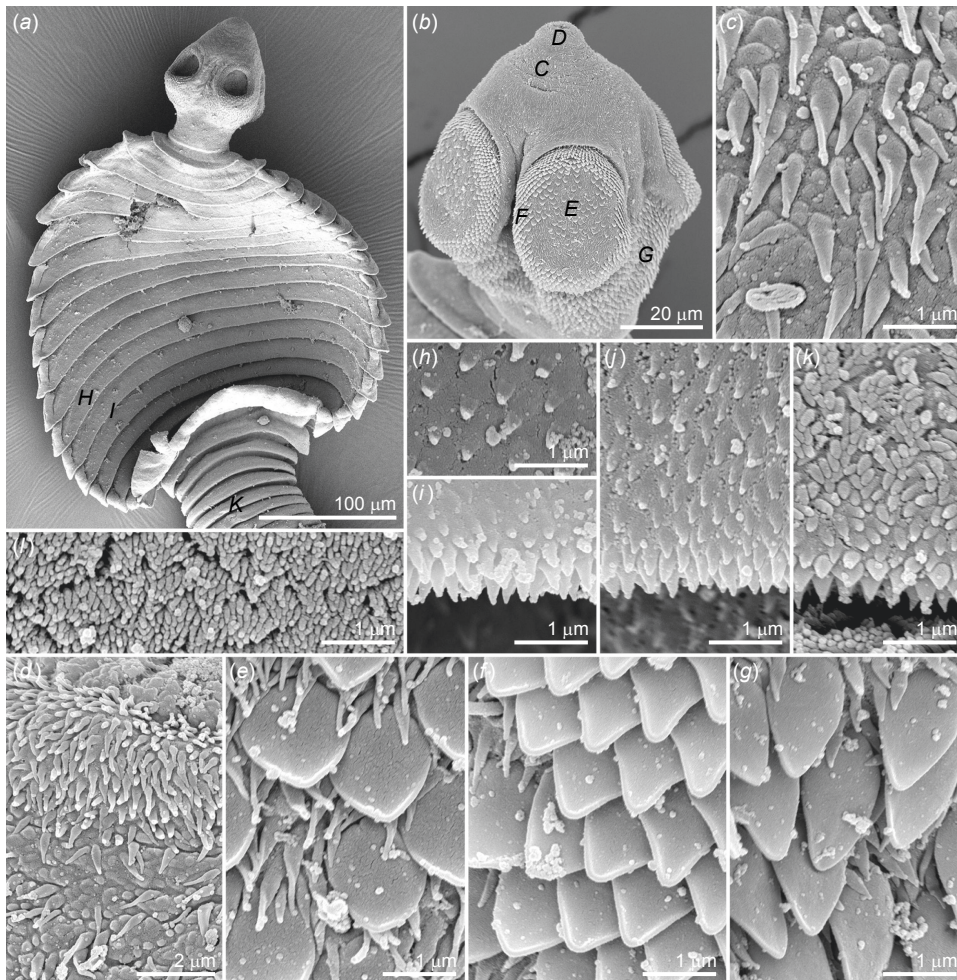


Fig. 9. Scanning electron micrographs of *Eniochobothrium overstreeti*, sp. nov. (a) Scolex and anterior region of strobila comprising barren, laterally expanded proglottids forming trough; uppercase italic letters indicate location of details shown in (h), (i) and (k). (b) Scolex; uppercase italic letters indicate location of details shown in (c–g). (c) Surface of scolex proper anterior to acetabula. (d) Surface of scolex proper near terminal aperture. (e) Distal acetabular surface. (f) Surface of acetabular rim. (g) Surface of scolex proper posterior to acetabula. (h) Concave surface of trough, anterior proglottid region. (i) Concave surface of trough, posterior proglottid margin. (j) Convex surface of trough. (k) Surface of posterior margin of reproductive proglottid. (l) Surface of reproductive proglottid away from margin.

Caira *et al.* 2014 and Jensen *et al.* 2016); GenBank number GQ470201 (hologenophore LRP number 7203) (as *Eniochobothrium* sp. n.; Jensen and Bullard 2010); GenBank number GQ470202 (hologenophore LRP number 7204) (as *Eniochobothrium* sp. n.; Jensen and Bullard 2010); GenBank number ON040628 (hologenophore LRP number 10829, KW242); GenBank number ON040619 (hologenophore LRP number 10820, JW742)

Etymology

This species name honours the late Robin Overstreet of the Gulf Coast Research Laboratory, University of Southern Mississippi, for generously providing the laboratory space and resources that facilitated collection of these worms from cownose rays in the Gulf of Mexico.

Remarks

Eniochobothrium overstreeti, sp. nov. is shorter in total length than *E. qatarense* (961–1215 v. 2886–5234 for mature worms; 1230–1798 v. 3706–7480 for gravid worms).

Furthermore, it has a smaller trough than *E. qatarense* (244–420 long by 131–296 wide v. 613–888 long by 456–852 wide). The total lengths of both mature and gravid worms of this new species are also shorter than those of *E. gracile* (961–1798 v. 3500–12 000). Beyond overall size, this new species is difficult to distinguish further from *E. gracile* owing to the lack of detail provided in Shipley and Hornell's (1906) original description. It does, however, appear that the two species also differ in total number of proglottids (23–33 v. at least 42 respectively). *Eniochobothrium overstreeti*, sp. nov. is easily distinguished from *E. euaxos* in lacking, rather than possessing, preporal testes on the poral side of the proglottid.

Two specimens of this species were included in the phylogenetic analysis of Jensen and Bullard (2010) as *Eniochobothrium* n. sp. In addition, one specimen of this new species was included in the phylogenetic analyses of Caira *et al.* (2014) and Jensen *et al.* (2016), in both cases as *Eniochobothrium* n. sp. 1. With this description we are providing a formal name for all three of these specimens.

***Eniochobothrium vegrande*, sp. nov.**

(Fig. 8d–f, 10)

ZooBank urn:lsid:zoobank.org:act:2D853847-3613-49FA-AD5F-2D682BA06FDE

Based on two mature worms, three mature strobilae, four gravid worms and one specimen prepared for SEM.

Worms apolytic; mature worms 1304–1610 long, mature strobilae 1130–1480 long, with 22–26 proglottids; gravid worms 1286–1840 long, gravid strobilae 1147–1708 long, with 21–23 proglottids. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough and posterior region of reproductive proglottids (Fig. 8d); circumcortical longitudinal muscle bundles absent.

Scolex 136–184 long, 68–101 wide, bearing four acetabula, apical modification of scolex proper and apical organ (Fig. 8e). Acetabula in form of suckers, sessile, 41–54 long, 28–41 wide. Apical modification of scolex proper in form of small cone with terminal aperture, housing apical organ. Apical organ small, internal, non-protrusible, non-eversible, primarily glandular, 48–60 long, 25–30 wide. Cephalic peduncle absent.

Distal surfaces (Fig. 10e) and rims of acetabula (Fig. 10f), and region of scolex proper posterior to acetabula (Fig. 10g) covered with spatulate spinitriches and small gladiate spinitriches. Apical modification of scolex proper and scolex proper anterior to acetabula (Fig. 10d) covered with small gladiate spinitriches. Barren proglottids of trough covered with scolopate spinitriches on both concave and convex (Fig. 10h) surfaces. Reproductive proglottids covered with

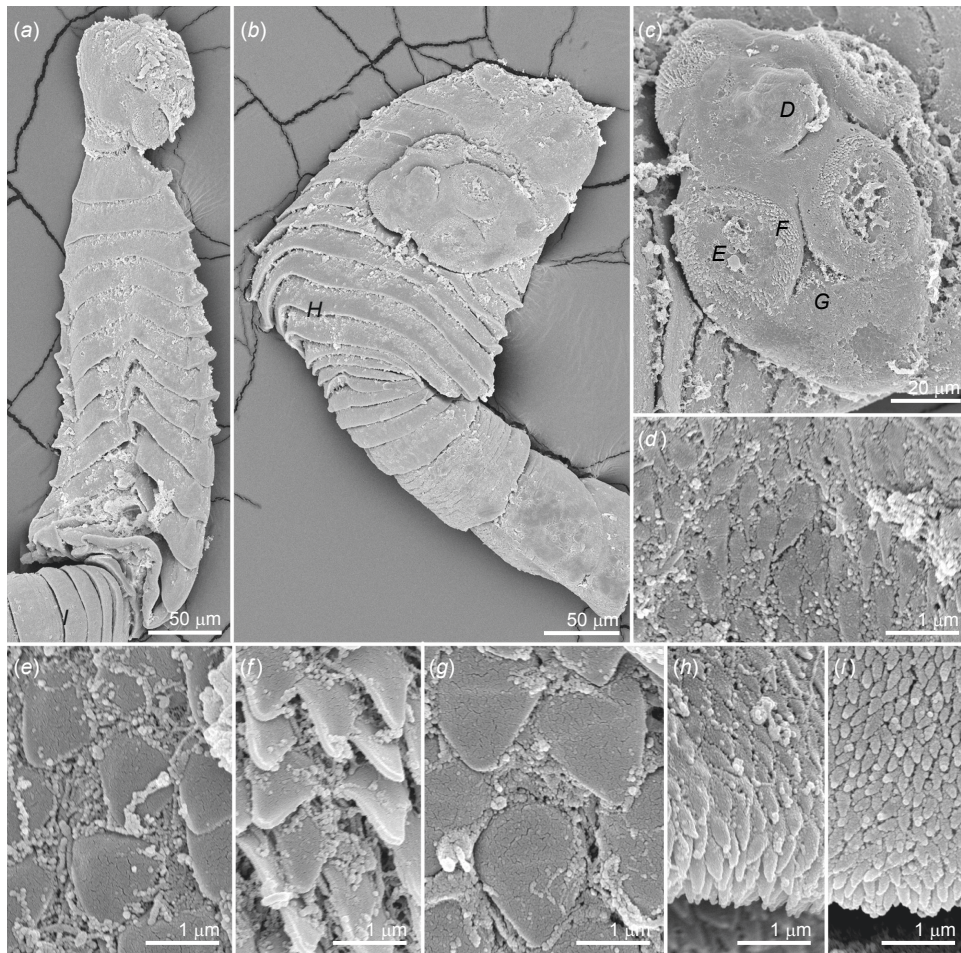


Fig. 10. Scanning electron micrographs of *Eniochobothrium vegrande*, sp. nov. (a) Scolex and anterior region of strobila comprising barren, laterally expanded proglottids forming trough; uppercase italic letter indicates location of detail shown in (i). (b) Scolex and anterior region of strobila comprising barren, laterally expanded proglottids forming trough; uppercase italic letter indicates location of detail shown in (h). (c) Scolex; uppercase italic letters indicate locations of details shown in (d–g). (d) Surface of scolex proper anterior to acetabula. (e) Distal acetabular surface. (f) Surface of acetabular rim. (g) Surface of scolex proper posterior to acetabula. (h) Convex surface of trough. (i) Surface of posterior margin of reproductive proglottid.

capilliform filitriches away from posterior proglottid margins and with scolopate spinitriches near or at posterior proglottid margins (Fig. 10i).

Proglottids craspedote, non-lacinate. Trough with 11–15 proglottids, 272–469 long, 118–197 wide. Reproductive proglottids in mature worms 9–12 in total number, in gravid worms 8–11 in total number; immature proglottids 6–10 in number, posterior-most immature proglottid 58–137 long, 129–187 wide; mature proglottids 1–2 in number; terminal mature proglottid 448–617 long, 172–215 wide; posterior-most mature proglottid in gravid worms 171–294 long, 144–176 wide; gravid proglottids 1 ($n = 4$) in number, 632–838 long, 186–216 wide.

Testes 14–17 in total number, 13–25 long, 11–32 wide, 1–2 layers deep in cross-section, extending from near anterior margin of proglottid to midlevel of vitelline field on aporal side, restricted to region posterior to pore between midlevel of cirrus sac and midlevel of vitelline field on poral side (Fig. 8f); aporal testes 10–14 in number; preporal testes absent; postporal testes on poral side 4–6 in number. Vas deferens conspicuous, thick walled, extending from level of ootype anteriorly along lateral margin of proglottid, then diagonally towards midline of proglottid to enter cirrus sac at its anteromedial margin. External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac U-shaped, thick walled, highly muscular, 305–422 long, 45–60 wide, containing cirrus. Cirrus armed. Ovary H-shaped in dorsoventral view (Fig. 8f), bilobed in cross-section, with smooth margins, symmetrical, 77–143 wide; ovarian lobes 92–214 long. Vagina absent; seminal receptacle conspicuous, located at anterior margin of ovary. Genital pores lateral, regularly alternating, 55–79% of proglottid length from posterior end in mature proglottids; genital atrium conspicuous. Uterus saccate, medial in position, extending from posterior margin of ovary to posterior margin of cirrus sac; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles medullary, arranged in two lateral fields (Fig. 8f); aporal field extending from midlevel of ovary to midlevel of cirrus sac, 133–176 long, 36–69 wide; poral field extending from midlevel of ovary to overlap posterior margin of cirrus sac, 98–138 long, 42–67 wide. Excretory vessels four, arranged in one dorsal and one ventral pair. Developing oncospheres subspherical, grouped in cocoons.

Type and only known host

Rhinoptera cf. *jayakari* (Myliobatiformes: Rhinopteridae).

Type locality

Magong, Penghu Island, Taiwan, Taiwan Strait (23°33'49"N, 119°34'31"E).

Site of infection

Spiral intestine.

Type specimens deposited

Holotype (NMNS number 8637-006) and two paratypes (NMNS numbers 8637-007–8637-008); four paratypes (USNM numbers 1672284–1672287); two paratypes and one SEM voucher (LRP numbers 10942–10944). Specimen prepared for SEM retained in the collection of K. Jensen.

Sequence data

GenBank number ON040626 (hologenophore LRP number 10827, KW235).

Etymology

The specific epithet, *vegrande* (little, small; L., neuter), refers to the fact that this is the smaller of the two members of this genus found parasitising *Rhinoptera* cf. *jayakari*.

Remarks

Eniochobothrium vegrande, sp. nov. is easily distinguished from *E. euaxos*, *E. qatarense* and *E. overstreeti* based on its possession of fewer testes (14–17 v. 35–48, 29–50 and 20–29 respectively). It can be further distinguished from *E. qatarense* in possessing fewer proglottids in mature worms (22–26 v. 39–50), and from *E. euaxos* and *E. overstreeti* in possessing a longer scolex (136–184 v. 88–101 and 82–105 respectively). Shipley and Hornell (1906) reported as many as 42 (or more) proglottids for *E. gracile* whereas *Eniochobothrium vegrande*, sp. nov. exhibits only 22–26 in mature worms and 21–23 in gravid worms. Moreover, whereas mature worms of *E. vegrande*, sp. nov. are 1304–1610 in total length and gravid worms 1286–1840 in total length, *E. gracile* was reported to be 3500–12 000 in total length by Shipley and Hornell (1906).

Genus *Amiculucestus*, gen. nov.

ZooBank urn:lsid:zoobank.org:act:4BC0EF94-DADA-45CB-90CB-60E2336CF490

Diagnosis

Family Eniochobothriidae. Worms apolytic, protandrous. Scolex with four acetabula and apical modification of scolex proper housing apical organ, weakly connected to strobila; acetabula in form of suckers; distal and proximal surfaces of acetabula covered with both spathulate and small gladiate spinitriches; apical modification of scolex proper cone shaped, with terminal aperture; apical organ small, glandular, non-eversible, non-protrusible. Cephalic peduncle absent. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough and posterior region of reproductive proglottids; circumcortical longitudinal muscle bundles absent. Proglottids craspedote, non-lacinate. Testes numerous,

one layer deep, arranged in two, or occasionally up to four, columns, extending from anterior margin or anterior fifth of proglottid to posterior margin of cirrus sac on aporal side, extending to cirrus sac on poral side, postporal testes usually absent. Vas deferens conspicuous, thick walled for most of its length, extending from posterior of proglottid to posterior margin of cirrus sac, expanded to form conspicuous external seminal vesicle. Internal seminal vesicle present. Cirrus sac inverted J-shaped, thick walled, highly muscular. Cirrus armed. Ovary H-shaped in dorsoventral view, bilobed in cross-section, with smooth or lobulated margins. Vagina and seminal receptacle absent. Genital pores lateral, irregularly or regularly alternating; genital atrium conspicuous. Uterus sac-like, medial, extending entire length of gravid proglottids. Vitellarium follicular; vitelline follicles medullary, in two lateral fields of multiple follicles, extending from near anterior margin of proglottid to anterior margin of ovary. Excretory vessels four, arranged in one dorsal and one ventral pair. Oncospheres grouped in cocoons. Parasites of batoids of the genus *Rhinoptera* (Rhinopteridae). Circumtropical.

Type species: Amiculucestus calli, gen. nov., sp. nov.

Additional species

Amiculucestus australiensis, sp. nov.; *Amiculucestus herzogae*, sp. nov.; *Amiculucestus penghuensis*, sp. nov.

Etymology

The name is derived from *amiculum* (mantle or cloak; L.) and *cestus* (worm; L., neuter) in reference to the laterally expanded region of the anterior strobila of this cestode.

Remarks

The presence of an anterior strobilar region consisting of laterally expanded, barren proglottids forming a trough, coupled with the lack of a vagina distinguishes *Amiculucestus*, gen. nov. from 24 of the 25 genera of lecanicephalideans recognised as valid by Jensen *et al.* (2017) and also identifies it as a member of the *Eniochobothriidae*. It conspicuously differs from *Eniochobothrium* – the only other genus in this family – in the following seven major respects: it is protandrous, rather than developing male and female reproductive organs that mature at approximately the same time; the cirrus sac is inverted J-shaped, rather than U-shaped; the uterus extends to near the anterior margin of the proglottid, rather than to the level of the genital pore; its vitelline follicles are arranged in lateral fields that begin near the anterior margin of the proglottid, rather than being restricted to the region between the ovary and the cirrus sac; the testes are arranged in regular columns and the postporal field of testes on the poral side is usually absent, rather than being arranged in more irregular aporal and postporal fields with preporal field of testes usually absent; it lacks, rather than possesses, a seminal receptacle; and it

possesses, rather than lacks, a prominent external seminal vesicle.

The species included in the phylogenetic analyses of Jensen *et al.* (2016) as *Eniochobothrium* n. sp. 2 and *Eniochobothrium* n. sp. 3 are in fact species of *Amiculucestus*. The latter specimen is assigned a formal name below.

Amiculucestus calli, gen. nov., sp. nov.

(Fig. 11, 12)

ZooBank urn:lsid:zoobank.org:act:2E6F70A7-1B18-477D-9C2D-5C62FFE04613

Based on two immature worms, two male-mature worms, five male-mature strobilae, two female-mature worms, three female-mature strobilae, one gravid worm, cross-sections of one worm and three worms prepared for SEM.

Worms apolytic, protandrous; mature and gravid worms with 32–45 proglottids; male-mature worms 1578–1928 ($n = 2$) long, male-mature strobilae 1360–2190 long; female-mature worms 2321–2349 ($n = 2$) long, female-mature strobilae 1856–2392 long; gravid worms 3226 ($n = 1$) long, gravid strobilae 3126 ($n = 1$) long. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough, and posterior region of reproductive proglottids (Fig. 11d); circumcortical longitudinal muscle bundles absent.

Scolex 94–136 long, 90–107 wide, bearing four acetabula, apical modification of scolex proper and apical organ. Acetabula in form of suckers, sessile, 43–53 long, 32–43 wide. Apical modification of scolex proper in form of small cone with terminal aperture, housing apical organ (Fig. 11a). Apical organ small, internal, non-protrusible, non-eversible, primarily glandular, 36–51 long, 23–30 wide. Cephalic peduncle absent.

Distal surfaces (Fig. 12d) and rims of acetabula (Fig. 12e) covered with spatulate spinitriches and small gladiate spinitriches. Apical modification of scolex proper and scolex proper anterior to acetabula covered with small gladiate spinitriches (Fig. 12c). Region of scolex proper posterior to acetabula (Fig. 12f) covered with large and small gladiate spinitriches. Barren proglottids of trough covered with scolopate spinitriches on both concave (Fig. 12g, h) and convex (Fig. 12i) surfaces. Reproductive proglottids covered with capilliform filitriches away from posterior proglottid margins and with scolopate spinitriches near or at posterior proglottid margins.

Proglottids craspedote, non-lacinate. Trough with 17–22 proglottids, 299–613 long, 226–318 wide. Reproductive proglottids 14–23 in total number; immature proglottids 13–22 in number, posterior-most immature proglottid 77–413 long, 165–281 wide; mature proglottids in mature worms 1 ($n = 12$) in number, in gravid worms 0 ($n = 1$) in number; terminal male-mature proglottid (Fig. 11b) 580–1228

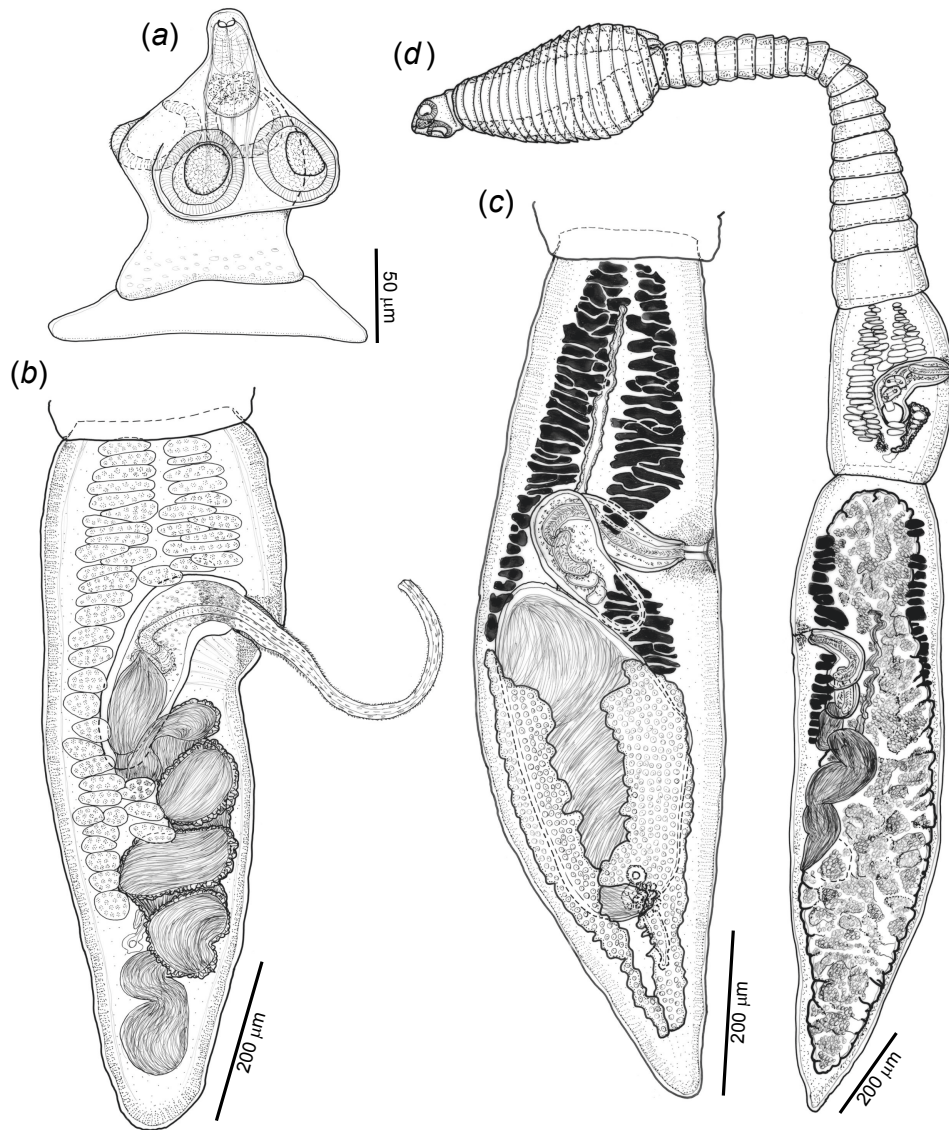


Fig. 11. Line drawings of *Amiculucestus calli*, sp. nov. (a) Scolex (paratype, USNM number I672241). (b) Terminal male-mature proglottid (paratype, USNM number I672237). (c) Terminal female-mature proglottid (paratype, USNM number I672241). (d) Gravid whole worm (holotype, USNM number I672244).

long, 251–293 wide; terminal female-mature proglottid (Fig. 11c) 944–1223 long, 293–350 wide; gravid proglottids 1 ($n = 1$) in number, 1383 ($n = 1$) long, 333 ($n = 1$) wide.

Testes 35–58 in total number, 10–41 long, 28–76 wide, one layer deep in cross-section, arranged in essentially two irregular columns (Fig. 11b), extending from anterior margin of proglottid to posterior quarter of proglottid on aporal side, extending from anterior margin of proglottid to anterior margin of cirrus sac and rarely slightly posterior to cirrus sac on poral side; aporal testes 17–37 in number; preporal testes 8–20 in number; postporal testes 0–4 in number, displaced medially by vas deferens. Vas deferens conspicuous, highly coiled, thick walled for much of its

length, extending from posterior margin of proglottid to enter cirrus sac at its posterior margin, expanded to form voluminous external seminal vesicle for much of its length. Internal seminal vesicle present. Cirrus sac inverted J-shaped (Fig. 11c), thick walled, highly muscular, 211–304 long, 63–97 wide, containing cirrus. Cirrus armed (Fig. 11b), coiled distally, at least 406 long ($n = 9$), 14–16 wide, 23–36 wide at base. Ovary H-shaped in dorsoventral view (Fig. 11c), bilobed in cross-section, with weakly lobulated margins, 150–263 wide, symmetrical; ovarian lobes 384–644 long. Vagina and seminal receptacle absent. Genital pores lateral, regularly alternating, 61–74% of proglottid length from posterior end in mature proglottid; genital atrium

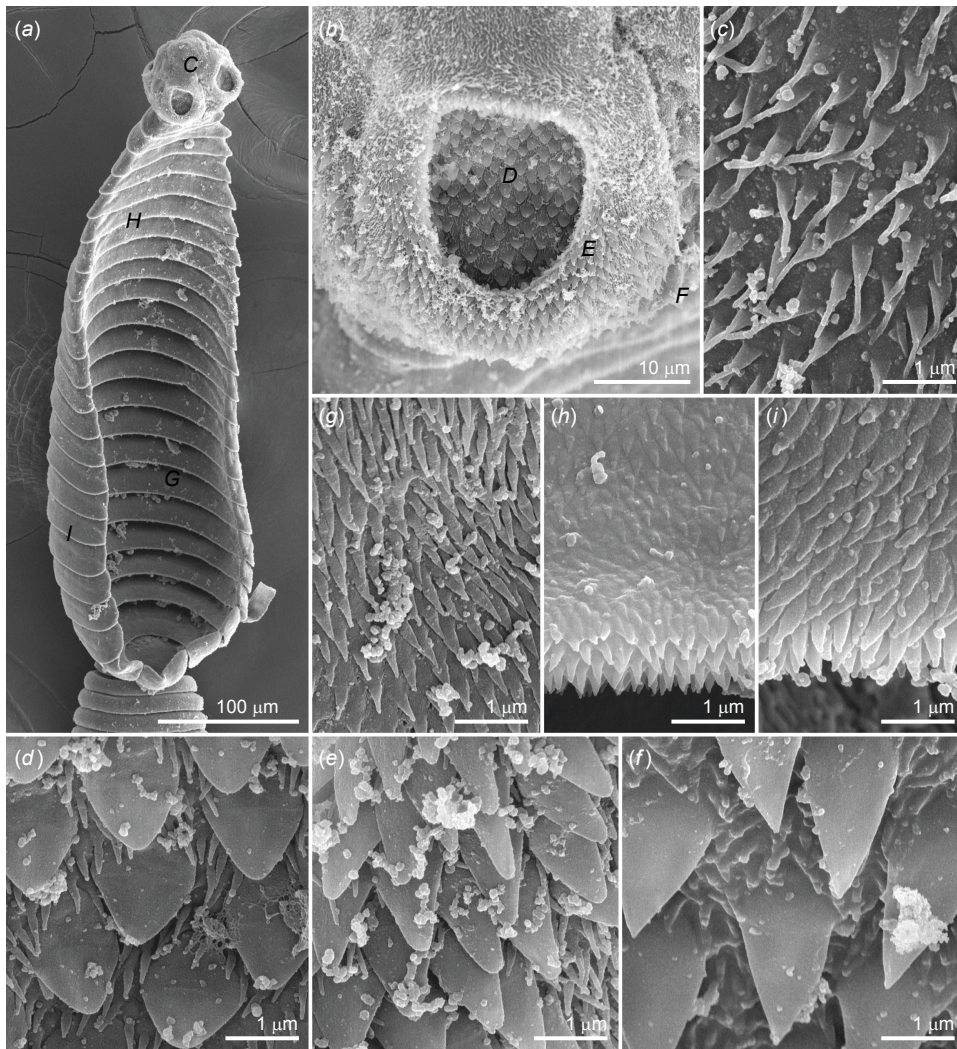


Fig. 12. Scanning electron micrographs of *Amiculucestus calli*, sp. nov. (a) Scolex and anterior region of strobila comprising barren, laterally expanded proglottids forming trough; uppercase italic letters indicate locations of details shown in (c) and (g–i). (b) Acetabulum; uppercase italic letters indicate locations of details shown in (d–f). (c) Surface of scolex proper anterior to acetabula. (d) Distal acetabular surface. (e) Surface of acetabular rim. (f) Surface of scolex proper posterior to acetabula. (g) Concave surface of trough, anterior proglottid region. (h) Concave surface of trough, posterior proglottid region. (i) Convex surface of trough. (j) Surface of reproductive proglottid away from margin.

conspicuous. Uterus saccate, medial, extending entire length of proglottid; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles medullary, 11–17 long, 33–79 wide, arranged in two lateral bands (Fig. 11c), extending from anterior margin of proglottid to anterior margin of ovary, interrupted by terminal genitalia on poral side. Excretory vessels four, arranged in one dorsal and one ventral pair. Developing oncospheres subspherical, grouped in cocoons.

Type and only known host

Rhinoptera brasiliensis Müller, Brazilian cownose ray (Myliobatiformes: Rhinopteridae).

Type locality

West tip of Horn Island, MS, USA, Gulf of Mexico (30°14'37.70"N, 88°46'37.62"W).

Additional locality

West of south tip of Chandeleur Island, LA, USA, Gulf of Mexico (29°57'9.54"N, 88°50'38.98"W).

Site of infection

Spiral intestine.

Type specimens deposited

Holotype (USNM number 1672244), and nine paratypes and cross-sections of one worm (USNM numbers 1672236–1672243, 1672245); six paratypes (LRP numbers 10908–10913). Specimens prepared for SEM retained in the collection of K. Jensen.

Sequence data

GenBank number ON040629 (hologenophore LRP number 10830, KW243).

Etymology

This species is named in honor of Garrett Call in recognition of his extensive research on tapeworms of cownose rays in the Gulf of Mexico.

Remarks

The description of the configuration of the testes in this species requires some explanation. This species is described as possessing postporal testes. However, in mature male proglottids, the testes of the postporal field are often displaced medially by the extensive vas deferens making them appear to be part of the aporal field of testes. That said, this feature seems to be somewhat variable in this species. Of the 10 worms in which testes were observed, 8 possessed between one and four testes in the postporal field, whereas postporal testes were not observed in the 2 remaining specimens.

Amiclucestus australiensis, sp. nov.

(Fig. 13, 14)

ZooBank urn:lsid:zoobank.org:act:33CC3147-AC61-475A-A78F-B58239AE59B2

Based on four male-mature worms, one male-mature strobila, three female-mature worms, two female-mature strobilae, one gravid worm, three gravid strobilae, cross-sections of two whole worms and two specimens prepared for SEM.

Worms apolytic, protandrous, mature and gravid worms with 21–33 proglottids; male-mature worms 1068–1201 long, male-mature strobila 1018–1138 long; female-mature worms 1598–1777 long, female-mature strobilae 1512–2023 long; gravid worms 1422 ($n = 1$) long, gravid strobilae 1342–1970 long. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough and posterior region of reproductive proglottids (Fig. 13c); circumcortical longitudinal muscle bundles absent.

Scolex 85–110 long, 61–83 wide, bearing four acetabula, apical modification of scolex proper and apical organ (Fig. 13a). Acetabula in form of suckers, sessile, 36–46 long, 24–34 wide. Apical modification of scolex proper in form of small cone with terminal aperture, housing apical organ. Apical organ small, internal, non-protrusible, non-eversible, primarily glandular, 30–37 long, 17–23 wide. Cephalic peduncle absent.

Distal surfaces (Fig. 14d) and rims of acetabula (Fig. 14e), and region of scolex proper posterior to acetabula (Fig. 14f) covered with spatulate spinitriches and small gladiate spinitriches. Apical modification of scolex proper and scolex proper anterior to acetabula (Fig. 14c) covered with small gladiate spinitriches. Barren proglottids of trough covered with scolopate spinitriches on both concave and convex (Fig. 14g) surfaces. Reproductive proglottids covered with

capilliform filitriches away from posterior proglottid margins and with scolopate spinitriches near or at posterior proglottid margins (Fig. 14h).

Proglottids craspedote, non-lacinate. Trough with 18–23 proglottids, 357–589 long, 177–244 wide. Reproductive proglottids 9–12 in total number; immature proglottids 8–11 in number, posterior-most immature proglottid 44–281 long, 71–129 wide; mature proglottids in mature worms 1 ($n = 10$) in number, in gravid worms 0 ($n = 4$) in number; terminal male-mature proglottid 467–653 long, 123–149 wide; terminal female-mature proglottid (Fig. 13c) 801–1172 long, 166–245 wide; gravid proglottids 1 ($n = 5$) in number, 627–1067 long, 184–282 wide.

Testes 11–16 in total number, 17–32 long, 18–31 wide, one layer deep in cross-section, arranged in essentially two regular columns (Fig. 13b), extending from anterior fifth of proglottid to posterior margin of ovary on aporal side, partially interrupted by cirrus sac, extending from anterior fifth of proglottid to cirrus sac on poral side; aporal testes 8–11 in number; preporal testes 3–6 in number; postporal testes absent. Vas deferens conspicuous, highly coiled, thick walled for much of its length, extending from posterior margin of proglottid to enter cirrus sac at its posterior margin, expanded to form voluminous external seminal vesicle. Internal seminal vesicle present. Cirrus sac inverted J-shaped (Fig. 13b), thick walled, highly muscular, 175–255 long, 51–64 wide, containing cirrus. Cirrus armed, coiled distally. Ovary H-shaped in dorsoventral view (Fig. 13c), bilobed in cross-section, with smooth margins, 98–161 wide, symmetrical; ovarian lobes 198–383 long. Vagina and seminal receptacle absent. Genital pores lateral, regularly alternating, 56–72% of proglottid length from posterior end in mature proglottid; genital atrium conspicuous. Uterus saccate, medial, extending entire length of proglottid; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles medullary, 11–16 long, 16–27 wide, arranged in two lateral bands (Fig. 13c), extending from anterior region of proglottid to anterior margin of ovary, interrupted by terminal genitalia on poral side. Excretory vessels four, arranged in one dorsal and one ventral pair. Developing oncospheres subspherical, grouped in cocoons.

Type and only known host

Rhinoptera neglecta Ogilby, Australian cownose ray (Myliobatiformes: Rhinopteridae).

Type locality

Dundee Beach, NT, Australia, Fog Bay, Timor Sea (12°45'33"S, 130°21'7"E).

Additional locality

None.

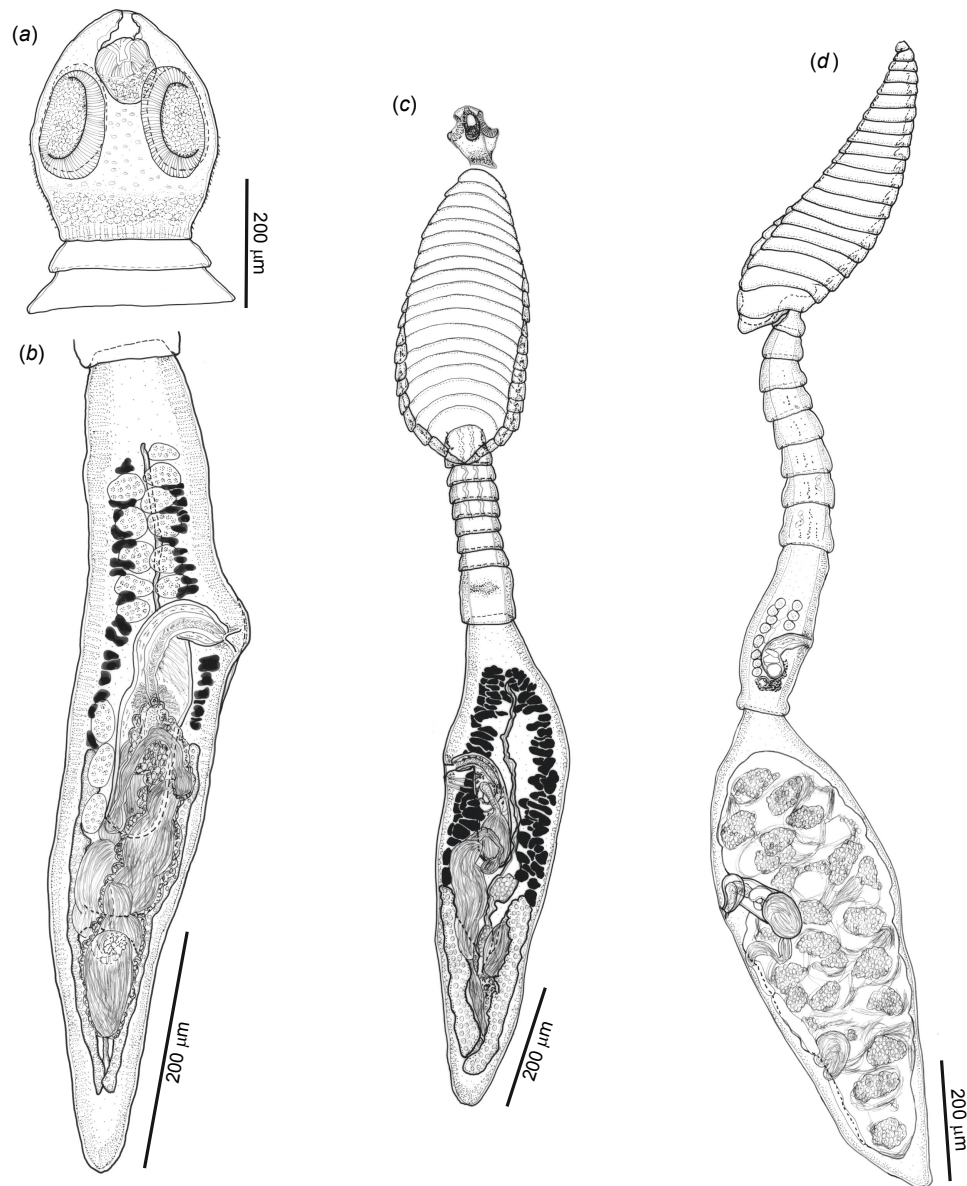


Fig. 13. Line drawings of *Amiculucestus australiensis*, sp. nov. (a) Scolex (paratype, QM number G240139). (b) Terminal male-mature proglottid with female reproductive organs incompletely developed (paratype, QM number G240138). (c) Whole worm with terminal female-mature proglottid (holotype, QM number G240137). (d) Gravid strobila with lateral view of trough (paratype, QM number G240143).

Site of infection

Spiral intestine.

Type specimens deposited

Holotype (QM number G240137) and six paratypes and cross-sections of one worm (QM numbers G240138–G240144); four paratypes (USNM numbers 1672232–1672235); three paratypes and cross-sections of one worm (LRP numbers

10903–10907). Specimens prepared for SEM retained in the collection of J. N. Caira.

Sequence data

GenBank number KU249056 (hologenophore LRP number 8786, PBI892) (as *Eniochobothrium* n. sp. 3; Jensen *et al.* 2016); GenBank number ON040631 (hologenophore LRP number 10832, KW247); GenBank number ON040632 (hologenophore LRP number 10833, KW248).

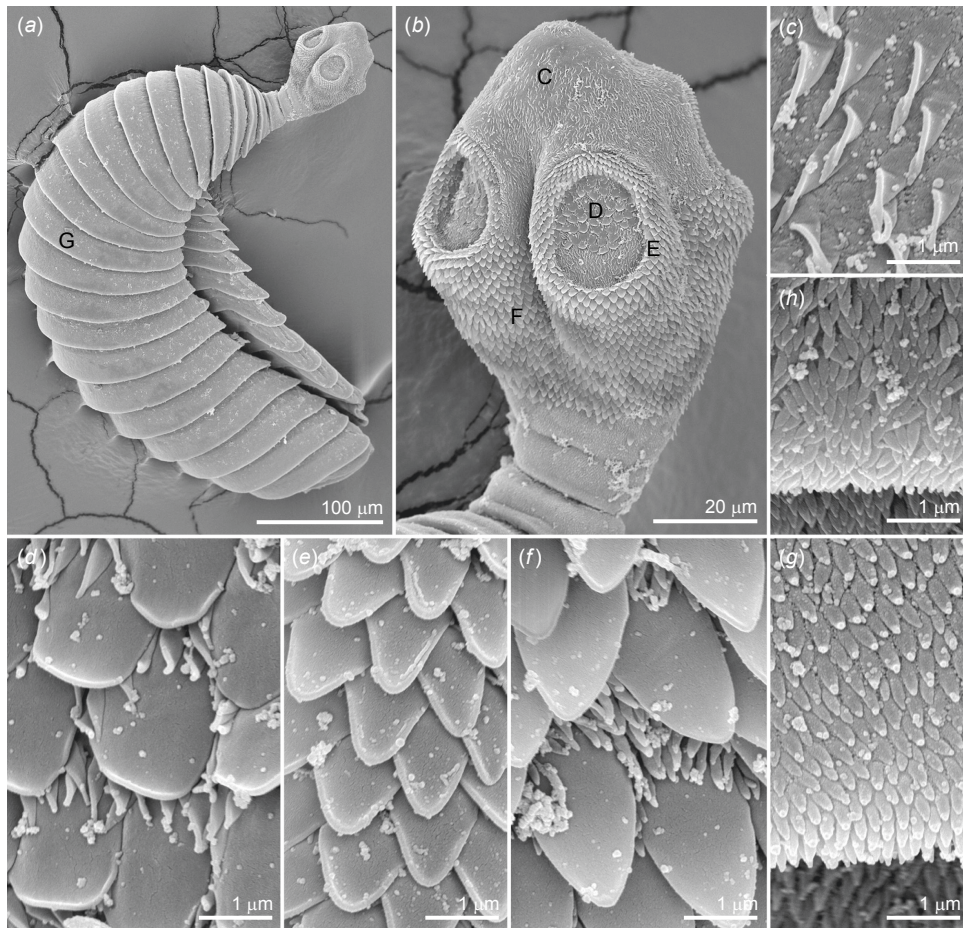


Fig. 14. Scanning electron micrographs of *Amiclucestus australiensis*, sp. nov. (a) Scolex and anterior region of strobila comprising barren, laterally expanded proglottids forming trough; uppercase italic letter indicates locations of detail shown in (g). (b) Scolex; uppercase italic letters indicate locations of details shown in (c–f). (c) Surface of scolex proper anterior to acetabula. (d) Distal acetabular surface. (e) Surface of acetabular rim. (f) Surface of scolex proper posterior to acetabula. (g) Convex surface of trough. (h) Surface of posterior margin of reproductive proglottid.

Etymology

The species is named for its type locality, northern Australia.

Remarks

Amiclucestus australiensis, sp. nov. is easily distinguished from *A. calli* based on its possession of fewer reproductive proglottids (9–12 v. 14–23) and substantially fewer testes (11–16 v. 35–58). In addition, gravid worms of *A. australiensis*, sp. nov. are shorter than those of *A. calli* (1422 v. 3126) and the fields of testes and vitelline follicles stop short of, rather than extend to, the anterior margin of the proglottid.

With the description of this species, we are providing a formal name for the specimen originally included in the phylogenetic analysis of [Jensen et al. \(2016\)](#) as *Eniochobothrium* n. sp. 3.

Amiclucestus herzogae, sp. nov.

(Fig. 15, 16)

ZooBank urn:lsid:zoobank.org:act:4FB19A2D-4A6A-4902-AF7F-8EF819D5F9A1

Based on one immature worm, eight male-mature strobilae, five female-mature strobilae, one gravid worm, two gravid strobilae, one semi-permanent preparation of eggs and one specimen prepared for SEM.

Worms apolytic, protandrous, mature and gravid worms with 32–38 proglottids; male-mature strobila 1324–1809 long; female-mature strobila 1816–2075 long; gravid worm 1972 ($n = 1$) long, gravid strobila 1872–1988 long. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough and posterior region of

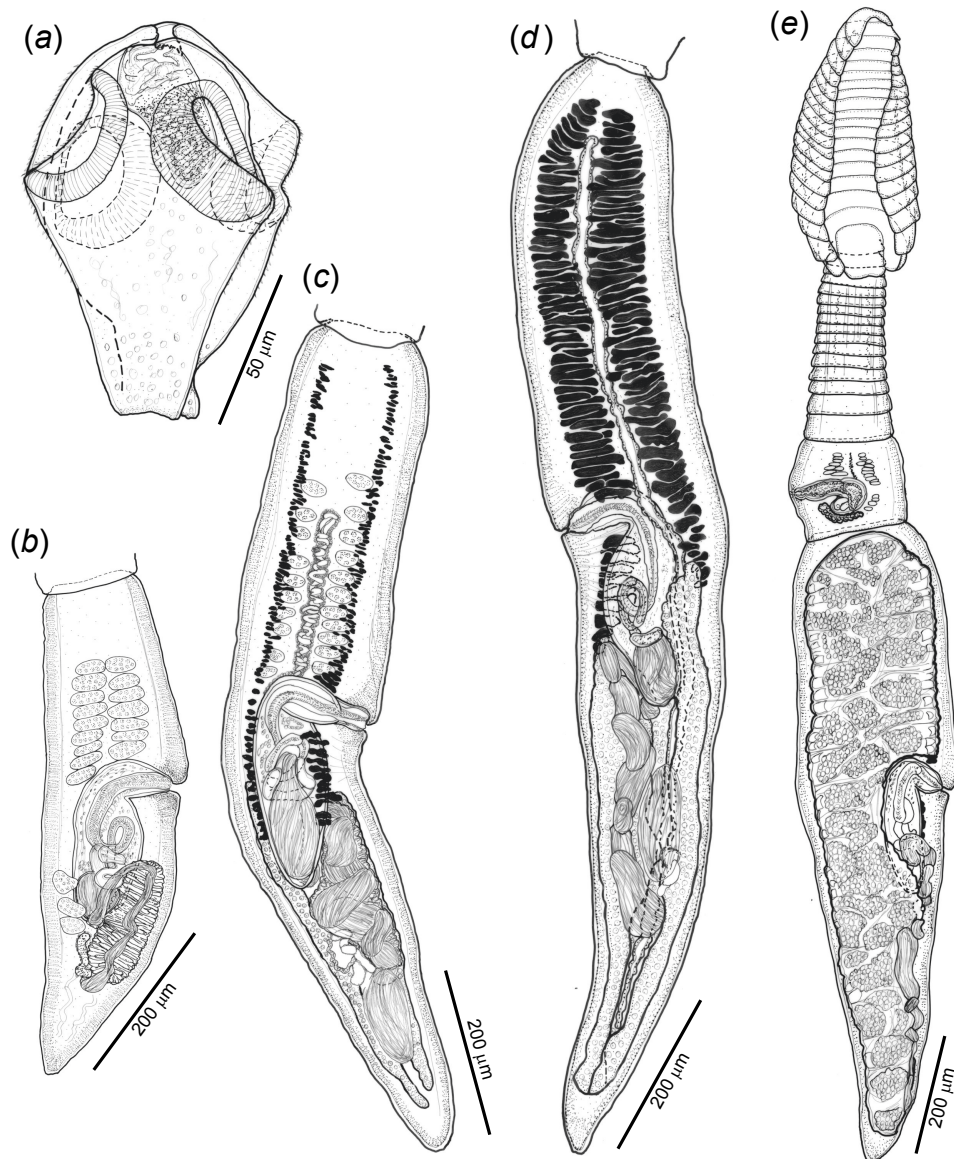


Fig. 15. Line drawings of *Amiculucestus herzogae*, sp. nov. (a) Scolex (paratype, USNM number 1672246). (b) Terminal male-mature proglottid (paratype, USNM number 1672247). (c) Terminal male-mature proglottid with female reproductive organs incompletely developed (holotype, USNM number 1672251). (d) Terminal female-mature proglottid (paratype, USNM number 1672254). (e) Gravid strobila (paratype, USNM number 1672256).

reproductive proglottids (Fig. 15e); circumcortical longitudinal muscle bundles absent.

Scolex 100–121 ($n = 2$) long, 87–100 ($n = 2$) wide, bearing four acetabula, apical modification of scolex proper and apical organ (Fig. 15a). Acetabula in form of suckers, sessile, 43–45 long, 26–35 wide. Apical modification of scolex proper in form of small cone with terminal aperture, housing apical organ. Apical organ small, internal, non-protrusible, non-eversible, primarily glandular, 32–34 ($n = 2$) long, 23–24 ($n = 2$) wide. Cephalic peduncle absent.

Distal surfaces (Fig. 16d) and region of scolex proper posterior to acetabula (Fig. 16f) covered with spatulate spinitriches and small gladiate spinitriches. Rims of acetabula (Fig. 16e) covered with large and small gladiate spinitriches. Apical modification of scolex proper and scolex proper anterior to acetabula (Fig. 16c) covered with small gladiate spinitriches. Barren proglottids of trough covered with scolopate spinitriches on both concave (Fig. 16g) and convex (Fig. 16h) surfaces. Reproductive proglottids covered with capilliform filitriches away from posterior proglottid margins (Fig. 16j)

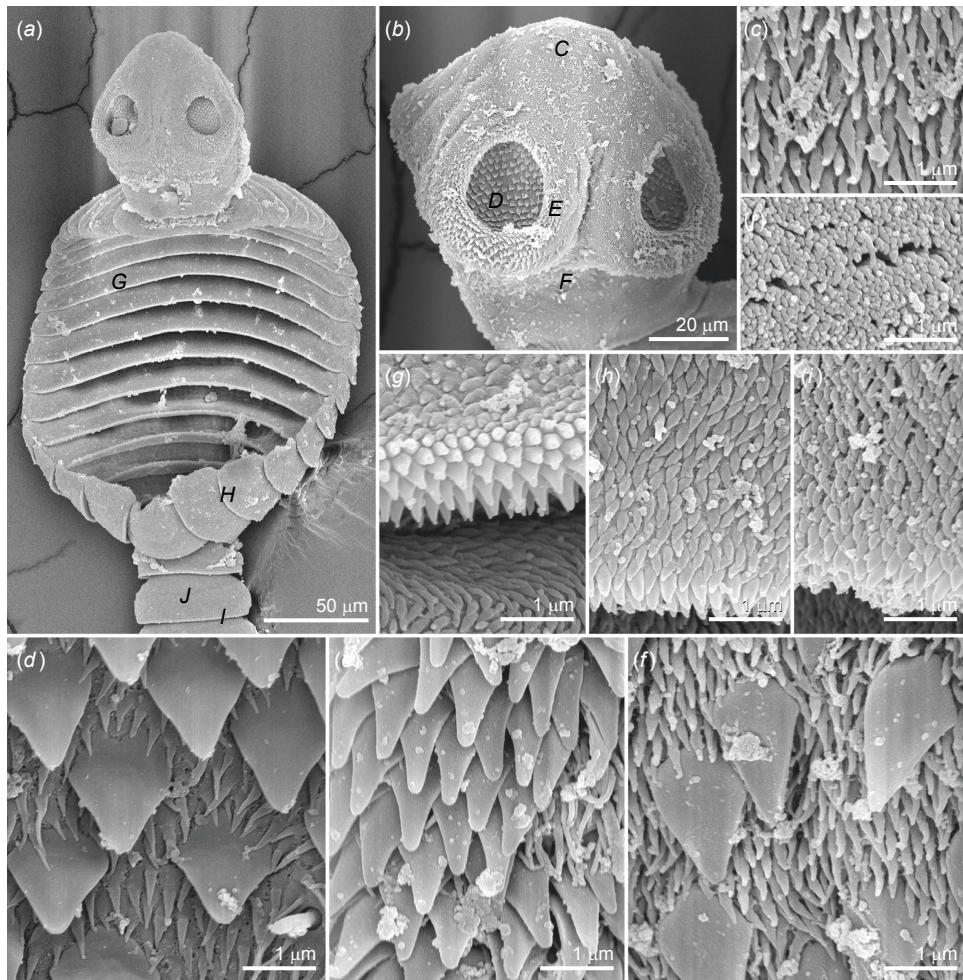


Fig. 16. Scanning electron micrographs of *Amiculucestus herzogae*, sp. nov. (a) Scolex and anterior region of strobila comprising barren, laterally expanded proglottids forming trough; uppercase italic letters indicate locations of details shown in (g–j). (b) Scolex; uppercase italic letters indicate locations of details shown in (c–f). (c) Surface of scolex proper anterior to acetabula. (d) Distal acetabular surface. (e) Surface of acetabular rim. (f) Surface of scolex proper posterior to acetabula. (g) Concave surface of trough (h) Convex surface of trough. (i) Surface of posterior margin of reproductive proglottid. (j) Surface of reproductive proglottid away from margin.

and with scolopate spinitriches near or at posterior proglottid margins (Fig. 16i).

Proglottids craspedote, non-laciniate. Trough with 17–21 proglottids, 333–444 long, 154–232 wide. Reproductive proglottids 12–19 in total number; immature proglottids 11–18 in number, posterior-most immature proglottid 90–214 long, 120–156 wide; mature proglottid in mature worms 1 ($n = 13$) in number, in gravid worms 0 ($n = 3$) in number; terminal male-mature proglottid (Fig. 15b) 595–1024 long, 167–205 wide; terminal female-mature proglottid (Fig. 15d) 1013–1259 long, 200–233 wide; gravid proglottids 1 ($n = 3$) in number, 951–1128 long, 251–282 wide.

Testes 19–29 in total number, 11–25 long, 22–45 wide, one layer deep in cross-section, arranged in essentially two regular columns (Fig. 15b, c), extending from anterior fifth

of proglottid to posterior margin of cirrus sac on aporal side, partially interrupted by cirrus sac, extending from anterior fifth of proglottid to anterior margin of cirrus sac on poral side; aporal testes 11–16 in number; preporal testes 7–13 in number; postporal testes absent. Vas deferens conspicuous, highly coiled, thick walled for much of its length, extending from region posterior in proglottid to enter cirrus sac at its posterior margin, expanded to form voluminous external seminal vesicle. Internal seminal vesicle present. Cirrus sac inverted J-shaped (Fig. 15b, c), thick walled, highly muscular, 228–339 long, 71–98 wide, containing cirrus. Cirrus armed, coiled distally, at least 88 long ($n = 3$), 18 ($n = 1$) wide, 24–26 wide at base. Ovary H-shaped in dorsoventral view (Fig. 15d), bilobed in cross-section, with smooth margins, 123–190 wide, symmetrical; ovarian lobes 328–490 long.

Vagina and seminal receptacle absent. Genital pores lateral, regularly alternating, 48–60% of proglottid length from posterior end in mature proglottid; genital atrium conspicuous. Uterus saccate, medial, extending entire length of proglottid; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles medullary, 7–15 long, 31–62 wide, arranged in two lateral bands (Fig. 15c, d), extending from near anterior margin of proglottid to anterior margin of ovary, somewhat interrupted by terminal genitalia on poral side. Excretory vessels four, arranged in one dorsal and one ventral pair. Developing oncospheres subspherical, grouped in cocoons.

Type and only known host

Rhinoptera jayakari Boulenger, Oman cownose ray (Myliobatiformes: Rhinopteridae).

Type locality

Tofo, Inhambane, Mozambique, Mozambique Channel (23°47'33.02"S, 35°31'16.38"E).

Site of infection

Spiral intestine.

Type specimens deposited

Holotype (USNM number 1672251), and 10 paratypes and 1 semi-permanent preparation of eggs (USNM numbers 1672246–1672250, 1672252–1672257); 6 paratypes and 1 SEM voucher (LRP numbers 10914–10920). Specimen prepared for SEM retained in the collection of K. Jensen.

Sequence data

GenBank number ON040623 (hologenophore LRP number 10824, KW229).

Etymology

This species is named for Kaylee Herzog, in appreciation of her assistance with obtaining scanning electron micrographs of this and the other species, as well as for valuable discussions about the morphology and anatomy of eniochobothriids.

Remarks

This new species is easily distinguished from its two congeners based on testis number; it possesses fewer testes than *A. calli* and more testes than *A. australiensis* (20–29 v. 35–58 and 11–16 respectively). *Amiclucestus herzogae*, sp. nov. further differs from *A. calli* in having a more posterior genital pore (48–60 v. 61–74%) and testes that stop short of, rather than extend to, the anterior margin of the proglottid. It further differs from *A. australiensis* in its possession of

a longer cirrus sac (264–339 v. 175–255) and vitelline follicles that extend well anterior to, rather than approximately to the level of, the anterior margin of the testicular field.

Amiclucestus penghuensis, sp. nov.

(Fig. 17)

ZooBank urn:lsid:zoobank.org:act:421F6E71-1F7F-418E-96ED-B02AF0B9CFC1

Based on three immature worms, two immature strobilae, one male-mature worm, one female-mature strobila and one gravid worm.

Worms apolytic, protandrous; male-mature and gravid worms with 35–47 ($n = 2$) proglottids; male-mature worm 1249 ($n = 1$) long, male-mature strobila 1206 ($n = 1$) long; female-mature strobila 1951 ($n = 1$) long; gravid worm 2506 ($n = 1$) long, gravid strobila 2398 ($n = 1$) long. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough and posterior region of reproductive proglottids (Fig. 17d); circumcortical longitudinal muscle bundles absent.

Scolex 108–195 long, 93–145 wide, bearing four acetabula, apical modification of scolex proper and apical organ (Fig. 17a). Acetabula in form of suckers, sessile, 44–77 long, 34–54 wide. Apical modification of scolex proper in form of small cone with terminal aperture, housing apical organ. Apical organ small, internal, non-protrusible, non-eversible, primarily glandular, 55–78 long, 23–45 wide. Cephalic peduncle absent. Microtriches not observed.

Proglottids craspedote, non-lacinate. Trough with 14–18 proglottids, 282–403 long, 92–215 wide. Reproductive proglottids 21–29 in total number; immature proglottids 20–28 in number, posterior-most immature proglottid 61–197 long, 139–153 wide; mature proglottids in mature worms 1 ($n = 2$) in number, in gravid worms 0 ($n = 1$) in number; terminal male-mature proglottids (Fig. 17c) 579 ($n = 1$) long, 180 ($n = 1$) wide; terminal female-mature proglottids 1139 ($n = 1$) long, 210 ($n = 1$) wide; gravid proglottids 1 ($n = 1$) in number, 1356 ($n = 1$) long, 326 ($n = 1$) wide.

Testes 24–31 in total number, 13–21 long, 33–37 wide, one layer deep in cross-section, arranged in essentially two regular columns (Fig. 17b, c), extending from near anterior margin of proglottid to posterior margin of cirrus sac on aporal side, partially interrupted by cirrus sac, extending from near anterior margin of proglottid to anterior margin of cirrus sac on poral side; aporal testes 13–16 in number; preporal testes 10–17 in number; postporal absent. Vas deferens conspicuous, thick walled for much of its length, extending from posterior margin of proglottid to enter cirrus sac at its posterior margin, expanded to form voluminous external seminal vesicle. Internal seminal vesicle present. Cirrus sac inverted J-shaped (Fig. 17c), thick walled, highly

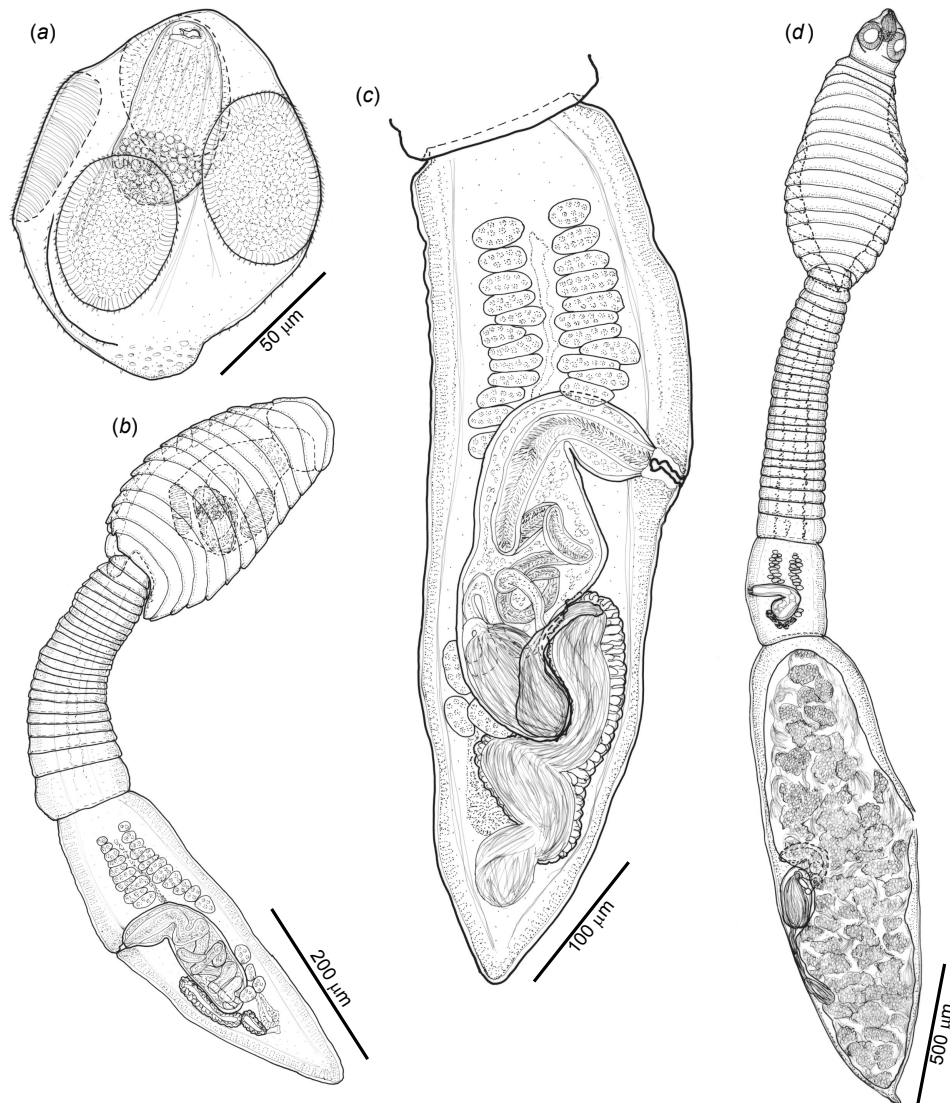


Fig. 17. Line drawings of *Amiculucestus penghuensis*, sp. nov. (a) Scolex (paratype, USNM number 1672258). (b) Whole worm with terminal male-mature proglottid (paratype, USNM number 1672259). (c) Terminal male-mature proglottid (holotype, NMNS number 8637-001). (d) Gravid whole worm (paratype, NMNS number 8637-003).

muscular, 301–349 long, 70–89 wide, containing cirrus. Cirrus armed, coiled distally. Ovary H-shaped in dorso-ventral view, bilobed in cross-section, with weakly lobulated margins, 151 ($n = 1$) wide, symmetrical; ovarian lobes 441–450 ($n = 1$) long. Vagina and seminal receptacle absent. Genital pores lateral, irregularly alternating, 59–61% ($n = 2$) of proglottid length from posterior end in mature proglottid; genital atrium conspicuous. Uterus saccate, medial, extending entire length of proglottid; uterine duct not observed; uterine pore absent. Vitellarium follicular; vitelline follicles medullary, 16–22 long, 37–65 wide, in two lateral band, extending from anterior margin of proglottid to anterior margin of ovary, somewhat interrupted by terminal genitalia. Excretory vessels four, arranged in one dorsal and one

ventral pair. Developing oncospheres subspherical, grouped in cocoons.

Type and only known host

Rhinoptera cf. *jayakari* (Myliobatiformes: Rhinopteridae).

Type locality

Magong, Penghu Island, Taiwan, Taiwan Strait (23°33'49"N, 119°34'31"E).

Site of infection

Spiral intestine.

Type specimens deposited

Holotype (NMNS number 8637-001) and two paratypes (NMNS numbers 8637-002–8637-003); two paratypes (USNM numbers 1672258–1672259); three paratypes (LRP numbers 10921-10922 and 10828, hologenophore).

Sequence data

GenBank number ON040627 (hologenophore LRP number 10828, KW236).

Etymology

The species is named for its type locality in the waters of the Taiwan Strait off Penghu Island.

Remarks

Amiculucestus penghuensis, sp. nov. conspicuously differs from *A. australiensis* and *A. herzogae* in that it possesses a greater number of reproductive proglottids (21–29 v. 9–12 and 12–19 respectively) and from *A. calli* in that it possesses fewer testes (24–31 v. 35–58). It can further be distinguished from all three species by its possession of a longer apical organ (55–78 v. 36–51, 30–37 and 32–34 in *A. calli*, *A. australiensis* and *A. herzogae* respectively).

The most recent diagnosis of the *Eniochobothriidae* (see Jensen *et al.* 2016) is amended below to accommodate new information presented here for *Eniochobothrium* and *Amiculucestus*, as well as to reflect updated terminology, particularly for the different strobilar regions and cirrus sac shape.

Family ENIOCHOBOTHRIIDAE Jensen, Caira, Cielocha, Littlewood & Waeschenbach, 2016, amended

Diagnosis

Worms apolytic, protandrous or not. Scolex with four acetabula and apical modification of scolex proper housing apical organ, usually weakly connected to strobila; acetabula in form of suckers; distal and proximal surfaces of acetabula covered with both spatulate and small gladiate spinitriches; apical modification of scolex proper cone shaped with terminal aperture. Apical organ small, glandular, non-eversible, non-protrusible. Cephalic peduncle absent. Strobila divided into anterior region of laterally expanded, barren proglottids forming trough, and posterior region of reproductive proglottids. Circumcortical longitudinal muscle bundles absent. Proglottids craspedote, non-lacinate. Testes numerous, arranged in two lateral fields or regular columns, postporal or preporal field of testes absent in some. Vas deferens thick walled, expanded or not to form voluminous external seminal vesicle. Internal seminal vesicle present. Cirrus sac

conspicuous, U-shaped or inverted J-shaped, thick walled. Cirrus armed. Ovary H-shaped in dorsoventral view, bilobed in cross-section, with smooth or lobulated margins. Vagina lacking; seminal receptacle present or absent. Genital pores lateral, irregularly or regularly alternating; genital atrium conspicuous. Uterus saccate, medial, extending from posterior margin of proglottid to anterior margin of proglottid or stopping short of genital pore. Vitellarium follicular; vitelline follicles medullary, arranged in two lateral fields or bands, restricted to region between cirrus sac and ovary or extending length of proglottid anterior to ovary. Excretory vessels in two lateral pairs. Oncospheres grouped in cocoons. Parasites of batoids of the genus *Rhinoptera* (Rhinopteridae). Circumtropical.

Type genus: *Eniochobothrium* Shipley & Hornell, 1906.

Additional genera: *Amiculucestus*, gen. nov.

Key to species of eniochobothriids

- 1 Cirrus sac U-shaped; uterus extending to anterior of proglottid..... 2
Cirrus sac inverted J-shaped; uterus extending to level of genital pore.....6
- 2 Preporal testes present*Eniochobothrium euaxos*
Preporal testes absent.....3
- 3 Worms (mature or gravid) greater than 2800 in total length.....4
Worms (mature or gravid) less than 2000 in total length.....5
- 4 Anterior-most proglottids of trough approximately as narrow as scolex ('short neck of three segments', Shipley and Hornell 1906, p. 64).....*Eniochobothrium gracile*
Anterior-most proglottids of trough conspicuously wider than scolex*Eniochobothrium qatarense*
- 5 Fewer than 18 testes.....*Eniochobothrium vegrande*
Twenty or more testes.....*Eniochobothrium overstreeti*
- 6 Fewer than 17 testes.....*Amiculucestus australiensis*
More than 18 testes.....7
- 7 Field of testes extending to anterior margin of proglottid; more than 34 testes.....*Amiculucestus calli*
Field of testes stopping well short of anterior margin of proglottid; fewer than 32 testes.....8
- 8 Nineteen or fewer reproductive proglottids.....*Amiculucestus herzogae*
Twenty-one or more reproductive proglottids.....*Amiculucestus penghuensis*

Discussion

The numerous morphological differences seen between species of *Eniochobothrium* and those of *Amiculucestus* were used to justify placing these taxa in separate genera. This taxonomic action was fully supported by the tree resulting from our phylogenetic analysis of 28S sequence data in which species in the two genera grouped in two subclades. The description of six new species and the erection of a second genus lend further support to the distinctive nature of the *Eniochobothriidae* relative to the seven other families of lecanicephalideans that emerged from the morphological and molecular work of Jensen *et al.* (2016).

The enigmatic nature of *Eniochobothrium* has been recognised since 1906 when Shipley and Hornell erected the genus for *E. gracile* noting that this species was so peculiar that it may even represent a new family – a nomenclatorial act that was not implemented until more than a century later by Jensen *et al.* (2016). For the most part, species of *Amiculucestus* share these peculiar features. Among these features is the complete lack of a vagina. This is puzzling because in essentially all cestodes of elasmobranchs this duct opens to the outside of these hermaphroditic worms in the genital atrium and serves as the conduit through which sperm from the cirrus of one proglottid reaches the female organs of another during fertilisation. The exception is *Dioecotaenia* Schmidt, 1969, which, although it possesses a vagina, that vagina lacks an external pore. Sperm is transferred by way of hypodermic insemination during which the cirrus of a male proglottid breaches the surface of a female proglottid and deposits sperm directly into the lumen of the vagina. From there sperm makes its way down to the seminal receptacle, which is essentially just an expanded region of the base of the vagina (see Schmidt 1969). The lack of a vagina in eniochobothriids raises the question of how fertilisation of any kind (cross or self) is accomplished. Adding to the intrigue is the fact that species of *Eniochobothrium* possess a sacciform seminal receptacle associated with the ovarian bridge that is filled with sperm in mature (Fig. 7a, d) and even in some immature (Fig. 7b) proglottids, but appears as a preformed empty cavity in less developed immature proglottids (Fig. 7b). The hypothesis that the sperm in the seminal receptacle came from a different worm is supported by the fact that seminal receptacles filled with sperm were observed in the proglottids of worms in which the testes had not yet developed to maturity (e.g. Fig. 7b). As in species of *Dioecotaenia*, the eniochobothriids possess an unusually large and robust cirrus sac and cirrus suggesting that hypodermic impregnation is possible. However, unlike the situation seen in *Dioecotaenia* by Schmidt (1969; fig. 9), worms with the cirrus of a male detached proglottid in the act of hypodermic impregnation still attached to a female proglottid have not been observed in the eniochobothriids. Nor have we seen any evidence of a breach in the surface of the receiving worm adjacent to its seminal receptacle. That said, it is interesting that Schmidt (1969) reported that holes made by cirri during sperm injection appear able to heal over in *Dioecotaenia*. Even more puzzling is the lack of evidence of a seminal vesicle in any of the species of *Amiculucestus*.

Another unusual feature of the eniochobothriids is their possession of a series of laterally expanded, barren proglottids at the anterior end of the strobila that form a trough. We believe that these proglottids are generated from a germinative zone located at the posterior margin of the scolex and that a second germinative zone located posterior to the last barren proglottid of the trough produces the reproductive proglottids that are more typical of those seen in other

elasmobranch tapeworms. Based on the tiny, fragile nature of the scolex of eniochobothriids and the fact that it is not uncommon to encounter worms that have lost their scolex, the scolex itself seems unlikely to play a major role in attachment of the worm to the surface of the mucosa of its host. Gross observations (Fig. 6a) and histological sections (Fig. 6b, c) of worms *in situ* indicate that attachment is accomplished by the trough consisting of barren proglottids, which appears to literally grasp a portion of the mucosal surface (Fig. 6a). Grasping is achieved by pulling the epithelial tissue of the host spiral intestine into the hollow space created by the concavity of the trough (Fig. 6b, c). Although the acetabula of the scolex also grasp host tissue, histological sections support the minor role this portion of the body plays in attachment relative to that of the trough (Fig. 6b). Histological sections indicate that the trough is embedded deeply into the mucosa down as far as the lamina propria (Fig. 6b). Furthermore, although some modification of the mucosal surface at the site of attachment in the form of expansion of the mucosal pits was observed, histological sections showed little evidence of severe histopathological change at the site of attachment (Fig. 6c).

The existence of two, rather than a single, germinative zone is highly unusual in cestodes. Most have only a single germinative zone located at the posterior margin of the scolex or neck that produces reproductive proglottids. The evolutionary pressures that may have led to development of a mechanism to co-opt the function of a series of proglottids from reproduction to attachment are interesting to consider. One of the factors that immediately comes to mind is the challenge these unusually tiny tapeworms (most are 2–6 mm in total length) must encounter in attempting to maintain their position in the active environment of the spiral intestine of their elasmobranch host against the forces of continual peristalsis.

This work has done much to expand our understanding of the Eniochobothriidae. The distribution of the family, which was known previously only from the northern coast of Australia, the north-eastern coast of Sri Lanka and the Arabian Gulf, has been expanded to include the western Atlantic Ocean off South Carolina, the eastern Atlantic Ocean off Senegal, the Gulf of Mexico, the Mozambique Channel and the Taiwan Strait. The distribution of the family appears to be restricted to the warmer waters of the globe and thus is considered to be circumtropical.

One of the advantages when working to estimate global diversity of parasite groups such as the eniochobothriids is their fidelity for a specific group of hosts. Despite extensive surveys of cestodes of elasmobranchs globally (Caira and Jensen 2014), which have included relatively dense sampling of elasmobranchs in the other genera of myliobatiforms, eniochobothriids are currently known to parasitise only members of the myliobatiform genus *Rhinoptera* (i.e. cownose rays). (The cestode from *Pastinachus sephen* (Forsskal), as *Trygon sephen*, described as *Eniochobothrium trygonis* Chincholikar

& Shinde, 1978 by Chincholikar and Shinde 1978 is likely a species of *Hornellobothrium* Shipley & Hornell, 1906.) This limited spectrum of realised host taxa greatly facilitates the assessment of the global diversity of this lecanicephalidean family. Taking stock of the current situation, at present eight valid species of *Rhinoptera* are recognised (Last *et al.* 2016) and a ninth (*R. cf. jayakari*) remains to be formally described. Five of these nine species host described eniochobothriids. *Rhinoptera brasiliensis* hosts *E. overstreeti* and *A. calli*; *R. javanica* hosts *E. gracile*; *R. jayakari* hosts *E. qatarense* and *A. herzogae*; *R. cf. jayakari* hosts *E. qatarense*, *E. vegrande* and *A. penghuensis*; and *R. neglecta* hosts *E. euaxos* and *A. australiensis*. Two additional species of *Rhinoptera* are known to host undescribed eniochobothriids that to date are known solely from molecular work. These are *R. bonasus*, which hosts *Amiculucestus* sp. nov. 5 (see Fig. 1); and *R. marginata*, which hosts *Eniochobothrium* sp. nov. 4 and *Amiculucestus* n. sp. 2 (see Fig. 1). In summary, five of these seven species of *Rhinoptera* host a species in both eniochobothriid genera and *R. cf. jayakari* hosts a species of *Amiculucestus* and two species of *Eniochobothrium*. Although not described here, we also found evidence of a second species of *Eniochobothrium* in *R. jayakari*. These data suggest that each species of *Rhinoptera* may host up to three species of eniochobothriids, bringing the global total to a maximum of twenty-seven species, assuming no new *Rhinoptera* species remain to be discovered.

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Data availability. The sequence data generated for this study are available in GenBank.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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