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Hyperapolytic species of *Acanthobothrium* (Cestoda: Onchoproteocephalidea) from batoids off Argentina

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ABSTRACT

Two hyperapolytic species of *Acanthobothrium* Blanchard, 1848 have been collected from *Discopyge tschudii* Heckel, 1846 and *Zapteryx brevirostris* (Müller et Henle) along the coast of Argentina. *Acanthobothrium stefaniae* sp. n. from *D. tschudii* is a category one species (i.e., it is less than 15 mm in total length, possesses fewer than 50 proglottids, fewer than 80 testes and essentially symmetrical ovary), and differs from all congeners by the following combination of features: proglottid hyperapolysis, hook morphology, size and shape of the cirrus sac, and by having spinitriches in the distal bothridial surface. This is the first record of *Acanthobothrium* in *Discopyge* Heckel, 1846. The specimens from *Z. brevirostris* conform to the morphology of *Acanthobothrium zapterycum* Ostrowski de Núñez, 1971. A redescription of this species is presented, which expands most ranges of measurements originally given, and provides details omitted in the original description, including the microthrix pattern. This study allowed us to observe the intraspecific variation in ovarian symmetry in *A. zapterycum*, which shed some doubt on the validity of this as a diagnostic feature. The reproductive strategy (apolysis) of several species of *Acanthobothrium* was reviewed and summarized.

1. Introduction

The order Onchoproteocephalidea includes species hosted by elasmobranchs as well species hosted by other vertebrate groups (i.e., freshwater fishes, snakes, amphibians and a mammal) [1,2]. Among the 11 genera that parasitize elasmobranchs, *Acanthobothrium* Blanchard, 1848 accounts for most of the diversity with 188 species [2]. Members of *Acanthobothrium* are parasites of a wide range of elasmobranchs, being particularly diverse in Myliobatiformes [3]. Most species are remarkably host specific for their definitive host, being oioxenous parasites [3,4]. Multiple infections by species of *Acanthobothrium* are common, with a record of seven species reported from the same host species (e.g. *A. campbelli* Marques, Brooks et Monks, 1995, *A. cimari* Marques, Brooks et Monks, 1995, *A. cleofanus* Monks, Brooks et Pérez Ponce de León, 1996, *A. costarricense* Marques, Brooks and Monks, 1995, *A. obuncus* Marques, Brooks et Barriga, 1997, *A. puntarenasense* Marques, Brooks et Monks, 1995 and *A. vargasi* Marques, Brooks et Monks, 1995 from *Hypanus longus* [Garman] [5]). In association with the diversity of hosts, *Acanthobothrium* has a broad geographic distribution, being reported from most oceans, including some rivers in South America and Malaysia [3,5,6,7].

Despite its diversity and ubiquity, few phylogenetic hypotheses involve a large number of species of *Acanthobothrium*. The tree presented by Campbell and Beveridge [8] for 34 Australian species based on 38 morphological characters was relatively poorly resolved and involved a significant degree of homoplasy. The molecular phylogeny by Fyler [3] included 49 morphologically diverse species of *Acanthobothrium* from a broad range of hosts and geographic localities. Fyler's phylogeny showed that *Acanthobothrium* is essentially monophyletic and that co-speciation has not been the predominant mechanism of evolution in this genus since there is little or no congruence between parasite and host clades [3]. Instead, some major clades within the *Acanthobothrium* phylogeny were restricted to major ocean basins, thus much of the phylogeny was explained by geography, regardless of host associations [3]. An interesting result from this analysis was that even if the species determined morphologically are generally corroborated by molecular data, several qualitative morphological features traditionally used in the descriptions have no phylogenetic significance. Although the four characters used by Ghoshroy and Caira [5] in their categorization system of species of *Acanthobothrium* are quite homoplastic (i.e. worm length, number of proglottids, number of testes and ovary symmetry) [3], the system is still useful for a prompt identification of species in the

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genus, in the absence of a more comprehensive phylogeny.

The pattern of maturation and subsequent release of proglottids from the strobila (apolysis) is a diagnostic feature for cestodes. Most species of *Acanthobothrium* are euapolytic [3], and only a few have been described as hyperapolytic or extremely hyperapolytic [9]. However, there have been some inconsistencies in the application of these terms, which were defined in several occasions with slight differences [10–13]. During a parasitological survey along the coast of Argentina, two hyperapolytic species of *Acanthobothrium* were collected, i.e. *A. zapterycum* Ostrowski de Núñez, 1971 from the rhinopristiform *Zapteryx brevirostris* (Müller et Henle) and a new species of *Acanthobothrium* from the torpediniform *Discopyge tschudii* Heckel. We describe herein the new species and present a redescription of *A. zapterycum* based on types and recently collected specimens. On the basis of the original drawings and descriptions, the reproductive strategy (apolysis) of most species of *Acanthobothrium* was reviewed (Table 1).

2. Materials and methods

2.1. Specimen collection

Cestodes were recovered from the spiral intestines of 13 specimens of *Discopyge tschudii* and seven specimens of *Zapteryx brevirostris* caught at several localities along the coast of Argentina. Eleven specimens of *D. tschudii* were caught off coastal waters of Buenos Aires Province as follows: one specimen at 35°50′S, 56°18′W (host field number PD5-112), one specimen at 37°29′S, 56°45′W (PD5-021), three specimens at 37°46′S, 56°56′W (PD5-005, PD5-244, PD5-245) in August 2012 and six specimens at 38°46′S, 57°56′W in July 2001 (VIPQ-045a, VIPQ-045b, VIPQ-053a, VIPQ-053b, VIPQ-053c, VIPQ-059). Two additional specimens of *D. tschudii* were caught off Patagonian waters: one specimen at 45°08′S, 65°19′W (PD7-267) in March 2013, and one specimen at 46°43′S, 66°04′W (PD4-254) in April 2012. Six specimens of *Z. brevirostris* were caught off coastal waters of Buenos Aires Province as follows: two specimens at 36°38′S, 56°15′W (PD5-072, PD5-074), three specimens at 37°29′S, 56°45′W (PD5-024, PD5-031, PD5-032) in August 2012 and one specimen at 38°46′S, 57°56′W (VIPQ-064) in July 2001. One additional specimen was caught off Patagonian waters, at 42°05′S, 62°50′W (PD4-284) in April 2012. Hosts labelled as PD4, PD5 and PD7 were caught with bottom trawls on board of the Oceanographic Vessel "Puerto Deseado" (CONICET), hosts labelled as VIPQ were caught by commercial trawlers. Additional information and images of each host specimen can be obtained by entering the host specimen number (e.g., PD4-254, PD7-267, etc.) in the specimen database at http://tapewormdb.uconn.edu. Additional five specimens of *D. tschudii* were also examined but no onchoproteocephalidean cestodes were recovered from them. Two of them were caught in April 2012 off Santa Cruz Province (46°43′S, 66°04′W), and three specimens were caught in March 2013 off Chubut Province (43°05′S, 64°07′W). Also three uninfected specimens of *Z. brevirostris* caught off Buenos Aires Province (38°46′S, 57°56′W) in August 2012 were examined for parasites.

2.2. Specimen preparation for light microscopy

All tapeworms were removed from the spiral intestine of their respective host, fixed in 10% formalin and transferred to 70% ethanol for storage. Specimens prepared for light microscopy were hydrated in a graded ethanol series, stained with Harris' hematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Detached mature proglottids were embedded in paraffin, and serially cross-sectioned at a thickness of 8 μm. Sections were stained with Harris' hematoxylin, counterstained with eosin, and mounted in Canada balsam. Eggs were obtained from broken detached gravid proglottids and prepared as temporary mounts using distilled water. Whole mounts, sections and temporary mounts were observed and measured using an Olympus BX 51 compound microscope. Drawings were made with the aid of a drawing tube. Measurements include the range, followed in parentheses by the mean, standard deviation, number of worms examined, and the total number of observations when more than one measurement per worm was taken. All measurements are in micrometres, unless otherwise stated. Hook measurements follow Ghoshroy and Caira [5] and Campbell and Beveridge [8].

2.3. Scanning electron microscopy

Worms prepared for scanning electron microscopy (SEM) were hydrated in a graded ethanol series, post-fixed in 1% osmium tetroxide overnight at room temperature, dehydrated in a graded ethanol series, and dried using hexamethyldisilazane. After dehydration, the specimens were mounted on stubs with carbon tape, coated with *c*. 40 nm of gold/palladium in a Thermo VG Scientific Polaron SC 7630 and examined in a Philips XL 30 scanning electron microscope.

2.4. Terminology and abbreviations

Terminology for microthrix shape follows Chervy [14]. Shape terminology for cirrus sac follows Clopton [15]. Terms on the reproductive strategy follows Fyler [9] and Caira et al. [13]. A tapeworm is considered to be anapolytic if the strobila consists of gravid proglottids followed by proglottids that had shed their eggs. The tapeworms that retain some gravid proglottids on the strobila, but which are never found to retain spent proglottids posterior to gravid proglottids are considered to be apolytic. When the terminal proglottids of a tapeworm are mature proglottids, and gravid proglottids are never seen, it is considered to be euapolytic. Taxa in which the strobila is never seen to possess proglottids that are either mature or gravid, are described as hyperapolytic. Tapeworms that bear terminal proglottids in which the reproductive organs are completely undeveloped are considered to be extremely hyperapolytic [9,13].

Valid host names follow Froese and Pauly [16]. To facilitate comparison between the new species of *Acanthobothrium* and the large number of species described, the categorization system of Ghoshroy and Caira [5] was followed. Museum abbreviations used are as follows: IPCAS, Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; LRP, Lawrence R. Penner Parasitology Collection, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA; MACN-Pa, Museo Argentino de Ciencias Naturales, Colección Parasitológica, Buenos Aires, Argentina.

2.5. Review of proglottids apolysis

The proglottids apolysis of all the valid species of *Acanthobothrium* was revised based on the original figures, descriptions and subsequent redescriptions, to unify the terminology across species of *Acanthobothrium* (Table 1). Only the species that needed to be corrected were included in the table.

3. Results

3.1. Acanthobothrium stefaniae sp. n. (Figs. 1–3)

3.1.1. Description (based on 21 specimens prepared as follows: whole mounts of 19 entire worms, 15 detached mature proglottids and 10 detached gravid proglottids, serial cross-sections of 1 detached mature proglottid, 2 worms and 4 detached mature proglottids examined with SEM)

Worms 1.14–1.92 mm (1.40 mm ± 0.26; 11) long, 228–320 (280 ± 37; 7) maximum width at level of scolex; 10–16 (13 ± 1; 14) proglottids, slightly craspedote; hyperapolytic (Fig. 1A). Scolex 285–400 (337 ± 40; 16) long, consisting of scolex proper with 4 bothridia and cephalic peduncle (Figs. 1A, C, 2A). Bothridia free posteriorly, 247–355

Table 1
Unified terminology in proglottid apolysis in species of *Acanthobothrium*.

Species of *Acanthobothrium*	Apolysis originally described	Apolysis following definitions by Caira et al. [13]	Reference
A. aetiobatis (Shipley, 1900) Southwell, 1925	Apolytic	Euapolytic	[45]
A. amazonensis Mayes, Brooks et Thorson, 1978	Apolytic	Euapolytic	[25]
A. angelae Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. arlenae Campbell et Beveridge, 2002	Apolytic	Euapolytic	[8]
A. atahualpai Marques, Brooks et Barriga, 1997	Apolytic	Euapolytic	[46]
A. bartonae Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. batailloni Euzet, 1955	Apolytic	Doubtful	[47]
A. brachyacanthum Riser, 1955	Hyperapolytic	Doubtful	[48]
A. brayi Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. campbelli Marques, Brooks et Monks, 1995	Apolytic	Euapolytic	[49]
A. cartagenensis Brooks et Mayes, 1980	Apolytic	Euapolytic	[50]
A. chengi Cornford, 1974	Apolytic	Doubtful	[51]
A. chisholmae Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. cimari Marques, Brooks et Monks, 1995	Apolytic	Euapolytic	[49]
A. cleofanus Monks, Brooks et Ponce de Leon, 1996	Anapolytic	Euapolytic	[52]
A. colombianum Brooks et Mayes, 1980	Apolytic	Euapolytic	[50]
A. costarricense Marques, Brooks et Monks, 1995	Apolytic	Euapolytic	[49]
A. dujardini van Beneden, 1850	Apolytic	Doubtful	[53]
A. edmondsi Campbell et Beveridge, 2002	Apolytic	Euapolytic	[8]
A. edwardsi Williams, 1969	Apolytic	Doubtful	[53]
A. electricolum Brooks et Mayes, 1978	Apolytic	Euapolytic	[54]
A. floridensis Goldstein, 1964	Apolytic	Euapolytic	[55]
A. fogeli Goldstein, 1964	Apolytic	Euapolytic	[55]
A. franus Marques, Centritto et Stewart, 1997	Apolytic	Doubtful	[56]
A. giganticum Sanaka, Vijaya Lakshmi et Rao, 1993	Apolytic	Euapolytic	[57]
A. gloveri Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. goldsteini Appy et Dailey, 1973	Apolytic	Euapolytic	[58]
A. gonzalesmugaburoi Severino et Sarmiento, 1979	Apolytic	Doubtful	[59]
A. himanturi Brooks, 1977	Apolytic	Euapolytic	[60]
A. hispidum Riser, 1955	Hyperapolytic	Doubtful	[48]
A. holorhini Alexander, 1953	Apolytic	Doubtful	[61]
A. jonesi Campbell et Beveridge, 2002	Euapolytic	Apolytic	[8]
A. karachiense Bilqees, 1980	Apolytic	Doubtful	[62]
A. lasti Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. laurenbrownae Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. lilium Baer et Euzet, 1962	Apolytic	Doubtful	[45]
A. lineatum Campbell, 1969	Apolytic	Euapolytic	[63]
A. lintoni Goldstein, Henson et Schlicht, 1968	Apolytic	Hyperapolytic	[20]
A. longipedunculata Maheswari, Lakshmi et Rao, 1985	Apolytic	Euapolytic	[64]
A. lusarmientoi Severino et Verano, 1980	Apolytic	Euapolytic	[65]
A. maculatum Riser, 1955	Hyperapolytic	Euapolytic	[48]
A. magnum Euzet, 1959	Anapolytic	Doubtful	[11]
A. marplatensis Ivanov et Campbell, 1998	Hyperapolytic	Euapolytic	[66]
A. martini Campbell et Beveridge, 2002	Apolytic	Euapolytic	[8]
A. maryanskii Caira et Burge, 2001	Euapolytic	Apolytic	[67]
A. microcephalum Alexander, 1953	Apolytic	Doubtful	[61]
A. minusculus Marques, Brooks et Barriga, 1997	Apolytic	Euapolytic	[46]
A. monksi Marques, Brooks et Barriga, 1997	Apolytic	Euapolytic	[46]
A. mooreae Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. mujibi Bilqees, 1980	Apolytic	Doubtful	[62]
A. nicoyaense Brooks et McCorquodale, 1995	Apolytic	Euapolytic	[68]
A. ningdense Yang, Sun, Zhi, Iwaki, Reyda et Yang, 2016	Anapolytic	Doubtful	[21]
A. obuncus Marques, Brooks et Barriga, 1997	Apolytic	Doubtful	[46]
A. ocallaghani Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. odonoghuei Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. olseni Dailey et Mudry, 1968	Apolytic	Euapolytic	[69]
A. puntarenasense Marques, Brooks et Monks, 1995	Apolytic	Euapolytic	[49]
A. quadripartitum Williams, 1968	Apolytic	Doubtful	[70]
A. quinonesi Mayes, Brooks et Thorson, 1978	Apolytic	Doubtful	[25]
A. rajaebatis (Rudolphi, 1810) Euzet, 1959	Apolytic	Doubtful	[11]
A. regoi Brooks, Mayes et Thorson, 1981	Apolytic	Euapolytic	[26]
A. rhinobati Alexander, 1953	Apolytic	Doubtful	[61]
A. robustum Alexander, 1953	Apolytic	Euapolytic	[61]
A. rohdei Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. rubrum Bilqees, 1980	Apolytic	Doubtful	[62]
A. stevensi Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. tasajerasi Brooks, 1977	Apolytic	Doubtful	[60]
A. thomasae Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. tripartitum Williams, 1969	Apolytic	Euapolytic	[53]
A. unilateralis Alexander, 1953	Apolytic	Euapolytic	[61]
A. urolophi Schmidt, 1973	Apolytic	Euapolytic	[71]
A. urotrygoni Brooks et Mayes, 1980	Apolytic	Euapolytic	[50]
A. vargasi Marques, Brooks et Monks, 1995	Apolytic	Euapolytic	[49]
A. walkeri Campbell et Beveridge, 2002	Hyperapolytic	Euapolytic	[8]
A. waltairensis Maheswari, Sanaka, Lakshmi et Rao, 1987	Apolytic	Doubtful	[72]

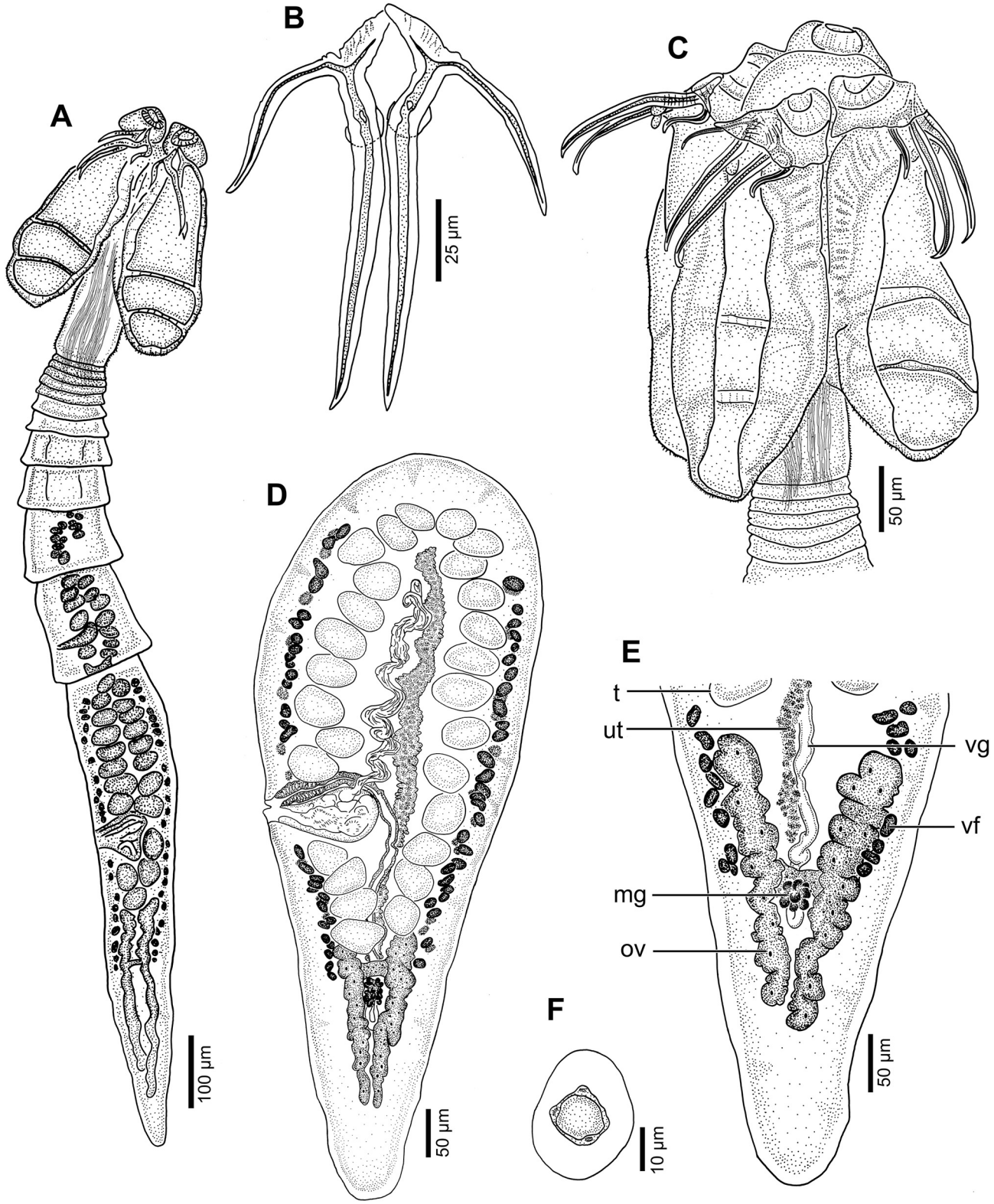

Fig. 1. *Acanthobothrium stefaniae* sp. n. from *Discopyge tschudii* Heckel. A – entire worm (paratype IPCAS No. C-786); B – detail of hooks (paratype MACN-Pa No. 625/4); C – scolex (paratype IPCAS No. C-786); D – detached mature proglottid (paratype MACN-Pa No. 627/1); E – detail of ootype region of a detached mature proglottid (paratype LRP No. 9408); F – egg. Abbreviations: mg – Mehlis' gland; ov – ovary; t – testes; ut – uterus; vf – vitelline follicle; vg – vagina.

(296 ± 27; 15) long, 130–180 (152 ± 14; 12) wide, each with 3 loculi (Figs. 1A, C, 2A) and specialized anterior region in form of muscular pad; muscular pad falciform with extended anterior and posterolateral margins (Fig. 2B), 40–55 (47 ± 5; 11) long, 83–103 (96 ± 7; 12) wide, bearing accessory sucker and one pair of hooks; accessory sucker 25–38 (29 ± 3; 13) long, 33–40 (38 ± 2; 10) wide (Figs. 1C, 2A, B). Bothridial anterior loculus 125–205 (175 ± 19; 13) long, middle loculus 30–60 (47 ± 8; 14) long, posterior loculus 52–75 (64 ± 8; 12) long; loculus length ratio (anterior: middle: posterior) 1: 0.2–0.3 (0.3 ± 0.03; 12): 0.3–0.4 (0.3 ± 0.04; 12) (Figs. 1C, 2A). Hooks bipronged, hollow, with

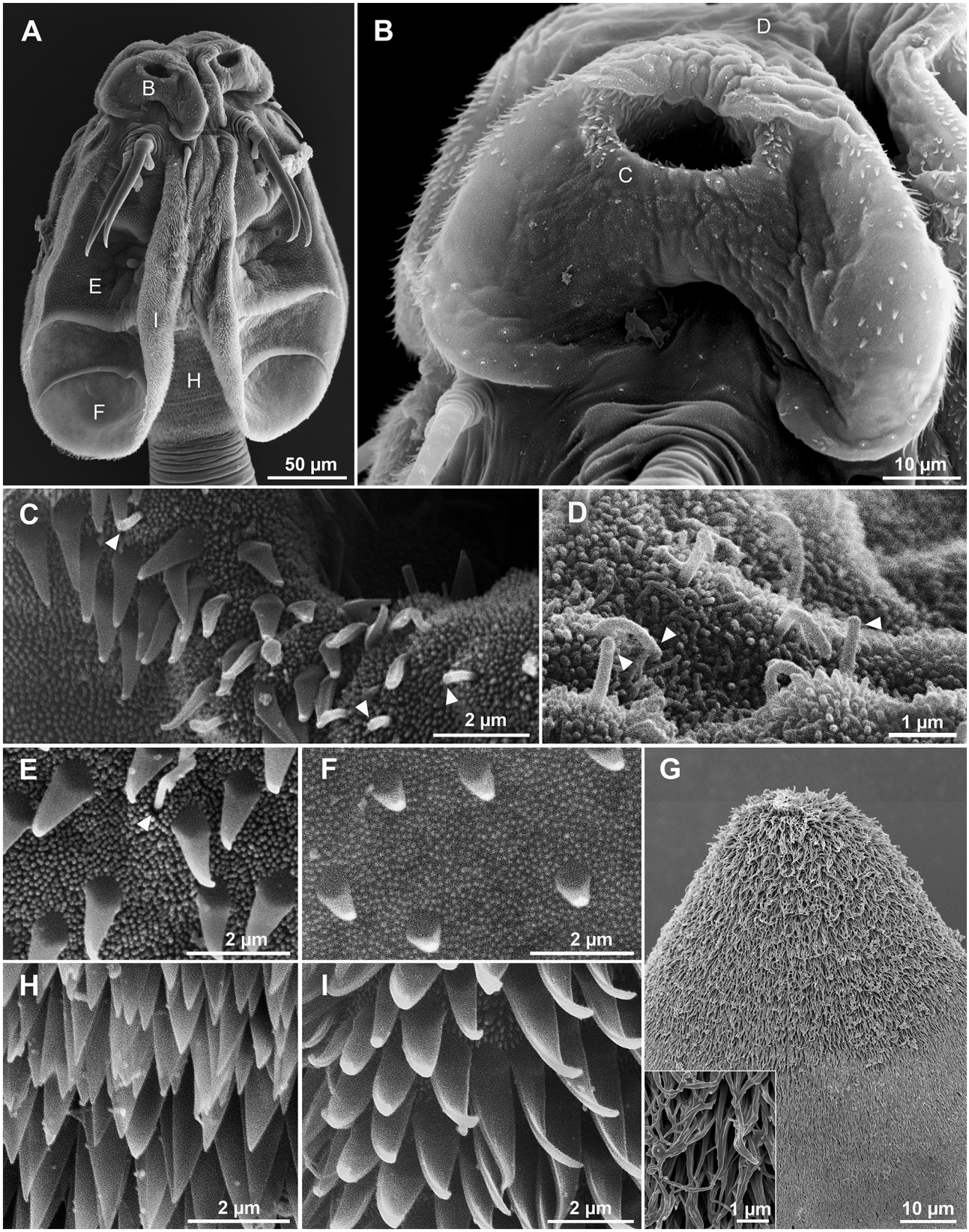

Fig. 2. *Acanthobothrium stefaniae* sp. n. from *Discopyge tschudii* Heckel, scanning electron micrographs. A – scolex, small letters indicate locations of details shown in Fig. 2B, E, F, H, I; B – bothridial muscular pad, small letters indicate locations of details shown in Fig. 2C, D; C – detail of distal surface of muscular pad and accessory sucker (arrows indicate cilia); D – surface of apex of the scolex (arrows indicate cilia); E – distal bothridial surface in the anterior loculus (arrow indicates cilium); F – distal bothridial surface in the posterior loculus; G – surface of the anterior third of a detached mature proglottid (inset shows a detail of the microtriches); H – surface of the cephalic peduncle; I – proximal bothridial surface.

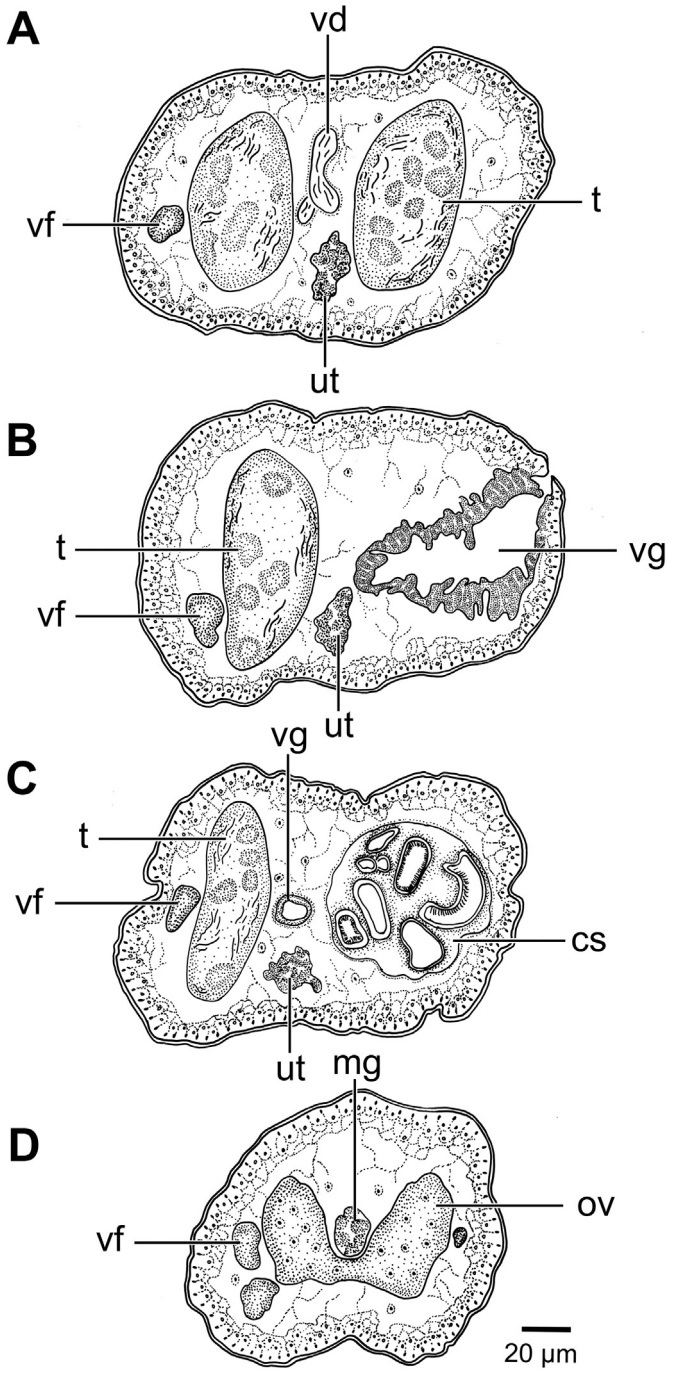

Fig. 3. *Acanthobothrium stefaniae* sp. n. from *Discopyge tschudii* Heckel, cross-sections of a detached mature proglottids (paratype MACN-Pa No. 628/2). A – cross section at level of the testes anterior to cirrus sac; B – cross section at level of the genital pore; C – cross section at level of the cirrus sac; D – cross section at level of the ovarian isthmus. Abbreviations: cs – cirrus sac; mg – Mehlis' gland; ov – ovary; t – testes; ut – uterus; vd – vas deferens; vf – vitelline follicle; vg – vagina.

tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, axial prongs longer than abaxial prongs, lateral and medial hooks equal in size (Fig. 1B). Lateral hook measurements (n = 9): A 25–32 (28 ± 3), B 102–112 (108 ± 4), C 32–52 (45 ± 6), D 122–135 (128 ± 4), E 55–82 (73 ± 8), W 30–45 (40 ± 6). Medial hook measurements (n = 9): A′ 25–32 (29 ± 2), B′ 97–112 (104 ± 5), C′ 42–50 (46 ± 3), D′ 117–135 (126 ± 6), E′ 65–80 (74 ± 4), W′ 30–47 (38 ± 6). Bases of lateral and medial hooks overlap interchangeably. Cephalic peduncle 105–185 (139 ± 25; 15) long, 80–120 (93 ± 11; 15) wide at posterior end. Apex of scolex covered with papiliform filitriches (Fig. 2D). Distal surface of muscular pads and accessory suckers with papilliform filitriches and gladiate spinitriches, spinitriches denser on accessory sucker rims (Fig. 2B, C). Proximal surface of muscular pads covered with papilliform filitriches and gladiate spinitriches, denser on lateral rims (Fig. 2B). Distal bothridial surfaces with papilliform filitriches interspersed with gladiate spinitriches decreasing in size and density posteriorly (Fig. 2E, F). Scolex proper and proximal surface of bothridia covered with papilliform filitriches and gladiate spinitriches (Fig. 2I). Cephalic peduncle covered with densely arranged gladiate spinitriches (Fig. 2H). Cilia were observed on apex, distal and proximal surfaces of muscular pads, accessory suckers and distal bothridial surfaces (Fig. 2C, D, E).

Immature proglottids initially wider than long, becoming longer than wide with maturity, covered with acicular to capilliform filitriches. Detached mature proglottids 710–1050 (847 ± 117; 13) long, 210–295 (252 ± 27; 15) wide, covered with capilliform filitriches, interspersed with densely arranged gladiate spinitriches on anterior end (Fig. 2G). Testes oval, 27–72 (38 ± 8; 11; 55) long, 37–77 (57 ± 8; 11; 55) wide, arranged in 2 columns from anterior margin of proglottid to anterior margin of ovary (Fig. 1D), 1 layer deep (Fig. 3A,B,C), 17–27 (23 ± 3; 13) in total number, 5–10 (7 ± 1; 13) in preporal field, 2–4 (3 ± 1; 13) in postporal field, 9–14 (12 ± 1; 13) antiporally (Fig. 1D). Cirrus sac pyriform, 85–125 (103 ± 11; 15) long, 55–105 (78 ± 16; 15) wide, containing coiled cirrus covered with spinitriches, vas deferens bulk occupying midline of proglottid from level of cirrus sac to almost anterior margin of proglottid, entering cirrus sac on its anterior margin (Fig. 1D). Genital pore marginal, 46–70% (56 ± 6; 13) of proglottid length from posterior end. Vagina extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally along anterior margin of cirrus sac to common genital atrium, with thick wall, surrounded by darkly stained cells distally, vaginal sphincter absent (Figs. 1D, 3B). Ovary near posterior end of proglottid, H-shaped in dorsoventral view (Fig. 1D, E), bilobed in cross-sections (Fig. 3D), ovarian lobes symmetrical, 155–355 (225 ± 45; 11; 22) long, width at ovarian isthmus 50–107 (79 ± 17; 12). Mehlis' gland posterior to ovarian isthmus, 25–30 (28 ± 3; 3) in diameter (Fig. 1E). Vitelline follicles in 2 columns (1 dorsal, 1 ventral) on each lateral margin of proglottid, extending from almost anterior margin of proglottid to level of ovarian isthmus, interrupted by vagina and cirrus sac dorsally and ventrally; vitelline follicles 7–27 (17 ± 6; 9; 45) long, 7–27 (16 ± 4; 9; 45) wide (Figs. 1D–E, 3A–D). Uterus preformed in mature proglottids, sacciform, occupying midline of proglottid, from level of ovarian isthmus to near anterior margin of proglottid (Fig. 1D). Detached gravid proglottids 1.34–1.97 mm (1.65 mm ± 0.18; 10) long, 405–600 (495 ± 55; 10) wide. Unembryonated eggs subspherical, 25–45 (38 ± 5; 6; 20) long, 22–37 (30 ± 4; 6; 20) wide (Fig. 1F).

3.1.2. Taxonomic summary

Type host: *Discopyge tschudii* Heckel, apron ray (Torpediniformes: Narcinidae).

Type locality: Coastal waters off Mar Chiquita City, Buenos Aires Province (37°46′S, 56°56′W) (PD5-005).

Additional locality: Coastal waters off Villa Gesell (37°29′S, 56°45′W) (PD5-021), off San Clemente del Tuyú (35°50′S, 56°18′W) (PD5-112) and off Camarones (45°08′S, 65°19′W) (PD7-267).

Site of infection: Spiral intestine.

Specimens deposited: Holotype MACN-Pa No 624 (entire worm), 19 paratypes MACN-Pa No. 625/1–6, 626/1–3, 627/1, 628/1–2 (8 entire worms, 7 detached mature proglottids, 3 detached gravid proglottids and histological sections of 1 detached mature proglottid); 12 paratypes IPCAS No. C-786 (5 entire worms, 4 detached mature proglottids and 3 detached gravid proglottids); 13 paratypes LRP Nos. 9403–9410 (5 entire worms, 4 detached mature proglottids and 4 detached gravid proglottids).

Prevalence of infection: 72% (13 hosts infected out of 18 examined).

Etymology: This species is named after Stefanía Franzese, first author's sister.

3.1.3. Remarks

Acanthobothrium stefaniae sp. n. is a category 1 species (i.e., it is less than 15 mm in total length, possesses fewer than 50 proglottids, fewer than 80 testes and an essentially symmetric ovary) [5]. The fact that we observed that the ovary can vary intraspecifically from symmetrical to asymmetrical in *A. zapterycum* (see below), sheds some doubts on the validity of this feature in the distinction of species. Therefore, the new species is compared to all the species previously described in category 1 and 2, since both categories share similar features with exception of the ovarian symmetry. Thus, *A. stefaniae* sp. n. can be distinguished from 42 species described so far in category one ([17–19] and citations therein) by being hyperapolytic instead of euapolytic as in *A. asnihae* Fyler et Caira, 2006, *A. asrinae* Maleki, Malek et Palm, 2015, *A. atahualpai* Marques, Brooks et Barriga,1997, *A. bartonae* Campbell et Beveridge, 2002, *A. clarkeae* Campbell et Beveridge, 2002, *A. dollyae* Caira et Burge, 2001, *A. fogeli* Goldstein, 1964, *A. foulki* Reyda et Caira, 2006, *A. fylerae* Maleki, Malek et Palm, 2015, *A. gnomus* Reyda et Caira, 2006, *A. himanturi* Brooks, 1977, *A. hypermekkolpos* Fyler et Caira, 2010, *A. jamesi* Maleki, Malek et Palm, 2015, *A. janineae* Maleki, Malek et Palm, 2015, *A. jeanneae* Fyler et Caira, 2010, *A. larsoni* Reyda et Caira, 2006, *A. laurenbrownae* Campbell et Beveridge, 2002, *A. lentiginosum* Vardo-Zalik et Campbell, 2011, *A. lepidum* Reyda et Caira, 2006, *A. lineatum* Campbell, 1969, *A. marplatensis* Ivanov et Campbell, 1998, *A. martini* Campbell et Beveridge, 2002, *A. marymichaelorum* Twohig, Caira et Fyler, 2008, *A. mathiasi* Euzet, 1959, *A. minusculus* Marques, Brooks et Barriga, 1997, *A. monksi* Marques, Brooks et Barriga, 1997, *A. nicoyaense* Brooks et McCorquodale, 1995, *A. oceanharvestae* Fyler, Caira et Jensen, 2009, *A. odonoghuei* Campbell et Beveridge, 2002, *A. paulum* Linton, 1890, *A. peruviense* Reyda, 2008, *A. rohdei* Campbell et Beveridge, 2002, *A. romanowi* Fyler, Caira et Jensen, 2009, *A. royi* Caira et Burge, 2001, *A. saliki* Fyler et Caira, 2006, *A. schalli* Vardo-Zalik et Campbell, 2011, *A. southwelli* Subhapradha, 1955, *A. ulmeri* Vardo-Zalik et Campbell, 2011, *A. urolophi* Schmidt, 1973, *A. westi* Vardo-Zalik et Campbell, 2011, *A. zainali* Fyler et Caira, 2006 and *A. zimmeri* Fyler, Caira et Jensen, 2009. In addition, *A. stefaniae* sp. n. differs from *A. jalalii* Maleki, Malek et Palm, 2013 and *A. nanogravidum* Zschoche, Caira et Fyler, 2011, which are apolytic. Among the species of *Acanthobothrium* in category 1, only *A. pearsoni* Williams, 1962 and *A. lintoni* Goldstein, Henson et Schlicht, 1968, are likely to be hyperapolytic. *Acanthobothrium lintoni* was originally described as apolytic; however, the original drawings show that the terminal proglottid might be immature in comparison to the mature detached proglottid (Figs. 4–5 in [20]). Both species are longer worms than *A. stefaniae* sp. n. (2.5–22.62, 12 vs. 1.14–1.92), have more testes per proglottid (30–46, 56–60 vs. 17–27), and a different hook morphology, being the axial and abaxial prongs of almost equal size in *A. lintoni* and *A. pearsoni*, whereas the axial prongs are conspicuously longer than the abaxial prongs in *A. stefaniae* sp. n. Also, most species in category 2 [21 and citations therein] are euapolytic rather than hyperapolytic (viz., *A. bobconniorum* Fyler et Caira, 2010, *A. brayi* Campbell et Beveridge, 2002, *A. brevissime* Linton, 1908, *A. bullardi* Ghoshroy et Caira, 2001, *A. campbelli*, *A. chisholmae* Campbell et Beveridge, 2002, *A. cimari*, *A. costarricense*, *A. dasi* Ghoshroy et Caira, 2001, *A. gloveri* Campbell et Beveridge, 2002, *A. guanghaiense* Yang, Sun, Zhi, Iwaki, Reyda et Yang, 2016, *A. lasti* Campbell et Beveridge, 2002, *A. masnihae* Fyler et Caira, 2006, *A. minus* Tazerouti, Kechemir-Issad et Euzet, 2009, *A. mooreae* Campbell et Beveridge, 2002, *A. ocallaghani* Campbell et Beveridge, 2002, *A. olseni* Dailey et Mudry, 1968, *A. popi* Fyler, Caira et Jensen, 2009, *A. puntarenasense*, *A. rajivi* Ghoshroy et Caira, 2001, *A. semnovesiculum* Verma, 1928, *A. sphaera* Maleki, Malek et Palm, 2013, *A. stevensi* Campbell et Beveridge, 2002, *A. tetabuanense* Reyda et Caira, 2006, *A. thomasae* Campbell et Beveridge, 2002, *A. tripartitum* Williams, 1969, *A. unilateralis* Alexander, 1953, *A. urotrygoni* Brooks et Mayes, 1980, *A. vargasi*, *A. walkeri* Campbell et Beveridge, 2002 and *A. woodsholei* Baer, 1948) (note that the apolysis of some of these species has been redefined in the present study, see Table 1). In addition, the category 2 species *A. annapinkiensis* Carvajal et Goldstein, 1971 and *A. coquimbensis* Carvajal et Jeges, 1980 are apolytic rather than hyperapolytic. *Acanthobothrium stefaniae* sp. n. differs from *A. zapterycum*, both hyperapolytic, by having fewer testes (17–27 vs. 29–41), and a smaller cirrus sac (85–125 vs. 130–200 long, 55–105 vs. 147–240 wide).The species in category 2 for which the apolysis could not be verified, differ from *A. stefaniae* sp. n. by having more testes (as *A. brachyacanthum* and *A. lilium*), fewer proglottids (as *A. edwardsi* and *A. quadripartitum*), spherical cirrus sac and vaginal sphincter (as *A. tasajerasi*), and axial and abaxial prongs of almost equal size (as in *A. batailloni* and *A. dujardini*).

3.2. Acanthobothrium zapterycum Ostrowski de Núñez, 1971 (Figs. 4–6)

3.2.1. Redescription (based on 34 specimens prepared as follows: whole mounts of 30 entire worms [holotype, 10 paratypes and 19 specimens recently collected] and 26 detached gravid proglottids [15 paratypes and 11 proglottids recently collected]; 4 entire worms and 4 detached gravid proglottids examined with SEM)

Worms 1.41–3.14 mm (2.15 mm ± 0.52; 28) long, 167–237 (219 ± 24; 10) maximum width at level of scolex, 5–10 (7 ± 1; 25) acraspedote proglottids per worm, hyperapolytic (Fig. 4A). Scolex 285–575 (428 ± 77; 25) long, consisting of scolex proper with 4 bothridia and cephalic peduncle (Figs. 4A, C, 5A). Bothridia posteriorly joined by velum (Figs. 4C, 5A), 212–350 (298 ± 34; 25) long, 65–137 (108 ± 24; 9) wide, each with 3 loculi and specialized anterior region in form of muscular pad (Figs. 4C, 5A); muscular pad falciform with extended anterior and postero-lateral margins (Fig. 5B), 50–95 (67 ± 11; 20) long, 67–112 (94 ± 11; 14) wide, bearing accessory sucker and one pair of hooks; accessory sucker 27–35 (31 ± 3; 13) long, 27–47 (34 ± 6; 12) wide. Bothridial anterior loculus 105–180 (138 ± 21; 19) long, middle loculus 57–80 (66 ± 7; 20) long, posterior loculus 50–85 (62 ± 10; 19) long; loculus length ratio (anterior: middle: posterior) 1: 0.4–0.6 (0.5 ± 0.07; 19): 0.3–0.7 (0.5 ± 0.11; 18) (Figs. 4C, 5A). Hooks bipronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous; axial and abaxial prongs approximately equal in size in lateral hook, axial prong slightly longer than abaxial prong in medial hook; medial hook slightly larger than lateral hook (Fig. 4E). Lateral hook measurements (n = 18): A 25–42 (35 ± 5), B 57–90 (71 ± 8), C 57–85 (71 ± 6), D 75–125 (101 ± 12), E 92–135 (108 ± 10), W 35–60 (49 ± 8). Medial hook measurements (n = 18): A′ 25–42 (34 ± 4), B′ 70–97 (84 ± 7), C′ 55–77 (68 ± 6), D′ 90–132 (112 ± 10), E′ 90–117 (103 ± 7), W′ 20–67 (47 ± 11). Base of medial hook overlaps base of lateral hook. Cephalic peduncle 100–337 (206 ± 65; 23) long, 42–100 (74 ± 14; 21) wide at anterior end. Apex of scolex, distal and proximal surfaces of muscular pad and accessory sucker, distal bothridial surface and scolex proper covered with papilliform filitriches (Fig. 5B–D, H). Proximal bothridial surface covered with papilliform to acicular filitriches interspersed with gladiate spinitriches (Fig. 5F). Cephalic peduncle covered with densely arranged gladiate spinitriches (Fig. 5E). Cilia were observed on distal surface of muscular pad and distal bothridial surface (Fig. 5D, H). Cilia on distal bothridial surface (anterior loculus) emerging from deep pits (Fig. 5D).

Immature proglottids initially wider than long, becoming longer than wide with maturity, covered with acicular filitriches. Mature proglottids not observed. Detached gravid proglottids 2.0–4.2 mm (2.9 mm ± 0.5; 26) long, 290–600 (428 ± 86; 24) wide. Anterior end of detached gravid proglottid covered with coniform spinitriches and long capilliform filitriches (Fig. 5G).Testes oval, 35–70 (52 ± 7; 17; 85) long, 50–102 (71 ± 12; 17; 85) wide, arranged in 2 columns from almost anterior margin of proglottid to anterior margin of antiporal

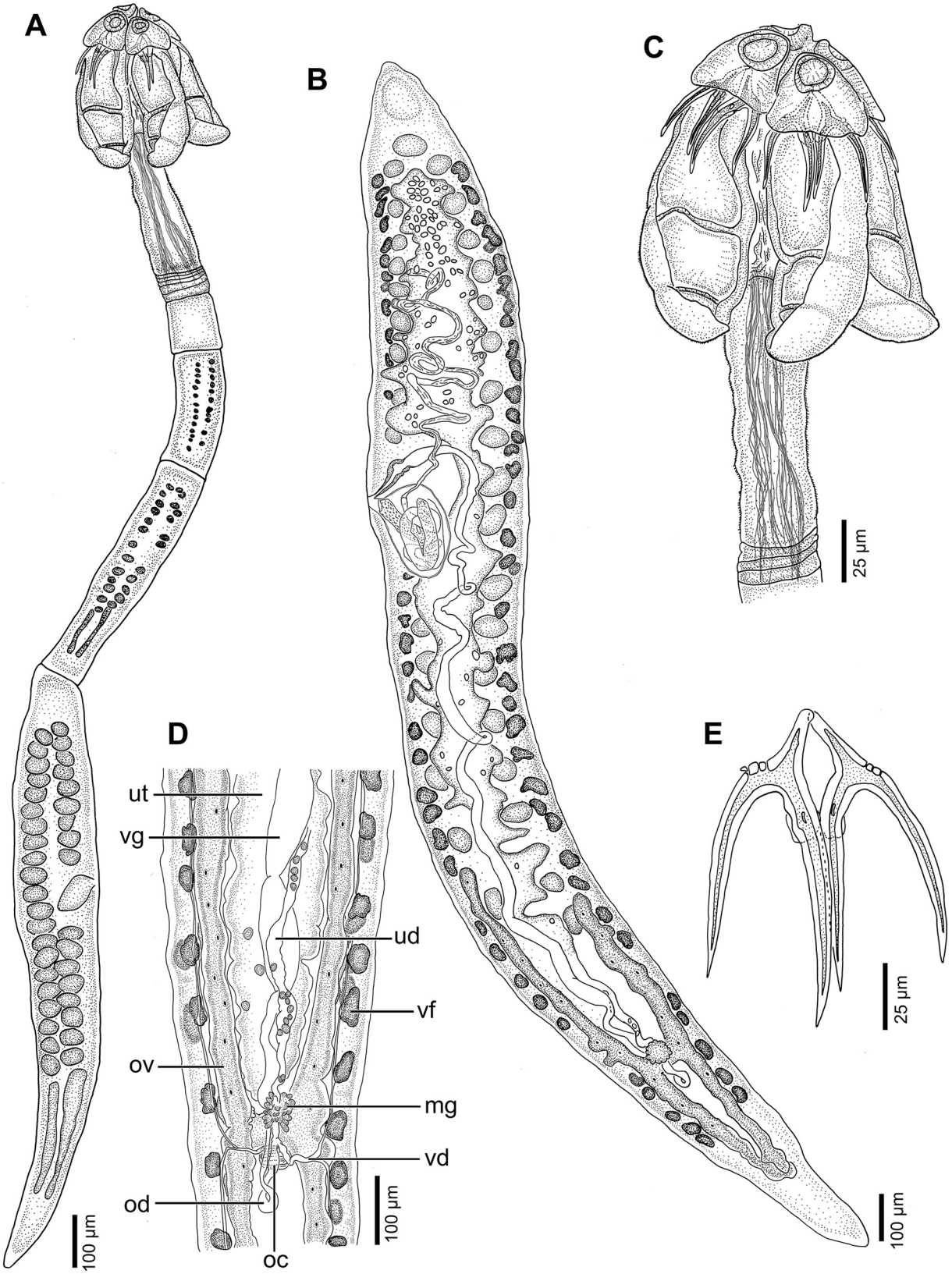

Fig. 4. *Acanthobothrium zapterycum* Ostrowski de Núñez, 1971 from *Zapteryx brevirostris* (Müller et Henle). A – entire worm (voucher LRP No. 9412); B – detached gravid proglottid (voucher IPCAS No. C-787); C – scolex (voucher LRP No. 9412); D – detail of ootype region in a detached gravid proglottid (voucher IPCAS No. C-787); E – detail of hooks (voucher MACN-Pa No. 629/1). Abbreviations: mg – Mehlis' gland; oc – oocapt; od – oviduct; ov – ovary; ud – uterine duct; ut – uterus; vd – vitelline duct; vf – vitelline follicle; vg – vagina.

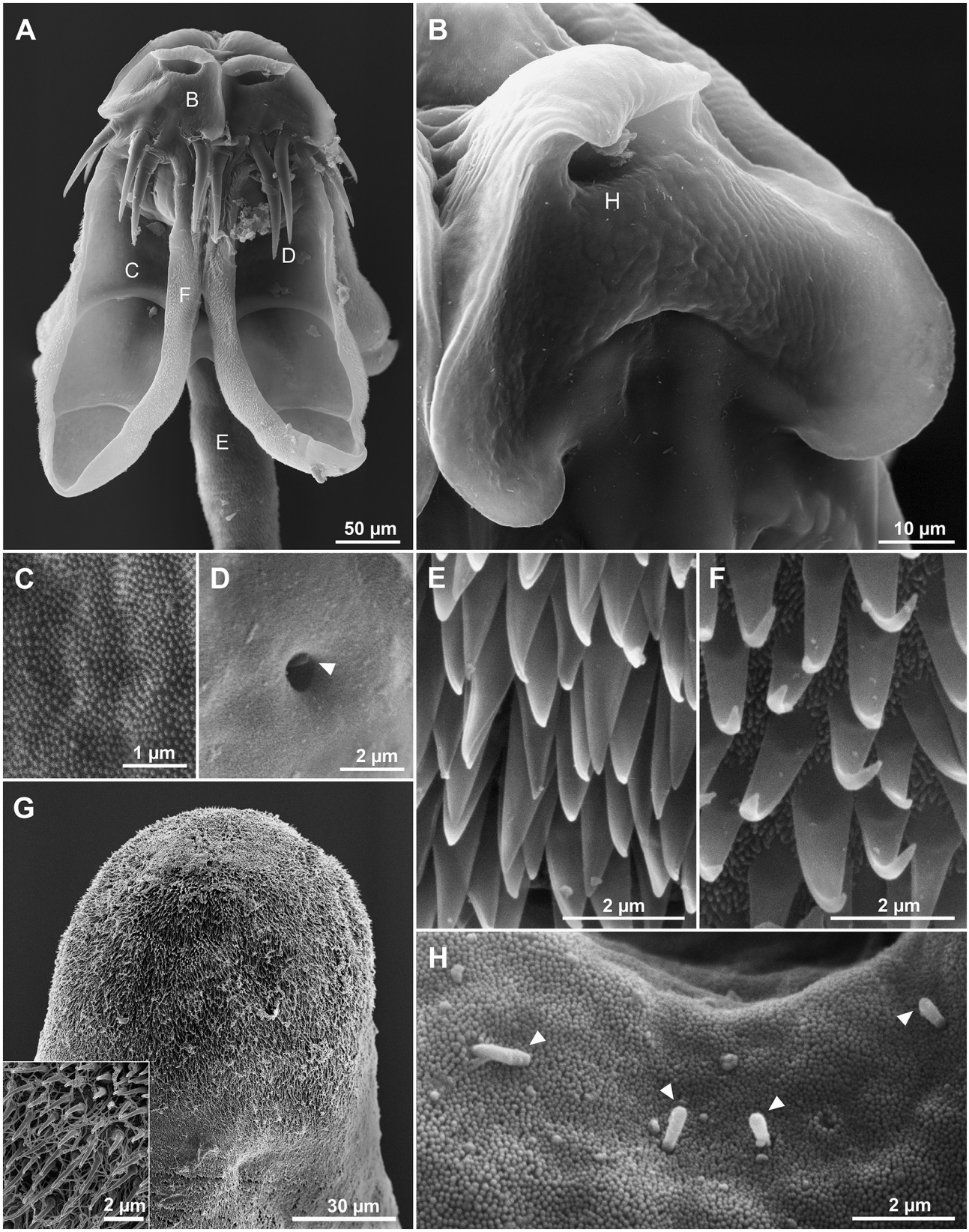

Fig. 5. *Acanthobothrium zapterycum* Ostrowski de Núñez, 1971 from *Zapteryx brevirostris* (Müller et Henle), scanning electron micrographs. A – scolex, small letters indicate locations of details shown in Fig. 5B–F; B – bothridial muscular pad, small letter indicates location of the detail shown in Fig. 5H; C – distal bothridial surface; D – distal bothridial surface with cilium emerging from a pit (arrow indicates cilium); E – surface of the cephalic peduncle; F – proximal bothridial surface; G – surface of the anterior region of a detached gravid proglottid (inset shows a detail of the microtriches); H – detail of the distal surface of the muscular pad and the accessory sucker (arrows indicate cilia).

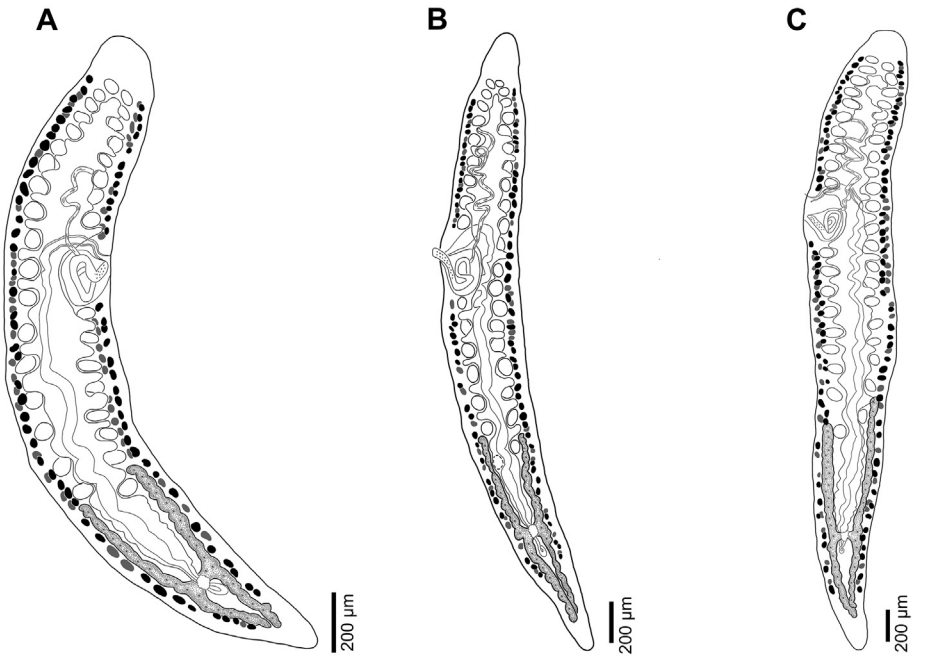

Fig. 6. *Acanthobothrium zapterycum* Ostrowski de Núñez, 1971 from *Zapteryx brevirostris* (Müller et Henle), detached gravid proglottids showing different shapes of ovary. A – symmetric ovary (LRP No. 9417); B – slightly asymmetric ovary, having the poral lobe larger than the aporal lobe (LRP No. 9416); C – asymmetric ovary having the poral lobe shorter than the aporal lobe (IPCAS No. C-787).

ovarian lobe, overlapping anteriorly poral ovarian lobe, 1 layer deep, 29–41 (35 ± 3; 25) in total number, 7–11 (9 ± 1; 25) in preporal field, 5–9 (7 ± 1; 25) in postporal field, 15–22 (19 ± 2; 25) antiporally (Fig. 4B). Cirrus sac oval, 130–200 (160 ± 19; 21) long, 147–240 (196 ± 25; 21) wide, containing coiled cirrus covered with spinitriches, vas deferens bulk occupying midline of proglottid anterior to cirrus sac, entering cirrus sac on its anterior margin (Fig. 4B). Genital pore marginal, 62–77% (69 ± 3; 26) of proglottid length from posterior margin. Vagina extending from ootype along midline of proglottid to anterior margin of cirrus sac, then laterally along anterior margin of cirrus sac to common genital atrium, vaginal sphincter absent (Fig. 4B). Ovary near posterior end of proglottid, H-shaped in dorsoventral view, bilobed in cross-sections, symmetric or asymmetric (35% of proglottids with symmetric ovary; 65% of proglottids with asymmetric ovary, poral or aporal lobe longer than opposite lobe) (Fig. 6), overlapping posteriorly (Fig. 4B), poral lobe 625–1255 (919 ± 170; 23) long, aporal lobe 645–1330 (917 ± 169; 23) long, width at ovarian isthmus 100–175 (133 ± 22; 23). Mehlis' gland at level of ovarian isthmus, 45–70 (52 ± 7; 16) in diameter (Fig. 4B, D). Vitelline follicles in 2 columns (1 dorsal, 1 ventral) on each lateral margin of proglottids, extending from almost anterior margin of proglottid to 3/4 of ovary length, interrupted by vagina and cirrus sac dorsally and ventrally (Fig. 4B); vitelline follicles 15–60 (33 ± 10; 16; 80) long, 17–42 (29 ± 5; 16; 80) wide. Uterus sacciform, occupying midline of proglottid, from level of ovarian isthmus to near anterior margin of proglottid (Fig. 4B). Unembryonated eggs subspherical, 22–30 (27 ± 2; 4; 20) long, 17–25 (21 ± 2; 4; 20) wide.

3.2.2. Taxonomic summary

Type host: *Zapteryx brevirostris* (Müller et Henle), lesser guitarfish (Rhinopristiformes: Rhinobatidae).

Type locality: Mar del Plata, Buenos Aires, Argentina (geographic coordinates not given in the original description).

Additional localities: Coastal waters off Villa Gessel (37°29′S, 56°45′W) (PD5-024), La Lucila del Mar (36°38′S, 56°15′W) (PD5-074), Puerto Quequén (38°46′S; 57°56′W) (VIPQ-064) and Puerto Pirámides (42°05′S, 62°50′W) (PD4-284).

Site of infection: Spiral intestine.

Specimens deposited: holotype MACN-Pa No. 214/1 (entire worm), paratypes MACN-Pa No. 214/1-2 (entire worms), paratypes MACN-Pa No. 214/4–5 (detached gravid proglottids), 14 voucher specimens MACN-Pa No. 629/1, 630/1–3, 631/1–4, 632/1–4 (9 entire worms and 5 detached gravid proglottids), 8 voucher specimens IPCAS No. C-787 (5 entire worms and 3 detached gravid proglottids), 8 voucher specimens LRP Nos. 9411–9417 (5 entire worms and 3 detached gravid proglottids).

Prevalence of infection: 70% (7 hosts infected out of 10 examined).

Etymology: not given, but presumably named after its host.

3.2.3. Remarks

The morphology of the specimens recently collected from *Z. brevirostris* agrees with *A. zapterycum*. Ostrowski de Núñez [22] presented an accurate but brief description of *A. zapterycum* based on 14 specimens from *Z. brevirostris* from Mar del Plata. Newly collected specimens allowed for a redescription, which expands most ranges of measurements (i.e., worm and scolex size, cirrus sac, number of proglottids and testes), and provides information omitted in the original description (i.e. measurements of all structures associated with the scolex, including some hook measurements, and details of the genitalia).The microthrix pattern of *A. zapterycum* is described for the first time, showing a similar pattern observed in other members of the genus (i.e., papilliform and/or acicular filitriches and gladiate spinitriches). It is important to note that among types and newly collected specimens, the morphology of the ovary presented great variation, from symmetric to conspicuously asymmetric, with the longer ovarian lobe being the poral lobe in some specimens and the aporal lobe in others. *Acanthobothrium zapterycum* was considered as having an asymmetric ovary by Ghoshroy and Caira [5], on the basis of the Fig. 5b–c in Ostrowski de Núñez [22] since the different morphologies were not mentioned in the original description. Therefore, the categorization by Ghoshroy and Caira [5] should be used with caution in the comparison of species of *Acanthobothrium* because categories 1 and 2, 3 and 4, 5 and 6, and 7 and 9, differ from one another only in ovarian symmetry, a feature that might have some intraspecific variation that could have remained unnoticed.

4. Discussion

4.1. Distribution and host diversity

Acanthobothrium has a worldwide distribution in warm and temperate marine waters. No species have been found in the cold waters of the Southern Ocean and Arctic realms [23,24]. The absence of reports of species of *Acanthobothrium* from the Temperate Southern Africa realm might be due to limited sampling of cestodes from elasmobranchs in that region. Actually, the genus is well represented in temperate waters around the globe, with about 92 species [24]. In addition, it is one of the few genera of cestodes from elasmobranchs known from the river systems of South America [6,25–27] and Malaysian Borneo [7,28].

Acanthobothrium has a broad host range and are particularly diversified in Myliobatiformes with more than half of the species described from this order of hosts [24]. In general, the reports of species of *Acanthobothrium* from different orders of batoids are proportional to the diversity of species of elasmobranchs in such particular orders. For example, 22% (47/210) of the species of Myliobatiformes, 13% (9/69) of the Torpediniformes and 16% (10/62) of the Rhinopristiformes have been reported as hosts for *Acanthobothrium* [24,29,30]. Thus, it is not possible to confirm that the Myliobatiformes are their preferred hosts or just the most frequent elasmobranchs to come across. This is the first record of the torpediniform genus *Discopyge* Heckel as host for a species of *Acanthobothrium*. As predicted by Caira et al. [2], *Discopyge* serves as a host for only one species of *Acanthobothrium* so far.

4.2. Microthrix pattern

The microthrix pattern is quite uniform among species in *Acanthobothrium*, having filitriches covering most surfaces of the worms, interspersed with gladiate spinitriches on proximal bothridial surface, scolex proper and cephalic peduncle ([9,17–19,31,32] and citations therein). Gladiate spinitriches are confined to proximal surfaces of the scolex in most species, however, they have also been observed on the distal bothridial surface in *A. puertecitence* Caira et Zahner, 2001, *A. rodmani* Fyler, Caira et Jensen, 2009 and *A. stefaniae* ([31,33], present study). The arrangement of these gladiate spinitriches differs among species, being scattered among filitriches in *A. puertecitense*, densely arranged in *A. rodmani*, and diminishing in density from anterior loculus to posterior loculus in *A. stefaniae*. Furthermore, in *A. stefaniae* the gladiate spinitriches were also found covering the distal surface of muscular pads and accessory suckers (Fig. 2B, C). This species is also peculiar by having cilia on the apex, muscular pad, accessory sucker and distal bothridial surface of the scolex (Fig. 2C–E). This feature was also observed in *A. zapterycum*, having cilia emerging from deep pits on the distal surface of the anteriormost bothridial loculus and muscular pad (Fig. 5D, H). Even if this is the first report of such structures in *Acanthobothrium*, cilia have been described in a wide range of tapeworms, including trypanorhynchs, diphyllideans, rhinebothriideans and other onchoproteocephalideans [34–38].

4.3. Proglottid apolysis

The term hyperapolytic refers to tapeworms that release the proglottids when they are still immature [13]. However, this term has been used with subtle differences by different authors, causing the overestimation of reports of hyperapolytic species in *Acanthobothrium*. As mentioned before, *A. lintoni* is most probably hyperapolytic, along with *A. indicum* Subhapradha, 1955, *A. pearsoni*, *A. stefaniae* and *A. zapterycum* ([20,22,39,40], present study). Fyler [9] described *A. margieae* Fyler, 2011, the only species of *Acanthobothrium* that is extremely hyperapolytic, with adult specimens that bear terminal proglottids in which the reproductive organs are completely indistinguishable. In all hyperapolytic and extremely hyperapolytic species studied with SEM, gladiate or coniform spinitriches have been described in the anterior region of the detached proglottids ([9], present study), which might help the proglottids to remain attached to the intestinal wall of their hosts. Also, detached mature proglottids of some euapolytic species of *Acanthobothrium* seem to live free in the intestine of their definitive host for some period of time, since similar structures have also been observed in *A. coronatum* (Rudolphi, 1819) [11], *A. dujardini*, and *A. edwardsi* (see Williams 1969). This feature was also observed in hyperapolytic species in other genera, as in the tetraphyllidean *Yorkeria* Southwell, 1927, the phyllobothriidean *Trilocularia* Olsson, 1867 and the lecanicephalidean *Anteropora* Subhapradha, 1955 [41–43], revealing that this reproductive strategy has evolved independently in different lineages of tapeworms.

It is interesting to note that three of the six hyperapolytic species of *Acanthobothrium* are parasites of Torpediniformes (i.e., *A. lintoni*, *A. indicum* and *A. stefaniae*). Torpediniform rays are also hosts of four hyperapolytic species of *Anteropora*, and the only species known of *Pentaloculum* Alexander, 1963, which is also hyperapolytic [44]. It seems that somehow this reproductive strategy in tapeworms might be related to the host biology, as suggested by Fyler [9] for the orectolobiform *Chiloscyllium punctatum* Müller et Henle and the squaliform *Squalus acanthias* Linnaeus, which also host hyperapolytic species of tapeworms.

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