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New species of *Echinobothrium* van Beneden, 1849 (Cestoda: Diphyllidea) from Indo-Pacific maskrays (*Neotrygon* Castelnau) off Australia and Borneo

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Abstract: Four new diphyllidean species of the genus *Echinobothrium* van Beneden, 1849 are described from Indo-Pacific maskrays (*Neotrygon* Castelnau, Dasyatidae). *Echinobothrium giraffaeous* sp. n. from *Neotrygon leylandi* (Last) off northern Australia, *Echinobothrium ivanovae* sp. n. from *Neotrygon orientalis* Last, White et Serét off Borneo, and *Echinobothrium bethae* sp. n. from *Neotrygon varidens* (Garman) off Borneo are distinguished from all but one of the 33 valid species of the genus by the possession of the outermost A hooks with an extended base into which the bases of the three outermost B hooks are inserted. *Echinobothrium rhynchobati* (Khalil et Abdul-Salam, 1989) is the only known species with this unique feature, but its rostellum has a system of interlocking knobs and sockets that articulate bases of the A and B type hooks with one another, which is not present in any of the newly described species. *Echinobothrium tyleri* sp. n. from *Neotrygon australiae* Last, White et Serét off northern Australia is distinguished from all known species of *Echinobothrium* by its unique rostellar hook formula {2–3 18/17 2–3}. With the present addition of four new species, the central Indo-Pacific realm becomes the major hotspot for *Echinobothrium*, from which 13 species have been reported.

Keywords: *Echinobothrium giraffaeous* sp. n., *Echinobothrium ivanovae* sp. n., *Echinobothrium bethae* sp. n., *Echinobothrium tyleri* sp. n., parasites, cestodes, new species.

Until recently, tapeworms of the order Diphyllidea van Beneden in Carus, 1863 were neglected and their diversity underestimated (Tyler 2006). However, a detailed revision of the group has been carried out recently (Kuchta and Caira 2010, Ivanov and Caira 2012), including a comprehensive molecular phylogenetic study that yielded three new genera and revealed genetic boundaries among members of the group (Caira et al. 2013, 2017, Abbott and Caira 2014). Since 2000, the number of recognised species has nearly doubled, increasing from 36 to the current 59 valid species (Caira et al. 2017).

Currently, six valid diphyllidean genera are recognised: *Echinobothrium* van Beneden, 1849, *Ditrachybothridium* Rees, 1959, *Ahamulina* Marques, Jensen et Caira, 2012, *Halysioncum* Caira, Marques, Jensen, Kuchta et Ivanov, 2013, *Coronocestus* Caira, Marques, Jensen, Kuchta et Ivanov, 2013, and *Andocadoncum* Abbot et Caira, 2014 (see Caira et al. 2017).

Among these genera, *Echinobothrium* is by far the most speciose, with 33 valid species reported from a wide range of batoids and, in particular, from the families Rajidae Bonaparte, Rhinobatidae Müller et Henle, and Dasyatidae Jordan (see Caira et al. 2017). Despite the high diversity of the latter family, with well over 100 valid species in 19 genera, little is known about their diphyllidean fauna. So far, only 24 species of diphyllideans were described from dasyatids, but expected number of species is at least 80 based on a study of Caira et al. (2017). In total only six species of *Echinobothrium* were described from dasyatids (Caira et al. 2017). Three of these species infect stingrays of the genus *Taeniura* Müller et Henle, and two species infect stingrays of the genus *Himantura* Müller et Henle (Caira et al. 2017). The sixth species, *Echinobothrium longicolle* Southwell, 1925, was described by Southwell (1925) from *Neotrygon kuhlii* (Müller et Henle) (as *Trygon kuhlii*) in Sri Lanka.

Since most diphyllideans are strict specialists (oioxenous host specificity) for their hosts (i.e., they parasitise only a single host species), examination of additional members of *Neotrygon* Castelnau seems likely to yield additional diphyllidean novelty. There are 16 valid species of the genus *Neotrygon*, nonetheless the exact number of known species remains unclear as recent morphological and molecular work has led to the recognition of previous-

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ly undescribed diversity in the genus (Naylor et al. 2012, Puckridge et al. 2013, Borsa et al. 2018). It seems clear that these unstudied species of *Neotrygon* likely harbour additional species of *Echinobothrium*. Indeed, examination of newly collected material and unidentified museum specimens of several previously unexamined species of *Neotrygon* off Australia and Borneo revealed several new species of *Echinobothrium*. The four species for which sufficient material was available are described herein.

MATERIALS AND METHODS

Each host specimen collected *de novo* was assigned a unique collection code and collection number combination (e.g., BO-336), and additional information and images for each specimen are available in the Global Cestode Database (GCD) (http://elasmobranchs.tapewormdb.uconn.edu). For each specimen, liver tissue samples were preserved in 95% ethanol for subsequent identity verification using NADH2 sequence data (see Naylor et al. 2012). With the exception of NT-8, newly collected host specimens were obtained in association with commercial trawlers. These consisted of: one specimen of *Neotrygon orientalis* Last, White et Serét (as *Neotrygon kuhlii* 1 of Naylor et al. 2012) (BO-336) from the South China Sea off Kuching, Borneo; one specimen of *Neotrygon varidens* (Garman) (as *N. kuhlii* 2 of Naylor et al. 2012) (BO-409) from the South China Sea off Kuching, Borneo; two specimens of *Neotrygon australiae* Last, White et Serét (as *N. kuhlii* 4 of Naylor et al. 2012) (NT-63 and NT-85) from the Arafura Sea off the Wessel Islands in the Northern Territory, Australia; one specimen of *Neotrygon picta* Last et White (NT-74) collected in the Arafura Sea off the Wessel Islands in the Northern Territory, Australia.

In addition, one specimen of *Neotrygon ningalooensis* Last, White et Puckridge (NT-8) was collected using a hand spear in the Gulf of Carpentaria, off Nhulunbuy in the Northern Territory, Australia. The identities of the hosts of the museum collections examined (SAM Nos. 17178–17232) are more problematic. This material was collected in 1986 by B.G. Robertson at Fog Bay and Cape van Dieman in the Northern Territory and off Bunbury, Western Australia. At that time, the hosts were identified as *Neotrygon leylandi* (Last). However, since several species of *Neotrygon* are now known to occur in these regions (see Last et al. 2010, 2016, Puckridge et al. 2013), and images or tissues are not available for the host specimens from which this material was collected, the identification of these hosts as *N. leylandi* has yet to be confirmed.

Tapeworms were prepared as whole mounts and for scanning electron microscopy (SEM) following Kuchta and Caira (2010). SEM was performed using a LEO/Zeiss DSM982 Gemini field emission scanning electron microscope at the University of Connecticut, Storrs, USA. Measurements were taken using an Infinity 2 digital camera system mounted on an Olympus BX51 and Quick Photo software and are presented as range followed by mean, standard deviation, number of samples measured, and total number of measurements when more than one measurement was made per sample, in parentheses. Measurements are in micrometres unless otherwise noted.

Hook terminology and numbering scheme follow Neifar et al. (2001), modified by Tyler (2006) and Kuchta and Caira (2010). Microthrix terminology follows Chervy (2009). The studied material is deposited in various museums (see remarks of each description) and the abbreviations used are as follows: IPCAS, Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic; LRP, Lawrence R. Penner Parasitology Collection, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA; MAGNT, Museum and Art Gallery of the Northern Territory; MZUM(P), Museum of Zoology, University of Malaya, Kuala Lumpur, Malaysia; SAM, South Australian Museum, Adelaide, South Australia, Australia; USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.

RESULTS

All six species found in maskrays represent new species of *Echinobothrium.* However, due to the limited availability of material, only four of them are described below as new. Description of putative new species from *Neotrygon ningalooensis* (i.e., NT-8) and *N. picta* (i.e., NT-74) will be possible only after additional material is collected.

Echinobothrium bethae sp. n. Figs. 1, 2

ZooBank number for species:
urn:lsid:zoobank.org:act:FDAA7A6F-3087-4220-9DCE-FBBE321E0D4D

Description (based on whole mounts of six mature specimens and one specimen examined with SEM): Worms 0.9–1.2 mm (1.1 ± 0.2; 4) long; greatest wide generally at level of terminal proglottid, 140–190 (162 ± 19; 5) wide; proglottids acraspedote, 5 (5 ± 0; 5) in number (Fig. 1A). Scolex consisting of scolex proper and cephalic peduncle (Figs. 1A, 2A). Scolex proper 188–236 (211 ± 20; 4) long by 111–164 (134 ± 27; 3) wide, composed of armed apical rostellum and 1 dorsal and 1 ventral bothrium; bothria 153–196 (177 ± 18; 4) long by 111–164 (134 ± 27; 3) wide.

Rostellum bearing 1 dorsal and 1 ventral group of 19 (19 ± 0; 3) solid apical hooks arranged in 2 rows and flanked on each side by 2–3 (2.5 ± 0.6; 3, 6) lateral hooklets; hooklets 12–27 (19 ± 5; 3, 15) long, with scalpel-like blade (Fig. 1B). Apical hooks gradually increasing in length towards centre of group, type B symmetry. First B hook after hooklets 31–40 (35 ± 3; 3, 5) long (33–75% longer than adjacent hooklet), remaining B hooks 45–95 (72 ± 16; 3, 30) long; first A hook after fourth B hook 60–71 (65 ± 6; 3, 4) long (0–13.4% shorter than fourth B hook), remaining A hooks 61–81 (72 ± 7; 3, 12) long. Bases of 3 outermost B hooks on each side inserted in extended arch of first A hook base (Fig. 1B). Hook formula {2–3 6/13 2–3}.

Cephalic peduncle 114–183 (149 ± 25; 5) long; greatest width at anterior end, 59–83 (74 ± 9; 5) wide, armed with 8 longitudinal columns of 12–16 (15 ± 2; 5) spines. Spines with triradiate bases, decreasing in length posteriorly; free prong of first 3 anterior spines 41–55 (49 ± 4; 5, 15) long; free prong of last 3 posterior spines 7–22 (14 ± 4; 5, 15) long.

Distal bothrial surfaces with conspicuous central triangular region; triangular region covered with capiliform filitriches (Fig. 2E); remainder of distal surfaces covered with trifurcate spinitriches with slender digits (Fig. 2D). Prox-

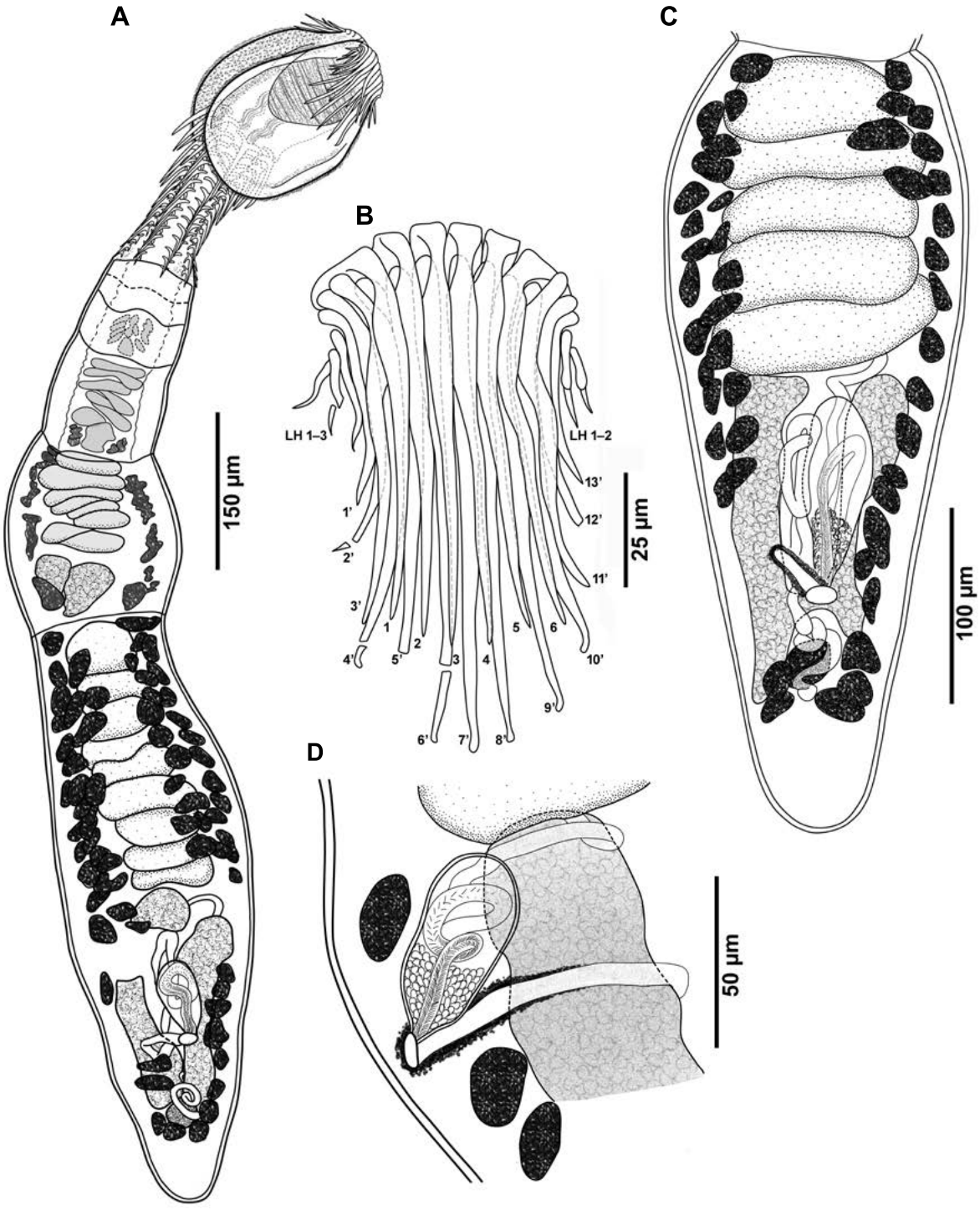

Fig. 1. Line drawings of *Echinobothrium bethae* sp. n. from *Neotrygon varidens* (Garman). **A** – entire specimen (holotype); **B** – apical hooks and lateral hooklets; **C** – mature proglottid, ventral view; **D** – detail of terminal genitalia, lateral view.

imal bothrial surfaces covered with palmate spinitriches with 5–7 digits interspersed with acicular filitriches (Fig. 2B,C). Apex of scolex proper apparently devoid of microtriches. Cephalic peduncle devoid of microtriches, but cilia present (Fig. 2F).

Immature proglottids 4 (4 ± 0; 5) in number, initially wider than long, becoming longer than wide with maturity. Mature proglottids 1 (1 ± 0; 6) in number, 311–572 (416 ± 88; 6) long by 140–190 (161 ± 20; 5) wide, length: wide ratio (1.8–3 : 1). No gravid proglottids observed (Fig. 1A).

Testes 5–8 (6.5 ± 1; 6) in number, extending from anterior margin of proglottid to approximately anterior margin of ovary, 29–45 (35 ± 5; 6, 18) long by 71–99 (87 ± 8; 6, 18) wide, arranged in 1 regular column and 1 row deep in cross section (Fig. 1A,C). Cirrus-sac oval, 59–86 (71 ± 12; 4) long by 27–46 (36 ± 9; 4) wide, length: width ratio (1.8–2.2 : 1), located anterior to vagina opening, containing robust coiled cirrus; cirrus covered with short spinitriches (Fig. 1D). Internal and external seminal vesicle not observed. Vas deferens directed anteriorly.

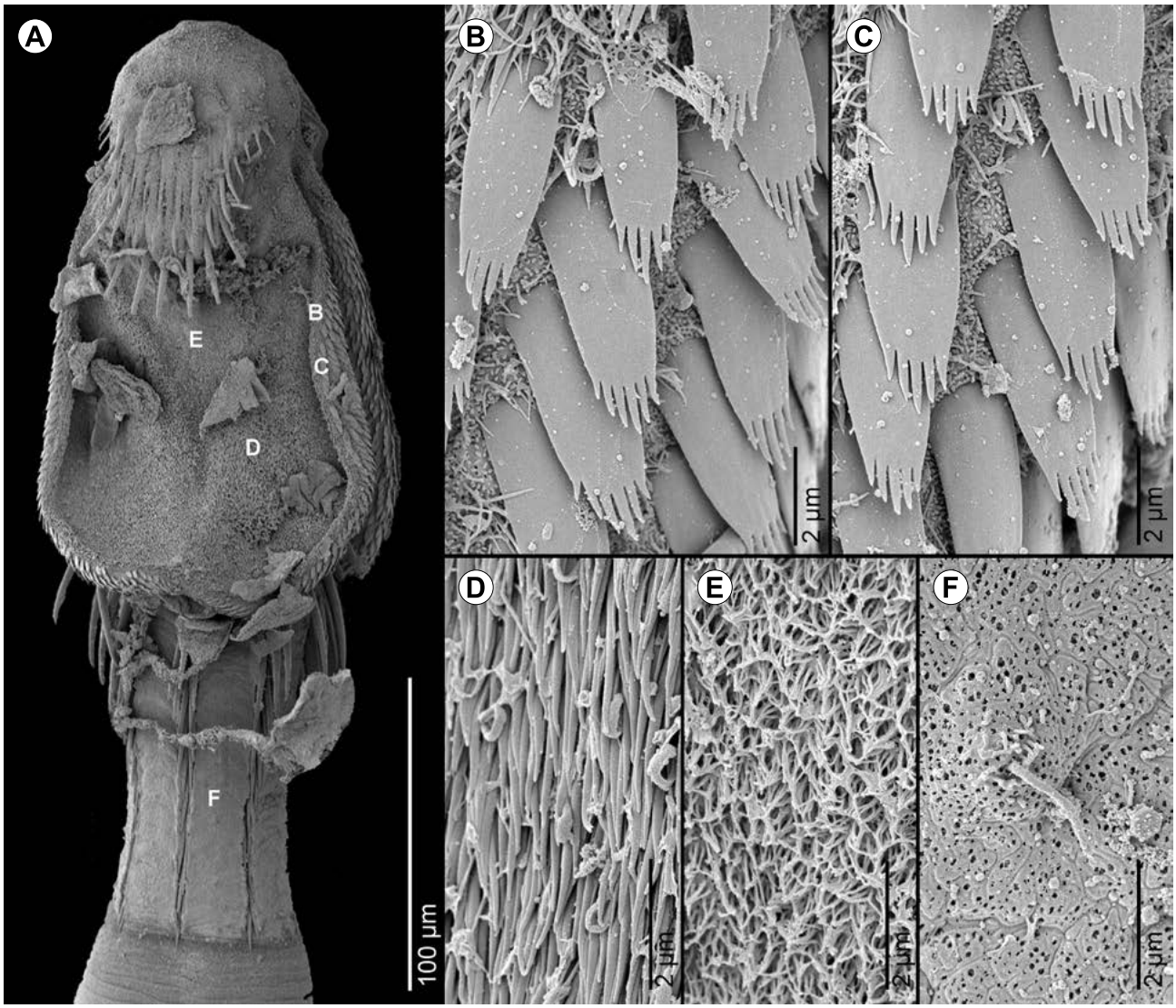

Fig. 2. Scanning electron micrographs of *Echinobothrium bethae* sp. n. from *Neotrygon varidens* (Garman). **A** – scolex. Note: small letters correspond to the figures showing higher magnification images of these surfaces; **B**, **C** – proximal bothrial surface; **D** – posterior region of distal bothrial surface; **E** – distal bothrial surface at margin of central triangular region; **F** – surface of cephalic peduncle.

Ovary near posterior margin of proglottid, H-shaped in frontal view, bilobed in cross section, 113–167 (130 ± 25; 4) long by 50–75 (60 ± 9; 5) wide at level of connection isthmus (Fig. 1A,C). Mehlis' gland posterior to ovarian isthmus, 27–32 (30 ± 2; 4) long by 23–27 (25 ± 2; 4) wide. Vagina parallel to cirrus-sac, directed anteriorly and then posteriorly between ovary upper lobes, coiled at posterior end, widening as approaching genital pore, distal portion 6–12 (9 ± 3; 3) in diameter, surrounded by glandular cells (Fig. 1D). Genital pore midventral; 26–30% from posterior margin of mature proglottid (Fig. 1A,C).

Vitellarium follicular; vitelline follicles 14–29 (20 ± 6; 4, 11) long by 15–34 (23 ± 7; 4, 11) wide, subcortical, in two lateral fields, distributed throughout length of proglottid, each band consisting of 1 dorsal and 1 ventral column of follicles, uninterrupted at level of ovary; vitelline fields confluent in posterior extremity of proglottid (Fig. 1A,C). Uterus and eggs not observed.

Type and only host: *Neotrygon varidens* (Garman) (Myliobatiformes: Dasyatidae).

Type locality: South China Sea, Borneo, Sarawak (2.81700000 110.87976667) (BO-409); collected by J.N. Caira on April 21, 2004.

Site of infection: Spiral intestine.

Prevalence and intensity: 1 of 2 examined hosts infected with 8 worms.

Type material: Holotype deposited at MZMU (No. 2024.1 (H)) and paratypes at USNM (Nos. 1606803, 1606804), MZMU (No. 2023.1 (P)), LRP (No. 11025), and IPCAS (No. C-935).

Etymology: The name of the new species is given after Elizabeth Barbeau of the University of Connecticut, USA, for her extraordinary help in building the Global Cestode Database.

Remarks. With respect to the structure of the rostellum, *Echinobothrium bethae* sp. n. differs from all other species of *Echinobothrium*, except *Echinobothrium rhynchobatid* (Khalil et Abdul-Salam, 1989), by the extended base of its

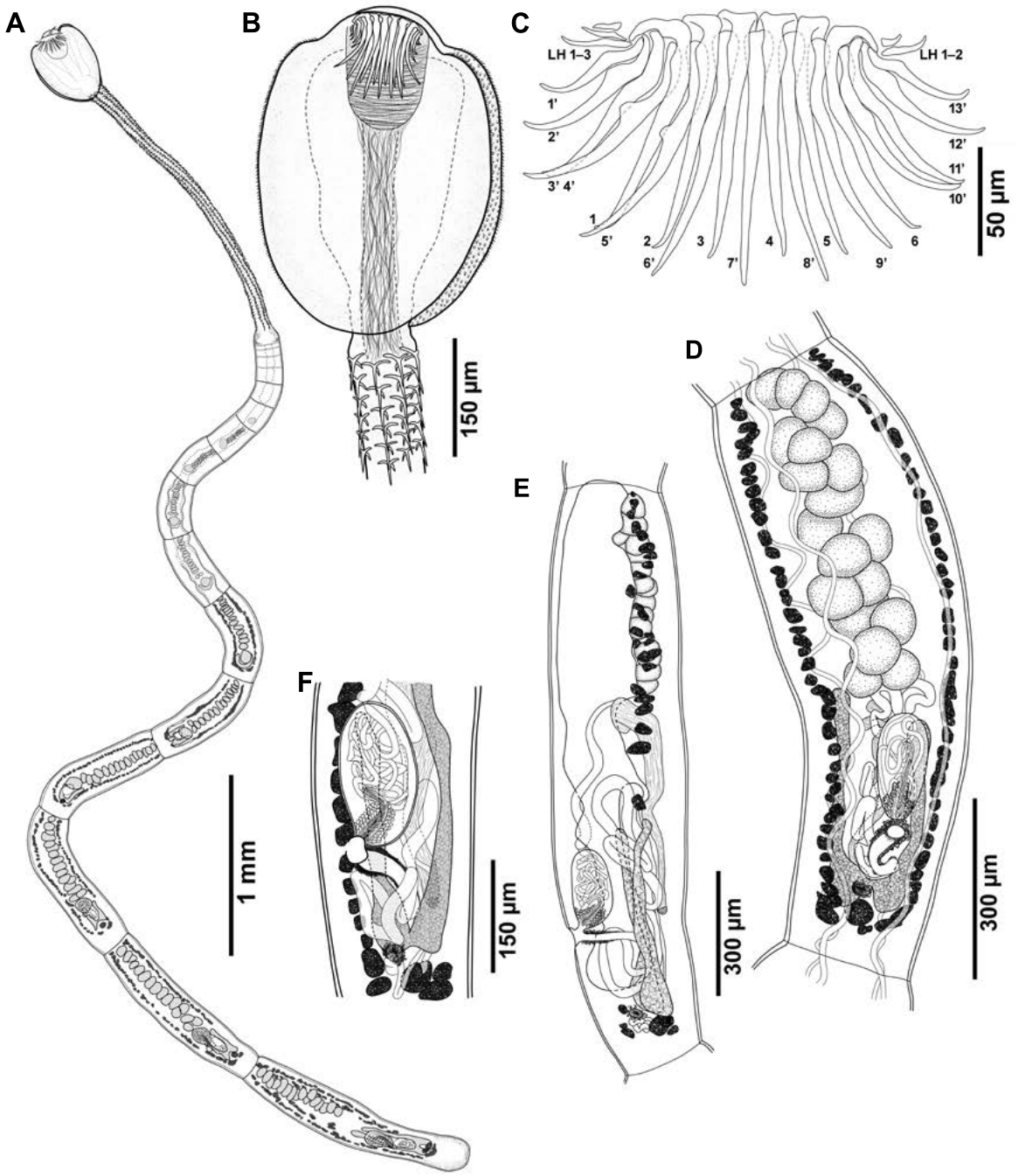

Fig. 3. Line drawings of *Echinobothrium giraffaeous* sp. n. from *Neotrygon leylandii* (Last). **A** – entire specimen (holotype); **B** – scolex, dorsoventral view; **C** – apical hooks and lateral hooklets; **D** – mature proglottid; **E** – gravid proglotti (eggs are not drawn), ventral (D) and lateral (E) views; **F** – detail of terminal genitalia, lateral view.

outermost A hook into which the bases of the three outermost B hooks are inserted (but see also species described below). However, the hook formula of *E. rhynchobati* is different from *E. bethae* sp. n. ({1–2 6/15 1–2} vs. {2–3 6/13 2–3}).

***Echinobothrium giraffaeous* sp. n.** Figs. 3, 4

ZooBank number for species:
urn:lsid:zoobank.org:act:BEA84A95-D382-4D80-B9AD-40461BB92549

Description (based on whole mounts of 15 mature and five immature worms, and two specimens examined with SEM): Worms apolytic, 6.9–14.1 mm (10.2 ± 1.7; 14) long; greatest width generally at level of terminal proglottid, 256–487 (356 ± 73; 14) wide; proglottids acraspedote, 11–22 (16 ± 3; 15) in number (Fig. 3A). Scolex consisting of scolex proper and cephalic peduncle. Scolex proper 308–486 (409 ± 47; 15) long by 250–334 (297 ± 25; 10) wide, composed of armed apical rostellum and 1 dorsal and 1 ventral bothrium; bothria 238–449 (365 ± 65; 16) long by 250–334 (297 ± 25; 10) wide (Figs. 3A,B, 4A).

Rostellum bearing 1 dorsal and 1 ventral group of 19 (19 ± 0, 5) solid apical hooks arranged in 2 rows and flanked on each side by 1–3 (2 ± 0.4; 9, 18) lateral hooklets; hooklets 9–36 (24 ± 7; 5, 20) long (Fig. 3C). Apical hooks gradually increasing in length towards centre of group, type B symmetry. First B hook after hooklets 26–54 (44 ± 8; 5, 10) long (21–31% longer than adjacent hooklet), remaining B hooks 43–124 (92 ± 21; 5, 25) long; first A hook after fourth B hook 82–110 (97 ± 9; 5, 10) long (0–20% longer than fourth B hook), remaining A hooks 92–116 (105 ± 8; 5, 20) long. Bases of 3 outermost B hooks on each side inserted in arch of first A hook base. Hook formula {1–3 6/13 1–3}.

Cephalic peduncle extremely long, 1.7–2.7 mm (2.2 ± 0.3; 15); greatest width at anterior end, 84–107 (94 ± 8; 15) wide, armed with 8 longitudinal columns of 83–123 (101 ± 11; 14) spines (Figs. 3A, 4A). Spines with triradiate bases, decreasing in length posteriorly; free prong of first 3 anterior spines 25–45 (36 ± 5; 15, 45) long; free prong of last 3 posterior spines 8–15 (11 ± 2; 15, 45) long.

Distal bothrial surfaces with conspicuous central triangular region; triangular region covered with capilliform filitriches (Fig. 4E); remainder of distal surfaces covered with trifurcate spinitriches, with slender digits (Fig. 4D). Anterior proximal bothrial surfaces covered with trifid spinitriches or with pectinate spinitriches with 4 digits interspersed with capilliform filitriches (Fig. 4B); posterior proximal bothrial surfaces with pectinate spinitriches with 4–5 digits interspersed with capilliform filitriches (Fig. 4C). Apex of scolex proper devoid of microtriches. Cephalic peduncle and proglottids covered with capilliform filitriches.

Immature proglottids 9–16 (12 ± 3; 15) in number, initially wider than long, becoming longer than wide with maturity (Fig. 3A). Mature proglottids 1–4 (3 ± 1; 15) in number, 643–1,596 (1,020 ± 211; 15, 38) long by 217–458 (294 ± 57; 15, 38) wide, length: width ratio (1.9–5 : 1). Gravid proglottids 0–3 (1 ± 1; 12) in number, 1,147–1,918 (1,525 ± 247; 12, 19) long by 256–487 (372 ± 59; 12, 18) wide, length: width ratio (2.6–5.1 : 1).

Testes 12–24 (17 ± 3; 15, 38) in number, 46–95 (62 ± 12; 15, 45) long by 60–113 (82 ± 15; 15, 45) wide, arranged in 1 regular column and 1 layer deep in cross section in immature proglottids extending from anterior margin of proglottid to anterior margin of cirrus-sac, but tending to overlap and become positioned in more columns and/or 2 rows in mature proglottids (Fig. 3D,E). Cirrus-sac pyriform, 154–235 (191 ± 21; 15) long by 75–142 (104 ± 20; 15) wide, length: width ratio (1.4–2.3 : 1), located anterior to vagina opening, containing elongate, coiled cirrus; cirrus covered with short spinitriches 5–9 (6 ± 1; 15) long (Fig. 3D–F). Internal seminal vesicle not observed. Vas deferens long, coiled anteriorly to cirrus-sac, ending in a long running parallel to cirrus-sac seminal vesicle (Fig. 3E).

Ovary asymmetrical, near posterior margin of proglottid, H-shaped in frontal view, bilobed in cross section, 84–203 (125 ± 35; 10) wide at level of connection isthmus, left ovarian lobe 236–462 (320 ± 75; 15) long, right ovarian lobe 199–438 (335 ± 69, 15) long (Fig. 3D,E). Mehlis' gland ventral to, at same level as, or slightly posterior to ovarian bridge, 22–30 (27 ± 3; 15) long by 23–31 (27 ± 2; 15) wide. Vagina long, parallel to cirrus-sac, undulating ventrally to ovary and coiled at posterior end, widening as approaching genital pore, distal portion 14–36 (22 ± 5; 15) wide, surrounded by glandular cells (Fig. 3D–F). Genital pore midventral, 19–29% from posterior margin of mature proglottids, 13–35% from posterior margin of gravid proglottids (Fig. 3D,E).

Vitellarium follicular; vitelline follicles 15–44 (27 ± 7; 15, 45) long by 9–45 (21 ± 7; 15, 45) wide, in two lateral bands; each band arranged in two columns, extending throughout length of proglottid, each band consisting of 1 dorsal and 1 ventral column of follicles, uninterrupted at level of ovary, confluent in posterior extremity of proglottid (Fig. 3A,D,E). Uterus saccate, originating as uterine duct in ootype region, extending anterodorsal to cirrus-sac, ventral to testes until filling all anterior part in gravid proglottids (Fig. 3E). Eggs oval in shape, non filamented, 12–20 (14.5 ± 2.1; 8, 36) long by 13–20 (14 ± 1.6; 8, 36) wide..

Type and only host: *Neotrygon leylandi* (Last) (Myliobatiformes: Dasyatidae); collected by B.G. Robertson on September 28, 1986.

Type locality: Fog Bay, Northern Territory, Australia (-12.832500, 130.282806)

Additional localities: Cape van Dieman, Northern Territory (-16.4734472334603, 139.7650869329647); Bunbury, Western Australia (-32.700000, 115.283333).

Site of infection: Spiral intestine.

Prevalence and intensity: unknown, 24 tapeworms were obtained from 27 specimens of *D. leylandi* (SAM).

Type material: Holotype is deposited at SAM (No. AHC37016) and paratypes at SAM (No. AHC37017–37030), IPCAS No. C-934), USNM (Nos. 1606819– 1606814), and LRP (Nos. 11026–11028).

Etymology: This species is named after its extremely long cephalic peduncle, which resembles the long neck of a giraffe (*Giraffa* Linnaeus).

Remarks. *Echinobothrium giraffaeous* sp. n. is easily distinguished from all but one of its 33 valid congeners by its extremely long cephalic peduncle (1.7–2.7 mm), bearing 83–123 spines in each of its 8 columns. The cephalic peduncle of these 32 other species reaches a maximum length of 1.66 mm and a maximum of 60 spines per column. The exception is *Echinobothrium longicolle* with a longer cephalic peduncle (2.95–4.9 mm) and 159–181 spines per column. The latter species is also much larger than *E. giraffaeous* sp. n. (20–30 mm vs. 7–14 mm), and the spines on its cephalic peduncle possess leaf-like bases rather than the typical triradiate bases found in other members of the genus.

Echinobothrium giraffaeous sp. n. is distinguished from its congeners, except for *E. bethae* and *E. rhynchobati*, by an extended base of its outermost A hook into which the bases of the three outermost B hooks are inserted. However, *E. giraffaeous* sp. n. can be easily distinguished from *E. bethae* sp. n. by having much longer cephalic peduncle (1.7–2.7 mm *vs*. 114–183 µm). As with *E. bethae* sp. n., the rostellum of the new species differs from that of *E. rhyn-*

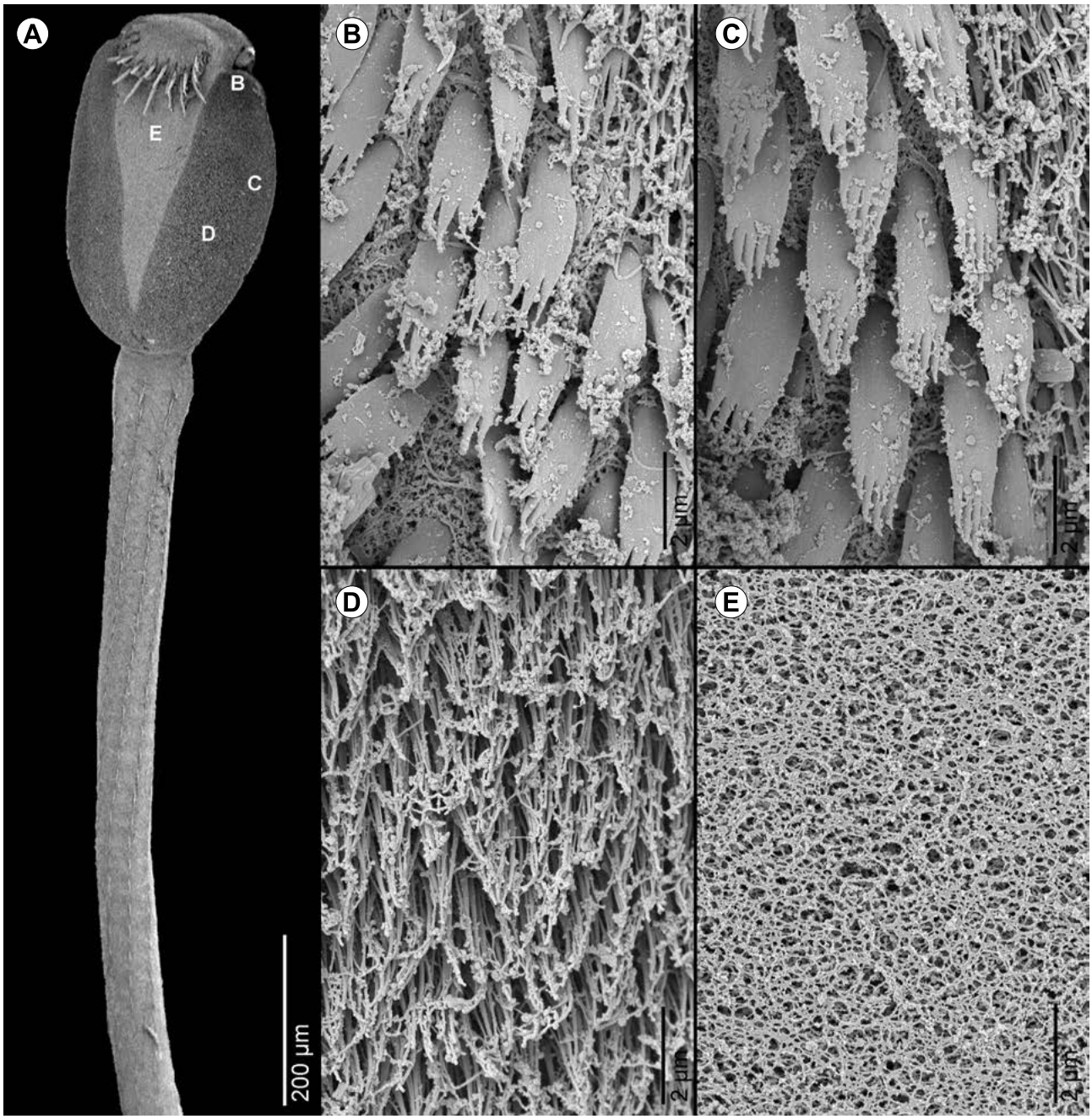

Fig. 4. Scanning electron micrographs of *Echinobothrium giraffaeous* sp. n. from *Neotrygon leylandi* (Last). **A** – scolex. Note: small letters correspond to the figures showing higher magnification images of these surfaces; **B**, **C** – proximal bothrial surface; **D** – posterior region of distal bothrial surface; **E** – distal bothrial surface at margin of central triangular region.

chobati in lacking, rather than possessing, the system of interlocking knobs and sockets that articulate bases of type A and B hooks with one another.

***Echinobothrium ivanovae* sp. n.** Figs. 5, 6

ZooBank number for species:
urn:lsid:zoobank.org:act:921E187E-53D6-48E1-BEBD-85265AF5D09F

Synonym: *Echinobothrium* sp. 4 by Caira et al. (2013).

Description (based on whole mounts of ten mature specimens and three specimens examined with SEM): Worms apolytic, 1.5–2.8 mm (2.3 ± 0.5; 8) long; greatest wide generally at level of terminal proglottid, 164–249 (195 ± 37; 8) wide; proglottids acraspedote, 5–8 (6 ± 1; 10) in number (Fig. 5A). Scolex consisting of scolex proper and cephalic peduncle. Scolex proper 174–217 (188 ± 13; 10) long by 108–130 (117 ± 9; 5) wide, composed of armed apical rostellum and 1 dorsal and 1 ventral bothrium; bothria 126–171 (158 ± 14; 10) long by 108–130 (117 ± 9; 5) wide (Figs. 5B, 6A).

Rostellum bearing 1 dorsal and 1 ventral group of 19 (19 ± 0; 4) solid apical hooks arranged in 2 rows and flanked on each side by 3 (3 ± 0; 4, 7) lateral hooklets; hooklets 9–27 (18 ± 7; 4, 20) long, with scalpel-like blade (Fig. 5D). Apical hooks gradually increasing in length towards cen-

tre of group, type B symmetry. First B hook after hooklets 33–43 (40 ± 3; 4, 6) long (22–69% longer than adjacent hooklet), remaining B hooks 42–97 (72 ± 15, 4, 28) long; first A hook after fourth B hook 63–74 (69 ± 4; 5, 6) long (7–13% shorter than fourth B hook), remaining A hooks 71–78 (74 ± 2; 3, 8) long. Bases of 3 outermost B hooks on each side inserted in arch of first A hook base. Hook formula {3 6/13 3}.

Cephalic peduncle 202–370 (259 ± 53; 9) long; greatest width at anterior end, 51–65 (58 ± 5; 10) wide, armed with 8 longitudinal columns of 13–17 (16 ± 1; 10) spines (Figs. 5B, 6A). Spines with triradiate bases, decreasing in length posteriorly; free prong of first 3 anterior spines 36–54 (45 ± 5; 10, 30) long; free prong of last 3 posterior spines 8–23 (14 ± 4; 10, 30) long.

Distal bothrial surfaces with conspicuous central triangular region; triangular region covered with acicular filitriches (Fig. 6E); remainder of distal surfaces covered with trifurcate spinitriches with slender digits (Fig. 6D). Proximal bothrial surfaces covered with palmate spinitriches with 6–8 digits interspersed with acicular filitriches (Fig. 6B,C). Apex of scolex proper apparently devoid of microtriches. Cephalic peduncle devoid of microtriches.

Immature proglottids 4–6 (4.5 ± 1; 10) in number, initially wider than long, becoming longer than wide with maturity. Mature proglottids 1–2 (1 ± 0.4; 10) in number, 420–792 (605 ± 108; 9, 11) long by 121–177 (152 ± 22; 10, 12) wide, length: wide ratio (3.3–5.9 : 1). Gravid proglottids 0–1 (0.5 ± 0.5; 10) in number, 1,110–1,177 (1,134 ± 30; 4) long by 228–249 (239 ± 11; 3) wide, length: width ratio (4.5–5 : 1) (Fig. 5A).

Testes 5–11 (8 ± 2; 10, 12) in number, extending from anterior margin of proglottid to approximately anterior margin of ovary, 37–56 (46 ± 6; 10, 30) long by 55–125 (90 ± 15; 10, 30) wide, arranged in 1 regular column and 1 row deep in cross section (Fig. 5A,C). Cirrus-sac pyriform, 60–97 (76 ± 12; 8) long by 28–51 (42 ± 8; 8) wide, length: width ratio (1.4–2.6 : 1), located anterior to vaginal opening, containing robust coiled cirrus; cirrus covered with short spinitriches (around 4 in length) (Fig. 5C). Internal and external seminal vesicle not observed. Vas deferens directed anteriorly, towards right first and then sharply towards left at approximately level of first testes.

Ovary near posterior margin of proglottid, H-shaped in frontal view, bilobed in cross section, 132–258 (194 ± 42; 9) long by 64–120 (81 ± 19; 7) wide at level of connection isthmus (Fig. 5C). Mehlis' gland at same level or slightly anterior and posterior to ovarian isthmus, 13–17 (16 ± 2; 4) long by 11–16 (13 ± 2; 4) wide. Vagina parallel to cirrus-sac, directed anteriorly and then posteriorly between ovary upper lobes, coiled at posterior end, widening as approaching genital pore, distal portion 8–14 (11 ± 2; 6) in diameter, surrounded by glandular cells (Fig. 5C). Genital pore midventral; 20–32% from posterior margin of mature proglottid, 18–22% from posterior margin of gravid proglottid (Fig. 5C).

Vitellarium follicular; vitelline follicles 14–29 (21 ± 4; 10, 30) long by 13–39 (21 ± 6; 10, 30) wide, subcortical, in two lateral fields, distributed throughout length of proglottid, each band consisting of 1 dorsal and 1 ventral column of follicles, uninterrupted at level of ovary; vitelline fields confluent in posterior extremity of proglottid (Fig. 5A,C). Uterus saccate, originating as uterine duct in ootype region, extending anterodorsal to cirrus-sac, ventral to testes until filling all anterior part in gravid proglottids. Eggs present, but not measured.

Type and only host: *Neotrygon orientalis* Last, White et Serét (Myliobatiformes: Dasyatidae).

Type and only locality: South China Sea, Borneo, Sarawak, off Kuching (2.502039, 110.671339) (BO-336); collected by K. Jensen and G. Yearsley on April 28, 2004.

Site of infection: Spiral intestine.

Prevalence and intensity: 2 of 10 examined hosts infected with 9 and 15 worms per host.

Type material: Holotype is deposited at MZUM (No. 2014.2 (H)) and paratypes at USNM (Nos. 1606796–1606802), IPCAS (No. C-936), MZUM (No. 2023.2 (P)), and LRP (Nos. 11029–11034).

Representative DNA sequences: *Echinobothrium* sp. 4 in Caira et al. (2013).

Etymology: The species is named after the late Veronica Ivanov from the Universidad de Buenos Aires for her extraordinary contribution to our knowledge of cestodes, including diphyllideans.

Remarks. *Echinobothrium ivanovae* sp. n. is distinguished from all other species of *Echinobothrium*, except for *E. bethae* sp. n. *E. giraffaeous* sp. n. and *E. rhynchobati*, in possessing outermost A hooks with an extended base into which the bases of the three outermost B hooks are inserted. However, it differs from *E. bethae* sp. n. in its longer body (length 1.5–2.8 mm vs. 0.9–1.2 mm), longer cephalic peduncle (202–370 µm vs. 114–183 µm), and smaller Mehlis' gland (16 × 13 µm vs. 30 × 25 µm). It is easily distinguished from *E. giraffaeous* sp. n. because the latter species has a much longer cephalic peduncle (1.7–2.7 mm vs. 202–370 µm). Finally, *E. ivanovae* sp. n. differs by the presence of an armed cephalic penduncle and the absence of a connection between apical hooks A and B at the base with an intricate system of knobs and sockets (see fig. 16 of Tyler 2006).

Echinobothrium ivanovae was included in the molecular phylogenetic study of diphyllideans published by Caira et al. (2013) under the name *Echinobothrium* sp. 4. This species was closely related to those of *Taeniura lymma* (Forsskål), but not all species were molecularly characteristed and nodal support in this part of the tree was relatively low (Caira et al. 2013).

***Echinobothrium tyleri* sp. n.** Figs. 7, 8

ZooBank number for species:
urn:lsid:zoobank.org:act:92E5148A-E34E-4475-9699-D294ECD481A3

Description (based on whole mounts of 13 mature and four immature specimens and two specimens examined with SEM): Worms apolytic, 4.1–9.7 mm (5.9 ± 2.0; 6) long; greatest width generally at level of terminal proglot-

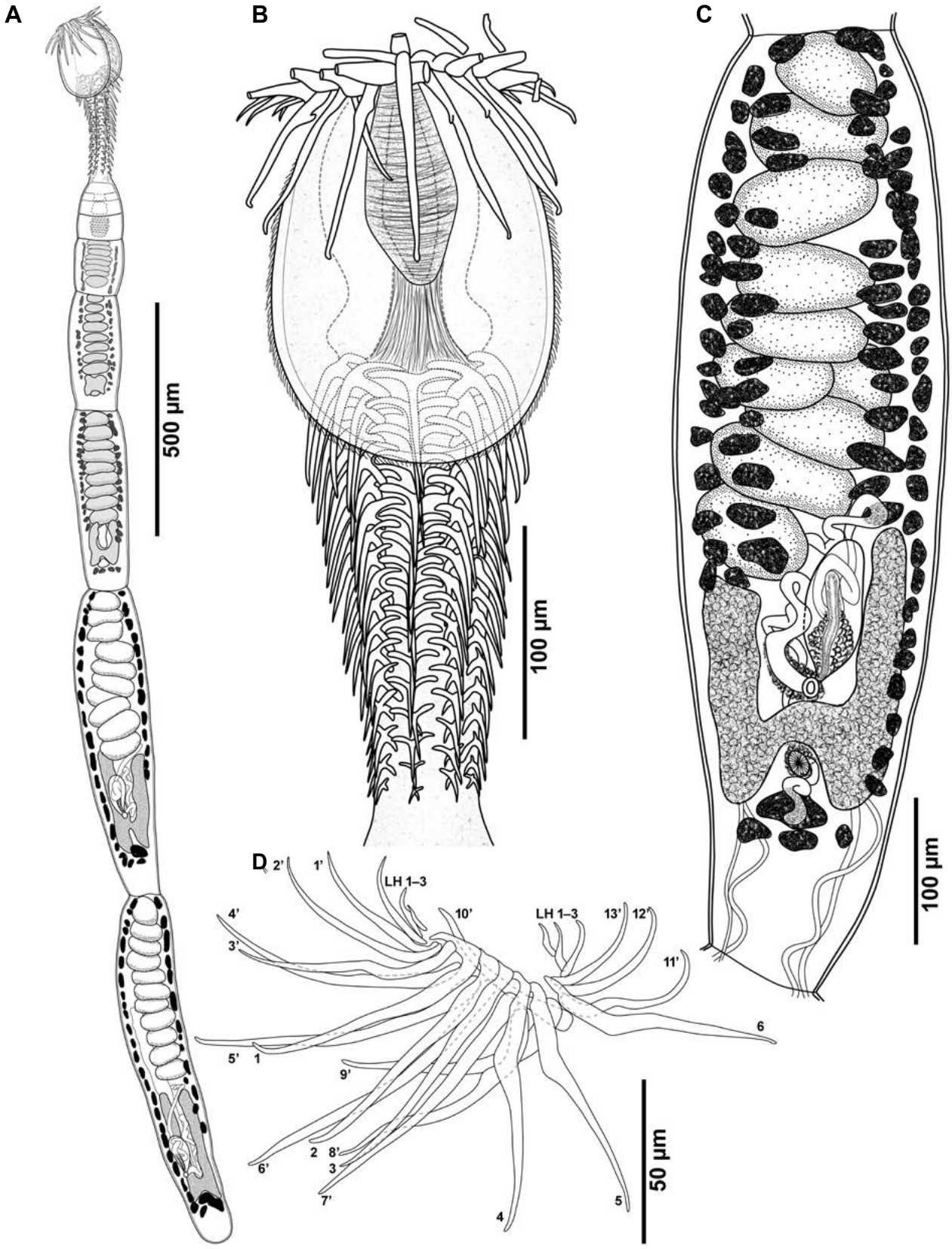

Fig. 5. Line drawings of *Echinobothrium ivanovae* sp. n. from *Neotrygon orientalis* Last, White et Serét. **A** – entire specimen (holotype) including last gravid proglottid (eggs not drawn); **B** – scolex, dorsoventral view; **C** – mature proglottid, ventral view; **D** – apical hooks and lateral hooklets.

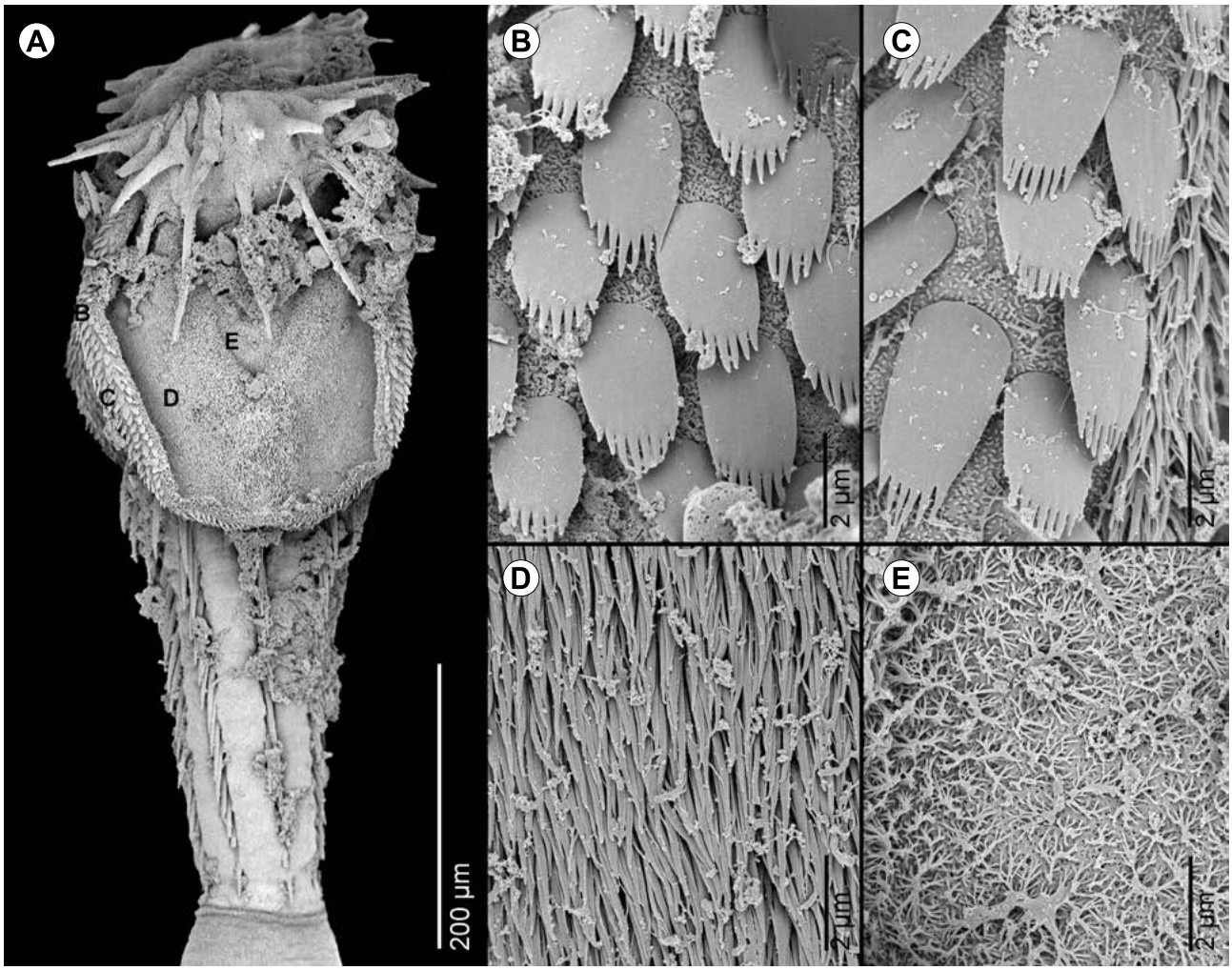

Fig. 6. Scanning electron micrographs of *Echinobothrium ivanovae* sp. n. from *Neotrygon orientalis* Last, White et Serét. **A** – scolex. Note: small letters correspond to the figures showing higher magnification images of these surfaces; **B**, **C** – proximal bothrial surface; **D** – posterior region of distal bothrial surface; **E** – distal bothrial surface at margin of central triangular region.

tid, 152–240 (183 ± 31; 7) wide; proglottids acraspedote, 14–26 (20 ± 4;, 14) in number (Fig. 7A). Scolex consisting of scolex proper and cephalic peduncle. Scolex proper 309–507 (425 ± 55; 12) long by 239–327 (280 ± 34; 9) wide, composed of armed apical rostellum and 1 dorsal and 1 ventral bothrium; bothria 277–423 (368 ± 47; 12) long by 239–327 (280 ± 34; 9) wide (Figs. 7B, 8A).

Rostellum bearing 1 dorsal and 1 ventral group of 35 (35 ± 0; 10) solid apical hooks arranged in 2 rows and flanked on each side by 2–3 (2.37 ± 0.5; 10, 19) lateral hooklets; hooklets 13–27 (20.6 ± 3.9; 5, 24) long, with scalpel-like blade (Fig. 7C). Apical hooks gradually increasing in length towards centre of group, type B symmetry. First A hook after hooklets 30–43 (38 ± 4; 5, 10) long (48–69% longer than adjacent hooklet), remaining A hooks 44–121 (95 ± 22; 5, 89) long; first B hook after second A hook 57–69 (63 ± 4; 5, 10) long (8–69% longer than second A hook), remaining B hooks 72–148 (118 ± 21; 5, 73) long. Hook formula {2–3 18/17 2–3}.

Cephalic peduncle 213–387 (303 ± 58; 12) long; greatest width generally at anterior end, 82–117 (101 ± 13; 12) wide, armed with 8 longitudinal columns of 13–15 (14 ± 1; 15) spines (Figs. 7B, 8A). Spines with triradiate bases, decreasing in length posteriorly; free prong of first 3 anterior spines 27–36 (32 ± 2; 15, 45) long; free prong of last 3 posterior spines 8–22 (14 ± 4; 15, 45) long.

Distal bothrial surfaces with conspicuous central triangular region; triangular region covered with acicular filitriches (Fig. 8F). Remainder of distal surfaces covered with trifurcate spinitriches with slender digits (Fig. 8E). Proximal bothrial surfaces covered with pectinate spinitriches with 4–8 digits interspersed with acicular filitriches; however, they bear trifid spinitriches in anterior part (Fig. 8C). Apex of scolex proper covered with sparse capilliform filitriches (Fig. 8D). Cephalic peduncle covered with papilliform filitriches (Fig. 8G).

Immature proglottids 9–20 (14 ± 3; 12) in number, initially wider than long, becoming longer than wide with maturity. Mature proglottids 0–10 (4 ± 3; 12) in number, 494–1,325 (880 ± 174; 7, 25) long by 116–240 (154 ± 26; 7, 25) wide, length: width ratio (3.3–9 : 1). Gravid proglottids 0–1 (0.1 ± 0.3; 12) in number, 867 (867 ± 0; 1) long by 182 (182 ± 0; 1) wide, length: width ratio (4.8 : 1) (Fig. 7A).

Testes 10–19 (14 ± 3; 7, 23) in number, extending from anterior margin of proglottid to approximately anterior margin of cirrus-sac, 25–51 (39 ± 8; 7, 21) long by 36–89

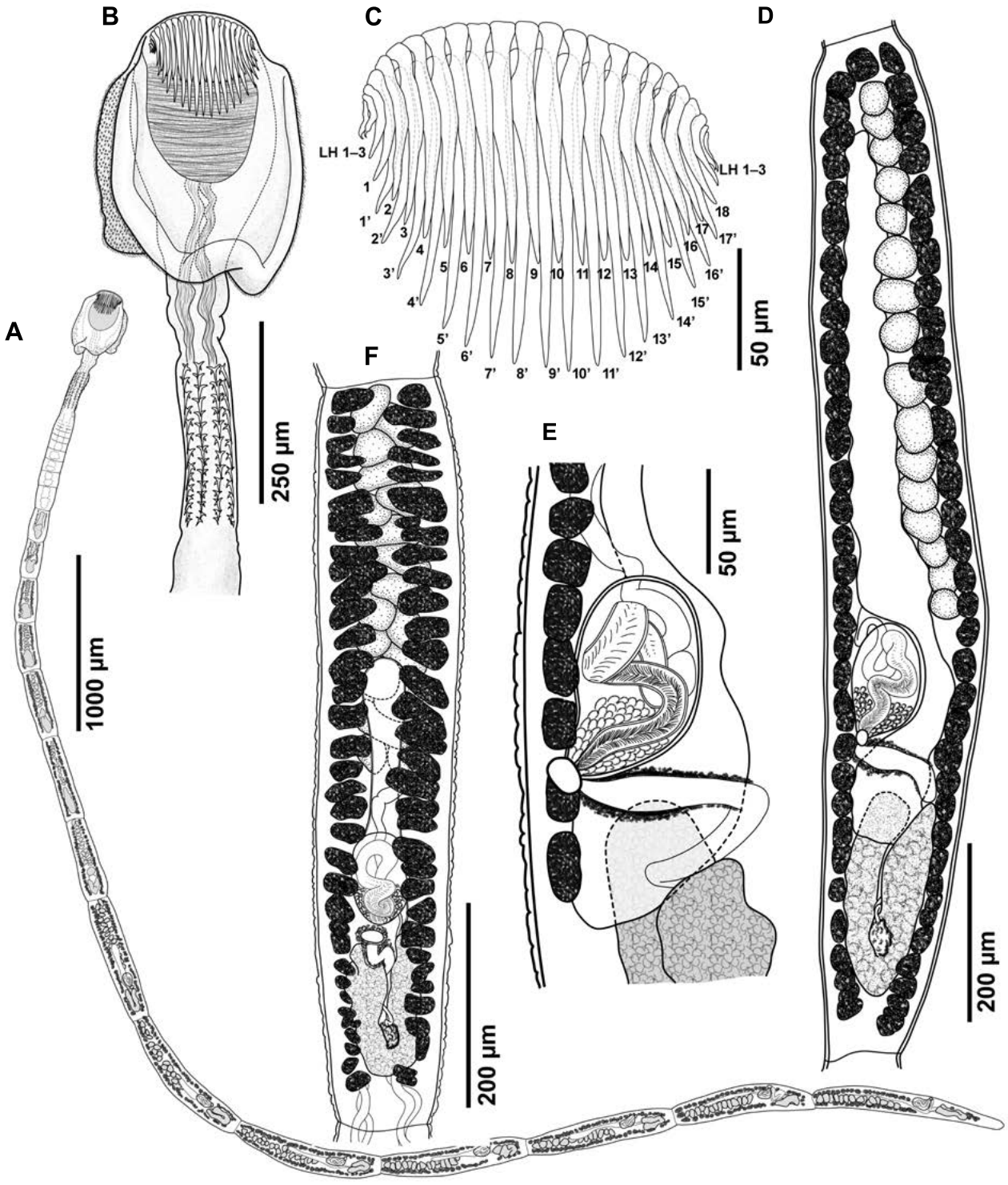

Fig. 7. Line drawings of *Echinobothrium tyleri* sp. n. from *Neotrygon australiae* Last, White et Serét. **A** – entire specimen (holotype); **B** – scolex, dorsoventral view; **C –** apical hooks and lateral hooklets; **D** – mature proglottid; **E** – gravid proglottis (eggs are not drawn), lateral (D) and ventral (F) views; **E** – detail of terminal genitalia, lateral view.

(54 ± 14; 7, 21) wide, arranged in 1 regular column and 1 row in cross section in immature proglottids, but tending to overlap and become positioned in more columns and/or 2 rows in mature proglottids (Fig. 7D,F). Cirrus-sac pyriform, 93–143 (108 ± 18; 7) long by 57–94 (68 ± 13; 7) wide, length: width ratio (1.4–2.1 : 1), located anterior to vagina opening, containing robust coiled cirrus; cirrus covered with short spinitriches (Fig. 7D–F). Internal and external seminal vesicles not observed. Vas deferens directed anteriorly, towards side first and then meandering in sinistral side.

Ovary near posterior margin of proglottid, U-shaped in frontal view, bilobed in cross section, 138–273 (201 ± 42; 7) long by 69–137 (87 ± 33; 4) wide (Fig. 7D,F). Mehlis'

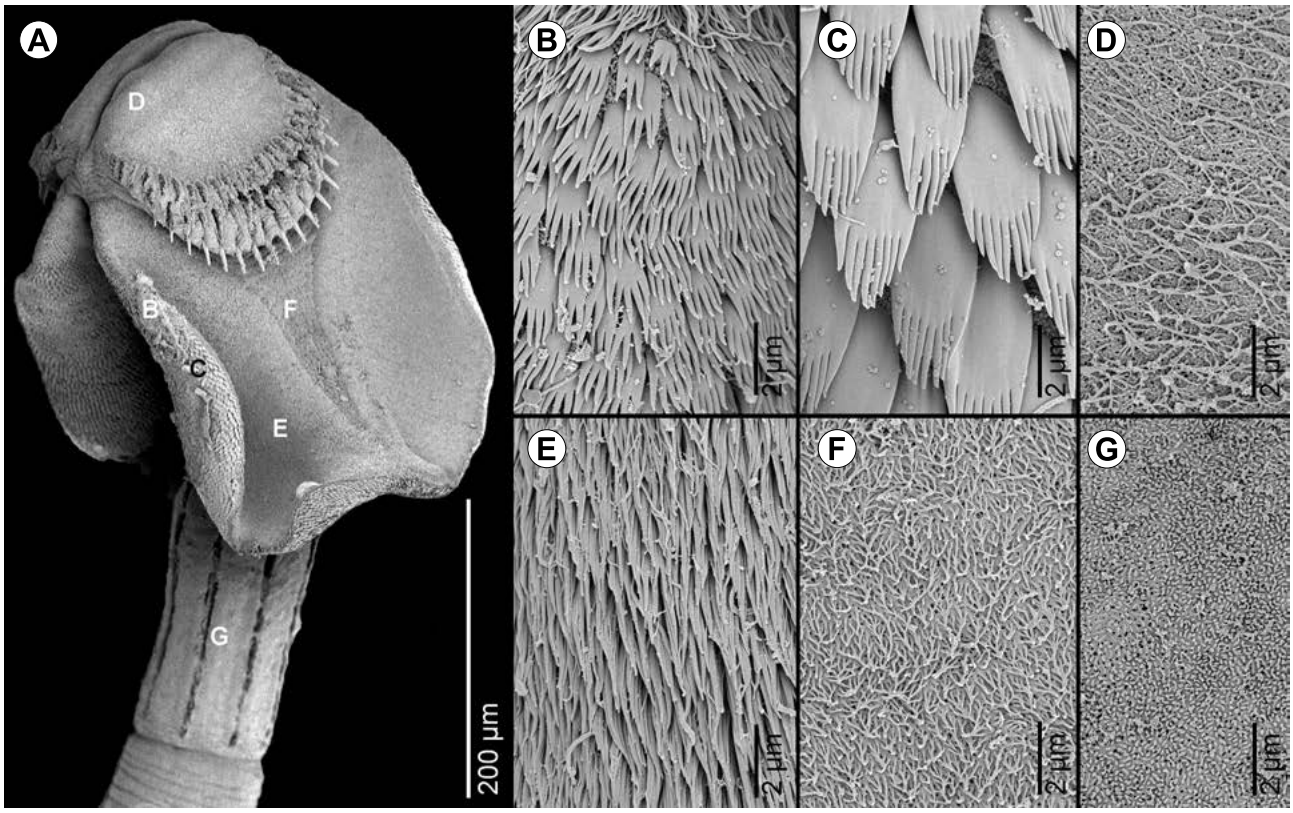

Fig. 8. Scanning electron micrographs of *Echinobothrium tyleri* sp. n. from *Neotrygon australiae* Last, White et Serét. **A** – scolex. Note: small letters correspond to the figures showing higher magnification images of these surfaces; **B** – anterior region of proximal bothrial surface; **C** – central proximal bothrial surface; **D** – surface of apical region of scolex; **E** – posterior region of distal bothrial surface; **F** – distal bothrial surface at margin of central triangular region; **G** – surface of cephalic peduncle.

gland follicular, located between ovarian lobes, just anterior to its junction, 55–59 (57 ± 2; 2, 3) long by 42–63 (52 ± 11; 2, 3) wide. Vagina paralell to cirrus-sac, directed posteriorly between ovarian lobes, widening as approaching genital pore, distal portion 9–21 (15 ± 5; 7) in diameter, surrounded by glandular cells (Fig. 7E). Genital pore midventral; 28–41% from posterior margin of mature proglottid (Fig. 7D,F).

Vitellarium follicular; vitelline follicles 21–40 (28 ± 6; 7, 21) long by 35–100 (59 ± 22; 7, 21) wide, subcortical, in two wide lateral fields, distributed throughout length of proglottid, each band consisting of 1 dorsal and 1 ventral column of follicles, uninterrupted at level of ovary; vitelline fields confluent in posterior extremity of proglottid (Fig. 7A,D,F). Uterus saccate, originating as uterine duct in ootype region, developing early in mature proglottids anterodorsally to cirrus-sac, ventrally to testes until filling all anterior part of proglottid, filling completely gravid proglottids (Fig. 7D). Eggs oval in shape, non filamented, 20–23 (22 ± 1; 2, 4) long by 13–16 (14 ± 1; 2, 4) wide.

Type and only host: *Neotrygon australiae* Last, White et Serét (Myliobatiformes: Dasyatidae).

Type and only locality: Arafura Sea, Australia, Northern Territory, east off Wessel Islands (-11.295556, 136.996667) (NT-63); collected by J.N. Caira on November 13, 1999 and November 15, 1999 (NT-85).

Site of infection: Spiral intestine.

Prevalence and intensity: 2 of 6 examined hosts infected with 2 and 32 worms.

Type material: Holotype is deposited at MAGNT (No. D001947) and paratypes at USNM (Nos. 1606805–1606808), IPCAS (No. C-933), and LRP (Nos. 11035–11042).

Etymology: The name of this new species is given in honour of Gaines Albert Tyler, who described several diphyllideans and wrote an exceptional monograph on diphyllideans (Tyler 2006).

Remarks. *Echinobothrium tyleri* sp. n. differs from *E. bethae* sp. n., *E. giraffaeous* sp. n., *E. ivanovae* sp. n., and *E. rhynchobati* in that the outermost A hooks do not have an extended base. Furthermore, it differs from all other known species of *Echinobothrium* by unique rostellar hook formula {2–3 18/17 2–3}. A characteristic feature of *E. tyleri* sp. n. that is shared by only three species of the genus (*Echinobothrium brachysoma* Pintner, 1889, *E. heroniense* Williams, 1964 and *E. minutamicum* Twohig, Caira et Fyler, 2008) is that the two outermost A hooks on each side of the rostellum are not interlocked with a B hook. However, as stated above, the new species can be easily distinguished from these three species by the hook formula (*E. brachysoma* {3–4 6/3 3–4}, *E. heroniense* {2–3 22/19 2–3}, and *E. minutamicum* {3–4 6/3 3–4}). In addition, the outermost A hooks of *E. minutamicum* are short and trifid rather than elongated and spiniform.

DISCUSSION

Three of the four new species described here have the peculiarity of possessing the outermost A hooks with an

extended base into which the bases of the three outermost B hooks are inserted. To date, *Echinobothrium rhynchobati* from the guitarfish *Glaucostegus granulatus* (Cuvier) was the only species in the genus that exhibited this unique feature. This species was used to establish the genus *Macrobothridium* Khalil et Abdul-Salam, 1998 within the new family Macrobothridiidae, but this genus was then synonymised with *Echinobothrium* by subsequent authors (see Tyler 2006, Caira et al. 2017). In his monograph on the Diphyllidea, Tyler (2006) called attention to the unique hook formula of *E. rhynchobati* and, based on re-examination of paratypes and voucher material, suggested that what had been interpreted as lateral hooklets in the original description was actually a series of type B hooks that were not interlocked with type A hooks.

However, the rostellum of *E. rhynchobati* has an intricate system of knobs and sockets that articulate type A and B hooks with one another (Tyler 2006), which is not the case in any of the three new species listed above. In contrast, in our species, the bases of the three outermost B hooks on each side are simply inserted into the concavities formed by the basal arches of the outermoust A hooks and do not form any articulating system. This essential difference would prevent the four species from being included in a common clade that would include species *Echinobothrium* with enlarged bases of the outermost A hooks.

Furthermore, this incompatibility becomes apparent when considering the hosts from which these different species have been described. *Echinobothrium rhynchobati* was described from *G. granulatus* (Rhinobatidae), while the three new species were found in members of the genus *Neotrygon* (Dasyatidae). According to available molecular data, *E. rhynchobati* and *E. ivanovae* sp. n. form two unrelated clades (see fig. 2 of Caira et al. 2013). Therefore, the possible similarities in rostellum structure between *E. rhynchobati* and *E. giraffaeous* sp. n., *E. ivanovae* sp. n. and *E. bethae* sp. n. are most likely the result of convergent evolution.

The host specificity of diphyllideans has been shown to be essentially strict (Caira et al. 2017). Although occasionally different diphyllideans parasitise the same host species (Kuchta and Caira 2010; Moghadam and Haseli 2014), the conspecifity of host specimens infected by different diphyllideans is questionable (Caira et al. 2017). The present results are in complete agreement with these data that each *Echinobothrium* species parasitises only a single species of *Neotrygon*. Therefore, our results support the hypothesis that most diphyllideans are strict specialists (Naylor et al. 2012, Caira et al. 2013, 2017).

Echinobothrium giraffaeous sp. n. is described from *Neotrigon leylandi*, which appears to be endemic to northwestern Australia since the description of the eastern form, the speckled maskray *Neotrygon picta*, was initially thought to be a colour variant of *N. leylandi* (see Last and White 2008).

Species of *Echinobothrium* are restricted to batoids and, with few exceptions, specifically to members of the families Rhinobatidae, Rajidae and Dasyatidae (Caira et al. 2013, 2017). Maskrays (genus *Neotrygon*) were previously considered a subgenus of *Dasyatis* Rafinesque until were revised by Last and White (2008) based on morphological and molecular evidence. The group comprises around 15 nominal species restricted to the Indo-West Pacific. However three of its members (*Neotrygon kuhlii*, *N. ningalooensis* and *N. annotate* [Last]) each form a complex of distinct cryptic species that have yet to be adequately characterised (Puckridge et al. 2013, Last et al. 2016).

To date, the only existing record of an *Echinobothrium* species from maskrays, *Echinobothrium longicolle*, was reported from *N. kuhlii* in Sri Lanka (Southwell 1925). Although specimens of the presumed type host were studied, the *E. longicolle* has not been collected since its description (Tyler 2006, Caira et al. 2017; present study). However, as Tyler (2006) noted and given the results of the comprehensive phylogenetic analysis by Naylor et al. (2012), specimens originally described as *E. longicolle* likely do not correspond to the same species as those collected later. Further collections are needed to determine the true type host of this species. Moreover, the morphological characterisation of *E. longicolle* deserves further attention, since its most characteristic feature (i.e., peduncular spines with leaflike bases instead of the typical triradiate ones) considered by Tyler (2006) as a possible developmental anomaly, might not have been fully developed in the type material and in many cases were directed anteriorly instead of posteriorly as usual.

With the present descriptions, the Central Indo-Pacific realm (see Spalding et al. 2007) becomes the major hotspot for *Echinobothrium* diversity with 13 species reported (*Echinobothrium chisholmae* Jones et Beveridge, 2001, *E. elegans* Tyler, 2006, *E. heroniense*, *E. minutamicum*, *E. rhynchobati*, *E. sematanense* Ivanov et Caira, 2012, *E. sinensis* (Li et Wang, 2007), *E. tetabuanense* Ivanov et Caira, 2012, and *E. weipaense* Ivanov et Caira, 2012 in addition to the four species described herein). Within this region, the Sahul Shelf Province of northern Australia harbours most of the recorded *Echinobothrium* diversity (i.e., six of the 13 species listed above). The Temperate Northern Atlantic realm, which includes the Mediterranean Sea, is the second most important realm, with 11 *Echinobothrium* species recorded. The present results also indicate members of the genus *Neotrygon* as suitable hosts for *Echinobothrium* species.

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REFERENCES

Abbott L.M., Caira J.N. 2014: Morphology meets molecules: a new genus and two new species of diphyllidean cestodes from the yellowspotted skate, *Leucoraja wallacei*, from South Africa. J. Parasitol. 100: 323–330.

Borsa P., Arlyza I.S., Hoareau T.B., Shen K.-N. 2018: Diagnostic description and geographic distribution of four new cryptic species of the blue-spotted maskray species complex (Myliobatoidei: Dasyatidae; *Neotrygon* spp.) based on DNA sequences. J. Ocean. Limnol. 36: 827–841.

Caira J.N., Ivanov A.V., Jensen K., Marques F.P.L. 2017: Diphyllidea. In: J.N. Caira and K. Jensen (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, Kansas, USA, pp. 149–166.

Caira J.N., Marques F.P.L., Jensen K., Kuchta R., Ivanov V. 2013: Phylogenetic analysis and reconfiguration of genera in the cestode order Diphyllidea. Int. J. Parasitol. 43: 621–639.

Chervy L. 2009. Unified terminology for cestode microtriches: a proposal from the participants of the International Workshops on Cestode Systematics in 2002–2008. Folia Parasitol. 56: 199–230.

Ivanov V.A., Caira J.N. 2012: Description of three new species of *Echinobothrium* (Cestoda: Diphyllidea) from Indo-Pacific elasmobranchs of the genus *Glaucostegus* (Rajiformes: Rhinobatidae). J. Parasitol. 98: 365–377.

Kuchta R., Caira J.N. 2010: Three new species of *Echinobothrium* (Cestoda: Diphyllidea) from Indo-Pacific stingrays of the genus *Pastinachus*. Folia Parasitol. 57: 185–196.

Last P.R., White W.T. 2008: Resurrection of the genus *Neotrygon* Castelnau (Myliobatoidei: Dasyatidae) with the description of *Neotrygon picta* sp. nov., a new species from the Northern Australia. In: Last P.R., White W.T. and Pogonoski J.J. (Eds.), Descriptions of New Australian Chondrichthyans. CSIRO Marine and Atmospheric Research Paper no. 22, pp. 315–325.

Last P.R., White W.T., Puckridge M. 2010: *Neotrygon ningalooensis* n. sp. (Myliobatoidei: Dasyatidae), a new maskray from Australia. Aqua. Int. J. Ichthyol. 16: 37–50.

Last P.R., White W.T., Seret B. 2016: Taxonomic status of maskrays of the *Neotrygon kuhlii* species complex (Myliobatoidei: Dasyatidae) with the description of three new species from the Indo-West Pacific. Zootaxa 4083: 533–561.

Moghadam M.M.M., Haseli M. 2014. *Halysioncum kishiense* sp. n. and *Echinobothrium parsadrayaiense* sp. n. (Cestoda: Diphyllidea) from the Banded eagle ray, *Aetomylaeus* cf. *nichofii* off the Iranian coast of the Persian Gulf. Folia Parasitol. 61: 133–140.

Naylor G.J.P., Caira J.N., Jensen K., Rosana K.A.M., White W.T., Last P.R. 2012: A DNA sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. Bull. Am. Mus. Nat. Hist. No. 367: 1–262.

Neifar L., Tyler G.A., Euzet L. 2001: Two new species of *Macrobothridium* (Cestoda: Diphyllidea) from rhinobatid elasmobranchs in the Gulf of Gabes, Tunisia, with notes on the status of the genus. J. Parasitol. 87: 673–680.

Puckridge M., Last P.R., White W.T., Andreakis N. 2013: Phylogeography of the Indo-West Pacific maskrays (Dasyatidae, *Neotrygon*): a complex example of chondrichthyan radiation in the Cenozoic. Ecol. Evol. 3: 217–232.

Southwell T. 1925: A monograph on the Tetraphyllidea with notes on related cestodes. Mem. Liverpool School Trop. Med. (New Series) 2: 1–368.

Spalding M.D., Fox H.E., Halpern B.S., McManus M.A., Molnar J., Allen G.R., Davidson N., Jorge Z.A., Lombana A.L., Lourie S.A., Martin K.D., McManus E., Molnar J., Recchia C.A., Robertson J. 2007: Marine ecoregions of the World: a bioregionalization of coastal and shelf areas. BioScience 57: 573–583.

Tyler G.A. 2006: A monograph on the Diphyllidea (Platyhelminthes, Cestoda). Tapeworms of Elasmobranchs. Part II. Bull. Univ. Nebraska State Mus. 20: 1–142.

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