

## Three new species of *Echinobothrium* (Cestoda: Diphyllidea) from Indo-Pacific stingrays of the genus *Pastinachus* (Rajiformes: Dasyatidae)

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**Abstract:** Three new species of diphyllidean cestodes are described from stingrays of the genus *Pastinachus* from the Indian and Pacific Oceans. All three new species differ from all but 10 of the 36 valid species of *Echinobothrium* van Beneden, 1849 in that they bear lateral hooklets arranged in a continuous band across each lateral surface of the rostellum joining the groups of dorsal and ventral apical hooks, rather than arranged in distinct dorsal and ventral groups, on each side of the apical hooks. *Echinobothrium nataliae* sp. n. ex *Pastinachus solocirostris* off Borneo differs from other relatives in the following combination of characters: number of spines in each column on the cephalic peduncle, number of lateral hooklets and number of apical hooks. *Echinobothrium reginae* sp. n. ex *Pastinachus* cf. *sephen* off Madagascar differs from all congeners in the following combination of characters: number of hooklets and number of spines on the cephalic peduncle. *Echinobothrium vojtai* sp. n. from an undescribed species of *Pastinachus* off Borneo differs from its congeners in the following combination of characters: number of hooklets, number of apical hooks and number of spines in each column on the cephalic peduncle. The hook formula was modified to accommodate species with lateral hooklets arranged in a continuous band as follows: {(LH) AH(A)/AH(B)}, where (LH) is the total number of lateral hooklets in a band. A table of all nominal species of *Echinobothrium*, showing their taxonomic status, type host and locality, number of peduncle spines in a column and hook formula, is presented. *Macrobothridium djeddensis* and *Macrobothridium sinensis* are transferred to *Echinobothrium*. In addition, *Echinobothrium deeghai* is considered as a *species inquirenda*.

**Keywords:** Borneo, *Echinobothrium nataliae*, *Echinobothrium reginae*, *Echinobothrium vojtai*, hook formula, Madagascar, *Macrobothridium*

Tapeworms belonging to the order Diphyllidea van Beneden in Carus, 1863 comprise a unique and small group of parasites of elasmobranchs. While the phylogenetic position of the order remains somewhat unclear, it is likely most closely related to the order Trypanorhyncha Diesing, 1863 (see Palm et al. 2009, Olson et al. 2010). To date, the order is considered to include 47 nominal species in two genera. The third nominal genus, *Macrobothridium* Khalil et Abdul-Salam, 1989, was synonymised with *Echinobothrium* van Beneden, 1849 by Tyler (2006). Here we follow Tyler (2006) and thus formally transfer both *Macrobothridium djeddensis* Pramanik et Manna, 2005 of Pramanik and Manna (2005) ex *Rhynchobatus djiddensis* (Forsskål) and *Macrobothridium sinensis* Li et Wang, 2007 of Li and Wang (2007) ex *Platyrrhina sinensis* (Bloch and Schneider) to *Echinobothrium*, creating the new combinations *Echinobothrium djeddensis* comb. n. and *Echinobothrium sinensis* comb. n.

While some species are known from sharks (e.g., *Echinobothrium diamanti* Ivanov et Lipshitz, 2006), the majority of *Echinobothrium* species are parasites of batoids, having been reported from a diversity of genera (Ivanov and Lipshitz 2006, Tyler, 2006; Table 1). The three new species described below represent the first verified records of diphyllideans from cowtail stingrays of the genus *Pastinachus* Rüppell and also expand the known distribution of *Echinobothrium* spp. to include the island of Madagascar.

### MATERIALS AND METHODS

In total, 13 stingrays belonging to three species of the genus *Pastinachus* Rüppell were dissected. These included a total of 9 specimens of *Pastinachus solocirostris* Last, Manjaji et Yearsley, from the island of Borneo, 7 of which were collected between May 2002 and April 2004 from the South China Sea in Malaysian Borneo as follows: 1 specimen (BO-11)

off Santubong (01°43'00.16"N, 110°19'13.55"E), 3 specimens (BO-164, BO-165, BO-177) off Semantan (01°48'15"N, 109°46'47"E), and 3 specimens (BO-256, BO-267, BO-464) off Mukah (02°53'52"N, 112°05'44"E). The remaining 2 specimens from Borneo were collected from Indonesian localities (i.e., off Kalimantan) between November 2006 and July 2007 as follows: 1 specimen (KA-44) from Muara Pasir (01°45'58"S, 116°23'36"E) and 1 specimen (KA-148) from Singkawang (00°55'06"N, 108°58'58"E). Two specimens (GA-16, GA-31), identified as *Pastinachus* cf. *sephen* (see Jensen and Caira 2008) were collected in September 1999 off La Mer d'Emeraude near Ramena (12°11'60"S, 49°22'00"E) and out of Nosy Be (13°25'S, 48°6'E), Madagascar. Finally, two specimens from an undescribed species of *Pastinachus*, which will be referred to here as *Pastinachus* sp. (thin-tail), were examined. These specimens were collected in May 2003 from the South China Sea, in Malaysian Borneo off Kampung Tetabuan (06°01'10"N, 117°42'15"E) (BO-99) and in July 2007 off Selakau (01°03'31"N, 108°58'25"E) in Kalimantan (KA-162). With the exception of the hosts from Madagascar, all hosts were caught using small bottom trawls or small-mesh gill nets in conjunction with local fishermen. The specimens from Madagascar were collected using a hand spear. In each case, the spiral intestine was removed, opened with a longitudinal midventral incision extending to the centre of the lumen of the spiral intestine to expose the upper and lower surfaces of each chamber, and fixed in 10% formalin in the field. Spiral intestines were transported to the lab in individual whirl-pak bags and were subsequently transferred to 70% ethanol for storage.

Tapeworms were prepared as whole mounts for light microscopy as follows. They were hydrated in a graded ethanol series, stained in Delafield's haematoxylin, differentiated in tap water, destained in acidic 70% ethanol, neutralized in basic 70% ethanol, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted on glass slides in Canada balsam. Compressed mounts of rostellar hooks and peduncle spines were prepared as follows. Specimens were hydrated in a graded ethanol series, compressed and transferred to Berlese's medium and subsequently mounted in Canada balsam on glass slides. Semi-permanent mounts of eggs were prepared using the lacto-phenol method described by Jensen (2005). Measurements were acquired using an optical reticle or with a SPOT Diagnostic Instrument digital camera system mounted on a Zeiss Axioskop 2 and SPOT software (version 4.5). Hook terminology, numbering scheme and measurements follow Tyler (2006). The hook formula presented is modified from that of Neifar et al. (2001) to more readily accommodate species with continuous bands of lateral hooklets. The modified formula is as follows: {(LH) AH(A)/AH(B)}, where (LH) is the total number of lateral hooklets in a band on one side. A more detailed description of the scheme is presented in the Discussion below. Measurements are given in the text as ranges followed in parentheses by the mean, standard deviation, and number of measurements made. All measurements are in micrometres unless otherwise noted. Microthrix terminology follows Chervy (2009).

Samples were prepared for scanning electron microscopy (SEM) as follows. They were hydrated in a graded ethanol series, transferred to 1% osmium tetroxide overnight, dehydrated in a graded ethanol series, transferred to hexamethyldisilazane (HMDS) (Ted Pella, Inc., Redding, California), and allowed to

air dry. They were subsequently mounted on aluminium stubs using double-sided carbon tape, sputter coated with 30-nm gold/palladium, and examined with a LEO/Zeiss DSM982 Gemini field emission scanning electron microscope.

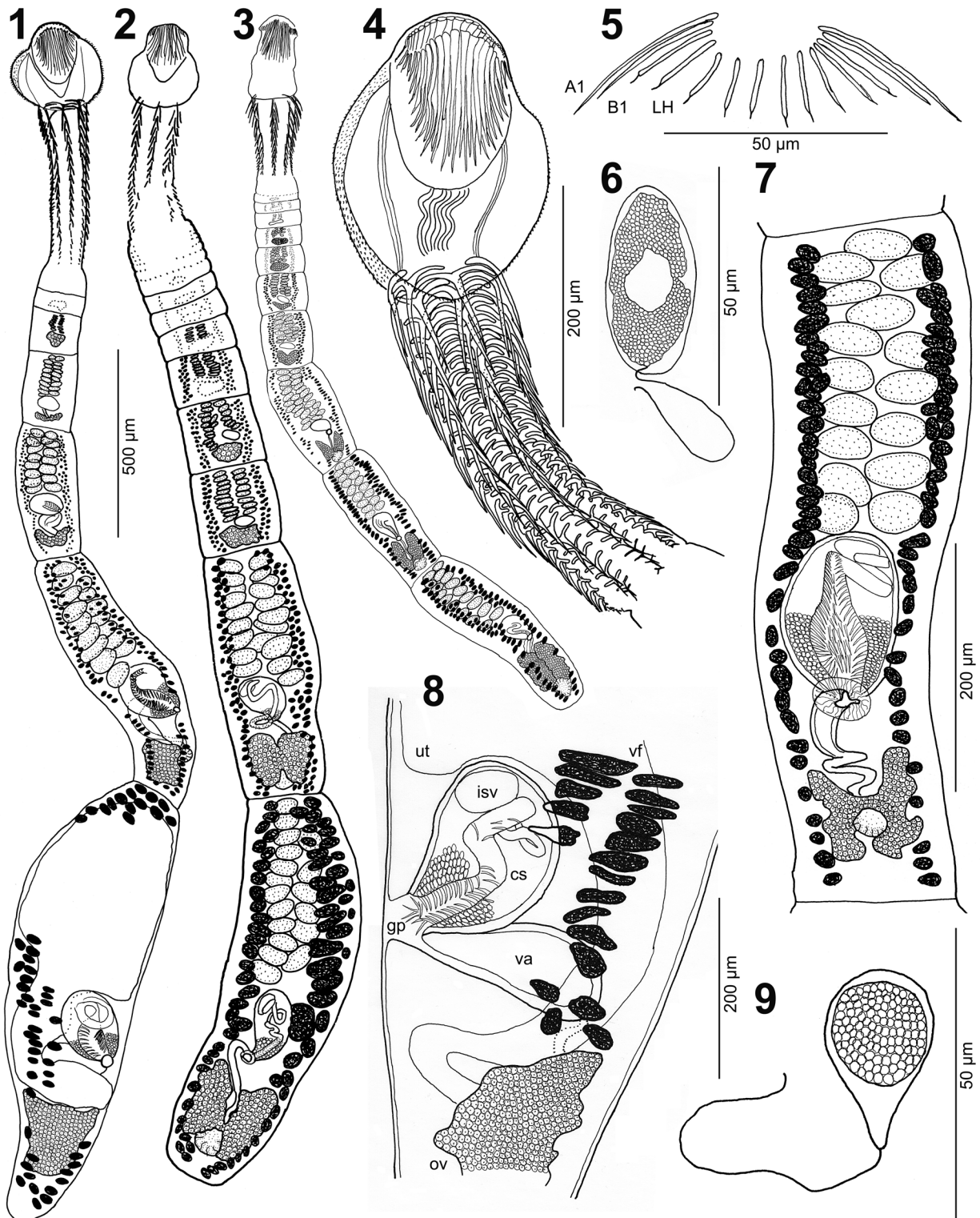
Museum abbreviations used are as follows: IPCAS, Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; LRP, Lawrence R. Penner Parasitology Collection, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut; MNHN, Muséum National d'Histoire Naturelle, Paris, France; MZB, Museum Zoologicum Bogoriense, Zoological Division, Research Center for Biology, Indonesian Institute of Science, Cibinong, Indonesia; MZUM(P) Muzium Zoologi, Universiti Malaya, Kuala Lumpur, Malaysia; SBC, Sarawak Biodiversity Center, Kuching, Sarawak, Malaysia; and USNPC, U.S. National Parasite Collection, Beltsville, Maryland.

For comparative purposes, the following material of species of *Echinobothrium* was examined: 1 paratype of *E. bonasum* Williams et Campbell, 1980 (LRP No. 7442); 3 whole mount vouchers (LRP Nos. 7443–7445) and 1 apical hook preparation of voucher of *E. fautleyae* Tyler et Caira, 1999 (LRP No. 7446); 4 whole mounts of vouchers (LRP Nos. 7447–7448) of *E. mexicanum* Tyler et Caira, 1999; 12 paratypes of *E. hoffmanorum* Tyler, 2001 (LRP Nos. 2038–2050); and 22 paratypes of *E. ray-allemangi* Tyler, 2001 (LRP Nos. 2000–2021).

## RESULTS

### *Echinobothrium nataliae* sp. n. Figs. 1, 4–8, 19–26

**Description** (based on whole mounts of 12 mature worms, 2 compressed scoleces, semi-permanent mount of 1 gravid proglottid in lactophenol and 5 specimens examined with SEM): Worms euapolytic, 1,714–3,098 (2,427 ± 426; 10) long; greatest width at level of terminal proglottid, 150–374 (246 ± 62; 10) wide; proglottids acraspedote, 6–7 in number (Fig. 1). Scolex consisting of scolex proper and cephalic peduncle (Figs. 1, 4, 19). Scolex proper 205–251 (227 ± 12; 10) long by 160–206 (185 ± 15; 10) wide, composed of armed apical rostellum and 1 dorsal and 1 ventral bothrium; bothria 146–187 (166 ± 12; 10) long by 159–206 (183 ± 16; 10) wide. Rostellum bearing 1 dorsal and 1 ventral group of 27–29 (28.5 ± 1; 7) solid apical hooks arranged in 2 rows flanked on each side by continuous band of 10–14 (13.5 ± 2; 7) small lateral hooklets; hooklets 18–34 (22 ± 3; 34) long, with scalpel-like blade (Figs. 5, 26). Hook formula {(10–14) 14/13–15}. Apical hooks gradually increasing in length towards centre of group, type B symmetry. First B hook after hooklets 30–36 (35 ± 7; 4) long (18–30% longer than adjacent hooklet), remaining B hooks 49–110 long (88 ± 19; 32); first A hook after first B hook 34–52 (42 ± 8; 5) long (4–5% longer than first B hook), remaining A hooks 50–118 (89 ± 19; 30) long. Cephalic peduncle 280–469 (386 ± 61; 10) long by 66–108 (95 ± 17; 10) wide, armed with 8 longitudinal columns of 24–29 (28 ± 2; 24) spines; spines with triradiate bases, decreas-



**Figs. 1–9.** Line drawings. **Figs. 1, 4–8.** *Echinobothrium nataliae* sp. n. **Fig. 1.** Whole worm. **Fig. 4.** Scolex. **Fig. 5.** Lateral hooklets. **Fig. 6.** Egg. **Fig. 7.** Mature proglottid. **Fig. 8.** Detail of terminal genitalia, lateral view. **Fig. 2.** *Echinobothrium reginae* sp. n., whole worm. **Figs. 3, 9.** *Echinobothrium vojtaei* sp. n. **Fig. 3.** Whole worm. **Fig. 9.** Egg. **Abbreviations:** A1 – first A (anterior) hook; B1 – first B (posterior) hook; cs – cirrus-sac; gp – genital pore; isv – internal seminal vesicle; LH – lateral hooklets; ov – ovary; ut – uterus; va – vagina; vf – vitelline follicles.



ing in length posteriorly; free prong of first 3 anterior spines 79–89 ( $84 \pm 3$ ; 6) long; free prong of last 3 posterior spines 10–25 ( $18 \pm 5$ ; 6) long (Fig. 4).

Distal bothrial surfaces with conspicuous central triangular region; triangular region covered with capilliform and acicular filitriches (Fig. 24); border between central triangular region and remainder of distal surfaces with alternating rows of large cilia  $\sim 2$  long (Figs. 23, 24); remainder of distal surfaces covered with trifurcate spinitriches with slender digits (Figs. 23, 24). Proximal bothrial surfaces covered anteriorly with small trifid spinitriches  $\sim 1$  long interspersed with acicular filitriches (Fig. 21) and posteriorly with robust pectinate spinitriches  $\sim 4$  long with 7–8 digits interspersed with acicular filitriches (Fig. 22). Apex of scolex proper apparently devoid of microtriches. Cephalic peduncle and proglottids covered with capilliform filitriches (Fig. 25).

Immature proglottids 4–5 in number, initially wider than long, becoming longer than wide with maturity (Fig. 1). Mature proglottids 1–2 in number, 415–748 ( $556 \pm 105$ ; 9) long by 138–288 ( $188 \pm 8$ ; 9) wide, length:width ratio 1.5–3.5:1. Gravid proglottid 1 in number, 764–1,194 ( $1,028 \pm 154$ ; 9) long by 150–373 ( $245 \pm 66$ ; 9) wide, length:width ratio 3.0–6.3:1. Testes 16–20 ( $18 \pm 1$ ; 10) in number, 25–46 ( $31 \pm 6$ ; 10) long by 45–75 ( $63 \pm 9$ ; 10) wide, arranged in 2 regular columns from anterior margin of proglottid to anterior margin of cirrus-sac, 1 row deep in cross-section (Fig. 7). Cirrus-sac ovoid, 118–213 ( $168 \pm 34$ ; 10) long by 97–242 ( $136 \pm 41$ ; 10) wide, length:width ratio 1.0–1.5:1, located anterior to vagina, containing robust cirrus; cirrus covered with large coniform spinitriches; spinitriches 16–23 ( $20 \pm 3$ ; 7) long (Figs. 7, 8). Internal seminal vesicle 53–71 ( $66 \pm 7$ ; 7) long by 44–65 ( $52 \pm 8$ ; 7) wide; external seminal vesicle not seen. Vas deferens minimal. Ovary near posterior margin of proglottid, H-shaped in frontal view (Fig. 7), 86–364 ( $164 \pm 87$ ; 10) long by 71–163 ( $99 \pm 29$ ; 10) at widest point, (Fig. 7). Mehlis' gland posterior and dorsal to ovarian isthmus, 28–65 ( $47 \pm 14$ ; 10) long by 24–53 ( $31 \pm 11$ ; 10) wide, (Fig. 7). Vagina short, posterior to cirrus-sac, with robust muscular sack-like distal portion opening into genital pore, 45–62 ( $49 \pm 6$ ; 10) long by 51–108 ( $81 \pm 17$ ; 10) wide (Fig. 8). Genital pore midventral, 29–45% (34%) from posterior margin of mature proglottid; 33–46% (36%) from posterior margin of gravid proglottid. Vitellarium follicular; vitelline follicles 15–29 ( $19 \pm 4$ ; 10) long by 33–69 ( $50 \pm 15$ ; 10) wide, in 2 lateral fields, distributed throughout length of proglottid, uninterrupted at level of ovary; vitelline fields not confluent in anterior and posterior extremities of proglottid (Fig. 7). Uterus saccate, originating as uterine duct in ootype region, extending anterodorsal to cirrus-sac, ventral to testes (Figs. 1, 8). Eggs oval, 34–39 ( $36 \pm 1$ ; 10) long by 16–17 ( $17 \pm 0.5$ ; 10) wide, with single terminal polar filament; terminal filament 45–50 long (Fig. 6); unembryonated.

Type and only host: *Pastinachus solocirostris* Last, Manjaji et Yearsley, 2005 (Rajiformes: Dasyatidae).

Type locality: South China Sea, Malaysian Borneo, Sarawak, off Semantan ( $01^{\circ}48'15.45''\text{N}$ ,  $109^{\circ}46'47.17''\text{E}$ ) (BO-164); May 14, 2003.

Additional locality: South China Sea, Malaysian Borneo, Sarawak, off Mukah ( $02^{\circ}53'52''\text{N}$ ,  $112^{\circ}05'44''\text{E}$ ).

Site of infection: Spiral intestine.

Prevalence and intensity: 6 of 9 (67%) examined, infected with 4 to 26 individuals per host; total of 128 diphyllideans.

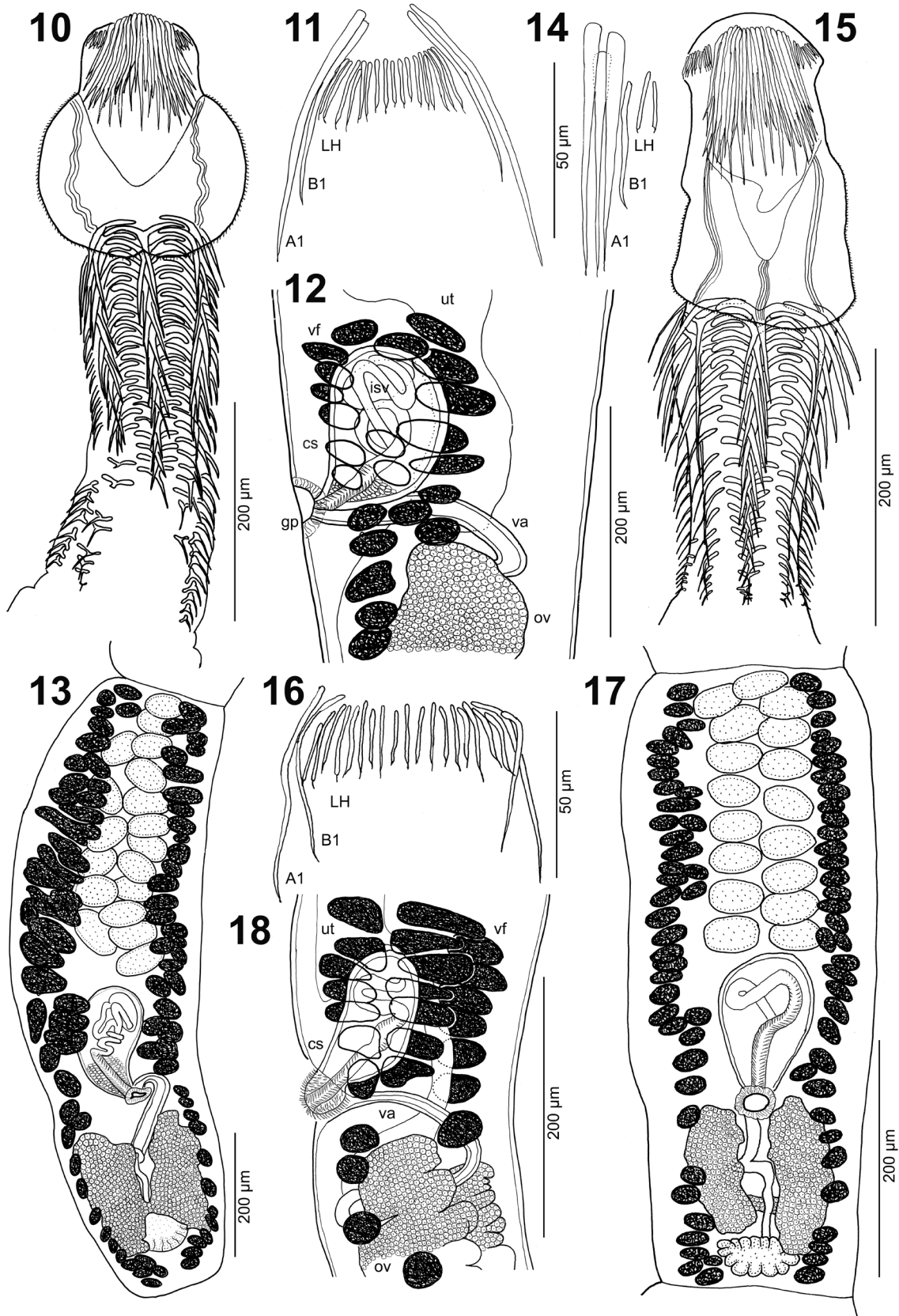
Specimens deposited: Holotype (MZUM(P) No. 1504) and 2 paratype (MZUM(P) Nos. 1505–1506); 1 paratype (SBC No. P-00030); 7 paratypes (LRP Nos. 7451–7457); 5 paratypes prepared for SEM retained in junior author's collection; 3 paratypes (USNPC Nos. 103001–103002); 4 paratypes (IPCAS No. C-558/1).

Etymology: This species is named in honour of the senior author's niece, Natálie (= Natalia; *nataliae*) Kuchtová.

**Remarks.** *Echinobothrium nataliae* is readily distinguished from all but 10 of the 36 valid species of the genus (Table 1) in its possession of lateral hooklets that are arranged in a continuous band rather than in dorsal and ventral groups, on each side of the scolex. With respect to the other species with lateral hooklets arranged in continuous bands, *E. nataliae* exhibits a greater number of spines in each cephalic peduncle column than *E. californiense*, *E. hoffmanorum*, *E. pigmentatum*, and *E. rayallemangi* (24–29 vs. 5–7, 14–16, 8–13, 2–5, per column, respectively) and fewer spines per column than *E. euzeti*, *E. megacanthum*, (24–29 vs. 100–107, 38–43, respectively). *Echinobothrium nataliae* possesses fewer lateral hooklets in a band than *E. bonasum*, *E. fautleyae*, and *E. raschii* (10–14 vs. 24–27, 20–21, 24–36, respectively). Finally, it differs from *E. mexicanum* in possessing a greater number of anterior and posterior apical hooks (14/13–15 vs. 12/11). Furthermore, *E. nataliae*, is one of only 5 species of *Echinobothrium* having remarkably large cirrus spinitriches. In this respect it resembles *E. bonasum*, *E. mathiasi*, *E. megacanthum* and *E. mexicanum*.

### *Echinobothrium reginae* sp. n. Figs. 2, 10–13, 27–33

**Description** (based on whole mounts of 9 mature worms, 2 compressed scoleces and 2 specimens examined with SEM): Worms euapolytic, 2,716–4,575 ( $3,245 \pm 604$ ; 9) long, greatest width at level of terminal proglottid, 217–337 ( $270 \pm 41$ ; 9) wide; proglottids acraspedote, 12–15 in number (Fig. 2). Scolex consisting of scolex proper and cephalic peduncle (Figs. 10, 27). Scolex proper 176–229 ( $203 \pm 18$ ; 9) long by 136–189 ( $153 \pm 18$ ; 9) wide, composed of armed apical rostellum and 1 dorsal and 1 ventral bothrium; bothria 102–147 ( $124 \pm 16$ ; 9) long by 130–189 ( $160 \pm 29$ ; 2) wide. Rostellum bearing 1 dorsal and 1 ventral group of 29 solid apical hooks arranged in 2 rows flanked on each side by single continuous band of



**Figs. 10–18.** Line drawings. **Figs. 10–13.** *Echinobothrium reginae* sp. n. **Fig. 14.** *Echinobothrium fautleyae*, detail of lateral-most hooks and hooklets. **Figs. 15–18.** *Echinobothrium vojtaei* sp. n. **Figs. 10, 15.** Scolex. **Figs. 11, 16.** Hooklets. **Figs. 12, 18.** Detail of terminal genitalia, lateral view. **Figs. 13, 17.** Mature proglottid. **Abbreviations:** A1 – first A hook; B1 – first B hook; cs – cirrus-sac; gp – genital pore; isv – internal seminal vesicle; LH – lateral hooklets; ov – ovary; ut – uterus; va – vagina; vf – vitelline follicles.



16–17 ( $16.5 \pm 1$ ; 5) small lateral hooklets; hooklets 22–30 ( $25 \pm 2$ ; 32) long, with scalpel-like blade (Figs 10, 11, 27, 28). Hook formula {(16–17) 14/15}. Apical hooks gradually increasing in length towards centre of group, type B symmetry. First B hook after hooklets 48–51 ( $50 \pm 2$ ; 2) long (41–44% longer than adjacent hooklet), remaining B hooks 75–104 ( $93 \pm 9$ ; 11) long; first A hook after first B hook 83–91 ( $86 \pm 4$ ; 2) long (42–43% longer than first B hook), remaining A hooks 97–105 ( $100 \pm 5$ ; 10) long. Cephalic peduncle 300–449 ( $367 \pm 40$ ; 9) long by 85–125 ( $101 \pm 14$ ; 9) wide, armed with 8 longitudinal columns of 30–33 ( $32.5 \pm 3$ ; 10) spines; spines with triradiate bases, decreasing in length posteriorly; free prong of first 3 anterior spines 74–88 ( $82 \pm 5$ ; 7) long; free prong of last 3 posterior spines 6–27 ( $18 \pm 9$ ; 7) long (Figs. 10, 27).

Distal bothrial surfaces with conspicuous central triangular region; triangular region covered with capilliform filitriches and some acicular filitriches (Fig. 31), remainder of distal surfaces covered with trifurcate spinitriches with slender digits (Fig. 31). Proximal bothrial surfaces covered with small trifid spinitriches ~1.5 long, interspersed with capilliform filitriches (Figs. 30, 33). Apex of scolex proper surface apparently devoid of microtriches, covered with irregular grooves (Fig. 29). Cephalic peduncle and proglottids covered with capilliform filitriches (Fig. 32).

Immature proglottids 9–13 in number, initially wider than long, becoming longer than wide with maturity (Fig. 2). Mature proglottids 1–2 in number, 398–801 ( $595 \pm 129$ ; 9) long by 184–308 ( $225 \pm 41$ ; 9) wide; length:width ratio 2.0–3.4:1. Gravid proglottid 1 in number, 678–1,166 ( $908 \pm 168$ ; 9) long by 220–337 ( $269 \pm 41$ ; 9) wide, length:width ratio 3.1–3.9:1. Testes 17–19 ( $18 \pm 1$ ; 9) in number, 31–50 ( $34 \pm 7$ ; 10) long by 54–80 ( $68 \pm 10$ ; 10) wide, arranged in 2 irregular columns from anterior margin of proglottid to anterior margin of cirrus-sac, 1 row deep in cross-section (Figs. 2, 13). Cirrus-sac pyriform, 96–177 ( $140 \pm 3$ ; 9) long by 54–110 ( $84 \pm 15$ ; 9) wide, length:width ratio 1.6–2.1:1, located anterior to vagina; cirrus covered with small slender spinitriches; cirrus spinitriches 4–7 ( $6 \pm 1$ ; 10) long (Figs. 12, 13). Internal seminal vesicle present, 56–60 long by 35–40 wide; external seminal vesicle not seen. Vas deferens minimal. Ovary near posterior margin of proglottid, H-shaped in frontal view (Fig. 13), 100–195 ( $139 \pm 37$ ; 9) long by 80–175 ( $125 \pm 32$ ; 9) wide at widest point, extending to genital pore. Mehlis' gland posterior-dorsal to ovarian isthmus, 42–99 ( $68 \pm 19$ ; 9) long by 47–93 ( $74 \pm 13$ ; 9) wide (Fig. 13). Vagina short, muscular, distal portion not expanded, 19–41 ( $26 \pm 7$ ; 9) in diameter (Figs. 12, 13). Genital pore midventral, 28–38% (32%) from posterior margin of mature proglottid, 31–45% (36%) from posterior margin of gravid proglottid. Vitellarium follicular; vitelline follicles 14–28 ( $21 \pm 4$ ; 10) long by 33–60 ( $45 \pm 8$ ; 10) wide, in 2 lateral fields, distributed throughout length of proglottid, uninterrupted at level of ovary; vitelline follicles confluent or not at anterior and posterior extremities of proglottid (Fig. 13). Uterus saccate, originating as

uterine duct in ootype region, extending anterodorsal to cirrus-sac, continuing as expanded sac ventral to testes. Eggs not observed.

Type and only host: *Pastinachus* cf. *sephen* (Forsskal, 1775) (Rajiformes: Dasyatidae) (GA-16).

Type locality: La Mer d'Emeraude near Ramena, Madagascar, Indian Ocean ( $12^{\circ}11'60''\text{S}$ ,  $49^{\circ}22'00''\text{E}$ ); September 20, 1999.

Site of infection: Spiral intestine.

Prevalence and intensity: 1 of 2 examined, infected with 14 individuals.

Specimens deposited: Holotype (MNHN(P) No. HEL 154); 4 paratypes (LRP Nos. 7460–7463); 2 specimens prepared for SEM retained in junior author's collection; 3 paratypes (USNPC Nos. 103003–103005); 4 paratypes (IPCAS No. C-557/1).

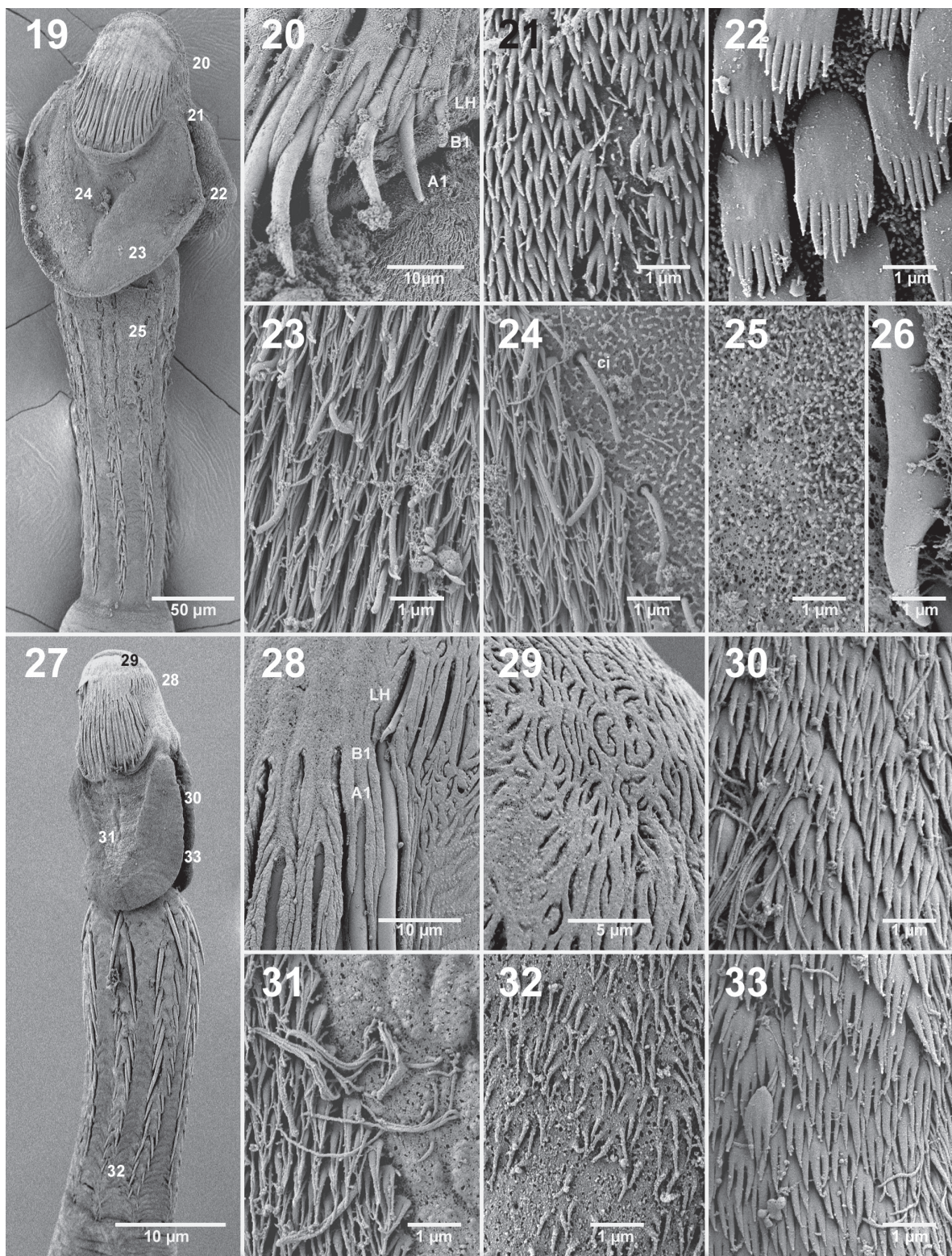
Etymology: This species is named in honour of the senior author's mother, Regina Kuchtová.

**Remarks.** *Echinobothrium reginae* differs from all but 11 of the 37 valid species of the genus, including *E. nataliae* (Table 1), in its possession of lateral hooklets arranged in a continuous band, rather than in distinct dorsal and ventral groups. With respect to the other species with continuous bands of lateral hooklets, it differs from all but *E. hoffmanorum* in number of lateral hooklets; it possesses a greater number of lateral hooklets than *E. euzeti*, *E. megacanthum*, *E. mexicanum*, and *E. nataliae* (16–17 vs. 13–14, 12, 10–13, 10–14, respectively) and fewer lateral hooklets than *E. bonasum*, *E. californiense*, *E. fautleyae*, *E. pigmentatum*, *E. raschii*, and *E. rayallemangi*, (16–17 vs. 24–27, 19–24, 20–21, 20–22, 24–36, 20–24, respectively). *Echinobothrium reginae* conspicuously differs from *E. hoffmanorum* in number of cephalic peduncle spines in each column; whereas *E. reginae* bears 30–33 spines per column, *E. hoffmanorum* has only 14–16.

*Echinobothrium vojtai* sp. n. Figs. 3, 9, 15–18, 34–40

**Description** (based on whole mounts of 12 mature worms, 5 compressed scoleces, semi-permanent mount of 1 gravid proglottid in lactophenol and 2 specimens examined with SEM): Worms euapolytic, 1,872–3,059 ( $2,607 \pm 322$ ; 10) long, greatest width at level of terminal proglottid, 217–258 ( $240 \pm 24$ ; 10) wide; proglottids acraspedote, 10–12 in number (Fig. 3). Scolex consisting of scolex proper and cephalic peduncle (Figs. 15, 34). Scolex proper 178–258 ( $222 \pm 23$ ; 10) long by 117–160 ( $141 \pm 19$ ; 10) wide, composed of armed apical rostellum and 1 dorsal and 1 ventral bothrium; bothria 110–171 ( $139 \pm 20$ ; 10) long by 115–137 ( $124 \pm 9$ ; 3) wide. Rostellum bearing 1 dorsal and 1 ventral group of 29 solid apical hooks arranged in 2 rows flanked on each side by continuous band of 18–20 ( $19 \pm 1$ ; 10) small lateral hooklets; hooklets 22–32 ( $26.5 \pm 2$ ; 36) long, with scalpel-like blade (Figs. 16, 35). Hook formula {(18–20) 14/15}. Apical hooks gradually increasing in length towards centre of group, type B symmetry. First B hook after hooklets





**Figs. 19–33.** Scanning electron micrographs. **Figs. 19–26.** *Echinobothrium nataliae* sp. n. **Fig. 19.** Scolex. Note: small numbers correspond to the figures showing higher magnification images of these surfaces. **Fig. 20.** Detail of first apical hooks and lateral hooklets. **Fig. 21.** Anterior region of proximal bothrial surface. **Fig. 22.** Posterior region of proximal bothrial surface. **Fig. 23.** Posterior region of distal bothrial surface. **Fig. 24.** Distal bothrial surface at margin of central triangular region. **Fig. 25.** Surface of cephalic peduncle. **Fig. 26.** Detail of hooklet. **Figs. 27–33.** *Echinobothrium reginae* sp. n. **Fig. 27.** Scolex. Note: small numbers correspond to the figures showing higher magnification images of these surfaces. **Fig. 28.** Detail of first apical hooks and lateral hooklets. **Fig. 29.** Surface of apical region of scolex. **Fig. 30.** Anterior region of proximal bothrial surface. **Fig. 31.** Distal bothrial surface at margin of central triangular region. **Fig. 32.** Surface of cephalic peduncle. **Fig. 33.** Posterior region of proximal bothrial surface. **Abbreviations:** A1 – first A (anterior) hook; B1 – first B (posterior) hook; ci – cilium; LH – lateral hooklet.



48–51 ( $50.5 \pm 2$ ; 3) long (37–44% longer than adjacent hooklet), remaining B hooks 72–104 ( $94 \pm 8$ ; 33) long; first A hook after first B hook 72–91 ( $82 \pm 8$ ; 3) long (30–50% longer than adjacent B hook), remaining A hooks 82–105 ( $95 \pm 8$ ; 31) long. Cephalic peduncle short, 181–243 ( $208 \pm 18$ ; 10) long by 68–110 ( $96 \pm 13$ ; 10) wide, armed with 8 longitudinal columns of 20–26 spines each; spines with triradiate bases, decreasing in length posteriorly; free prongs of first 3 anterior spines 62–75 ( $70 \pm 3$ ; 10) long; free prongs of last 3 posterior spines 7–12 ( $11 \pm 2$ ; 10) long (Figs. 15, 34).

Distal bothrial surfaces with conspicuous central triangular region; triangular region covered with capilliform filitriches (Fig. 37), remainder of distal surfaces covered with trifurcate spinitriches with slender digits (Fig. 38). Proximal bothrial surfaces covered with small trifid spinitriches  $\sim 1.5$  long (Fig. 36), interspersed with capilliform filitriches. Apex of scolex proper surface apparently devoid of microtriches, covered with grooves. Cephalic peduncle (Fig. 39) and proglottids (Fig. 40) covered with capilliform filitriches,

Immature proglottids 7–9 in number, initially wider than long, becoming longer than wide with maturity (Fig. 3). Mature proglottids 1–2 in number, 429–598 ( $517 \pm 56$ ; 10) long by 177–282 ( $212 \pm 28$ ; 10) wide; length:width ratio 2.1–2.9:1. Gravid proglottid 1 in number, 660–736 ( $709 \pm 27$ ; 5) long by 217–278 ( $240 \pm 22$ ; 5) wide; length:width ratio 2.6–3.3:1. Testes 16–18 ( $17 \pm 1$ ; 7) in number, 40–69 ( $54 \pm 8$ ; 10) long by 22–47 ( $32 \pm 8$ ; 10) wide, arranged in 2 regular columns from anterior margin of proglottid to anterior margin of cirrus-sac, 1 row deep in cross-section (Fig. 17). Cirrus-sac pyriform, 85–122 ( $110 \pm 12$ ; 9) long by 61–84 ( $71 \pm 7$ ; 9) wide, length:width ratio 1.3–2.0:1, located anterior to lobes of ovary; cirrus covered with small slender spinitriches; cirrus spinitriches 5–7 ( $6 \pm 1$ ; 7) long (Figs. 17, 18). Internal and external seminal vesicles not seen. Vas deferens minimal. Ovary near posterior margin of proglottid, H-shaped in frontal view (Fig. 17), 101–203 ( $147 \pm 37$ ; 10) long by 101–158 ( $121 \pm 21$ ; 10) wide at widest point, extending to genital pore. Mehlis' gland posterior-dorsal to ovarian isthmus, 52–66 ( $60 \pm 6$ ; 5) long by 36–77 ( $52 \pm 17$ ; 5) wide (Fig. 17). Vagina short, posterior to cirrus-sac, muscular, 8–24 ( $14 \pm 4$ ; 9) in diameter (Figs. 17, 18). Genital pore midventral, 30–58% (41%) from posterior margin of mature proglottid, 29–40% (35%) from posterior margin of gravid proglottid. Vitellarium follicular; vitelline follicles 13–22 ( $17 \pm 3$ ; 10) long by 21–53 ( $41 \pm 11$ ; 10) wide, in 2 lateral fields, distributed throughout length of proglottid, uninterrupted at level of ovary; vitelline fields not confluent in anterior or posterior extremities of proglottid (Fig. 17). Uterus saccate, originating as uterine duct in ootype region, extending anterodorsal to cirrus-sac, continuing as expanded sac ventral to testes. Eggs (Fig. 9) pyriform, 32–37 ( $34 \pm 2$ ; 10) long by 16–22 ( $20 \pm 2$ ; 10)

wide, with single terminal polar filament on one pole, 45–57 long (Fig. 9); unembryonated.

Type and only host: *Pastinachus* sp. (thin-tail) (Rajiformes: Dasyatidae) (KA-162).

Type locality: Java Sea, Kalimantan, Selakau ( $01^{\circ}03'30.60''\text{N}$ ,  $108^{\circ}58'24.60''\text{E}$ ); November 7, 2007.

Site of infection: Spiral intestine.

Prevalence and intensity: 1 of 2 examined, infected with 20 diphyllideans.

Specimens deposited: Holotype (MZB No. Ca 135) and 1 paratype (MZB No. Ca 136); 4 paratypes (LRP Nos. 7464–7467); 5 paratypes prepared for SEM retained in junior author's collection; 3 paratypes (USNPC Nos. 103006–103008); 4 paratypes (IPCAS No. C-556/1).

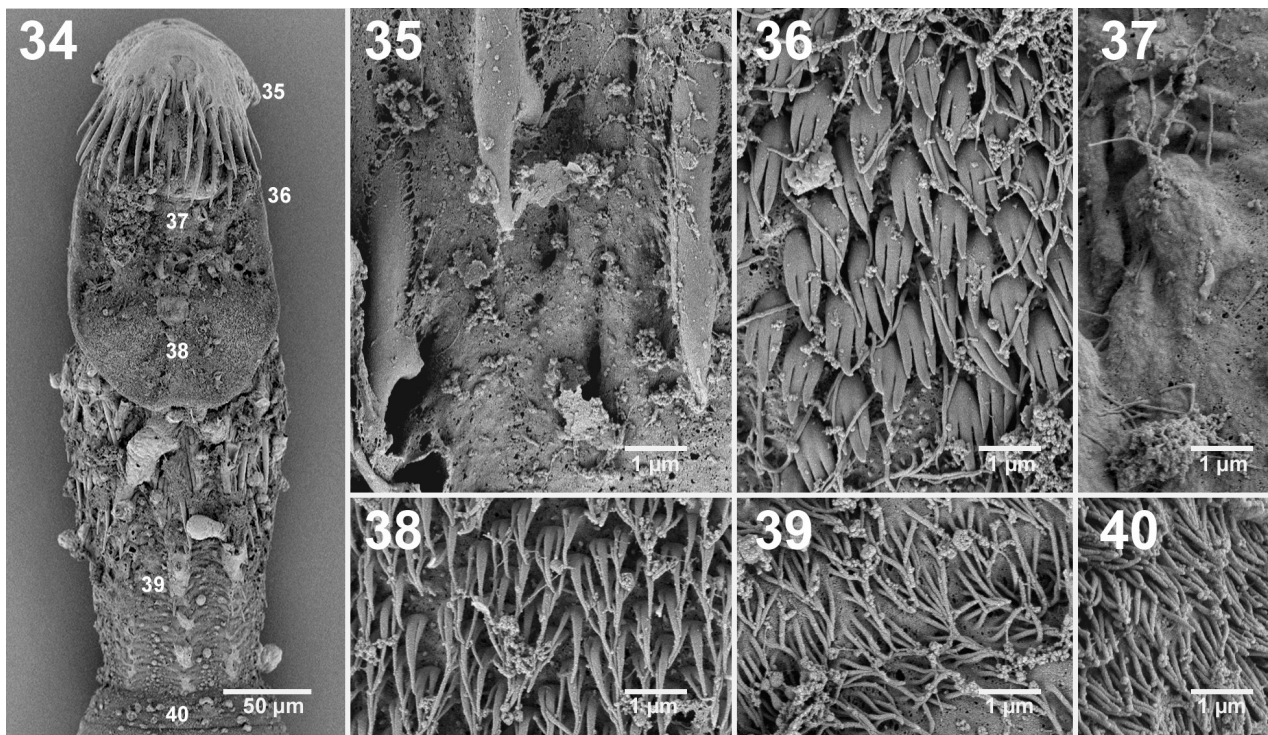
Etymology: This species is named in honour of the senior author's nephew, Vojtěch (= Vojta; *vojtai*) Kuchta.

**Remarks.** *Echinobothrium vojtai* is easily distinguished from all but 12 of the 38 valid species of the genus, including *E. nataliae* and *E. reginae* (Table 1) in its possession of lateral hooklets arranged in a continuous band, rather than in dorsal and ventral groups on each side of the scolex. Among the species with continuous bands of lateral hooklets on each side, it has fewer cephalic peduncle spines per column than *E. euzeti*, *E. megacanthum*, and *E. reginae* (20–26 vs. 100–107, 38–43, 30–33, respectively) and a greater number of cephalic peduncle spines per column than *E. californiense*, *E. hoffmanorum*, *E. pigmentum*, and *E. rayallemangi* (20–26 vs. 5–7, 14–16, 8–13, 2–5, respectively). Furthermore, it bears fewer lateral hooklets per band than *E. bonasum*, *E. raschii* (18–20 vs. 24–27, 24–36), a greater number of lateral hooklets per band than *E. mexicanum*, *E. nataliae* (18–20 vs. 10–13, 10–14), and a greater number of apical hooks than *E. fautleyae* (14/15 vs. 6/7).

## DISCUSSION

The diphyllideans are a relatively small order of elasmobranch tapeworms. The taxonomy of the order emphasizes the morphology of the armature of the scolex. In an attempt to facilitate comparisons among diphyllidean species, Neifar et al. (2001) suggested a standardized formula for representing the number and arrangement of rostellar hooks and hooklets. In that system, the armature of the rostellum is considered to consist of two groups of large apical hooks (dorsal and ventral), which may or may not be flanked on each side by smaller lateral hooklets. Jones and Beveridge (2001) proposed that the anterior apical hooks in both the dorsal and ventral groups be assigned the designation A and the posterior apical hooks in the dorsal and ventral groups be assigned the designation B. Based on the assumption that the apical hooks of the dorsal and ventral faces of the bothria are symmetrical, the formula of Neifar et al. (2001) is as follows: {LH AH(A)/AH(B) LH}, where AH(A) is the number of type A apical





**Figs. 34–40.** Scanning electron micrographs of *Echinobothrium vojtaei* sp. n. **Fig. 34.** Scolex. Note: small numbers correspond to the figures showing higher magnification images of these surfaces. **Fig. 35.** Detail of hooklets. **Fig. 36.** Proximal bothrial surface. **Fig. 37.** Distal bothrial surface at margin of central triangular region. **Fig. 38.** Posterior regions of distal bothrial surface. **Fig. 39.** Surface of cephalic peduncle. **Fig. 40.** Surface of proliferation zone.

hooks on a bothrial face, AH(B) is the number of type B apical hooks on a bothrial face, and LH is the number of lateral hooklets flanking the apical hooks on each side. In species in which the apical hooks clearly differ from the lateral hooklets in size and/or form, and the lateral hooklets on each side are arranged in distinct dorsal and ventral groups, this formula can be applied without ambiguity. However, when one or both of these conditions is violated, application of the formula can be somewhat problematic.

Additional criteria are required in instances in which size and form make the distinction between apical hooks and lateral hooklets unclear, particularly at the point of transition between the smaller apical hooks and lateral hooklets. For example, in *E. fautleyae*, there exist armature elements at the boundary between those that are clearly hooks and those that are hooklets that are difficult to classify as either armature type for they are conspicuously smaller than the hooks, but larger than the hooklets (Fig. 14). Tyler and Caira (1999), not without some trepidation, ultimately considered these to be lateral hooklets. However, it now seems clear that the distinction between apical hooks and lateral hooklets should be defined as suggested by Rees (1961), who proposed that elements bearing distal scalpel-like blade should be considered hooklets. Although the hooklets of some species may not have scalpel-like blade, this criterion is useful in the cases

of taxa with hooklet that do. Using this criterion, given that the elements of concern in *E. fautleyae* lack distal scalpel-like blade, these elements are more appropriately considered to represent apical hooks than lateral hooklets. This species is herein redescribed to consist of 6–7, rather than 5–6, apical hooks in a group, with only 10–11, rather than 11–12, lateral hooklets (see Table 1).

Here we propose a modification of the formula of Neifar et al. (2001) to more readily accommodate taxa bearing lateral hooklets that are arranged in a continuous band between the dorsal and ventral sets of apical hooks on each side (e.g., Figs. 5, 11, 16). For in such taxa, it is difficult to distinguish between the hooklets associated with the dorsal and ventral groups of apical hooks without ambiguity. Tyler (2006) suggested that in such instances, the number expressed in the armature formula as LH should be half that found in the entire band of hooklets on each side. While this solution at least partially addresses the issue, the uninterrupted nature of the band of lateral hooklets is not clearly described. We propose that the total number of hooklets in a band on a side should be presented as a single number (or range if appropriate) in parentheses to mark that it represents the total number of hooklets in one band. This number should precede the apical hook numbers in the formula. Thus, the hook formula would be as follows: {(LH) AH(A)/AH(B)}. We have employed this new formula in the descriptions of each of the new species

Table 1. List of nominal species of the genus *Echinobothrium*.

Species	Taxonomic status*	Hook formula§	Number of spines	Type host	Type locality	Body water
<i>E. acanthinophyllum</i> Rees, 1961	valid	{2-4 12/11 2-4}	10-13	<i>Raja montagui</i>	English Channel; Roscoff (FR)	Atlantic
<i>E. acanthocolle</i> Wojciechowska, 1991	valid	{3 16/15 3}	2-5	<i>Raja</i> (= <i>Amblyraja</i> ) <i>georgiana</i>	South Georgia Island (UK)	Atlantic
<i>E. affine</i> Diesing, 1863	valid	{2-3 6/5 2-3}	20-30	<i>Raja asperina</i> (= <i>R. radula</i> )	Nice (FR)	Atlantic
<i>E. benedeni</i> Ruskowski, 1927	valid	{4 14/12 4}	–	<i>Hippolyte varians</i> (and immature in <i>Raja punctata</i> )	Roscoff (FR)	Atlantic
<i>E. boisi</i> Southwell, 1911	sp. <i>inq.</i>	9-12?	24	<i>Aetobatis</i> (= <i>Aetobatus</i> ) <i>narinari</i>	Portugal Bay (IN)	Indian
<i>E. bonasum</i> Williams et Campbell, 1980	valid	{(24-27) 6/5}	22-26	<i>Rhinoptera bonasus</i>	Chesapeake Bay (USA)	Atlantic
<i>E. brachysoma</i> Pintner, 1889	valid	{3-4 6/3 3-4}	14-18	"Rothenarten" (skates)	Trieste (IT)	Atlantic
<i>E. californiense</i> Ivanov et Campbell, 1998	valid	{(19-24) 10/11}	(6?)5-7	<i>Platyrrhinoides triseriata</i>	Newport Beach (USA)	Pacific
<i>E. chisholmae</i> Jones et Beveridge, 2001	valid	{3-6 6/5 3-6}	21-29	<i>Rhinobatos</i> (= <i>Glaucoctegus</i> ) <i>typus</i>	Heron Island (AU)	Pacific
<i>E. clavatum</i> Probert et Stohart, 1989	valid	{3-4 8/7 3-4}	11-16	<i>Raja clavata</i>	off Anglesey, Irish Sea (UK)	Atlantic
<i>E. coenophorum</i> Alexander, 1963	valid	{1 9/8 1}	11-15	<i>Raja</i> (= <i>Zearaja</i> ) <i>nasuta</i>	Cook Strait (NZ)	Pacific
<i>E. coronatum</i> Robinson, 1959	valid	14 20 14?	32	<i>Mustelus lenticalatus</i>	Wellington Harbor (NZ)	Pacific
<i>E. deeghai</i> Gupta et Parmar, 1988	sp. <i>inq.</i> †	14?	–	<i>Trygon</i> (= <i>Pastinachus</i> ) <i>sephen</i>	Deegha (IN)	Indian
<i>E. djeddensis</i> (Pramanik et Lipshitz, 2006)	valid†	{7-11 14-15/13-14 7-11}	95-118	<i>Iago omanensis</i>	Gulf of Aqaba (IS)	Indian
<i>E. elegans</i> Tyler, 2001	sp. <i>inq.</i> †	22?	–	<i>Rhynchobatus djeddensis</i> (= <i>R. djeddensis</i> )	Digha coast (IN)	Indian
<i>E. euterpes</i> (Neifar, Tyler et Euzet, 2001) Tyler, 2006	valid	{3-4 10/9 3-4}	10-14	<i>Taeniura lymma</i>	Zarzis (TU)	Pacific
<i>E. euzeiti</i> Campbell et Carvajal, 1980	valid	{3-5 13-15/14-16 3-5}	–	<i>Rhinobatos rhinobatos</i>	Constitution (CH)	Pacific
<i>E. fautleyae</i> Tyler et Cair, 1999	valid	{(13-14) 13/12}	100-107	<i>Psammobatis</i> (= <i>Sympterygia</i> ) <i>lima</i>	Puertecitos (MX)	Pacific
<i>E. fautleyae</i> Tyler et Cair, 1999	valid	{(20-21) 6/7}†	19-36	<i>Rhinoptera steindachneri</i>	Aberdeen (UK)	Atlantic
<i>E. harfordi</i> McVicar, 1976	valid	{2-4 6/5 2-4}	11-14	<i>Raja naevus</i>	Al-Ghardaga (EG)	Indian
<i>E. helmy Mohamedi</i> Saoud, Ramadan et Hassan, 1982	valid	2 29 2?	16-17	<i>Taeniura lymma</i>	Heron Island (AU)	Pacific
<i>E. heroniensis</i> Williams, 1964	valid	{2-3 22/19 2-3}	24-32	<i>Taeniura lymma</i>	Isla San Esteban (MX)	Pacific
<i>E. hoffmanorum</i> Tyler, 2001	valid	{(12-22) 10/9-11}	14-16	<i>Urolophus maculatus</i>	Waltair (IN)	Indian
<i>E. lateroporum</i> Subhadrappa, 1948	<i>nomen nudum</i>	5 30 5?†	?	<i>Mustelus manazo</i> by Tyler (2006)	Atlantic coast of France by Tyler (2006)	Atlantic
<i>E. levicolle</i> Lespes, 1857	sp. <i>inq.</i>	more than 20?	?	<i>Nassa reticulata</i>	Ceylon Pearl Banks (IN)	Indian
<i>E. longicollis</i> Southwell, 1925	valid	{9/10}?	159-181	<i>Trygon</i> (= <i>Dasyatis</i> ) <i>kahlui</i>	Sete (FR)	Atlantic
<i>E. madhiasi</i> Euzet, 1951	valid	{3-4 13/14 3-4}	57-60	<i>Leiobatis</i> (= <i>Myliobatis</i> ) <i>aquila</i>	San Antonio Oeste (AR)	Atlantic
<i>E. megacanthum</i> Ivanov et Campbell, 1998	valid	{(12) 14/13}	38-43	<i>Myliobatis goodei</i>	Bahia de Los Angeles (USA)	Pacific
<i>E. mexicanum</i> Tyler et Cair, 1999	valid	{(10-13) 12/11}	23-24	<i>Myliobatis longirostris</i>	Off Sematan (MA)	Indian
<i>E. minutum</i> Twobig, Cair et Fyler, 2008	valid†	{3-4 6/3 3-4}	7-9	<i>Himantura walga</i>	Trieste (IT) by Tyler (2006)	Atlantic
<i>E. musteli</i> Pintner, 1889	valid	{3-4 16/15 3-4}†	20-22	"Hundshaie" ( <i>Mustelus mustelus</i> by Tyler 2006)	Ranagiri (IN)	Indian
<i>E. nagabhushani</i> (Chincholikar et Shinde, 1976) Tyler, 2006	sp. <i>inq.</i>	?	–	<i>Trygon</i> sp.	off Semantan (MA)	Pacific
<i>E. nataliae</i> sp. n.	valid†	{(10-14) 14/13-15}	24-29	<i>Pastinachus solocrostris</i>	Madras (IN)	Indian
<i>E. nigracanthum</i> Reimer, 1975	sp. <i>inq.</i> †	5 30 5?	–	<i>Bullia melanoides</i>	Mar del Plata (AR)	Atlantic
<i>E. notoguidoi</i> Ivanov, 1997	valid	{13 16/15 13}	24-26	<i>Mustelus schmitti</i>	Quebec (CA)	Atlantic
<i>E. pigmentatum</i> Ostrowski de Nùñez, 1971	valid	{(20-22) 10/9}	8-13	<i>Zapteryx brevirostris</i>	Bering Sea (USA)	Pacific
<i>E. raji</i> Heller, 1949	valid	at least 40?	5-9	<i>Raja scabrata</i> (= <i>Amblyraja radiata</i> ?)	Bahia de Los Angeles (USA)	Pacific
<i>E. raschii</i> Campbell et Andrade, 1997	valid	{(24-36) 12-13/11-12}	21-26	<i>Rhinoraja longi</i>	Waltair (IN)	Indian
<i>E. royaldemangui</i> Tyler, 2001	valid	{(20-24) 12/11}	2-5	<i>Rhinobatos leucorhynchus</i>	Ramen (MNG)	Indian
<i>E. reeseae</i> Ramadevi, 1969	valid	{2 7/6 2}	–	<i>Himantura walga</i>	Sri Lanka (IN)	Indian
<i>E. reginae</i> sp. n.	valid†	{(16-17) 14/15}	30-33	<i>Pastinachus cf. arnis</i>	Kuwait City (KU)	Indian
<i>E. rhinoptera</i> Shipley et Hornell, 1906	sp. <i>inq.</i>	?	12-13	<i>Rhinoptera javanica</i>	Xiamen (CHINA)	Pacific
<i>E. rhynchobati</i> (Khalil et Abdul-Salam, 1989) Tyler, 2006	valid	{1-2 6/15-17 1-2}	–	<i>Rhynchobatus</i> [sic] (= <i>Glaucoctegus</i> ) <i>granularis</i>	Gulf of Gabès (TU); Zarzis (TU); Sfax (TU)	Atlantic
<i>E. scoliodoni</i> Sanaka, Lakshmi et Rao, 1986	sp. <i>inq.</i>	{10-12 10-13 10-12}	2-3	<i>Chiloscyllium indicum</i>	Belgine coast by Tyler (2006)	Pacific
<i>E. sinensis</i> (Li et Wang, 2007) comb. n.	valid†	{8-10 13-17/13-17 8-10}	–	<i>Platyrrhina sinensis</i>	Java Sea (Indonesia)	Pacific
<i>E. syrtensis</i> (Neifar, Tyler et Euzet, 2001) Tyler, 2006	valid	{4-5 6/5 4-5}	16-18	<i>Rhinobatos cemiculus</i>		
<i>E. typus</i> van Beneden, 1849	valid	{2-4 4/3 2-4}	20-26	"raie boudée" (= <i>Raja clavata</i> )		
<i>E. vojiai</i> sp. n.	valid†	{(18-20) 14/15}	–	<i>Pastinachus</i> sp. (thin-tail)		

\*Follows Tyler (2006) unless otherwise indicated; † not treated or different status of Tyler (2006); § Names and formulae in bold indicate taxa in which lateral hooklets are arranged in a continuous band on each side; sp. *inq.* – species inquirenda; ? not clear from literature or unknown.



described above, all three of which exhibit lateral hooklets arranged in a continuous band. In order to facilitate interspecific comparisons, the rostellar armature formulae of the nominal species of *Echinobothrium* for which these data are available are presented in Table 1. In the cases of species that bear a continuous band of lateral hooklets on each side, the formula presented has been modified from that of the original description (and/or that of Tyler, 2006) to conform to the modified version of the formula proposed here; these are indicated in bold in Table 1.

It is interesting to place the hosts of the three new species described here into a broader perspective relative to the hosts of other species of *Echinobothrium* for our results formally expand the range of host genera known to host diphyllideans. All three of the new diphyllidean species described here were found to parasitize stingrays of the genus *Pastinachus*. Although previously considered to be monotypic, including only *P. sephen* (Forsskål) from the Red Sea, *Pastinachus* has recently been determined to include additional species. For example, Last et al. (2005) described *Pastinachus solocirostris* Last, Manjani et Yearsley from a diversity of localities in the Indo-Pacific, and Last and Stevens (2009) formally recognized *Pastinachus atrus* (Macleay) from Australia. Although *Echinobothrium nataliae* was described from one of these newly recognized cowtail species, both *E. reginae* and *E. vojtai* were hosted by potentially undescribed species of *Pastinachus* (see <http://tapewormdb.uconn.edu/hosts.php>). The host of *E. vojtai* represents a second species from Borneo bearing a shallow ventral fin-fold. Given that this species has not yet been described, it has been referred to here as *Pastinachus* sp. (thin-tail). The identity of the host of *E. reginae* is potentially even more problematic. This host specimen (GA-16) was also found to host *Uncibilocularis lorenii* Jensen et Caira, 2008 by Jensen and Caira (2008). The issues associated with the identity of this animal were discussed by Jensen and Caira (2008), who presented a figure (fig. 46) in the hopes that this would aid in the ultimate identification of this specimen once the taxonomy of *Pastinachus* is better understood. Given that it bears a well-developed ventral fin fold, we recognize that this specimen may ultimately be determined to be *P. sephen*. However, in the absence of detailed taxonomic work on

the cowtail stingrays of Madagascar, for consistency sake, we will follow Jensen and Caira (2008) and continue to refer to this specimen as *P. cf. sephen*.

To date, only a single other species of *Echinobothrium* has potentially been described from a species of *Pastinachus*. This species, *Echinobothrium deeghai* Gupta et Parmar, 1988, was described from *Trygon sephen* [sic] from the coast of India. Unfortunately, the description of Gupta and Parmar (1988) is superficial and the illustrations are schematic. No hook formula is presented and the description and illustrations suggest that the species not only lacks cephalic peduncle spines, but also lacks lateral hooklets. Perhaps most importantly, no mention is made of type material in the original description. Given these deficiencies, despite its recognition as a valid species by Tyler (2006), we concur with Campbell and Andrade (1997) that this species should be considered a *species inquirenda*.

Our results suggest that species of *Pastinachus* are viable hosts of *Echinobothrium* species. It seems likely that species of *Pastinachus* that have not yet been examined for tapeworms will be found to also serve as hosts for this diphyllidean genus. Furthermore, our results suggest that *Echinobothrium* is more globally widespread than previously thought.

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