

***Echinobothrium megacanthum* sp. n. (Cestoda: Diphyllidea) from the eagle ray *Myliobatis goodei* (Chondrichthyes: Rajoidei) from the Patagonian shelf of Argentina**

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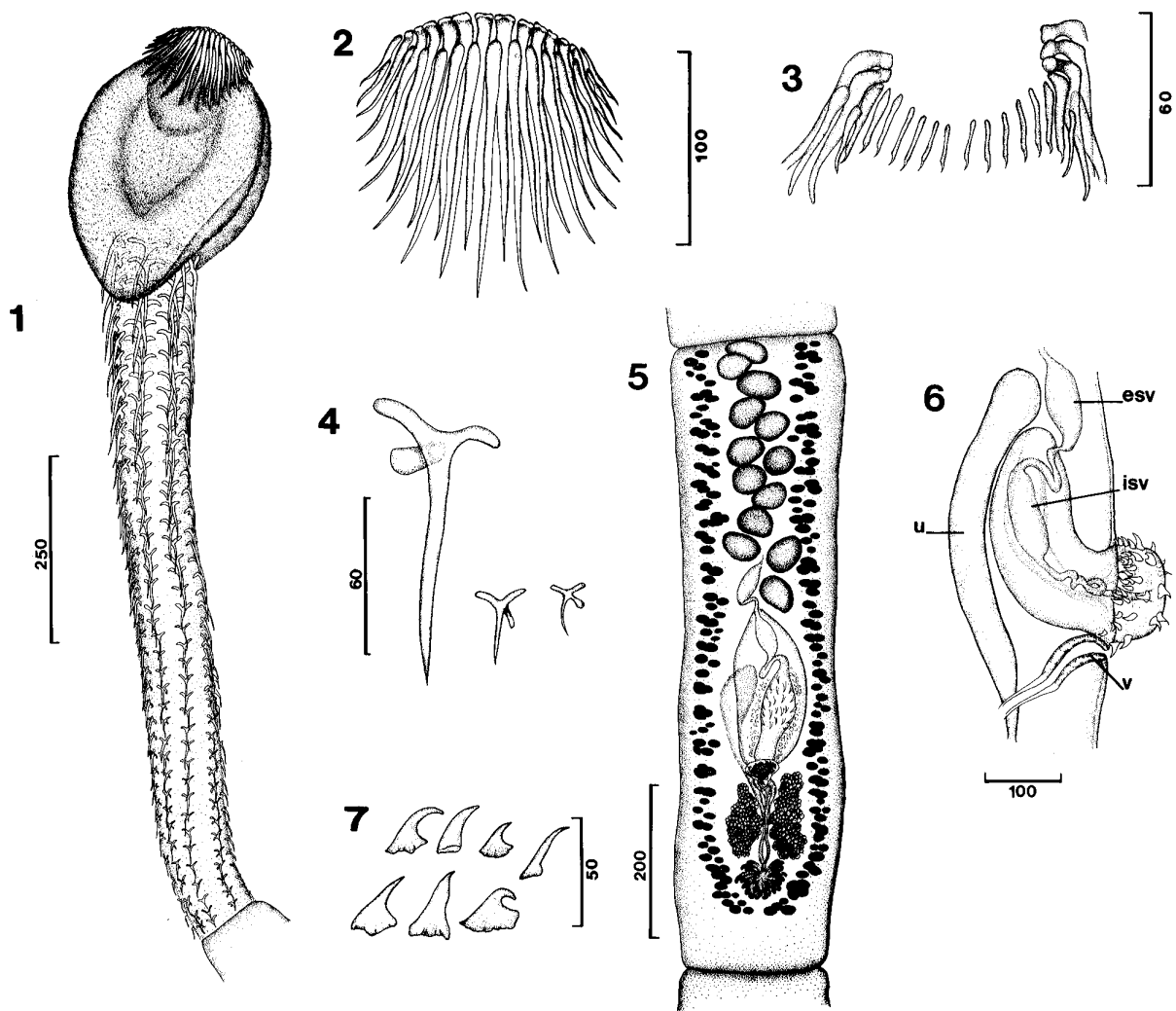
Abstract. A new species of *Echinobothrium* is described from the spiral intestine of the eagle ray *Myliobatis goodei* Garman, 1885 from the San Matías Gulf, Argentina. *Echinobothrium megacanthum* sp. n. is distinguished from all others by the following combination of characters: 27 large hooks per apical group, a continuous row of 12 hooklets on each lateral surface of the rostellum, 38-43 spines per row on the cephalic peduncle and 13-15 testes per segment. The new species is most similar to *E. euzeti* Campbell et Carvajal, 1980, *E. mathiasi* Euzet, 1951 and *E. raschii* Campbell et Andrade, 1997. *Echinobothrium megacanthum* can be differentiated from *E. euzeti* by the number of spines per row on the cephalic peduncle (100-107 in *E. euzeti*), number of lateral hooklets (6-7), and number of testes (37-42). It can be distinguished from *E. mathiasi* by the number of lateral hooklets (4 in *E. mathiasi*), number of spines per row on the cephalic peduncle (50-60), number of testes (25-30), and from *E. raschii* by the number of lateral hooklets (27-36 in *E. raschii*), number of spines per row on the cephalic peduncle (21-26), and number of testes (17-23). Differences in the scolex armature clearly distinguish *E. megacanthum* from *E. pigmentatum* Ostrowski de Núñez, 1971, *E. acanthocolle* Wojciechowska, 1991 and *E. notoguidoi* Ivanov, 1997, the only species described previously from hosts in the southwestern Atlantic. *Echinobothrium pigmentatum* differs in possessing 20 large apical hooks, a continuous row of 21-22 lateral hooklets and 9-13 spines on the cephalic peduncle. The scolex armature of *E. acanthocolle* lacks lateral hooklets, and possesses only 5 spines per row on the cephalic peduncle. *Echinobothrium notoguidoi* differs in the possession of a wide corona of spines posterior to the rostellum, number of spines per row on the cephalic peduncle (24-26), number of hooklets disposed in lateral groups (13 per group), and number of large apical hooks (31).

The genus *Echinobothrium* van Beneden, 1849 is the most species-rich genus of diphyllidean cestodes. Two species of *Echinobothrium*, *E. pigmentatum* Ostrowski de Núñez, 1971 from the rhinobatid *Zapteryx brevirostris* and *E. notoguidoi* Ivanov, 1997 from the triakid shark *Mustelus schmitti*, have been reported in previous studies of the helminth fauna of elasmobranchs from Mar del Plata on the north coast of Argentina. A third species, *E. acanthocolle* Wojciechowska, 1991 from the skate *Raja georgiana* has been described from South Georgia Island to the South of Argentina. Another new species of *Echinobothrium*, described herein, was recently discovered in the spiral intestine of a myliobatid ray, *Myliobatis goodei* taken during an ichthyological survey of fishes from the Patagonian shelf off southern Argentina.

MATERIALS AND METHODS

Three specimens of *Myliobatis goodei* captured in trawls during ichthyological surveys of the San Matías Gulf on the Patagonian coast of Argentina were identified and dissected by Dr. Edgardo Di Giacomo from the Instituto de Biología

Marina y Pesquera "Almirante Storni" San Antonio Oeste, Argentina. Worms were fixed *in situ* by injecting the spiral intestine with 10% formalin. After 48 hrs the preserved spiral valves were transferred to 70% ethanol for storage. Whole mounts of worms were stained with Harris' hematoxylin, dehydrated in ethanol, cleared in methyl salicylate and mounted in Canada balsam. The numbers of rostellar hooks were determined from permanent whole mounts and from specimens temporarily mounted in glycerine. Details of internal anatomy were determined from transverse serial sections of a specimen embedded in paraffin, cut 8 µm thick, stained with Harris' hematoxylin, and counterstained with eosin. Two specimens were prepared for scanning electron microscopy as follows: they were fixed in 1% osmium tetroxide, dried in Peldri II (Ted Pella Inc., Redding, California), mounted on stubs with adhesive tape, coated with conductive silver paint, sputter coated with gold in a Hummer VI-A sputter coater, and examined in a JSM 820 (Jeol) scanning electron microscope. Measurements are preceded by the sample size (n) and include the range followed in parentheses by the mean and standard deviation. All measurements are in micrometres unless otherwise stated. Figures were drawn with the aid of a drawing tube on a Nikon Optiphot-2 microscope. Type specimens have been deposited in the Museo de Ciencias Naturales de La Plata, Departamento



Figs. 1-7. *Echinobothrium megacanthum* sp. n. **Fig. 1.** Scolex. **Fig. 2.** Large apical hooks. **Fig. 3.** Detail of hooklets from lateral view of scolex. **Fig. 4.** Spines on the cephalic peduncle. **Fig. 5.** Mature segment, ventral view. **Fig. 6.** Detail of terminal genitalia (holotype), lateral view, note partially everted cirrus. **Fig. 7.** Detail of cirrus spines.

Abbreviations: esv = external seminal vesicle, isv = internal seminal vesicle, u = uterus, v = vagina.

de Zoología Invertebrados (Parasitología) (MLP), Argentina, Institute of Parasitology, Academy of Sciences of the Czech Republic (IPCAS), České Budějovice, and the United States National Parasite Collection (USNPC) in Beltsville, Maryland, USA.

RESULTS

Echinobothrium megacanthum sp. n. Figs. 1-14

Type host: *Myliobatis goodei* Garman, 1885 (Chondrichthyes: Myliobatidae).

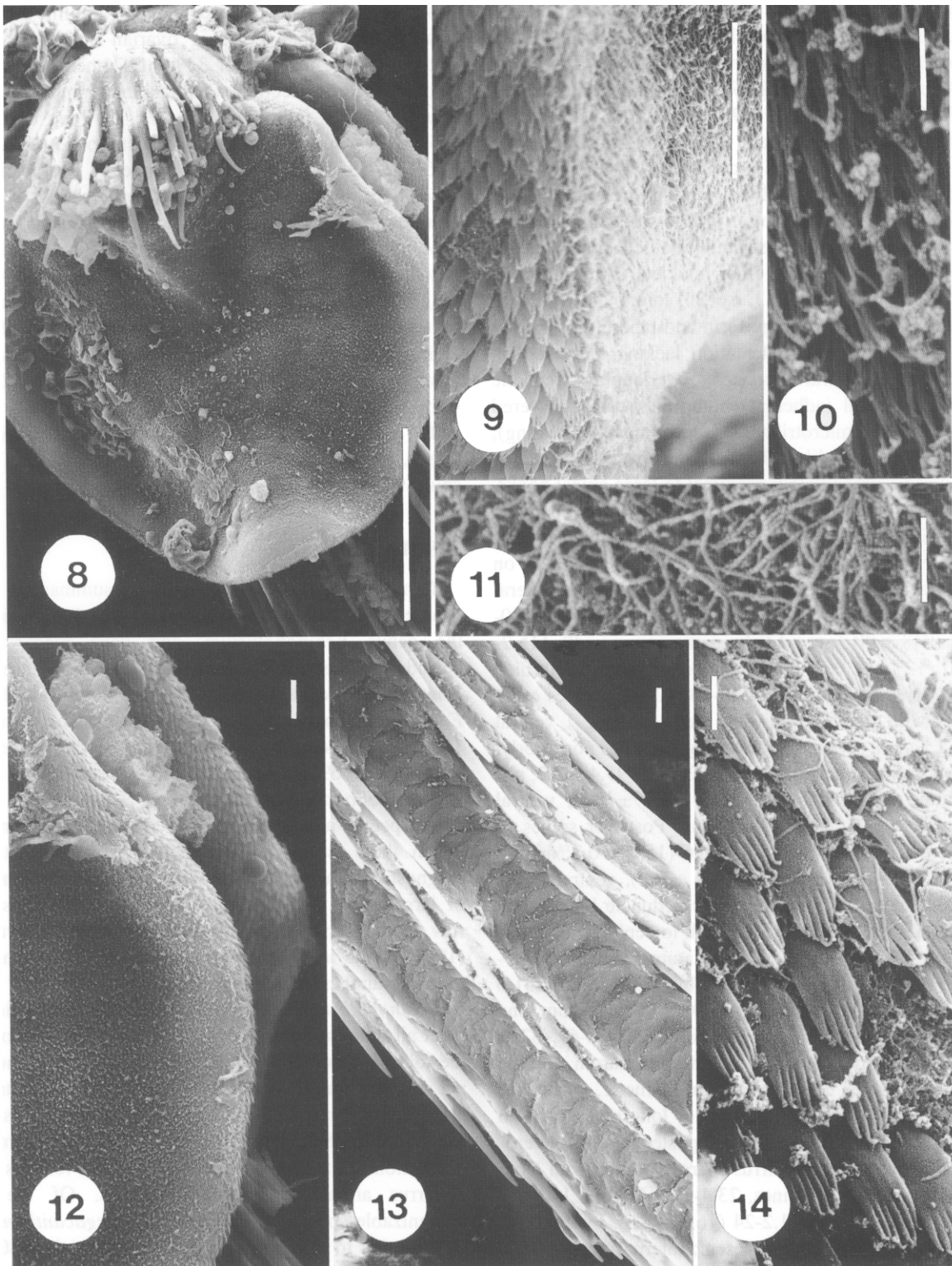
Type locality: San Antonio Oeste (40°44'S, 64°56'W), San Matías Gulf, Argentina.

Site of infection: spiral intestine.

Holotype: MLP No. 3958.

Paratypes: IPCAS No.C-288, USNPC No. 87474

Description (based on 6 complete specimens and 7 fragmented specimens with scolices): Strobila 4.45-6.64 (5.84 ± 0.9 ; $n = 6$) mm long, maximum width 205-288 (243 ± 31.4), acraspedote, apolytic, composed of 9-12 (11 ± 1 ; $n = 6$) segments; 1-2 (2 ± 1) segments mature, terminal segment gravid. Scolex 1.14-1.31 (1.23 ± 0.06 ; $n = 12$) mm long, consisting of armed apical rostellum, 2 oval bothridia and armed cephalic peduncle. Rostellum 160-176 (168 ± 8 ; $n = 12$) long by 144-208 (160 ± 27.7) wide. Rostellar armature consisting of one dorsal and one ventral group of 27 large apical hooks flanked by a continuous row of lateral hooklets. Each group of 27 large hooks arranged in 2 rows; 14 hooks



Figs. 8-14. Scanning electron micrographs of *Echinobothrium megacanthum* sp. n. **Fig. 8.** Scolex. **Fig. 9.** Edge of bothridium showing transition from filiform microtriches on distal surface (right) to palmate microtriches on proximal surface (left). **Fig. 10.** Filiform microtriches on distal surface of bothridia. **Fig. 11.** Meshlike branching filaments on anterior median region of bothridium, distal surface. **Fig. 12.** Bothridia showing views of proximal and distal surfaces. **Fig. 13.** Cephalic peduncle surface showing rows of spines. **Fig. 14.** Palmate microtriches on proximal surface of bothridium. Scale bars 100 μm (Fig. 8), 10 μm (Figs. 9, 12, 13) and 1 μm (Figs. 10, 11, 14).

in anterior row, 13 in posterior row, largest hooks at middle of rows. Hook lengths: anterior row 31.5-34.6 (32.9 ± 1 ; $n = 84$) to 115.2-124.8 (120.5 ± 3.5) long; posterior row 23.7-28.8 (26.7 ± 1.5 ; $n = 78$) to 123.8-131.2 (127.1 ± 3) long. A total of 12 lateral hooklets, 18.6-27.5 (22.7 ± 2.8 ; $n = 48$) long, forms a continuous row across each lateral surface of rostellum joining groups of large hooks; hooklets decreasing in size toward centre of row, space between sixth and seventh hooklets slightly larger than separation between other hooklets. Two bothridia, 288.0-329.6 (297.2 ± 16.3 ; $n = 20$) long by 227.0-281.6 (258.1 ± 22.8) wide, linguiform and tapering to a point posteriorly, marginal indentation lacking, disposed on dorsal and ventral surfaces, overlapping cephalic peduncle. Distal (adherent) bothridial surface covered with filiform microtriches, (approximately 2 long); branching rhizoid-like filaments occupy central region of bothridia immediately proximal to large apical hooks. Proximal bothridial surface covered with palmate microtriches, 3.0-3.5 long by 1.2-1.4 maximum width, each bearing a single row of 5-7 slender processes on the free margin; central process 1.0-1.2 long, lateral processes shorter, 0.5-0.8 long. Cephalic peduncle 780-1027 (948 ± 90.3 ; $n = 13$) long, maximum width 115.2-144.0 (127.2 ± 9.6) near junction of bothridia, armed with 8 longitudinal rows of 38-43 (40 ± 2) spines each. Spines with slender spiniform blade, bearing 1 ventral and 2 lateral prongs, decreasing in size posteriorly. Largest spines anterior, blade 92.2-99.2 (95.4 ± 2.2 ; $n = 30$) long, base width 51.2-68.2 (61.7 ± 5.3), ventral prong 22.4-27.2 (24.7 ± 1.5) long. Posterior spines of each row smaller, blade 14.6-16.6 (15.7 ± 0.7 ; $n = 20$) long, base width 12.8-15.4 (14.0 ± 0.9), ventral prong 7.0-10.2 (8.9 ± 1) long. Cephalic peduncle longer than bothridia, ratio of bothridial length to cephalic peduncle length 1 : 2.7-1 : 3.6 (1 : 3.2). Mature segments 929.5-1200 (1090 ± 112.1 ; $n = 5$) long by 205.0-253.5 (231.6 ± 16.3) wide; gravid segment 1.59-1.60 ($n = 2$) mm long by 202-288 wide. Genital pore midventral, 60-67 (62; $n = 6$) % of segment length from anterior margin. Genital atrium 75-80 (78 ± 2.4 ; $n = 6$) in diameter. Cirrus sac 134.5-292.0 (215.1 ± 55.6 ; $n = 16$) long by 73.6-144.0 (108.0 ± 17.7) wide, elongated, postequatorial, antero-medial to genital pore. Cirrus sparsely armed with large uncinata and falcate spines, 23.4-27.3 (25.6 ± 1.4 ; $n = 10$) long, base length 11.2-24 (16.9 ± 3.6). Internal and external seminal vesicles present; internal vesicle 132-138 (135 ± 2.2 ; $n = 6$) long and 51.3-54.2 (52.6 ± 1.1) wide; external vesicle 133-137.5 (135.1 ± 1.7 ; $n = 6$) long and 54.5-56.4 (55.7 ± 0.7). Testes subspherical, 41.6-51.2 (44.9 ± 3.2 ; $n = 20$) long by 51.3-60.8 (54.7 ± 3.3) wide, in median field, arranged in two irregular longitudinal rows extending from anterior margin of cirrus sac to anterior segment margin, 13-15 (14 ± 1 ; n

= 10) per segment. Vagina entering genital atrium posterior to cirrus sac, descending in midline between ovarian lobes, then crossing ovarian isthmus to join ootype. Ovary in posterior 33% of segment, H-shaped, bilobed in cross section. Ovarian lobes approximately equal, 136-227 (178.1 ± 31.9 ; $n = 10$) long by 40-51 (45.2 ± 3.4) wide. Mehlis' gland posterior to ovary, 56-136 (82.4 ± 26.7 ; $n = 6$) in diameter. Uterus linear, ascending dorsal to cirrus sac, terminating slightly anterior to cirrus sac in early gravid segments, uterus development in fully gravid segments not observed. Vitelline follicles subspherical, 16-29 (21 ± 4.4 ; $n = 12$) long by 16-36 (25 ± 6.4) wide, forming 2 bands lateral to testes (2 follicles deep in each), extending almost entire length of segment, arching medially in postovarian space. Eggs oval, 17.3-19.2 (18.4 ± 0.8 ; $n = 4$) by 8-11.3 (10.1 ± 1.2), filaments not observed.

E t y m o l o g y : the specific epithet is from the Greek, mega - very large - acantha - spine, for the large spines of the cirrus armature.

DISCUSSION

Campbell and Andrade (1997) summarized the characters of a total of 19 clearly recognizable species in the genus *Echinobothrium*, and gave reasons for considering 8 species as *species inquirendae* (i.e., *E. benedeni* Ruzskowski, 1927, *E. boisii* Southwell, 1911, *E. deeghai* Gupta et Parmar, 1988, *E. laevicolle* Lespes, 1857, *E. lateroporum* Subhapradha, 1948, *E. reesae* Ramadevi, 1969, *E. rhinoptera* Shipley et Hornell, 1906 and *E. scoliiodoni* Sanaka, Lakshmi et Rao, 1986). The latter species are based on inadequate descriptions in which there is uncertainty about characters that are diagnostic in the separation of species, i.e., the number of large apical hooks, lateral hooklets and spines per row in the cephalic peduncle. More recently, Ivanov (1997) added another species, *E. notoguidoi*, bringing the total number of recognizable species to 20.

Echinobothrium megacanthum can be distinguished from all other species in this genus by its possession of the following combination of characters: 27 large hooks per apical group, a continuous row of 12 hooklets on each lateral surface of the rostellum, 38-43 spines per row on the cephalic peduncle, 13-15 testes per segment, possession of external and internal seminal vesicles and a cirrus armature with large spines. Of the 20 recognizable species in the genus, *E. megacanthum* is most similar to *E. euzeti* Campbell et Carvajal, 1980, *E. mathiasi* Euzet, 1951 and *E. raschii* Campbell et Andrade, 1997. *Echinobothrium megacanthum* resembles *E. euzeti* in possessing a similar number of large apical hooks per group in the rostellum (27 vs. 25 in *E. euzeti*), but differs in having fewer spines per row on the cephalic peduncle (38-43 vs. 100-107), more

lateral hooklets (12 vs. 6-7) and fewer number of testes (13-15 vs. 37-42). *Echinobothrium megacanthum* is like *E. mathiasi* in the number of large apical hooks per group (27 vs. 26), and the presence of external and internal seminal vesicles, but the two species are easily differentiated by the number of lateral hooklets (12 vs. 4 in *E. mathiasi*), number of spines per row on the cephalic peduncle (38-43 vs. 50-60) and number of testes (13-15 vs. 25-30). The number of large apical hooks of *E. megacanthum* is near that of *E. raschii* (27 vs. 23-25), but *E. megacanthum* differs in the number of lateral hooklets (12 vs. 27-36 in *E. raschii*), number of spines per row on the cephalic peduncle (38-43 vs. 21-26) and in the number of testes (13-15 vs. 17-23).

Three species of *Echinobothrium* previously described in the southwestern Atlantic are *E. pigmentatum*, *E. acanthocolle* and *E. notoguidoi*. *Echinobothrium megacanthum* can be distinguished from *E. pigmentatum* by the number of large apical hooks (27 vs. 20 in *E. pigmentatum*), number of lateral hooklets (12 vs. 20-22), spines per row on the cephalic peduncle (38-43 vs. 9-13), and number of testes per segment (13-15 vs. 5-7). These characters also separate *E. megacanthum* from *E. acanthocolle* in which *E. megacanthum* possesses more large apical hooks (27 vs. 24 in *E. acanthocolle*), more lateral hooklets (12 vs. 0), more spines per row on the cephalic peduncle (38-43 vs. 5), and fewer testes per segment (13-15 vs. 23-25). Finally, *E. megacanthum* differs from *E. notoguidoi* in having fewer large apical hooks (27 vs. 31 in *E. notoguidoi*), fewer lateral hooklets (a continuous row of 12 vs. 2 lateral groups of 13), absence of a corona of spines posterior to the rostellum in *E. megacanthum*, and fewer spines per row on the cephalic peduncle (38-43 vs. 24-26).

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