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Six Species of *Acanthobothrium* (Eucestoda: Tetraphyllidea) in Stingrays (Chondrichthyes: Rajiformes: Myliobatoidei) from Ecuador

Fernando Marques
University of Toronto

Daniel R. Brooks
University of Toronto, dnlbrooks@gmail.com

Ramiro Barriga
Escuela Politecnica Nacional (Quito, Ecuador)

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SIX SPECIES OF *ACANTHOBOTHRIUM* (EUCESTODA: TETRAPHYLLIDEA) IN STINGRAYS (CHONDRICHTHYES: RAJIFORMES: MYLIOBATOIDEI) FROM ECUADOR

Fernando Marques, Daniel R. Brooks, and Ramiro Barriga*
Center for Historical Ecology and Biodiversity, Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 3G5

ABSTRACT: Six species of *Acanthobothrium,* 4 described as new, are reported in stingrays from southern Ecuador. *Acanthobothrium atahualpai* n. sp. in *Gymnura afuerae* most closely resembles *Acanthobothrium fogeli* and *Acanthobothrium parviuncinatum* by having bothridial hooks with recurved prongs and short handles. It differs from *A. fogeli* by having bothridial hooks 163–195 μm vs. 78–114 μm long and averaging 25 vs. 32 testes per proglottis; it differs from *A. parviuncinatum* by having bothridial hooks 163–195 μm vs. 87 μm long and averaging 25 vs. 13 testes per proglottis. *Acanthobothrium minusculus* n. sp. in *Urolophus tumbesensis* most resembles *Acanthobothrium campbelli* and *Acanthobothrium vargasi* by being no more than 3 mm long and having 6–30 testes per proglottis. It can be distinguished from them by having bothridial hooks averaging 86 μm vs. 108–111 μm and 130–133 μm long, and 6–10 vs. 15–23 and 22–29 testes per proglottis, respectively. *Acanthobothrium monksi* n. sp. in *Aetobatus narinari* resembles *Acanthobothrium tasajerasi* from *Himantura schmardae* by having a prominent genital atrium and a large globose cirrus sac; it differs by averaging 21 vs. 35 testes per proglottis and having bothridial hooks averaging 150 μm vs. 165 μm long. *Acanthobothrium obuncus* n. sp. in *Dasyatis longus* resembles a group of species characterized by wider than long to square immature and mature proglottides, bothridia at least partially fused to the scolex at their posterior ends, and asymmetrical ovarian arms with aporal arms extending anteriorly to the vaginal level. It resembles *Acanthobothrium americanum* by averaging 73 vs. 72 testes per proglottis, but differs by having bothridial hooks averaging 120–131 μm vs. 151 μm long; it resembles *Acanthobothrium chilensis* by having bothridial hooks averaging 120–131 μm vs. 130 μm long, but differs by averaging 73 vs. 90 testes per proglottis. *Acanthobothrium campbelli* in *Urotrygon chilensis* and *Acanthobothrium costarricense* in *Dasyatis longus,* previously known in those hosts from the Pacific coast of Costa Rica, are reported from Ecuador for the first time.

The tetraphyllidean cestode genus *Acanthobothrium* van Beneden 1849, represented by over 100 nominal species, is the most species-rich genus within the Onchobothriidae. Recent descriptions of new species of cestodes from the Eastern Pacific have discussed morphological similarities between them and species in the Caribbean (Marques et al., 1995, 1996), suggesting that they might constitute pairs of sister species that diverged from their common ancestors as a result of the formation of the Isthmus of Panama or some other significant geographic isolation event. These assertions have been based either on unique morphological features (Marques et al., 1996) or on hypotheses that their hosts are sister species, e.g., *Himatura pacifica* (Beebe & Tee-Van) and *Himatura schmardae* (Werner) (Lovejoy, 1996). At present, there is no robust phylogenetic analysis of *Acanthobothrium* to serve as a test for these postulates. Nevertheless, we consider them explicit predictions that can be submitted to direct test once such a phylogenetic hypothesis is produced. In this paper, we describe 4 new species of *Acanthobothrium* inhabiting stingrays from Ecuador and compare them with other morphologically similar species within the genus with an emphasis on their biogeographical distributions.

MATERIALS AND METHODS

During November of 1993, 2 *Gymnura afuerae* Hildebrand, 5 *Urolophus tumbesensis* Chirichigno and McEachran, 1 *Aetobatus narinari* (Euphrasen), and 5 *Dasyatis longus* Garman were captured in various localities in El Oro Province, Ecuador, using gill nets. Worms were removed from the host intestine, killed with hot tap water, transferred immediately to AFA for 24–48 hr, and then stored in 70% ethanol. Specimens were stained with Mayer's hematoxylin and mounted in Canada balsam for examination as whole mounts. Measurements are in μm unless otherwise stated; for some traits, ranges are given, followed in parentheses by mean values ± 1 standard deviation and the sample size (n), except for samples of 2 for which only the range is provided. Hook measurements follow Marques et al. (1995). Figures were drawn with the aid of a drawing tube. The tetralobed nature of the ovaries was confirmed from lateral views of unmounted specimens and from whole mounts because sufficient material for sectioning was not available. CHIOC refers to the Coleção Helmintológica do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; MEPN refers to the Museum of the Escuela Politécnica Nacional, Quito, Ecuador; MNHG refers to the Museum of Natural History, Geneva, Switzerland; HWML refers to the University of Nebraska State Museum, Harold W. Manter Laboratory, Division of Parasitology, Lincoln, Nebraska, USA; and USNPC refers to United States National Parasite Collection, Beltsville, Maryland, USA. Comparisons were based on the latest publication for a given species unless an accession number is specified.

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* Departamento de las Ciencias Biológicas, Escuela Politecnica Nacional, Apartado 2759, Quito, Ecuador.

DESCRIPTIONS

Acanthobothrium atahualpai n. sp.
(Fig. 1A–D)

Description (based on 2 complete and 1 incomplete specimens): Strobila acraspedote, apolytic, up to 2.1 mm long; composed of up to 17 proglottides. Scolex 316–426 wide, composed of 4 triloculate bothridia free at posterior end with velum between adjacent bothridia; each bothridium with apical sucker and pad, armed with pair of bifid hooks. Bothridia 422–435 (429 ± 5, n = 3) long by 150–205 (173 ± 23, n = 4) wide; anterior loculi 272–310 (295 ± 17, n = 3) long, middle loculi 70–74 (71 ± 1, n = 3) long, posterior loculi 51–80 (62 ± 13, n = 3) long. Ratio of locular lengths 1:0.23:0.20. Muscular pads 189–227 (211 ± 15, n = 4) wide, with median indentation, bearing apical sucker 48 (n = 2) in diameter. Hook formula for lateral hooks (n = 2):

$$\frac{32\text{–}35 \quad 144\text{–}147 \quad 154\text{–}166}{193\text{–}195}$$

Hook formula for medial hooks (n = 2):

$$\frac{32 \quad 134\text{–}141 \quad 128\text{–}134}{163\text{–}166}$$

Ratio of bothridial length to total hook length 1:0.41 for lateral hooks and 1:0.38 for medial hooks. Cephalic peduncle spined 358–563 (457 ± 84, n = 3) long, 106 (n = 1) wide at insertion to scolex. Immature

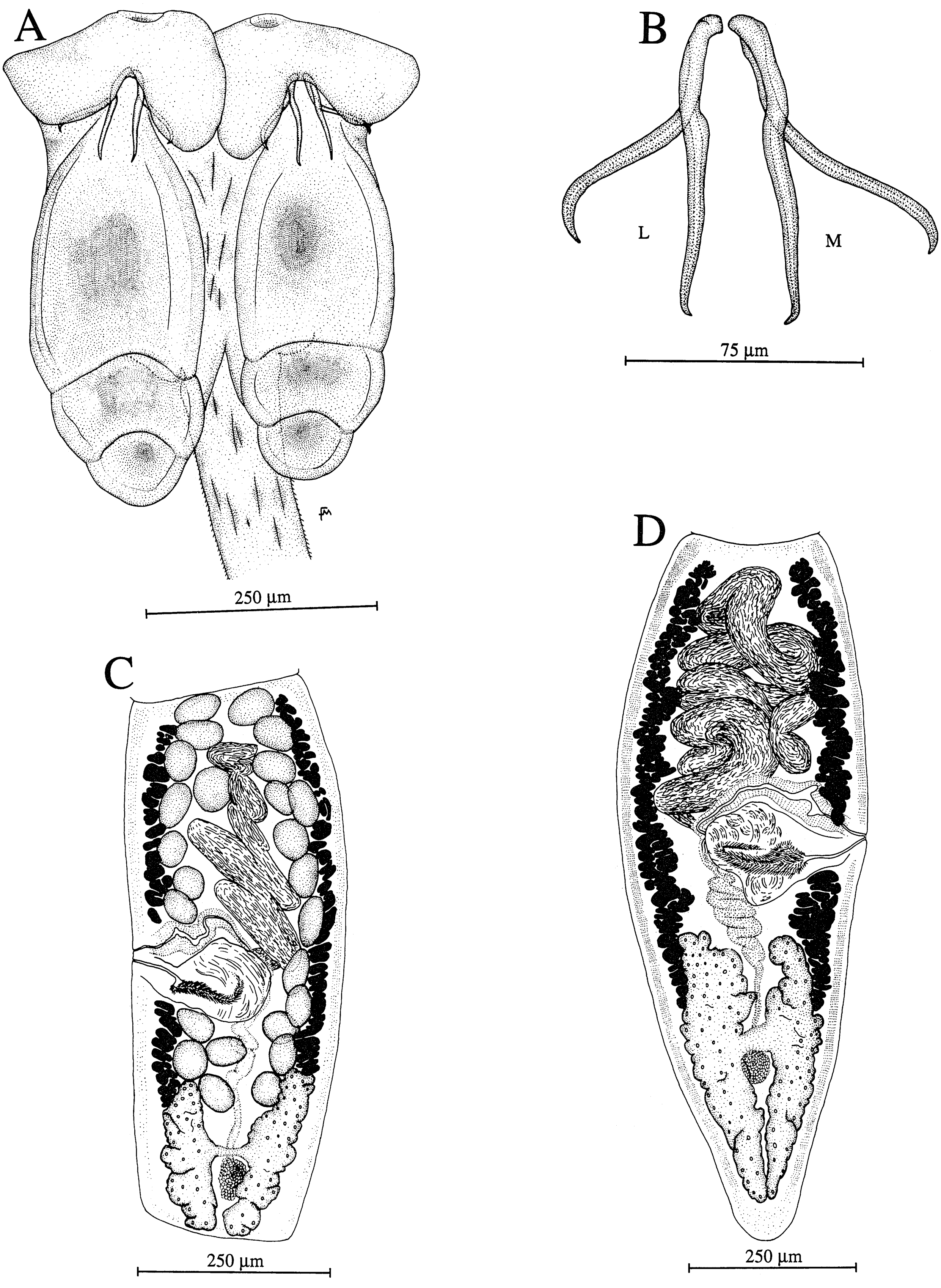

FIGURE 1. *Acanthobothrium atahualpai* n. sp. A. Scolex. B. Medial and lateral hooks. C. Mature proglottis. D. terminal proglottis. M = medial hook; L = lateral hook.

proglottides wider than long. Mature terminal proglottides 918–1,168 (1,042 ± 102, n = 3) long by 355–426 wide. Testes 35–61 (51 ± 8, n = 6) in diameter; 20–30 (25 ± 3, n = 5) in number, 4–10 (7 ± 2, n = 5) preporally, 4–6 (5 ± 1, n = 5) postporally, 12–15 (13 ± 1, n = 5) antiporally. Cirrus sac near midproglottis, 160–250 long by 128–157 wide, containing spined eversible cirrus. Genital atrium indistinct. Genital pore 42–47% of total length from anterior end, irregularly alternating. Vagina anterior to cirrus sac; vaginal sphincter not observed. Ovary near posterior end of proglottis, ∀-shaped in frontal view, X-shaped in cross section; 158–269 (210 ± 34, n = 7) wide at isthmus. Ovarian arms unequal in length, not reaching posterior margin of cirrus sac; aporal arm 371–592 long; poral arm 394–550 long. Uterus not observed. Vitelline follicles extending from level of ovarian isthmus to near anterior end of proglottis; follicles approximately oval, 29–51 (40 ± 7, n = 9) wide.

Taxonomic summary

Host: *Gymnura afuerae* Hildebrand (Rajiformes: Myliobatidae).
Site of infection: Spiral valve.
Locality: Puerto Bolívar, Provincia de El Oro, Ecuador (3°16′S: 80°19′W).
Holotype: MEPN no. 3029.
Paratypes: MNHG no. 22098.
Etymology: The species is named for the last Incan Emperor, Atahualpa.

Remarks

Acanthobothrium atahualpai, Acanthobothrium parviuncinatum Young, 1958 in *Gymnura marmorata* (Cooper) and *Urolophus halleri* (Cooper) from California, and *Acanthobothrium fogeli* Goldstein, 1964 in *Gymnura micrura* (Bloch and Schneider) from the Gulf of Mexico, are the only members of the genus possessing recurved tips of the bothridial hook prongs and short handles. *Acanthobothrium atahualpai* differs from these species by having longer hooks (up to 195 μm) and terminal proglottides (up to 1,042 μm) than *A. fogeli* (98 μm and 517 μm, respectively) and *A. parviuncinatum* (87 μm and 640 μm, respectively). *Acanthobothrium atahualpai* differs from *A. fogeli* by having a strobila up to 2.1 mm rather than 4.8 mm long, by having up to 17 rather than up to 35 proglottides per strobila, bothridial apical suckers averaging 48 μm rather than 80 μm in diameter, an average of 25 rather than 46 testes per proglottis, and slightly longer cirrus sacs (up to 246 μm vs. up to 228 μm). Further, *A. fogeli* differs from *A. atahualpai* by lacking postovarian testes and having a genital pore posterolateral to the ovary rather than preovarian. *Acanthobothrium atahualpai* differs from *A. parviuncinatum* by having extremely slender rather than relatively robust bothridial hook prongs and longer hooks (averaging 146 μm (inner) and 160 μm (outer) long vs. 70 μm (inner) and 62 μm (outer) long), and by having 20–35 rather than 12–14 testes per proglottis.

Acanthobothrium minusculus n. sp.
(Fig. 2A–C)

Description (based on 20 specimens): Strobila acraspedote, apolytic, 1–2 mm (1.4 ± 0.3, n = 18) long; composed of up to 11 (7 ± 2, n = 19) proglottides. Scolex 157–214 (187 ± 18, n = 16) wide, composed of 4 triloculate bothridia free at posterior end; each bothridium with apical sucker and pad, armed with pair of bifid hooks. Bothridia 179–227 (203 ± 13, n = 34) long by 77–109 (90 ± 8, n = 35) wide; anterior loculi 115–147 (130 ± 9, n = 34) long, middle loculi 26–42 (34 ± 4, n = 34) long, posterior loculi 32–48 (39 ± 4, n = 34) long. Ratio of locular lengths 1:0.27:0.30. Muscular pads 45–80 (60 ± 9, n = 29) wide, bearing apical sucker 29–37 (33 ± 2, n = 20) in diameter. Hook formula for lateral hooks (n = 26):

$$\frac{26 \pm 1\ (22\text{–}29) \quad 64 \pm 4\ (56\text{–}74) \quad 54 \pm 4\ (48\text{–}68)}{86 \pm 5\ (78\text{–}98)}$$

Hook formula for medial hooks (n = 27):

$$\frac{25 \pm 2\ (22\text{–}29) \quad 63 \pm 4\ (58\text{–}72) \quad 54 \pm 3\ (51\text{–}61)}{86 \pm 4\ (78\text{–}96)}$$

Ratio of bothridial length to total hook length 1:0.44 for lateral and medial hooks. Cephalic peduncle and scolex with spines. Peduncle 138–234 (188 ± 26, n = 20) mm long, 48–77 (61 ± 9, n = 19) wide at insertion to scolex. Immature proglottides wider than long. Mature terminal proglottides 378–538 (463 ± 49, n = 9) long by 96–157 (135 ± 20, n = 9) wide. Testes 6–10 (8 ± 1, n = 10) in number, 29–58 (39 ± 6, n = 32) in diameter; 1–2 (2 ± 0.4, n = 10) preporally, 1–2 (2 ± 0.5, n = 10) postporally, 4–6 (5 ± 1, n = 10) antiporally. Cirrus sac near midproglottis, 58–83 (71 ± 7, n = 9) long by 58–77 (66 ± 6, n = 9) wide, containing spined eversible cirrus. Genital atrium indistinct. Genital pore 10–37% (27 ± 6, n = 18) of total length from anterior end, irregularly alternating. Vagina anterior to cirrus sac; vaginal sphincter not observed. Ovary near posterior end of proglottis, ∀-shaped in frontal view, X-shaped in cross section; 57–130 (78 ± 19, n = 25) wide at isthmus. Ovarian arms unequal in length but not reaching posterior margin of cirrus sac; aporal arm 141–224 (175 ± 31, n = 7) long; poral arm 128–227 (163 ± 36, n = 7) long. Uterus indistinct in mature proglottis. Vitelline follicles extending from level of ovarian isthmus to near anterior end of proglottis; follicles roughly spherical, 9–35 (16 ± 5, n = 22) in diameter.

Taxonomic summary

Host: *Urolophus tumbesensis* Chirichigno and McEachran (Rajiformes: Urolophidae).
Site of infection: Anteriormost chamber of spiral valve.
Locality: Puerto Hualtaco, Provincia de El Oro, Ecuador (3°16′S: 80°19′W).
Holotype: MEPN no. 3030.
Paratypes: MNHG no. 22099; HWML no. 39178.
Etymology: The specific epithet refers to the tiny size, small bothridial hooks, and small number of testes per proglottis that characterize this species.

Remarks

Acanthobothrium minusculus resembles *Acanthobothrium brevissime* Linton, 1908 in *Dasyatis sabina* (LeSueur), *Dasyatis sayi* (LeSueur), *Dasyatis americana* Hildebrand and Schroeder, and *Raja eglanteria* Bosc from the Gulf of Mexico and the Chesapeake Bay, Virginia, USA; *Acanthobothrium vargasi* Marques, Brooks and Monks, 1995 in *D. longus* Garman from the Pacific coast of Costa Rica; *Acanthobothrium campbelli* Marques, Brooks and Monks, 1995 in *Urotrygon chilensis* (Günther) from the Pacific coast of Costa Rica; *Acanthobothrium urotrygoni* Brooks and Mayes, 1980 in *Urotrygon venezuelae* Schultz from the Caribbean coast of Colombia; *Acanthobothrium quadripartitum* Williams, 1968 in *Raja naevus* Müller & Henle from North Sea and English Channel; and *Acanthobothrium tripartitum* Williams, 1969 in *Raja microcellata* Montagu from the English Channel by being very small worms, with strobilae not longer than 5 mm and having fewer than 30 proglottides, and with spinose peduncles, short bothridial hooks, and an average of fewer than 30 testes per proglottis. The new species is distinct from the other 6 by having fewer testes, 6–10, per proglottis: *A. vargasi* has 22–29, *A. brevissime* has 19–40, *A. campbelli* has 15–23, *A. urotrygoni* has 24–33, *A. quadripartitum* has 18, and *A. tripartitum* has 13–16. In the new species, the genital pore averages 27% of proglottis length from the anterior end rather than 44–50% for *A. brevissime, A. vargasi,* and *A. campbelli,* 38% for *A. urotrygoni,* and approximately 50% for *A. quadripartitum* and *A. tripartitum. Acanthobothrium minusculus* further differs from *A. urotrygoni* by having relatively shorter middle bothridial loculi, with a ratio of bothridial locular lengths of 1:0.27:0.30 rather than approximately 1:0.22:0.36 (USNPC no. 75163, 2 paratypes). Further, *A. minusculus* can be distinguished from *A. brevissime* and *A. vargasi* by having shorter bothridia, from 176 to 224 μm long rather than 260–360 μm long for *A. brevissime* and 293–362 μm long for *A. vargasi,* shorter bothridial hooks, 80–100 μm long rather than 96–151 μm long and 123–145 μm long, respectively, and shorter terminal proglottides, up to 529 μm long rather than 1,000 μm long and 825 μm long, respectively. *Acanthobothrium minusculus* differs from *A. campbelli* by having shorter bothridial hooks, avaraging 86 μm long (lateral and medial) rather than 108 μm (lateral) and 111 μm (medial) long, and slightly shorter bothridia, up to 203 μm long vs. up to 264 μm long. The new species is shorter, 1.3 mm long vs. 2.4 mm long, and has larger bothridia, 224 μm vs. 196 μm long, and 76–107 μm vs. 60–61 μm wide, than *A. urotrygoni.* The new spe-

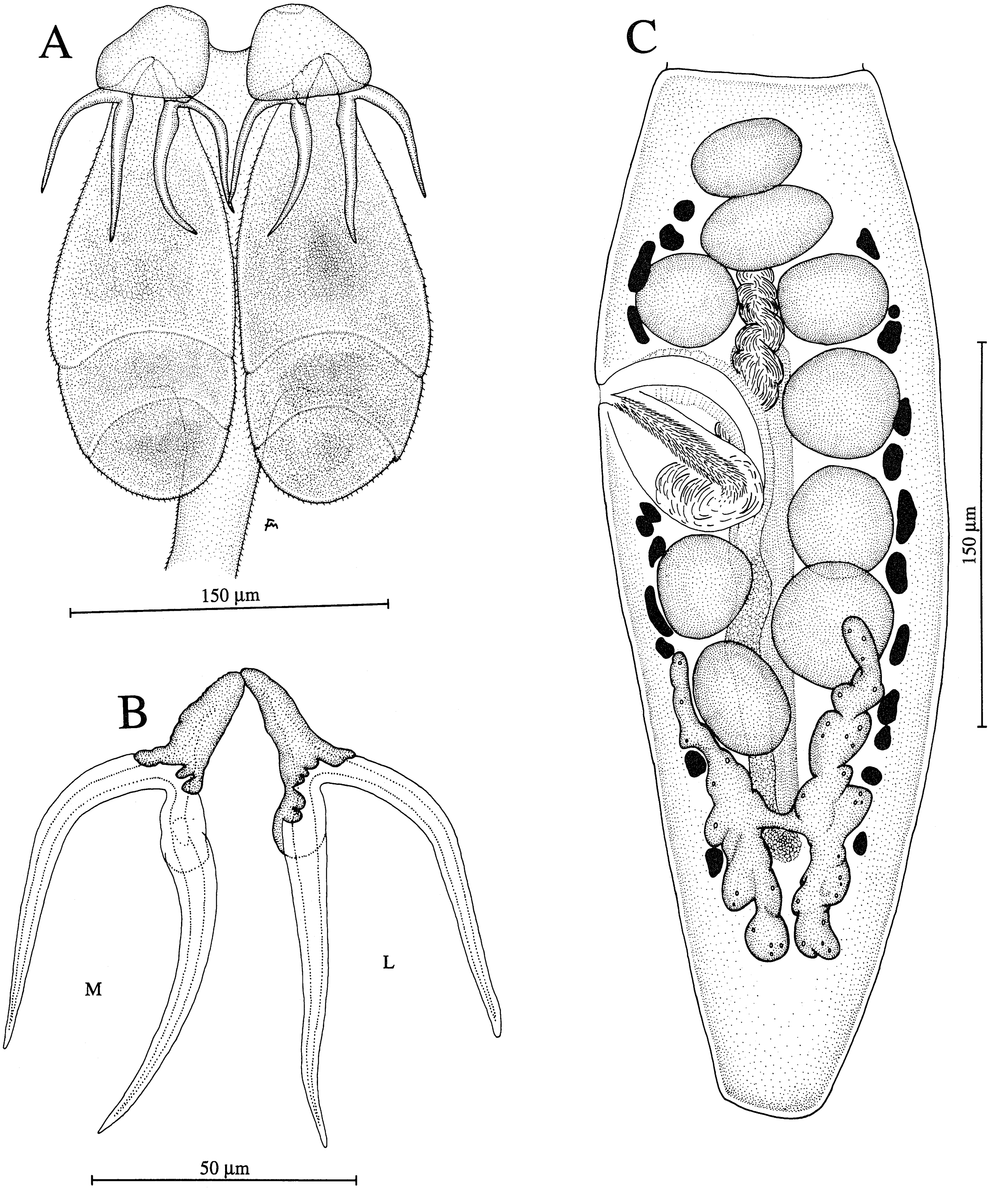

FIGURE 2. *Acanthobothrium minusculus* n. sp. A. Scolex. B. Medial and lateral hooks. C. Mature proglottis. M = medial hook; L = lateral hook.

cies can be distinguished from *A. quadripartitum* by the general shape of the bothridial hooks; in *A. quadripartitum* the handle is vertically aligned with the inner prong, whereas in *A. minusculus* the handle is not aligned with either prong. Finally, *A. minusculus* differs from *A. tripartitum* by possessing 6–10 testes per proglottis rather than 13–16, and an average of 7 rather than 3 proglottides.

Acanthobothrium minusculus was the only species of *Acanthobothrium* encountered in this study for which it was possible to determine a specific localization in the intestine; specimens were found only in the anteriormost chamber of the spiral valve of all 5 specimens of *U. tumbesensis* examined.

Acanthobothrium monksi n. sp.
(Fig. 3A–C)

Description (Based on 10 specimens): Strobila acraspedote, apolytic, 3.4–7.6 (5.4 ± 1.3, n = 8) mm long; composed 24–48 (33 ± 8, n = 9) of proglottides. Scolex 307–390 (356 ± 28, n = 5) wide, composed of 4 triloculate bothridia free at posterior end with velum between adjacent bothridia; each bothridium with apical sucker and pad, armed with pair of bifid hooks. Bothridia 368–445 (401 ± 28, n = 9) long by 166–189 (179 ± 8, n = 6) wide; anterior loculi 224–262 (244 ± 13, n = 9) long, middle loculi 64–96 (79 ± 10, n = 9) long, posterior loculi 58–96 (79 ± 11, n = 9) long. Ratio of locular lengths 1:0.33:0.33. Muscular pads 131–144 (136 ± 5, n = 5) wide, bearing apical sucker 48–64 (60 ± 5, n = 8) in diameter. Hook formula for lateral hooks (n = 16):

$$\frac{51 \pm 5\ (42\text{–}58) \quad 109 \pm 4\ (102\text{–}115) \quad 93 \pm 5\ (83\text{–}106)}{151 \pm 7\ (141\text{–}160)}$$

Hook formula for medial hooks (n = 16):

$$\frac{50 \pm 5\ (44\text{–}60) \quad 106 \pm 6\ (98\text{–}113) \quad 91 \pm 4\ (85\text{–}98)}{149 \pm 8\ (132\text{–}161)}$$

Ratio of bothridial length to total hook length 1:0.38 for lateral and medial hooks. Cephalic peduncle and scolex with spines. Peduncle 192–384 (290 ± 65, n = 9) mm long, 112–256 (199 ± 43, n = 9) wide at insertion to scolex. Immature proglottides wider than long. Mature terminal proglottides 282–570 (475 ± 86, n = 7) long by 218–374 (297 ± 48, n = 7) wide. Testes 26–42 (36 ± 4, n = 21) in diameter, 14–26 (21 ± 4, n = 11) in number; 4–7 (5 ± 1, n = 11) preporally, 2–6 (4 ± 1, n = 11) postporally, 8–15 (11 ± 2, n = 11) antiporally. Cirrus sac near midproglottis, 102–214 (156 ± 33, n = 8) long, not reaching midline of proglottis, 128–163 (146 ± 14, n = 8) wide, containing spined eversible cirrus. Genital atrium prominent. Genital pore 34–47% (37 ± 4, n = 7) of total length from anterior end, irregularly alternating. Vagina anterior to cirrus sac; vaginal sphincter present. Ovary near posterior end of proglottis, V-shaped in frontal view, X-shaped in cross section; 88–183 (135 ± 24, n = 20) wide at isthmus. Ovarian arms unequal in length; aporal arm 102–253 (173 ± 37, n = 9) long, extending anteriorly to posterior margin of cirrus sac; poral arm 106–189 (157 ± 26, n = 9) long, not reaching posterior margin of cirrus sac. Uterus indistinct in mature proglottis. Vitelline follicles extending from level of ovarian isthmus to near anterior end of proglottis; follicles roughly spherical, 13–32 (21 ± 6, n = 10) in diameter.

Taxonomic summary

Host: *Aetobatis narinari* (Euphrasen) (Rajiformes: Myliobatidae).
Site of infection: Spiral valve.
Locality: Puerto Jelí, Provincia de El Oro, Ecuador (3°16′S: 80°19′W).
Holotype: MEPN no. 3031.
Paratypes: MNHG no. 22100; HWML no. 39179.
Etymology: The species is named for Mr. Scott Monks.

Remarks

Acanthobothrium monksi is closely resembles *Acanthobothrium tasajerasi* Brooks, 1977 in *Himantura schmardae* (Werner) from the Caribbean coast of Colombia, in having a large globose cirrus sac, large genital atrium, a ratio of bothridial loculi length of approximately 1: 0.33:0.33, and an average of 20–25 testes per proglottis. However, the new species differs from *A. tasajerasi* by having an average of 21 vs. 35 testes per proglottis, and 24–48 vs.11–18 proglottides per strobila. Additionally, *A. monksi* has smaller bothridial hooks (handle, 42–60 μm; inner prong, 98–115 μm; outer prong, 83–106 μm; and total length, 132–161 μm) than *A. tasajerasi* (handle, 52–64 μm; inner prong, 110–130 μm; outer prong, 90–114 μm; and total length, 152–178 μm).

Other species of *Acanthobothrium* that are similar to *A. monksi* in scolex morphology and relative size of the cirrus sac include: *Acanthobothrium colombianum* Brooks and Mayes, 1980 in *A. narinari* (Euphrasen) from the Caribbean coast of Colombia; *Acanthobothrium himanturi* Brooks, 1977 in *H. schmardae* (Werner) also from the Caribbean coast of Colombia; *Acanthobothrium lineatum* Campbell, 1969 in *D. americana* Hilderbrand & Schroeder from Chesapeak Bay, Virginia; and *Acanthobothrium nicoyaense* Brooks and McCorquodale, 1995 in *A. narinari* from the Pacific coast of Costa Rica. *Acanthobothrium monksi* can be distinguished from these species by possessing a prominent genital atrium that is lacking in the species above. *Acanthobothrium monksi* further resembles *A. colombianum* by having 31–48 proglottides per strobila but differs by having an average of 36 rather than 46 testes per proglottis, and by having bothridial hooks with longer inner and outer prongs, averaging 185 μm rather than 149 μm in total length. *Acanthobothrium monksi* differs from *A. himanturi* by having shorter terminal proglottides (277–561 μm long vs. 780–1,260 μm), fewer testes per proglottis (14–26 vs. 38–40), and more proglottides per strobila (24–48 vs. 17–26). The new species differs from *A. lineatum* by having shorter bothridial hooks (132–161 μm long vs. 118–216 μm), shorter terminal proglottides (277–561 μm vs. 700–1,500 μm), fewer testes per proglottis (14–26 vs. 28–45), and more proglottides per strobila (24–48 vs. 6–19). Finally, *A. monksi* can be distinguished from *A. nicoyaense* by having more proglottides per strobila (24–48 vs. 13–19) and longer terminal proglottides (277–561 μm long vs. 710–1,261 μm long).

Acanthobothrium obuncus n. sp.
(Fig. 4A–C)

Description (based on 3 specimens): Strobila acraspedote, apolytic, up to 75.6 mm long; composed of up to 258 proglottides. Scolex up to 439 wide, composed of 4 triloculate sessile bothridia end with velum between adjacent bothridia; each bothridium with apical sucker and pad, armed with pair of bifid hooks. Bothridia 368–426 (399 ± 22; n = 6) long by 134–147 (140 ± 5, n = 5) wide; anterior loculi 234–256 (245 ± 8, n = 7) long, middle loculi 64–90 (78 ± 10, n = 7) long, posterior loculi 70–80 (76 ± 4, n = 7) long. Ratio of locular lengths to total bothridial length 1:0.33:0.31. Muscular pad 141–170 (158 ± 9, n = 4) wide, bearing apical sucker 33–48 (39 ± 5, n = 5) in diameter. Hook formula for lateral hooks (n = 2):

$$\frac{66 \quad 60\text{–}64 \quad 63\text{–}64}{126\text{–}130}$$

Hook formula for medial hooks (n = 2):

$$\frac{62\text{–}66 \quad 56\text{–}58 \quad 56\text{–}58}{115\text{–}120}$$

Ratio of bothridial length to total hook length 1:0.32 for lateral hooks and 1:0.30 for medial hooks. Cephalic peduncle and scolex without spines. Peduncle 26.7–27.8 mm long, 406–419 wide at insertion to scolex. Immature proglottides wider than long 174–374 (263 ± 48, n = 28) long by 561–1,090 (762 ± 177, n = 28) wide; mature proglottides 426–683 (540 ± 82, n = 14) long by 709–987 (800 ± 68, n = 14) wide; terminal proglottides 709–774 (750 ± 24, n = 4) long by 645–677 (658 ± 13, n = 4) wide. Testes 70–77 (73 ± 2, n = 11) in number, 45–90 (67 ± 10, n = 41) in diameter; 17–29 (21 ± 4, n = 10) preporally, 10–16 (13 ± 2, n = 10) postporally, 36–43 (39 ± 2, n = 10) antiporally. Cirrus sac near midproglottis, 258–322 (293 ± 22, n = 14) long, not reaching midline of proglottis, by 116–271 (163 ± 43, n = 14) wide, containing spined eversible cirrus. Genital atrium indistinct. Genital pore 47–56% (51 ± 3, n = 14) of the total length from anterior end, irregularly alternating. Vagina anterior to cirrus sac; vaginal sphincter absent. Ovary near posterior end of proglottis, U-shaped in frontal view, X-shaped in cross section; 645–1,245 (763 ± 216, n = 16) wide at isthmus. Ovarian arms unequal in length; aporal arm 258–484 (415 ± 75, n = 6) long, extending anteriorly to middle of cirrus sac; poral arm 161–258 (221 ± 35, n = 6) long, extending anteriorly to posterior

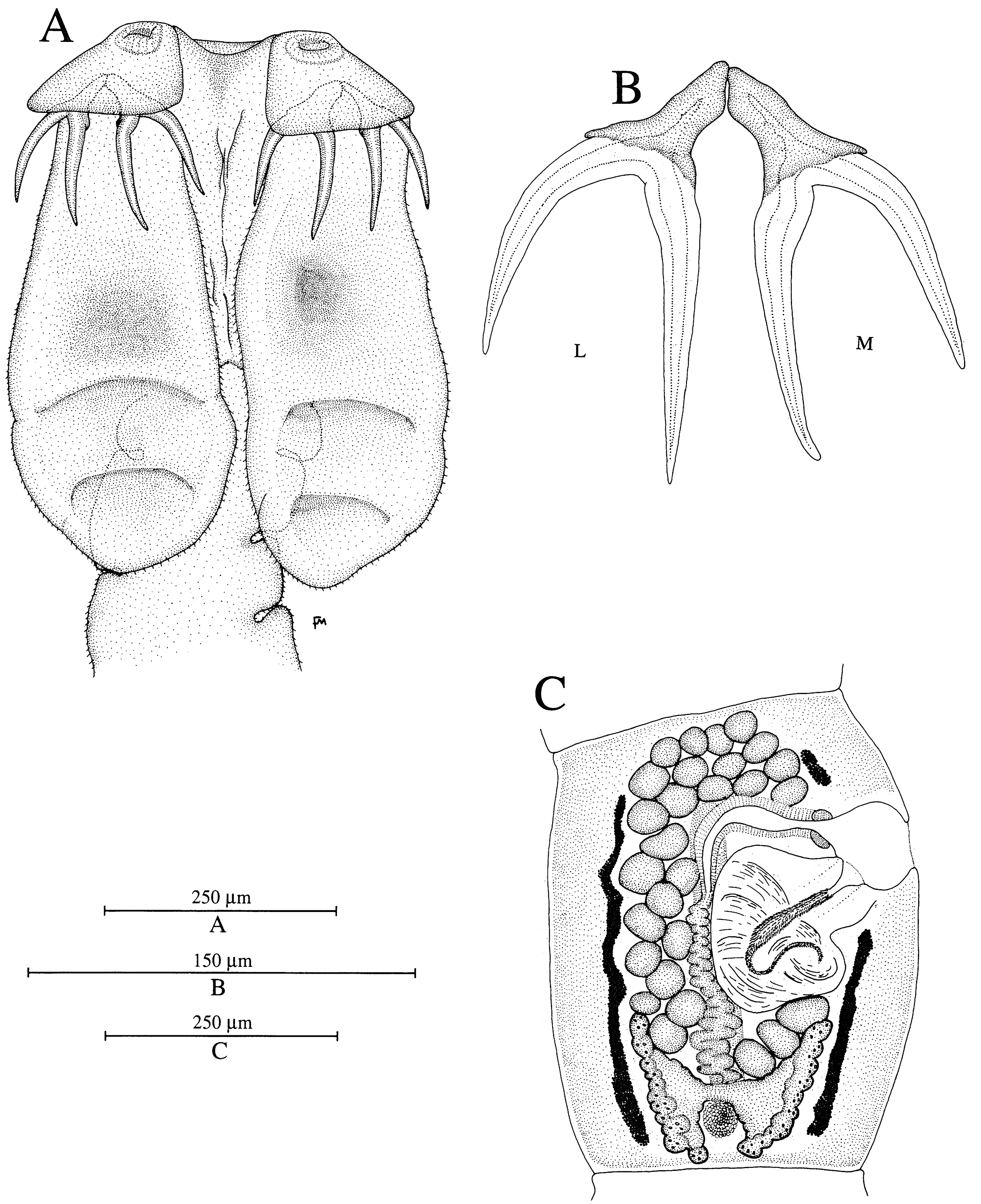

FIGURE 3. *Acanthobothrium monksi* n. sp. A. Scolex. B. Medial and lateral hooks. C. Mature proglottis. M = medial hook; L = lateral hook.

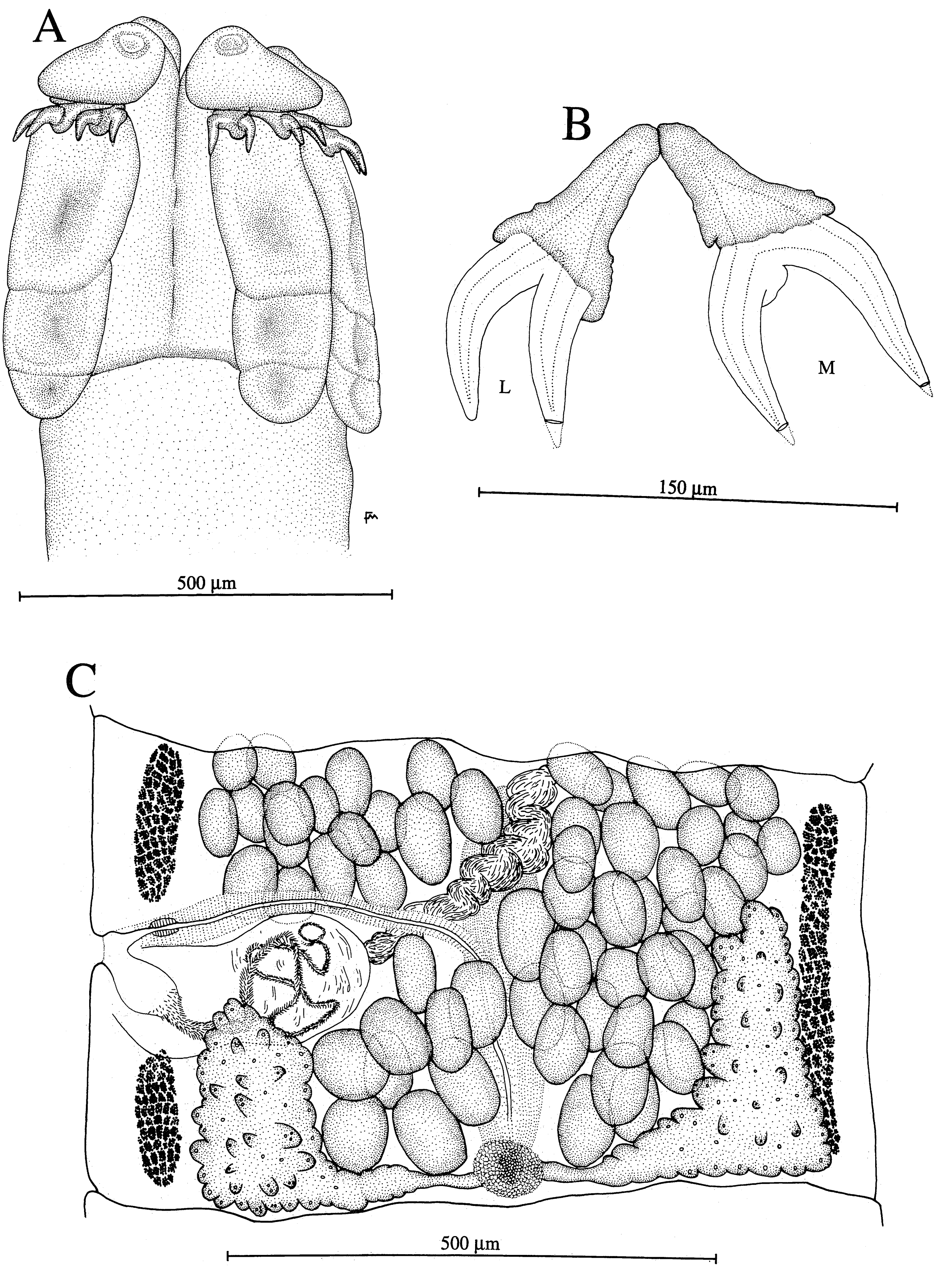

FIGURE 4. *Acanthobothrium obuncus* n. sp. A. Scolex. B. Medial and lateral hooks. C. Mature proglottis. M = medial hook; L = lateral hook.

margin of cirrus sac. Uterus indistinct in mature proglottis. Vitelline follicles extending from level of ovarian isthmus to near anterior end of proglottis; follicles roughly spherical, 13–23 (17 ± 3, n = 10) in diameter.

Taxonomic summary

Host: *Dasyatis longus* Garman (Rajiformes: Dasybatididae).

Site of infection: Spiral valve.

Locality: Puerto Hualtaco, Provincia de El Oro, Ecuador (3°16′S: 80°19′W).

Holotype: MEPN no. 3032.

Paratypes: MNHG no. 22101; HWML no. 39180.

Etymology: From the Latin "obuncus" which means "hook bent in" in reference to the hook shape of this species.

Remarks

Acanthobothrium obuncus resembles those species of *Acanthobothrium* with aspinose scoleces, muscular bothridia at least partially fused to the scolex at their posterior ends, bothridial hooks usually less than ⅓ the length of the bothridial, flat or low H- to V-shaped ovaries with symmetrical to asymmetrical arms extending anteriorly to, or nearly to, the cirrus sac, and vitellaria arranged in fields rather than a single or double row of follicles (see also Monks et al., 1996): *A. americanum* Campbell, 1969 in *D. americana* Hildebrand & Schroeder from Virginia; *Acanthobothrium chengi* Cornford, 1974 in *Dasyatis lata* Jordan & Everman from Hawaii; *Acanthobothrium chilensis* Rêgo, Vicente, and Ibáñez, 1968 in *Sarda chilensis* (Cuvier) from Peru; *A. crassicole* Wedl, 1885 in *Dasyatis pastinaca* (L.), *Dasyatis kuhlii* Müller and Henle, *Raja radiata* Donovan (= *Raja clavata*), and *Torpedo marmorata* Risso throughout the eastern North Atlantic and Mediterranean Sea (see Goldstein, 1967); *Acanthobothrium dasybati* Yamaguti, 1934 in *Dasyatis akajei* Müller & Henle, *Urolophus fuscus* Garman, and *Raja kenojei* Müller & Henle from Japan; *Acanthobothrium dighaensis* Srivastav and Capoor, 1980 in *Trygon marginatus* (Blyth) from India; *Acanthobothrium icelandicum* Nanger, 1972 in *Raja batis* L. from Iceland; *Acanthobothrium latum* Yamaguti, 1952 in *D. akajei* from Japan; *Acanthobothrium micrantha* Yamaguti, 1952 in *D. akajei* from Japan; *Acanthobothrium microcephalum* Alexander, 1953 in *Myliobatis californicus* (Gill) from California; *Acanthobothrium rubrum* Bilqees, 1980 in *Myrmyllo manazo* (BLK) from Pakistan; *Acanthobothrium tortum* (Linton, 1916) in *A. narinari* (Euphrasen) from Florida; and *Acanthobothrium wedli* Robinson, 1959 in *Raja nasuta* Müller & Henle from New Zealand. By exhibiting asymmetrical ovarian arms with the aporal arm extending anteriorly to the vaginal level and an average of 73 testes per proglottis, the new species resembles *A. americanum* (averaging 72 testes per proglottis), *A. chilensis* (80–100 testes per proglottis), *A. rubrum* (60–87 testes per proglottis), and *A. wedli* (80–100 testes per proglottis). All but 1 of the remaining species, some of which also exhibit asymmetrical ovarian arms, can be readily distinguished from *A. obuncus* by averaging more than 100 testes per proglottis. *Acanthobothrium dasybatis* can be readily distinguished from *A. obuncus* by having 40–70 testes per proglottis. The new species differs from *A. americanum* by having shorter bothridial hooks (averaging 120 μm [medial] and 131 μm [lateral] vs. 151 μm long) and fewer proglottides per strobila (258 vs. 507). *Acanthbothrium obuncus* differs from *A. chilensis* by having shorter bothridia (362–419 μm vs. 706–840 μm long; CHIOC 30308a, b—paratypes) and possessing slightly fewer testes per proglottis (70–77 vs. 80–100). The new species differs from *A. rubrum* by having shorter hooks (130–133 μm [lateral] and 118–123 μm [medial] vs. 470–490 μm) and fewer proglottides per strobila (up to 258 vs. 596). Finally, *A. obuncus* differs from *A. wedli* by having larger bothridial hooks (averaging 120 μm [medial] and 131 μm [lateral] vs. 90–105 μm long) and slightly fewer testes per proglottis (70–77 vs. 80–100).

Acanthobothrium campbelli Marques, Brooks, and Monks, 1995

Taxonomic summary

Host: *Dasyatis longus* Garman (Rajiformes: Dasybatididae), new host.

Site of infection: Spiral valve.

Locality: Puerto Hualtaco, Provincia de El Oro, Ecuador (3°16′S: 80°19′W), new locality.

Voucher specimens: MEPN no. 3033.

Acanthobothrium costarricense Marques, Brooks, and Monks, 1995

Taxonomic summary

Host: *Dasyatis longus* Garman (Rajiformes: Dasybatididae).

Site of infection: Spiral valve.

Locality: Puerto Hualtaco, Provincia de El Oro, Ecuador (3°16′S: 80°19′W), new locality.

Voucher specimens: MEPN no. 3034.

Both of these species were previously known only from their type locality, near Punta Morales in the Gulf of Nicoya, Costa Rica; this report thus constitutes new geographic distribution records for both species. *Acanthobothrium campbelli* was described originally in *U. chilensis,* so *D. longus* represents a new host record.

DISCUSSION

This study raises to 27 the number of species of *Acanthobothrium* reported from the eastern Pacific: 12 from California, 1 from Mexico, 4 from Costa Rica, 4 from Ecuador, 2 from Costa Rica and Ecuador, 1 from Peru, and 3 from Chile (Table I). *Acanthobothrium coronatum* (Rudolphi, 1819) and *A. dujardini* Benede, 1949, which have been reported from California, are European endemics, and thus the reports from California are undoubtedly misidentifications (see Goldstein, 1967). At present, only *A. campbelli* and *A. costarricense* are known from more than a single locality. We assume that additional inventories will add new species to this list and extend the geographical ranges of previously known species. Despite the undoubtedly incomplete nature of our database, it is clear that the species-rich nature of *Acanthobothrium* makes the group an excellent model system for a variety of evolutionary studies. Although we do not have a formal phylogenetic hypothesis for the entire genus at present, some components of relationships have begun to emerge.

First, we have now identified 6 probable pairs of sister species of *Acanthobothrium* occurring on each side of the Isthmus of Panama (geminate species sensu Jordan, 1908): *A. nicoyaense* and *A. colombianum* in *A. narinari*; *A. vargasi* in *D. longus* and *A. brevissime* in *D. sayi, D. sabina, D. americana,* and *Raja eglanteria*; *A. campbelli* in *U. chilensis* and *A. urotrygoni* in *U. venezuelae*; *A. monksi* in *D. longus* and *A. tasajerasi* in *H. schmardae*; *A. atahualpai* in *G. afuerae* and *A. fogeli* in *G. micrura*; and *A. obuncum* in *D. longus* and *A. americanum* in *D. americana.* It is also possible that either *A. costarricense* or *A. cimari,* both in *D. longus,* is the sister species of *A. himanturi* in *H. schmardae* or *A. lineatum* in *D. americana.* Marques et al. (1996) described *Acanthobothroides pacificus* and *Rhinebothrium geminum* in *Himantura pacifica,* listing synapomorphic traits indicating that they are the sister species of *Acanthobothroides thorsoni* and *Rhinebothrium magniphallum* in *Himantura schmardae* and *Dasyatis guttata* (Bloch and Schneider) from the Caribbean coasts of Colombia and Venezuela. As discussed by Marques et al. (1996), these apparent connections between the eastern Pacific and Caribbean helminth fauna of elasmobranchs probably date from the most recent formation of the Panamian Isthmus in the Pliocene.

A second major component of the eastern Pacific *Acanthobothrium* fauna is a morphologically similar species that exhibit

TABLE I. *Acanthobothrium* species reported from the Eastern Pacific.

Species
California
A. bajaensis Appy and Dailey, 1973
A. brachyacanthum Riser, 1955
A. goldsteini Appy and Dailey, 1973
A. hispidum Riser, 1955
A. holorhini Alexander, 1953
A. maculatum Riser, 1955
A. microcephalum Alexander, 1953
A. olseni Dailey and Mudry, 1968
A. parviuncinatum Young, 1954
A. rhinobati Alexander, 1953
A. robustum Alexander, 1953
A. unilateralis Alexander, 1953
Mexico
A. cleofanus Monks, Brooks, and Pérez Ponce de León, 1996
Costa Rica
A. campbelli Marques, Brooks, and Monks, 1995
A. cimari Marques, Brooks, and Monks, 1995
A. costarricense Marques, Brooks, and Monks, 1995
A. nicoyaense Brooks and McCorquodale, 1995
A. puntarenasense Marques, Brooks, and Monks, 1995
A. vargasi Marques, Brooks, and Monks, 1995
Ecuador
A. atahualpai n. sp.
A. campbelli Marques, Brooks, and Monks, 1995
A. costarricense Marques, Brooks, and Monks, 1995
A. minusculus n. sp.
A. monksi n. sp.
A. obuncum n. sp.
Peru
A. chilensis Rêgo, Vicente, and Inbáñez, 1968
Chile
A. annapinkiensis Carvajal and Goldstein, 1971
A. coquimbensis Carvajal and Jeges, 1980
A. psammobati Carvajal and Goldstein, 1969

antitropical distributions, e.g., *A. cleofanus* from Mexico is similar to *A. terezae* from southern Brazilian freshwater habitats (Monks et al., 1996), *A. minusculus* from Ecuador resembles *A. campbelli* from Costa Rica, and *A. atahualpai* is similar to *A. parviuncinatum* from California. If these morphological similarities are shown to include synapomorphic traits indicating a close relationship between these species, the pattern observed could be explained by White's (1982) hypothesis that temperature changes during the Cenozoic determined most of the antitropical distributions seen today. According to his hypothesis, a decrease in the earth's temperature in the middle Eocene dropped the tropical marine surface to about 20 C during the entire Oligocene and early Miocene, and the reappearance of tropical conditions in the middle Miocene caused a symmetrical disruption in the distribution of organisms unable to tolerate the increased tropical temperatures in low latitudes. If this hypothesis is correct, we would expect that a phylogenetic hypothesis for the genus *Acanthobothrium* would be congruent with the pattern that we recognize. This would imply that the sister species with antitropical distribution are no older than 20 million yr. Species representing both the northern and southern antitropical faunas, as well as species with continuous distribution in the eastern Pacific, e.g., *A. campbelli* and *A. costarricense,* might have sister species in the Caribbean due to the second formation of the Panamian Isthmus in the Pliocene, after the thermal events hypothesized for disrupting the low-latitude fauna during the Miocene. Thus, the factors that potentially determined the biogeographic patterns of amphi-isthmus *Acanthobothrium* species are complex. A more complete phylogenetic hypothesis for *Acanthobothrium* (and for other taxa) is needed to characterize additional sister species components and to test hypotheses of their origins.

Finally, when we search for similarities between species from this eastern Pacific/southern Caribbean faunal complex, we are always drawn to comparisons with species reported from the western Pacific and Indian Ocean: *Acanthobothrium electricolum* Brooks and Mayes, 1978 in *Narcine brasiliensis* Olfers from the Caribbean coast of Colombia is similar to *Acanthobothrium indicum* Subraphada, 1955 in *Narcine brunnea* Annadale from India (Brooks and Mayes, 1978); *A. costarricense, Acanthobothrium cimari,* and *Acanthobothrium puntarenense* in *D. longus* from Costa Rica (and Ecuador for *A. costarricense*) are similar to *Acanthobothrium lilium* Baer and Euzet, 1962 in *Dasyatis* sp. from Sri Lanka and *Acanthobothrium semnovesiculum* Verma, 1928 in *Dasyatis sephen* Müller and Henle from India; and *Acanthobothrium obuncus* from Ecuador is similar to *A. rubrum* from India and *A. wedli* from New Zealand. The hypothesis that these general similarities among species of *Acanthobothrium* might indicate phylogenetic relationships is supported by phylogenetic studies of the gnathostomid nematode genus *Echinocephalus* Molin, 1858 and the phyllobothriid tetraphyllidean cestode genus *Rhinebothrium* Linton, 1890 (Brooks and Deardorff, 1988). Such circum-Pacific biogeographical relationships date from the breakup of Pangaea at the beginning of the Cretaceous (Dietz and Holden, 1970; see also Brooks and McLennan, 1993).

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