A New Species of *Acanthobothrium* Van Beneden, 1849 (Eucestoda: Tetraphyllidea: Onchobothriidae) in *Dasyatis longus* Garman (Chondrichthyes: Myliobatiformes: Dasyatidae) from Chamela Bay, Jalisco, Mexico

Scott Monks

Daniel R. Brooks
*University of Toronto, dan.brooks@utoronto.ca*

Gerardo Pérez-Ponce de León
*Universidad Nacional Autonoma de Mexico, ppdleon@servidor.unam.mx*

Follow this and additional works at: [http://digitalcommons.unl.edu/parasitologyfacpubs](http://digitalcommons.unl.edu/parasitologyfacpubs)

Part of the Parasitology Commons


[http://digitalcommons.unl.edu/parasitologyfacpubs/281](http://digitalcommons.unl.edu/parasitologyfacpubs/281)
A NEW SPECIES OF ACANTHOBOOTHRIUM VAN BENEDEN, 1849
(EUCESTODA: TETRAPHYLLIDEA: ONCHOBOTHRIIDAE) IN
DASYATIS LONGUS GARMAN (CHONDRICHTHYES:
MYLIOBATIFORMES: DASYATIDIDAE) FROM
CHAMELA BAY, JALISCO, MEXICO

Scott Monks, Daniel R. Brooks, and Gerardo Pérez Ponce de León*
Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1

ABSTRACT: A new species of Acanthobothrium in Dasyatis longus from Chamela Bay, Jalisco, Mexico, is a member of a presumed clade of species diagnosed by being anapolytic or nearly so, having more than 100 testes per proglottis, with immature and mature proglottides wider than long to square, aspinose scolex, muscular bothridia fused to the scolex at their posterior ends, H- to V-shaped ovaries, relatively short symmetrical to asymmetrical ovarian arms that extend anteriorly to, or nearly to, the cirrus sac, and vitellaria arranged in fields rather than a single row of follicles. The new species most closely resembles Acanthobothrium terezae from the freshwater stingray Potamotrygon motoro in the following characters: bothridial hooks longer than 200 mm with inner hooks having bent asymmetrical prongs, an average of 130–140 testes per proglottis, and shallow genital atria located posterior to midline of proglottis. The new species differs from A. terezae by having outer hooks approximately the same size and shape as the inner hooks, inner hooks averaging 230 mm rather than 313 mm in total length, and cirrus sacs averaging 255 mm rather than 450 mm in length. The new species is unique among all described species of Acanthobothrium by having a cleft in the posterior margin of each apical bothridial pad. The apparent close relationship of the new species to one inhabiting a Neotropical freshwater stingray provides support for the hypothesized Pacific marine ancestry of Neotropical freshwater stingrays and raises the possibility that the Neotropical freshwater stingrays may not be monophyletic.

One of the most species-rich genera of tetraphyllidean eucestodes is Acanthobothrium van Beneden, 1849. During the course of an inventory of the parasite fauna of fish from Chamela Bay, Jalisco State, Mexico, we collected numerous specimens of a new species of Acanthobothrium inhabiting Dasyatis longus Garman. The new species adds to our understanding of the evolution of Neotropical freshwater stingrays (family Potamotrygonidae).

MATERIALS AND METHODS

Commercial fishermen captured specimens of Dasyatis longus with long lines 1–2 km from the shore of Chamela Bay, Jalisco State, Mexico (19°31’N, 105°04’E). Cestodes were removed from the spiral valve of the host, killed with hot tap water, transferred immediately to AFA for 24–48 hr, then stored in 70% ethanol. Specimens were stained either with Mayer’s carmalum or Ehrlich’s hematoxylin and mounted in Canadian balsam for examination as whole mounts. Serial sections of proglottides were cut 7–10 mm thick, stained with Masson’s trichrome, and processed in Peldri II (Pelco International, Riverdale, California) prior to sputter coating with gold. Specimens were examined using a Hitachi S-2500 scanning electron microscope. Figures were drawn with the aid of a drawing tube. Measurements are in micrometers unless otherwise stated; for some traits, ranges are given, followed by, in parentheses, its range. CHIBUNAM refers to the Colección Helminthológica del Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico; UNSMHWML refers to the Museum of Natural History, Geneva, Switzerland, Division of Invertebrates; FIOCruz Helmc Col refers to the Coleção Helminthológica, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil.

DESCRIPTION

**Acanthobothrium cleofanus n. sp.**

*(Fig. 1A, B, D, E)*

Description (based on 40 specimens): Strobila acraspedote, anapolytic, 3.7–6.7 (5.0 ± 1.0, n = 10) cm long; composed of 97–150 (120 ± 15, n = 10) proglottides. Scolex 731–1,030 (919 ± 75; n = 39) wide, composed of 4 sessile triloculate bothridia; each bothridium with apical sucker and pad, armed with pair of bifid hooks. Bothridia with thick muscular wall: 477–719 (636 ± 51; n = 40) long by 286–477 (417 ± 38, n = 40) wide. Locular wall dividing anterior and middle loculi relatively thick and muscular, between middle and posterior loculi relatively thin. Anterior loculus 127–235 (172 ± 26, n = 40) long, middle loculus 70–140 (113 ± 17, n = 40) long, posterior loculus 25–76 (54 ± 15, n = 40) long. Ratio of loculi lengths 1:0.6:0.3. Apical sucker 108–159 (136 ± 11; n = 40) in diameter; pads muscular, 232–331 (289 ± 22, n = 40) wide. Posterior edge of apical muscular pads extending posteriorly over handles of hooks, divided in middle to form 2 curved extensions, 1 over each hook. Outer prongs of bothridial hooks shorter than inner prongs; inner prong of hooks twisted. Hook formula for external hooks (n = 40):

\[
\begin{align*}
106 ± 5 & (95–117) \\
145 ± 10 & (117–170) \\
57 ± 6 & (47–69) \\
227 ± 13 & (198–258)
\end{align*}
\]

Hook formula for internal hooks (n = 40):

\[
\begin{align*}
105 ± 7 & (88–117) \\
146 ± 9 & (121–164) \\
58 ± 9 & (38–72) \\
232 ± 14 & (192–263)
\end{align*}
\]

Cephalic peduncle unspined, 1,150–3,530 (1,770 ± 51, n = 39) long. Immature proglottides wider than long; terminal proglottides 1,050–2,230 (1,700 ± 310, n = 31) long by 331–750 (539 ± 115, n = 29) wide. Testes 114–172 (140 ± 12, n = 38) in number, 32–64 (44 ± 8, n = 37) in diameter; 43–64 (14 ± 2, n = 39) preporally, 6–11 (8 ± 1, n = 39) postporally, 19–33 (26 ± 3, n = 39) antiporally. Testis size and number greatly reduced or lacking in terminal proglottides. Cirrus sac slightly posterior to midproglottis, containing spined eversible cirrus; extending medially to mid-proglottis, curved posteriorly in terminal proglottides. Cirrus sac 134–337 (255 ± 49, n = 36) long, 127–235 (170 ± 26, n = 36) wide. Cirrus 1,197 mm long when extended, slightly...
**Figure 1.** Acanthobothrium cleofanus n. sp. A. Scolex (scale bar = 500 μm). B. Enlargement of pair of bothridial hooks (scale bar = 100 μm). C. Acanthobothrium terezae, enlargement of pair of bothridial hooks (scale bar = 100 μm). Acanthobothrium cleofanus n. sp. D. Terminal attached proglottis (scale bar = 500 μm). E. Mature nonterminal attached proglottis (scale bar = 300 μm). In B and C, inner hooks are on the left, outer hooks on the right.
swollen at base; spines thin, averaging 13 long basally, 3 long at tip. Vas deferens coiled, extending anteriorly from cirrus sac to near anterior end of proglottis, filled with sperm in terminal proglottides. Genital atrium shallow. Genital pore located 54-65% (60 ± 3%, n = 36) of total length of proglottis from anterior end, irregularly alternating. vagina anterior to cirrus sac, vaginal wall glandular, vaginal sphincter present as a muscular thickening of the distal portion of the vagina. Vagina looping posteriorly on aporal side of midline, slightly coiled in terminal proglottides. ovary near anterior end of proglottis, X-shaped in cross section, with foliose branches. vaginal secretions from low changing proglottide mature: nearly U-shaped in immature proglottides, H-shaped in mature proglottides (Fig. 1E), and inverted A- or V-shaped in terminal proglottides (Fig. 1D). ovary 26-51 (44 ± 7, n = 9) wide at isthmus; arms unequal in length. Aporal arm 445-840 (616 ± 99, n = 32) long, extending to anterior margin of cirrus sac; poral arm 295-738 (501 ± 103, n = 32) long, extending to level of posterior margin of cirrus sac. Mehlis' gland immediately posterior to ovarian isthmus, seminal receptacle at level of or slightly anterior to isthmus. Vitelline follicles elongate oval in shape, 13-32 (19 ± 6, n = 34) wide, 35-69 (51 ± 10, n = 34) long, extending as narrow bands on each side of proglottis. Uterus saccate, narrowly elongate in terminal attached proglottides, expanded laterally filling available preovarian space in detached proglottides. Eggs not observed.

Taxonomic summary

Host: Dasyatis longus Garman (Chondrichthyes: Myliobatiformes: Dasyatidae).
Site of infection: Spiral valve.
 Locality: Chamela Bay, Jalisco, México (19°31'N, 105°04'E); June 1994.
Holotype: CHIBUNAM no. 2670.
Paratypes: CHIBUNAM no. 2671 (25 specimens); MNHG INV 38576 (5 specimens); MNHG INV 38576 (5 specimens); UNSHMWML 38576 (5 specimens).
Etymology: The specific epithet, meaning “cleft,” refers to the distinct structure of the apical pad, which is diagnostic for this species.

Remarks

Acanthobothrium van Beneden, 1849 currently contains more than 80 nominal species. Within that collection, there is a group characterized by the following features: (1) strobilae anapolytic or nearly so; (2) more than 100 proglottides per strobila; (3) bothridial hooks fusing to the scolex at their posterior ends; (4) bothridial hooks usually large, more than 150 μm long, with prongs short relative to the handle, giving them a stumpy appearance or with asymmetrical prongs, the outer prong being much shorter than the inner one; (5) more than 100 testes per proglottis, usually averaging 150 or more; (6) all except terminal proglottides wider than long or square; (7) foliose ovarian arms, usually flat or low H to V-shaped, extending anteriorly to the level of the cirrus sac, sometimes with asymmetrical arms; (8) genital pores indistinct and located at or posterior to midline of proglottis; and (9) vitellaria in fields rather than extending as narrow bands on each side of proglottis. Uterus saccate, narrowly elongate in terminal attached proglottides, expanded laterally filling available preovarian space in detached proglottides. Eggs not observed.

Acanthobothrium cleofanus (inhabiting D. akajei) and A. crassicolle (inhabiting D. pastinaca) are often found in Dasyatis pastinaca (Linnaeus, 1758) from the northeast Atlantic Ocean and Mediterranean Sea, Acanthobothrium herdmani Southwell, 1912 (inhabiting Dasyatis akajei Lindberg and Legeza, 1959) from the western Pacific Ocean, Ceylon, and the eastern Atlantic Ocean, Acanthobothrium grandiceps Yoshida, 1917 (inhabiting Dasyatis akajei Lindberg and Legeza, 1959) from the Sea of Ariake, Japan, Acanthobothrium micracanthum Yamaguti, 1952 (inhabiting D. akajei from the Sea of Ariake, Japan).
for *A. cleofanus* is 1:0.6:0.5, whereas for *A. terezae* it is approximately 1:0.6:0.8 (the excessively flattened condition of the paratypes of *A. terezae* did not permit precise measurements). In the new species, the apical sucker averages 136 μm in diameter; precise measurements of the apical suckers of the paratypes of *A. terezae* could not be obtained, but in the original description, Rêgo and Dias (1976) state a diameter of 87 μm. Strobilae of *A. cleofanus* are shorter than those of *A. terezae* (50 vs. 99 mm), largely because they have fewer proglottides (120 vs. 230).

*Acanthobothrium cleofanus* further differs from *A. terezae* by having inner and outer bothridial hooks that are virtually identical in shape (Fig. 1B), whereas those of *A. terezae* differ both in size and shape (Fig. 1C). The shape of the outer hooks of *A. terezae* are typical of most species of *Acanthobothrium*, and the shape of the inner hooks is highly similar to both hooks of *A. cleofanus*. The condition exhibited by *A. terezae* may thus be an evolutionary transition from the condition exhibited by most members of the genus to that found in *A. cleofanus*. The hooks on each bothridium of *A. cleofanus* are approximately equal in total length, a condition common to most members of the genus. In *A. terezae*, by contrast, the total length for the inner hooks of *A. terezae* (average 313 μm) is substantially greater than that of the outer hooks (average 226 μm) or the hooks of *A. cleofanus* (average 230 μm). This appears to be an autapomorphic trait for *A. terezae*. Finally, as mentioned above, *A. cleofanus* differs from *A. terezae* and all other reported species of *Acanthobothrium* by having a cleft in the posterior margin muscular apical pads of each bothridium (Figs. 1A, 2A, B). If *A. terezae* is the sister species of *A. cleofanus*, it might exhibit a similar structure, but we could not confirm the details of the structure of the bothridial apical pads of *A. terezae* due to the excessively flattened nature of the scoleces of the paratypes we examined. This character is readily visible using scanning electron microscopy and should be re-evaluated by examination of additional specimens of *A. terezae*.

**DISCUSSION**

If *A. cleofanus* is the sister species of *A. terezae*, as suggested above, it offers 2 significant pieces of information about the evolution the helminth fauna of Neotropical freshwater stingrays (family Potamotrygonidae). First, *A. cleofanus* provides further corroboration for the hypothesis that potamotrygonids have a Pacific marine ancestry (Brooks et al., 1981; Brooks and Deardorff, 1988; Brooks, 1992, 1995; Brooks and McLennan, 1991, 1993), because it occurs in a marine host inhabiting the eastern Pacific coast. Second, *A. cleofanus* offers at least partial refutation of the hypothesis that potamotrygonids are derived from a urolophid ancestor (Brooks et al., 1981). This study presents the first evidence that *A. terezae*, which inhabits *Potamotrygon motoro* (Natterer, 1841) and *Paratrygon aerieba*, is not the sister species of the clade containing *Acanthobothrium quinonesi* Mayes, Brooks, and Thorson, 1978, *Acanthobothrium regoi* Brooks, 1981, and *Acanthobothrium amazonensis* Mayes, Brooks, and Thorson, 1978, all of which inhabit species of *Potamotrygon*. The latter 3 species are highly similar to species of *Acanthobothrium* inhabiting species of *Urolophus* (including *Urobatis* of some authors), suggesting a relationship between *Urolophus* and *Potamotrygon*. The putative sister species relationship between *A. cleofanus*, which inhabits a member of *Dasyatis*, and *A. terezae* suggests a relationship between *Dasyatis* and the monotypic *Paratrygon*. In fact, none of the species of the putative clade of *Acanthobothrium* species of which *A. ter-
ezae and A. cleofanus are members inhabit urolophid stingrays. This provides a strong indication that the helminth fauna of potamotrygonids is not monophyletic, suggesting 1 of 2 possibilities. First, it is possible that the helminth fauna of potamotrygonids represents a mixture of resident and colonizer clades, involving parasites from urolophid and dasyatidid hosts. In this case, current phylogenetic hypotheses indicating closer relationships between dasyatids and potamotrygonids than between urolophids and potamotrygonids would suggest that the colonizers are from urolophids. The second possibility is that the helminths of freshwater stingrays represent 2 resident faunas, suggesting that potamotrygonids themselves are not monophyletic, perhaps with Paratrygon Duméril, 1865 (and possibly Plesiorygon Rosa, Castello and Thorson, 1987, for which no parasites are as yet reported) being most closely related to dasyatids, and Potamotrygon Garman, 1877 being most closely related to urolophids. In this case, the current high levels of homoplasy depicted in phylogenetic studies of stingray taxa might result from the polyphyletic status of the Potamotrygonidae. In either case, additional studies of helminths inhabiting marine stingrays along the eastern Pacific coast, those inhabiting species of Potamotrygon, and especially of those inhabiting the monotypic Plesiorygon and Paratrygon, will be necessary to resolve the original source of each of the parasite groups. Furthermore, new phylogenetic analyses of stingray relationships considering the possibility that potamotrygonids are not monophyletic should be investigated.

ACKNOWLEDGMENTS

We gratefully acknowledge the help of Felipe Noguera, Chief of the Estación de Biologia Chamela, for assistance in allowing us access to station facilities; the estimable Don Toño, who collected the fish examined in this study; and Deborah McLennan, University of Toronto, who helped collect parasite specimens. We thank Fernando Marques for his preparation of the illustrations and Dely Noronha, Instituto Oswaldo Cruz, for loan of the specimens of A. terezae. This study was funded by operating grants A7696 from the Natural Sciences and Engineering Council (NSERC) of Canada to D.R.B. and IN201593 from the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica-UNAM to G.P.P.dL.

LITERATURE CITED