J. Parasitol., 89(5), 2003, pp. 1018–1024

CESTODES OF CESTODES OF PERUVIAN FRESHWATER STINGRAYS

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ABSTRACT: Adult tetraphyllidean tapeworms (Platyhelminthes: Eucestoda) from the spiral intestines of 3 species of potamotrygonid stingrays (*Paratrygon aiereba*, *Potamotrygon castexi*, and *Potamotrygon motoro*) in the Madre de Dios river in Peru were found to host numerous cysts embedded in their parenchymal tissues. Histological sections of the cysts revealed the presence of a scolex bearing 4 suckers and an unarmed apical organ consistent with larval stages of both Cyclophyllidea and Proteocephalidea. To further elucidate their identities, partial 28S ribosomal DNA (rDNA) sequences were characterized from 3 cysts and 4 adult *Rhinebothrium* spp. 'host' worms and screened against all available cestode 28S rDNA data. Initial BLAST screening and subsequent alignment ruled out the possibility that the cysts were cyclophyllidean, and the cyst and adult sequences were thus aligned together with all available lecanicephalidean, litobothriidean, proteocephalidean, and tetraphyllidean sequences. Sequences from all 3 cysts were identical, and phylogenetic analysis clearly placed them among derived members of the Proteocephalidea, although no exact match was found. Sequences from the adult host worms formed 2 identical pairs and grouped together with other tetraphyllidean species from rays. These results are compared with records of hyperparasites of South American catfish cestodes. This is the first confirmed record of a proteocephalidean cestode parasitizing a tetraphyllidean cestode.

Parasites such as protozoans, nematodes, acanthocephalans, trematodes, monogeneans, copepods, and cestodes are themselves known to be hosts to other parasites. For example, parasitic protozoans have been reported to be hosts to other protozoans, including ciliates, flagellates, and sarcodines (Dogiel, 1965). Metazoan parasites may be infected with other metazoans; for example, nematodes parasitize amphistome trematodes (Platyhelminthes) of cyprinid fishes (Sey and Moravec, 1986). Albeit rare, tapeworms have been reported to be infected with other metazoan parasites, including nematodes (El-On et al., 1998), trematodes (Chiriac et al., 1975), acanthocephalans (Amin and Cowen, 1990), and even other tapeworms (Riggenbach, 1896; Gayevskaya, 1978; Rego and Gibson, 1989). In fact, there are 7 reports of tapeworms infecting fish tapeworms in South America, all of which involve unidentified larval cestodes from adult proteocephalideans collected from catfishes (Siluriformes) (Riggenbach, 1896; Rego and Pavanelli, 1985; Fortes and Hoffman, 1987; Rego and Pavanelli, 1987; Rego and Gibson, 1989; de Chambrier and Rego, 1995; Fortes and Hoffman, 1995). In none of these instances was the identity of the cysts confirmed, although several authors agreed that the cysts were consistent with either proteocephalidean or cyclophyllidean larval forms.

South American freshwater stingrays belong to the Potamotrygonidae (Elasmobranchii: Batoidea); all are endemic to South America's Atlantic draining rivers (Rosa, 1985). An initial survey of the parasites of the potamotrygonids of southeastern Peru resulted in the discovery of strobilae and scolices of several tetraphyllidean tapeworm species bearing numerous cysts that appeared to be larval cestodes. Larval cestodes have not been reported from cestodes in elasmobranchs. This new finding prompted a detailed study of these cysts using morphological and molecular methods.

MATERIALS AND METHODS

Collection and preparation of specimens

Freshwater stingrays were collected with hook and line in conjunction with local fisherman using a diversity of catfishes (Siluriformes) as bait. All collections were made in the Madre de Dios Department of Peru close to Boca Manu, a point where the Manu and Alto Madre de Dios rivers merge (12°16′350″S, 70°55′789″W). Four specimens of *Potamotrygon motoro* (Müller & Henle, 1841) Garman 1877, 6 of *Potamotrygon castexi* Castello & Yagolkowski, 1969, and 3 of *Paratrygon aiereba* (Müller & Henle, 1841) Garman 1877, were caught and examined for parasites from 25 to 31 May 2001. Stingray collections and export of parasites to the United States for further study were conducted under the guidelines of a permit granted to F.B.R. by the Peruvian Ministry of Fisheries in Lima (Resolución Directoral CE-00152002).

The spiral intestine of each stingray was removed, opened with a ventral incision down the midline of the intestine, and examined for worms. Tapeworms were removed and either placed in 95% ethanol or fixed in formalin diluted to 4% by mixing with 0.6% saline and subsequently transferred to 70% ethanol for storage. Specimens prepared for light microscopy were hydrated in a graded ethanol series, stained in Delafield's hematoxylin, dehydrated, and cleared in methyl salicylate, and mounted on glass slides in Canada balsam. Histological sections of a subsample of the specimens of each host tapeworm species found were prepared by embedding in paraplast and sectioning at 5-μm inter-

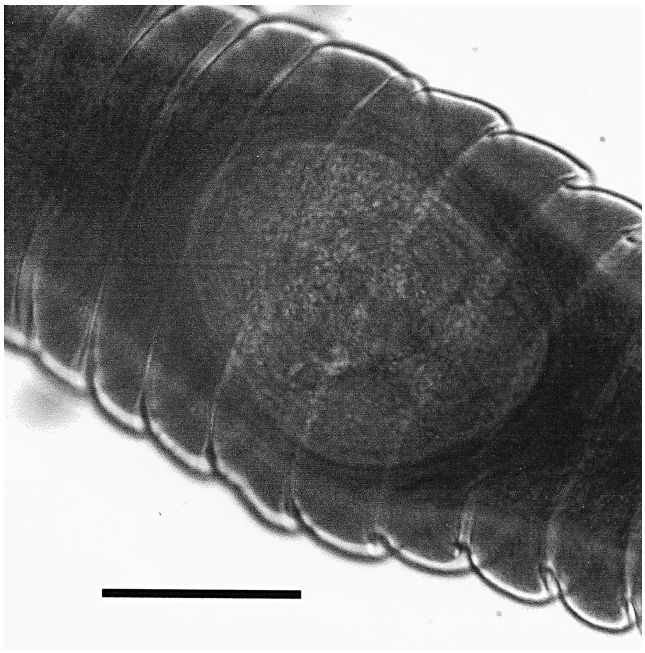

FIGURE 1. Cestode cyst in situ in strobila of *Rhinebothrium* sp. Bar = 120 μm.

Received 9 December 2002; revised 28 April 2003; accepted 28 April 2003.

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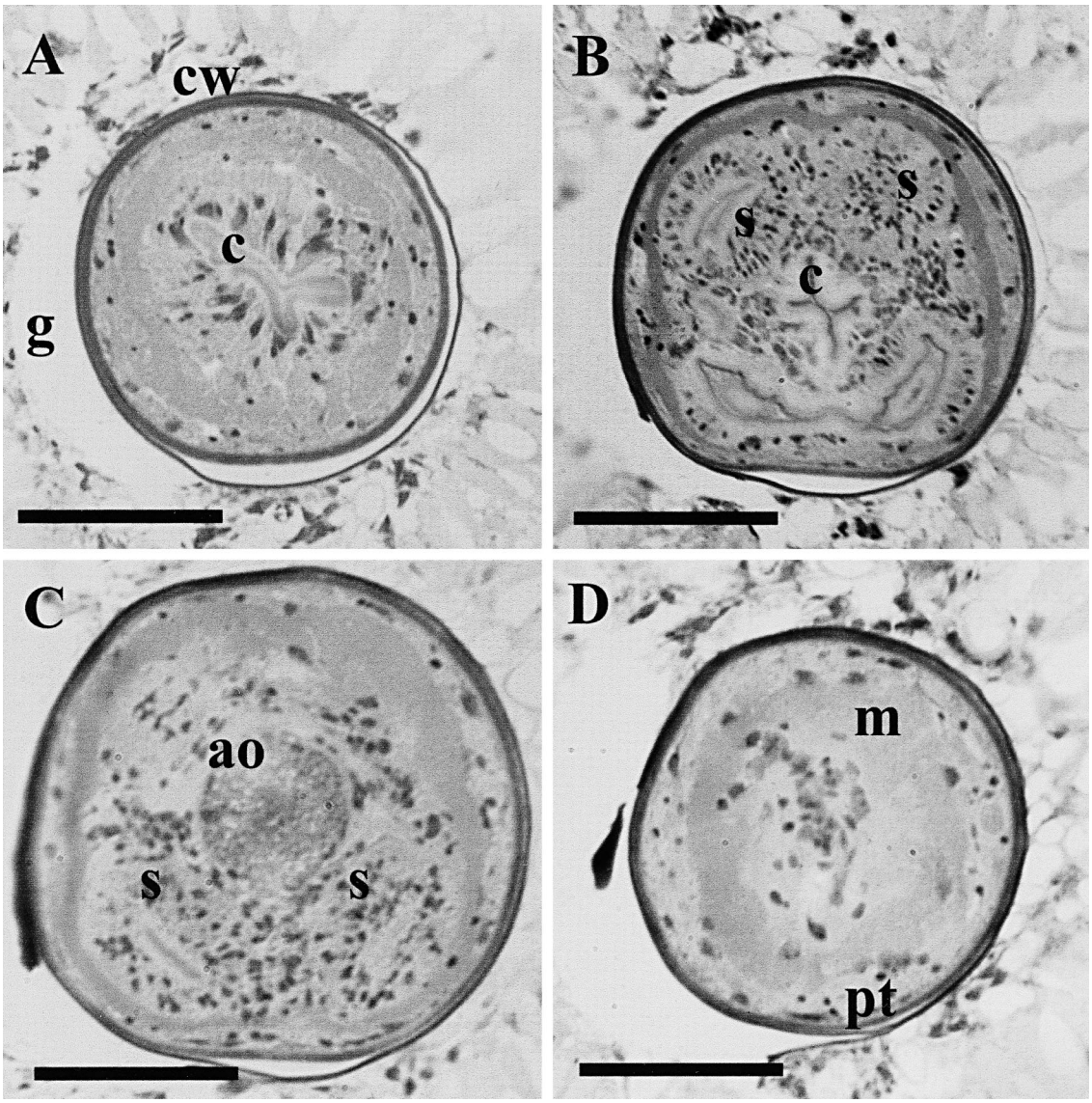

FIGURE 2. Serial thin cross sections of a cyst within a single *Rhinebothrium* sp. host tapeworm. s, suckers; c, cavity; ao, apical organ; pt, parenchymal tissue; m, muscle; cw, cyst wall; g, gap. Bar = 50 μm.

vals using a rotary microtome. Sections were stained in Gill's hematoxylin, counterstained in eosin, and cleared in xylene. In addition, specimens of each tapeworm species exhibiting cysts were embedded in standard EM epoxy resin (Araldite 6005, SPI-pon 812, and dodecenyl succinic anhydride) after infiltration with propylene oxide and equal parts of DMP-30 accelerator and resin, and semithin serial sections were prepared at 2-μm intervals with an ultramicrotome. Thin sections were stained with methylene blue and azure II and counterstained with fuchsin (Chien, 1992).

The mucosal walls of stingray spiral intestines were examined with a dissection microscope for the presence of cysts similar to those found in the tapeworms.

DNA isolation, polymerase chain reaction amplification, sequencing, and analysis

Adult specimens of 2 tetraphyllidean species of *Rhinebothrium* collected from *P. aiereba* were fixed in 95% ethanol, and the cysts found within were dissected from the host parenchymal tissue. Both the cysts and tissues from the adult host worms were prepared for genomic DNA (gDNA) extractions as follows. Ethanol was replaced with 1M Tris–ethylenediamine-tetraacetic acid (pH 8) buffer by repeated washings, and the gDNA was extracted using a Qiagen® DNeasy™ tissue kit following manufacturer-recommended protocols, with the exception that they were left overnight in proteinase K in a rotating incubator, and the final elution volume was 200 μl. The gDNA was further concentrated to a volume of ~20 μl using Millipore Microcon® columns. Polymerase chain reaction (PCR) amplifications of the D1–D3 regions (~1,400 bp) of the 28S ribosomal DNA (rDNA) gene were performed as described by Olson et al. (2001) using the primers LSU5 (5′-TAGGTCGAC CCGCTGAAYTTAAGCA-3′) and 1200R (5′-GCATAGTTCACCATC TTTCGG-3′). PCR amplicons were purified using Qiagen Qiaquick™ columns, cycle-sequenced directly from both strands using ABI BigDye™ chemistry, alcohol-precipitated, and run on an ABI Prism 377™ automated sequencer. Both the PCR primers as well as the internal primers ECD2 (5′-CTTGGTCCGTGTTTCAAGACGGG-3′), 300F (5′-CAAGTACCGTGAGGGAAAGTTG-3′), 400R (5′-GCAGCTTGA CTACACCCG-3′), and 900F (5′-CCGTCTTGAAACACGGACCAA G-3′) were used for sequencing. Ribosomal DNA sequences were characterized from 4 adult tetraphyllidean worms and from cysts from 3 of the 4 adult worms (GenBank AY193880-83 and AY193877-79, respectively).

Contiguous sequences were assembled using Sequencher™ version

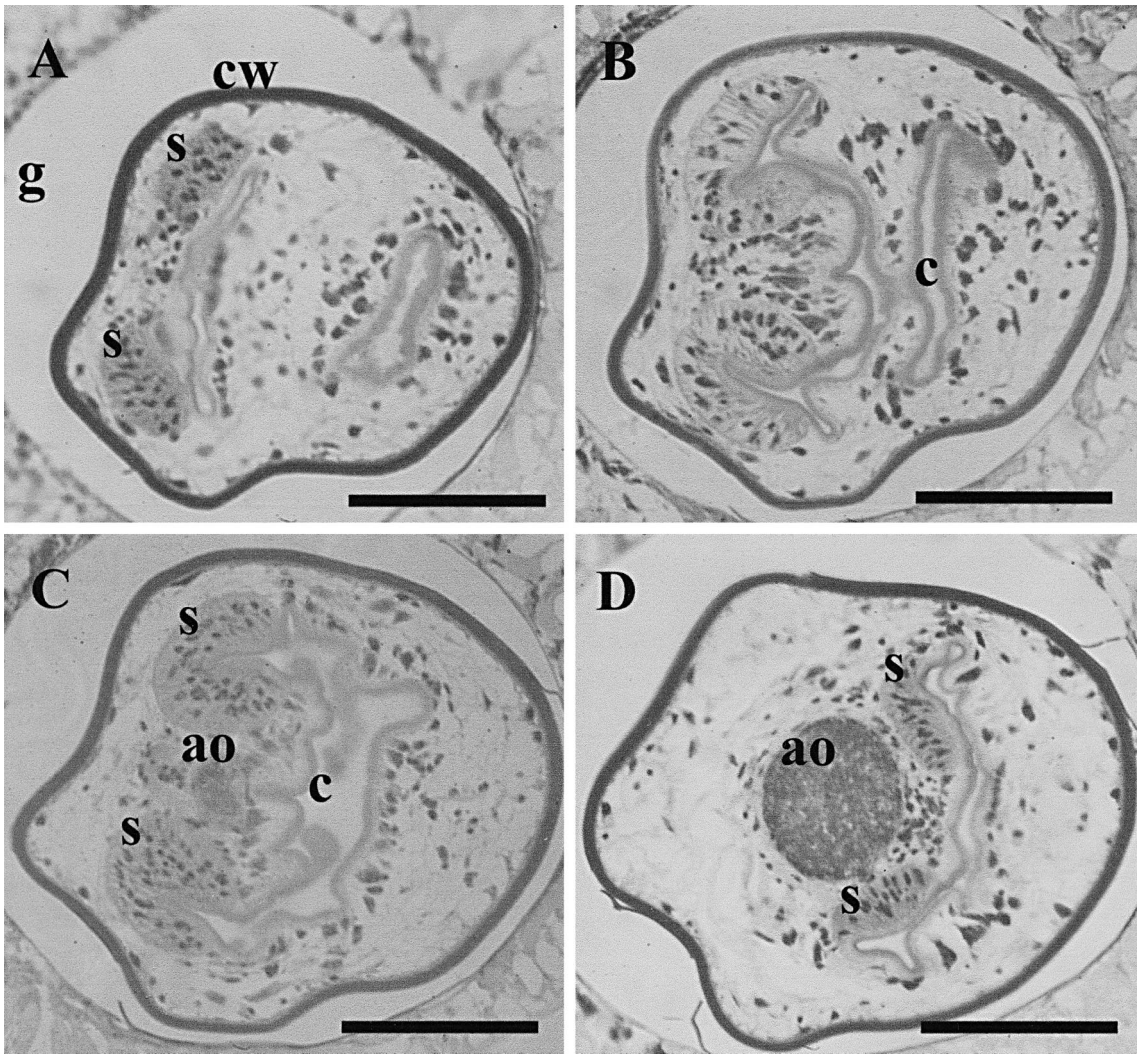

FIGURE 3. Serial thin transverse sections of a cyst within a single *Rhinebothrium* sp. host tapeworm. s, suckers; c, cavity; ao, apical organ; cw, cyst wall; g, gap. Bar = 50 μm.

3.1.1 (GeneCodes Corp., Ann Arbor, Michigan), screened using BLAST (Altshul et al., 1997), and aligned manually using MacClade (D.R. Maddison and W.P. Maddison, 2000), together with all available lecanicephalidean (3), litobothriidean (2), tetraphyllidean (17), and proteocephalidean (65) 28S rDNA sequences (see Zehnder and Mariaux, 1999; Zehnder and de Chambrier, 2000; Zehnder et al., 2000; Olson et al., 2001; Brickle et al., 2002 and for host and collection information regarding previously published cestode sequences). Because the 65 proteocephalidean sequences of Zehnder and Mariaux (1999) were shorter (~1,100 bps) than those generated in this study (~1,400 bps), the analysis was necessarily restricted to the sites for which data were available for all the taxa. Regions of ambiguity were excluded (160 of 1,112 alignment sites), and the reduced data set was analyzed by maximum parsimony using PAUP* (Swofford, 1998), as described by Olson et al. (2001). Resulting trees were rooted with the lecanicephalidean taxa on the basis of previous analyses of cestode interrelationships (Olson et al., 2001).

RESULTS

Hyperparasitism and cyst morphology

Tapeworms in 5 of 13 stingray individuals of 3 stingray species were found to possess cysts embedded in the scolex, neck, or strobila. The cysts were readily visible and were generally associated with an enlargement of the host tapeworm at the site of their inclusion (Fig. 1). The tapeworms parasitizing *P. aiereba* were most heavily infected. For example, 10 of 26 tapeworms from an individual of *P. aiereba* possessed cysts, with an overall combined intensity of 4.1 cysts per worm. No cyst was detected in the intestinal wall of any stingray specimen.

At least 8 species of tetraphyllideans representing both the Onchobothriidae and Phyllobothriidae were collected from the 3 stingray species. Tapeworms bearing cysts included *Anindobothrium guariticus* Marques, Brooks & Lasso, 2001, *Rhinebothrium* sp., and *Potamotrygonocestus* sp. in *P. aiereba*; *Rhinebothroides* sp. in *P. motoro*; and *Rhinebothroides* sp. in *P. castexi*.

Because of the small size of the cysts (120–230 μm in length), semithin epoxy sections proved to be more informative than paraplast sections. Semithin sections through 2 different cysts with different orientations are shown in Figures 2, 3. The cysts were found to bear a scolex with 4 suckers and lacked armature (Figs. 2, 3). Sections (Figs. 2A, B, 3) reveal a central

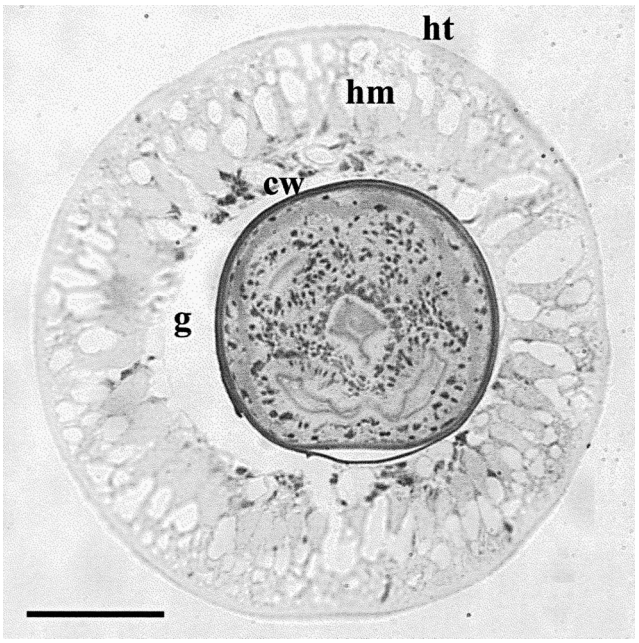

FIGURE 4. Semithin section of a cyst within the host tapeworm, *Rhinebothrium* sp. cw, cyst wall; g, gap; ht, host tegument; hm, host muscle. Bar = 50 μm.

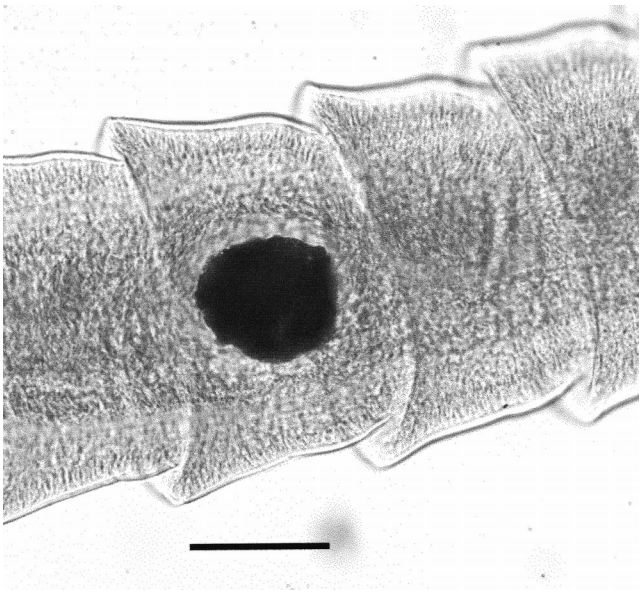

FIGURE 5. Host tapeworm, *Rhinebothrium* sp. containing a fully darkened cyst. Bar = 100 μm.

cavity that expands and is contiguous with the region of the body bearing the suckers (Fig. 3). A darkly staining anucleate concentration of spheres, consistent with an apical organ, was visible in the center of the cyst (Figs. 2C, 3C, D). Anterior and posterior portions of the cysts consisted entirely of parenchymal tissue and muscle (Fig. 2A, D). No evidence of primary lacunae was visible (Figs. 2, 3). The scolices appear to be withdrawn and inside out, or invaginated (Figs. 2, 3).

Host tapeworms retained intact tegument and muscle layers (Fig. 4). The cysts were delimited from the host cestode by a lamellar cyst wall, apparently of cyst origin (Figs. 2–4). However, cyst walls were generally surrounded by a conspicuous gap, visible as a halo (Figs. 2–4). A number of cysts appeared to be completely or partially surrounded by dark, granular pigmentation, possibly consistent with melanization (Fig. 5).

The presence of 4 suckers and an apical organ allowed identification of the cysts as cestodes belonging either to the Cyclophyllidea or to the Proteocephalidea.

Molecular analyses

The rDNA sequences of the 3 cysts were identical, whereas those of the 4 adult 'host' worms formed 2 identical pairs of sequences, differing from each other by 7 transition and 3 transversion substitutions (1,263 total bp compared), supporting the observation based on morphology that 2 different species of *Rhinebothrium* were present among the infected adults characterized genetically. Initial screening of the sequences against the GenBank public database using BLAST showed that the cyst sequences were most similar to those from proteocephalidean cestodes. A small number of tetraphyllidean species were also returned, albeit as low-scoring matches. No cyclophyllidean sequence showed sufficient similarity to be returned by the BLAST results, and subsequent manual inspection readily showed the disparity between the 28S sequences of the cysts and those of cyclophyllideans. Phylogenetic analysis was thus restricted to sequences from members of those orders including and most closely allied to the Proteocephalidea (Fig. 6) to maximize the number of alignable positions. This served both to increase the amount of usable data as well as to reduce homoplasy that would be introduced by including less closely related taxa. The analysis resulted in a single most parsimonious tree (Fig. 6) and showed the cyst sequences to be deeply nested within the Proteocephalidea, whereas sequences of the adult host worms grouped in the basal lineages of the 'Tetraphyllidea' among other species that infect batoid elasmobranchs (Fig. 6).

DISCUSSION

The possession of 4 suckers and an apical organ suggests that the cysts represent either larval cyclophyllidean or proteocephalidean cestodes. Cestode larval stages having invaginated scolices and lacking primary lacunae are merocercoids, as defined by Chervy (2002). The histological work implies that the invaginated scolex would evaginate and evert during progression to the adult stage.

Morphological identification of tapeworms is best done with fully developed adult specimens; very few features facilitate identification of larvae. Thus, molecular analysis was essential in allowing us to exclude the Cyclophyllidea as a possibility and to more precisely determine the identity of the cysts as members of the Proteocephalidea. However, failing an exact, or near-exact (see Brickle et al., 2002), genetic match, the specific identity of the cysts remains unknown, and even generic designation is made difficult by the fact that many common proteocephalidean genera have been shown to be polyphyletic, e.g., *Nomimoscolex*, *Ophiotaenia*, and *Proteocephalus* (see Zehnder and Mariaux, 1999 and Fig. 6). Yet, phylogenetic analysis suggests a close affinity of the cysts to species such as *Ageneiella brevifillis* de Chambrier & Vaucher, 1999 from Brazil and *Nomimoscolex admonticellia* (Woodland, 1934), *Ophiotaenia para-*

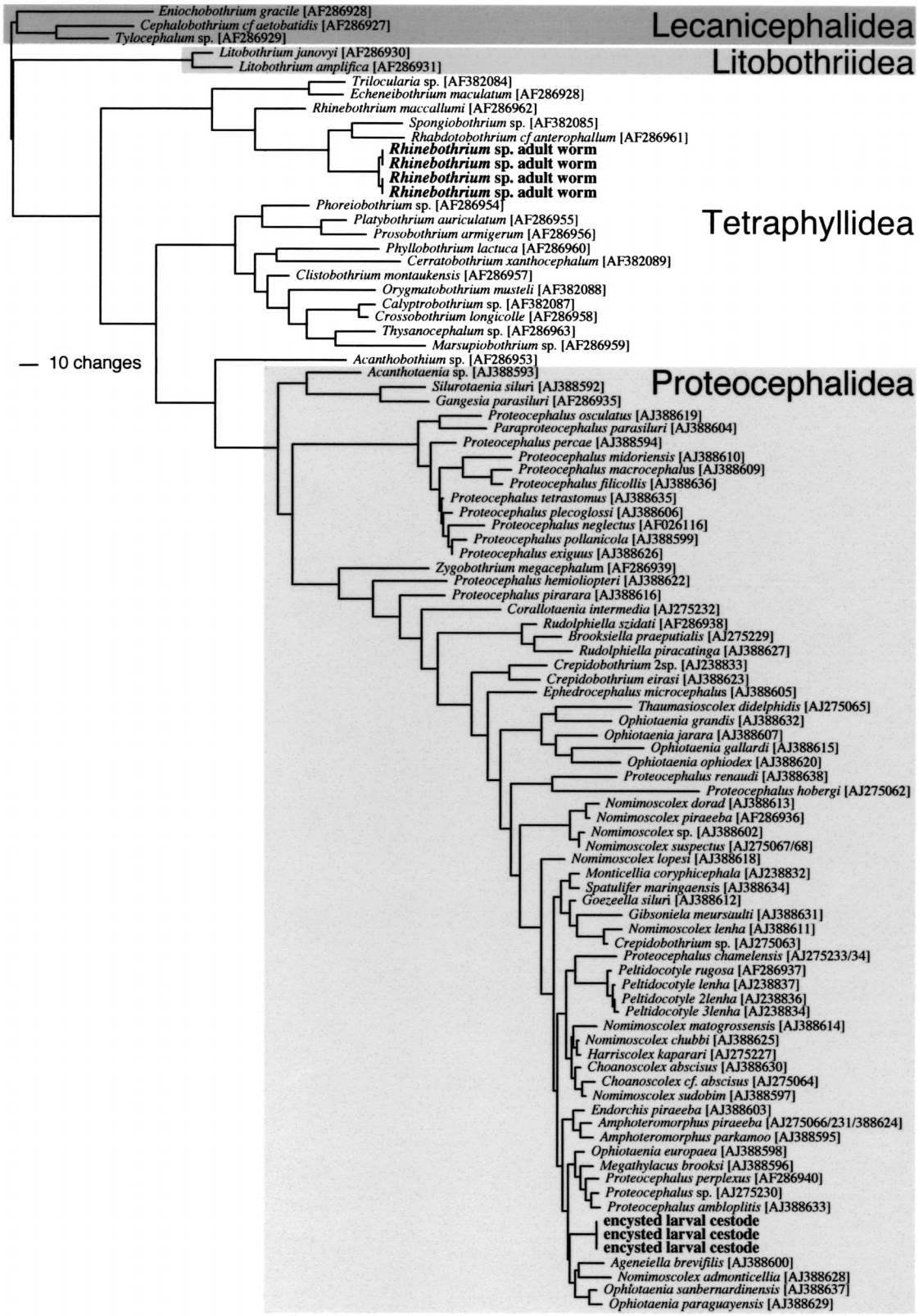

FIGURE 6. Phylogram based on maximum parsimony analysis of 28S rDNA (962 characters, consistency index = 0.35, retention index = 0.71, rescaled consistency index = 0.25, homoplasy index = 0.65). GenBank sequence accession numbers shown in brackets.

guayensis (Rudin, 1917), and *O. sanbernardinensis* (Rudin, 1917) from Paraguay, which, as adults, parasitize water snakes and catfishes, respectively (see Fig. 6). The tree in Figure 6 is presented for diagnostic purposes; readers interested specifically in the interrelationships of the Proteocephalidea are referred to Zehnder and Mariaux (1999) for a detailed account.

Cestode infections of cestodes have been reported previously from freshwater fishes of South America. Riggenbach (1896) encountered cestode larvae in the proteocephalidean *Rudolphiella lobosa* (Riggenbach, 1895) Fuhrmann, 1916 from pimelodid fishes (Siluriformes) in the Paraguay River (Paraná drainage). Fortes and Hoffmann (1987, 1995), Rego and Pavanelli (1985, 1987), Rego and Gibson (1989), and de Chambrier and Rego (1995) encountered larval cestodes encysted in the parenchyma of several different adult proteocephalidean cestodes of siluriform fishes in the Paraná drainage and Atlantic estuaries. On the basis of their observations of whole mounts, Rego and Gibson (1989) stated that the cysts they found were either proteocephalideans or cyclophyllideans but were more likely proteocephalideans. In a different study, de Chambrier and Rego (1995) referred to the cysts they found infecting the proteocephalideans they were describing as proteocephalideans. However, neither the conspecificity nor the ordinal identity of the cysts referred to in previous studies have been confirmed. Rego and Gibson (1989) provided detailed observations that allow comparison with the cysts described in this study. Although we treated a cestode and vertebrate system that was entirely different from that examined by Rego and Gibson (tetraphyllideans of stingrays vs. proteocephalideans of catfish), the 2 systems have features in common. For example, the cestode cysts in both studies were of similar size: 150–240 μm (Rego and Gibson, 1989) versus 120–230 μm in diameter (present study). In addition, cysts were found in all regions of the body of the host worms in both studies. Moreover, in both studies, the host tapeworms were enlarged around the cysts, and the parenchymal tissue of the host cestode was separated from the cyst wall by a gap, resulting in what Rego and Gibson (1989) termed a 'halo.' They also noted that the cysts appear to be brown (Rego and Gibson, 1989); in the present study, different degrees of pigmentation were observed.

Associations between the cysts and the vertebrate host appear to be different between the present study and that of Rego and Gibson (1989). They noted finding the cysts that they had discovered in tapeworms associated with the peritoneum of the teleost hosts of those tapeworms. The cestode larvae encysted in host tissue had much thicker cyst walls than those parasitizing worms. Rego and Gibson (1989) attributed this difference to the ability of the tapeworm cysts to develop in an environment protected from the immune system of the fish. In the present study larvae were not found within the tissues of the elasmobranch hosts.

Examining the proteocephalidean life cycle with respect to the food chain helps place this phenomenon within an ecological context. The general life cycle of species of *Proteocephalus* was discussed by Scholz (1999). Oncospheres are acquired by a copepod intermediate host in which larvae develop (termed procercoids, plerocercoids, or merocercoids). In most European species, the subsequent host is a fish, serving as definitive host after consuming an infected copepod (Scholz, 1999). In North America, the life cycle of *Proteocephalus ambloplitus* requires a second intermediate host, usually a small fish (Hoffman, 1967). Although no South American proteocephalidean life cycle has been resolved, larval stages of unidentified proteocephalideans have been reported in fishes, introducing the possibility of a second intermediate or paratenic host (Békési et al. 1992).

Catfish are a common element in the diet of South American freshwater stingrays, so one could imagine that a stingray (and its tapeworms) could obtain proteocephalidean larvae by ingesting 1 of several stages of the proteocephalidean life cycle—specifically, either a catfish infected with merocercoids, or a lower trophic level host such as a copepod, infected perhaps with procercoids. The explanation that a stingray acquired sediment with oncospheres that then developed into procercoids prior to the merocercoid stage is not beyond the realm of possibility. It is not possible to determine whether the cysts are, by infecting a tetraphyllidean, able to continue their life cycles. However, all proteocephalideans require a vertebrate definitive host (other than a stingray) to reach sexual maturity, and because freshwater stingrays are apex predators in their environment, it is unlikely that the cysts would ever encounter the appropriate definitive host. It therefore seems that the merocercoids infecting tapeworms of freshwater stingrays are at a dead end.

In many cases, including the present study, it is not known whether hyperparasitism is an obligatory, or even possible, route for life cycle completion of an organism. It is unlikely that the proteocephalidean merocercoids reported in this study to be encysting in tapeworms in freshwater stingrays are able to complete their life cycle. It may be that the proteocephalidean larvae are acting opportunistically and avoiding the unsuitable habitat of the elasmobranch's intestine by sequestering themselves within the body of other cestodes. Unlike other regions of the world, this appears to be a widespread phenomenon among the proteocephalideans of South America.

ACKNOWLEDGMENTS

Janine Caira provided F.B.R. with valuable advice during this study. Wendy Velazco-Weiss assisted with the acquisition of collecting permits. Steve Daniels helped with staining techniques. Tomás Scholz offered helpful comments and literature references. Felipe Gomez, Oliver Valenti, and Juan Canales caught stingrays. F.B.R. was supported by funds from the Lawrence Penner Endowment to the Department of Ecology and Evolutionary Biology and the Connecticut State Museum of Natural History, the Judith Shaw Parasitology Fund, and by an NSF PEET grant (DEB 9521943) awarded to J. N. Caira (The University of Connecticut, Storrs, Connecticut). P.D.O. gratefully acknowledges the support of a Wellcome Trust Fellowship (043965/Z/95/Z) awarded to D. T. J. Littlewood (The Natural History Museum, U.K.).

LITERATURE CITED

ALTSHUL, S. F., T. L. MADDEN, A. A. SCHAFFER, Z. ZHANG, W. MILLER, AND D. J. LIPMAN. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Research **25:** 402.

AMIN, O. M., AND M. COWEN. 1990. Cestoda from lake fishes in Wisconsin: The ecology of *Proteocephalus ambloplitis* and *Haplobothrium globuliforme* in bass and bowfin. Journal of the Helminthological Society of Washington **57:** 120–131.

BÉKÉSI, L., V. A. FEITOSA, AND F. A. B. CABRAL. 1992. Metacestodosis caused by plerocercoids of Proteocephalidea (Cestoda) in fish fry cultured in large scale in the Brazilian Northeast. Parasitologia Hungarica **25:** 9–13.

BRICKLE, P., P. D. OLSON, D. T. J. LITTLEWOOD, A. BISHOP, AND A. ARKHIPKIN. 2002. Parasites of *Loligo gahi* from waters off the Falkland Islands, with a phyllogenetically based identification of their cestode larvae. Canadian Journal of Zoology **79:** 2289–2296.

CHERVY, L. 2002. The terminology of larval cestodes or metacestodes. Systematic Parasitology **52:** 1–33.

CHIEN, K. 1992. Heat and epoxy resin. Electron Microscopy Society of America Bulletin **22:** 91–94.

CHIRIAC, E., S. BOLDOR, AND E. VADINEANU. 1975. Un cas d'hyperparasitisme chez la perche (*Perca fluviatilis* L.). Revue Roumaine de Biologie **20:** 241–243.

DE CHAMBRIER, A., AND A. A. REGO. 1995. *Mariauxiella pimelodi* n. g., n. sp. (Cestoda: Monticellidae): A parasite of pimelodid siluroid fishes from South America. Systematic Parasitology **30:** 57–65.

DOGIEL, V. A. 1965. General Protozoology. Clarendon Press, Oxford, U.K., 747 p. [English translation.]

EL-ON, J., R. SNEIER, M. FURTH, G. HOIDA, AND P. SCHANTZ. 1998. *Echinococcus granulosus*: Contamination of hydatid cysts with eggs and larvae of the nematode *Muellerius capillaris*. Parasitology Today **14:** 124.

FORTES, E., AND R. P. HOFFMAN. 1987. Hiperparasitismo de cestódeos de *Rhamdia sapo* Valenciennes, 1840 do estuário do Guaíba, Rio Grande do Sul, Brasil. Revista Brasileira de Medicina Veterinaria **9:** 114.

———, AND ———. 1995. Levantamento da fauna parasitária de peixes do Lago Guaíba, Porto Alegre, Rio Grande do Sul, Brasil. Revista Brasileira de Medicina Veterinaria **17:** 107–111.

GAYEVSKAYA, A. V. 1978. Several cases of accidental hyperparasitism in cestodes. Zoologicheskii Zhurnal **57:** 1262–1263.

HOFFMAN, G. L. 1967. Parasites of North American freshwater fishes. University of California Press, Berkeley, California, 486 p.

MADDISON, D. R., AND W. P. MADDISON. 2000. MacClade 4: Analysis of phylogeny and character evolution, version 4. Sinauer Associates, Inc., Sunderland, Massachusetts.

OLSON, P. D., D. T. J. LITTLEWOOD, R. A. BRAY, AND J. MARIAUX. 2001. Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). Molecular Phylogenetics and Evolution **19:** 443–467.

REGO, A. A., AND D. I. GIBSON. 1989. Hyperparasitism by helminths: New records of cestodes and nematodes in proteocephalid cestodes from South American siluriform fishes. Memorias do Instituto Oswaldo Cruz **84:** 371–376.

———, AND G. PAVANELLI. 1985. *Jauella glandicephalus* gen. n. sp. n. e *Megathylacus brooksi* sp. n., cestoídes proteocefalídeos patogênicos para o jaú, *Paulicea lutkeni*, peixe pimelodídeo. Revista Brasileira Biologica **45:** 643–652.

———, AND ———. 1987. Cestoídes proteocefalídeos do jaú, *Paulicea Luetkeni*, peixe pimelodídeo do Brasil. Revista Brasileira Biologica **47:** 357–361.

RIGGENBACH, E. 1896. Das Genus *Ichthyotaenia*. Revue Suisse de Zoologie **4:** 165–277.

ROSA, R. S. 1985. A systematic revision of the South American freshwater stingrays (Chondrichthyes: Potamotrygonidae). Ph.D. Dissertation. The College of William and Mary, Williamsburg, Virginia, 542 p.

SCHOLZ, T. 1999. Life cycles of species of *Proteocephalus*, parasites of fishes in the Palearctic Region: A review. Journal of Helminthology **73:** 1–19.

SEY, O., AND F. MORAVEC. 1986. An interesting case of hyperparasitism of the nematode *Spironoura babei* Ha Ky, 1971 (Nematoda: Kathlanidae). Helminthologia-Bratislava **23:** 173–177.

SWOFFORD, D. L. 1998. PAUP*: Phylogenetic analysis using parsimony (and other methods), version 4.0b10. Sinauer Associates, Inc., Sunderland, Massachusetts.

ZEHNDER, M. P., AND A. DE CHAMBRIER. 2000. Morphological and molecular analyses of the genera *Peltidocotyle* Diesing 1850 and *Othinoscolex* Woodland 1933, and morphological study of *Woodlandiella* Freze, 1965 (Eucestoda, Proteocephalidea), parasites of South American siluriform fishes (Pimelodidae). Systematic Parasitology **46:** 33–43.

———, AND J. MARIAUX. 1999. Molecular systematic analysis of the order Proteocephalidea Mola, 1928 (Eucestoda) based on mitochondrial and nuclear rDNA sequences. International Journal for Parasitology **29:** 1841–1852.

———, ———, C. VAUCHER, AND J. MARIAUX. 2000. *Nomimoscolex suspectus* n. sp. (Eucestoda: Proteocephalidea: Zygobothriinae) with morphological and molecular phylogenetic analysis of the genus. Systematic Parasitology **47:** 157–172.