A NEW ACANTHOCEPHALAN SPECIES (ARCHIACANTHOCEPHALA: OLIGACANTHORHYNCHIDAE) FROM CERDOCYON THOUS, A CRAB-EATING FOX IN THE BRAZILIAN PANTANAL WETLANDS

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ABSTRACT: A new species of Oligacanthorhynchidae (Acanthocephala) Prosthenorchis cerdocyonis n. sp. is described from 17 specimens collected from the small intestine of the crab-eating fox Cerdocyon thous Linnaeus, 1766 (Canidae: Carnivora) found in the Brazilian Pantanal wetlands. Specimens were studied using light and scanning electron microscopy. Characteristic features distinguishing the new species from others already described are presented, such as size of the body, the position of lemnisci, size of the eggs, host, and geographical distribution. Details of the body surface obtained by scanning electron microscopy, such as the presence of 2 lateral papillae in the proximal region of the proboscis, the presence of bars in hooks, and a robust and festooned collar, helped to identify the species. Until now, specimens belonging to Prosthenorchis reported from Cerdocyon thous were not identified to species. Furthermore, the new species is the first to be recorded in C. thous found in the Pantanal wetlands.

The family Oligacanthorhynchidae Southwell and Macfie, 1925, includes 12 genera (Amin, 1985, 2013) and includes Prosthenorchis Travassos, 1915. Species of Prosthenorchis have been reported as follows: in South America as Prosthenorchis elegans (Diesing, 1851) Travassos (1915) from primates; in Africa as Prosthenorchis lenmari Machado (1950) from lemurs in Madagascar, Prosthenorchis pardalis Southwell and Macfie (1925) from Felis pardus in Sierra Leone, and Prosthenorchis fraterna (Baer, 1959) Schmidt, 1972, from felid Panthera pardus in Congo; and in Asia as Prosthenorchis sinicus Hu-Jiand, 1990, from a dog in China (Schmidt, 1972, Amin et al., 2008; Amin, 2013).

The crab-eating fox, Cerdocyon thous, is a wild canid that inhabits savannas and woodlands in South America (Silveira, 1999; Courtenay and Maffei, 2004). Cerdocyon thous (Linnaeus, 1766) has been the subject of a research program conducted by Embrapa/Pantanal and the Institute Oswaldo Cruz Foundation (FIOCRUZ-RJ) that studies the ecology and health of wild carnivores in the Brazilian Pantanal wetlands. This research program also includes the study of helminth parasites, and specimens of helminths were made available to parasitologists at Fiocruz by Embrapa/Pantanal researchers. Although specimens of acanthocephalans have been reported from C. thous in Brazil (Griese, 2007; Ruas et al., 2008; Vieira et al., 2008; Lima, 2009), species of the genus Prosthenorchis had not been previously identified.

In this report, we describe a new species of the genus Prosthenorchis collected from the wall of the small intestine of crab-eating foxes (C. thous) from the Pantanal wetlands, Mato Grosso do Sul State, Brazil.

MATERIALS AND METHODS

Two adult crab-eating female foxes (C. thous) were found dead in 2007 on Nhumirim Ranch (18°59’S, 56°39’W), located in the Nhecolândia sub-region of the Pantanal in Brazil. The animals were necropsied, and acanthocephalans were collected from the small intestine of each fox for taxonomic study. The study area is characterized by sandy soil with mosaic vegetation of semi-deciduous forest with open grassy areas (Redela, 2006). Animal procedures were approved by the Brazilian Federal Environmental Agency (IBAMA, first license #183/2005, CFGAU/LIC; last license #11772-2).

Acanthocephalans were collected, washed in physiological saline (0.95% NaCl), and stored in 70% alcohol. At the laboratory, only adult specimens of both sexes were cleared in 90% phenol to study the hooks of the proboscis. To describe the internal structures, the specimens were stained with acid carmine, destained in acid (HCl) alcohol, dehydrated in an ascending acetone series, dried by the critical point method with CO2, and post-fixed for 3 hr at room temperature in 1% osmium tetroxide in M Na-cacodylate buffer. After being washed in the same buffer, specimens were post-fixed for 3 hr at room temperature in 1% osmium tetroxide in 0.1 M Na-cacodylate buffer. After being washed in the same buffer, specimens were post-fixed for 3 hr at room temperature in 1% osmium tetroxide in 0.1 M Na-cacodylate buffer. The material was then dehydrated in an ascending acetone series, dried by the critical point method with CO2, mounted with silver and gold, and then sputter-coated with a 20-nm-thick layer of gold. Samples were examined using a Jeol JSM-6390 LV microscope at an accelerating voltage of 15 kV at the Electron Microscopy Platform of the Oswaldo Cruz Institute.

DESCRIPTION

Prosthenorchis cerdocyonis n. sp. (Figs. 1–15)

General: Proboscis globular, armed with 36 hooks (12 rows of 3 hooks); showing 3 different morphologies varying from robust hooks at top to short hooks at bottom. Hooks are similar in size in both sexes. First type of apical hook posteriorly curved, presenting complex manubria and root immersed in matrix of proboscis, expanded laterally in T-shape, with top of hook bearing chisel-shaped bars. Second and third types of hooks in...
proximal rows have small, discoid roots. The lengths of the anterior hooks, measured from tip to posterior end, were 0.41–0.22 (0.32), 0.27–0.08 (0.15), the lengths of the hook roots measured 0.28–0.11 (0.18), 0.06–0.03 (0.05), and the posterior hooks measured 0.11–0.05 (0.08) with simple roots.

One papillae on each side at base of proboscis, presenting elevated border with smooth surface and medial pore; neck surrounded by robust, irregular, festooned collar; cylindrical body curved ventrally, thick and furrowed, not transparent, showing marked wrinkles transversely; long lemnisci, sometimes bent on themselves, usually reaching the posterior region of the body.

**Male holotype and 6 paratypes:** Body 9.60–6.8 (8.53) long, 1.97–1.26 (1.58) wide. Globular proboscides 1.1–0.56 (0.9) long, 0.98–0.72 (0.86) wide, bearing 36 hooks (12 spiral rows of 3 hooks). Collar 1.26–0.68 (0.84) long, 0.98–0.82 (0.88) wide, neck short, with longitudinal pleats. Two testicle ellipsoids, located in anterior region. Anterior testis 1.0–0.87 (0.94) long, 0.49–0.29 (0.39) wide; posterior testis 0.9–0.65 (0.76) long, 0.45–0.41 (0.44) wide (n = 2). Behind testicles, 8 ellipsoid, aggregated cement glands. Group of cement glands 1.23–0.96 (1.09) (n = 2) long. Ejaculatory duct 1.21–0.88 (1.05) (n = 2). Bursa located at end of body, 0.96 long (n = 1). Long lemnisci extend to posterior testis and reach the end of the body. Posterior region with reproductive vestibule closed by retracted copulatory bursa.

**Female allotype and 9 paratypes:** Body 12.18–5.34 (7.67) long, 2.08–1.4 (1.71) wide. Globular proboscides 1.0–0.68 (0.87) long, 1.04–0.78 (0.95) wide. Egg ellipsoid shape and with 3 membranes, Outer membrane (Om) is thick and granular, inner membrane (Im) is thin and transparent, embryo (Em) is surrounded by a thin membrane, 2 poles (Po) in each side.
wide, bearing 36 hooks (12 spiral rows of 3 hooks). Collar 1.16–0.34 (0.74) long, 1.18–0.72 (0.97) wide. In most specimens, lemnisci covered by eggs. Uterine bell 1.11–0.75 (0.90) long (n = 3; Fig. 3). The eggs (n = 22) ellipsoidal, 0.1–0.074 (0.09) long, 0.07–0.03 (0.05) wide, with 3 membranes. Outer membrane thick, granular, with hyaline poles; inner membrane thin, membrane covering embryo thin and transparent. Posterior end of body possessing smooth vulvar vestibule with prominent edges.

**Taxonomic summary**

*Type host:* *Cerdocyon thous* Linnaeus, 1766 (Crab-eating fox).

*Type locality:* Nhumirim Ranch (18°59′S, 56°39′W), Mato Grosso do Sul State, Brazil.

*Type material:* Holotype: 1 male (CHIOC: 35804a); allotype: 1 female (CHIOC: 35804b); paratype 5 females and 6 males (CHIOC: 35804c) deposited in the Helminth Collection of the Oswaldo Cruz Institute (Coleção Helmintológica do Instituto Oswaldo Cruz-CHIOC), Rio de Janeiro, Brazil.
Etymology: The new species is named after the genus of the host.

Remarks

The genus *Prosthenorchis* was created by Travassos (1915) for the type-species *Prosthenorchis elegans* (Diesing, 1851) (syn. *Echinorhynchus elegans*) from *Callithrix chrysoleuca* Wagner, 1842 (Schmidt, 1972). Stunkard (1965) included the genus *Prosthenorchis* in a new subfamily, Prosthenorchinae Travassos, 1917, together with 2 other species: *Prosthenorchis luhei* Travassos, 1917, a parasite from the carnivore *Nasua nasua*, and *Prosthenorchis avicola* Travassos, 1917, a parasite from the bird *Nettion brasiliense*.

In 1950, Machado Filho revised the species described by Travassos and included 13 new species parasitizing primates, carnivores, birds, and lizards from different geographic regions of the world. Yamaguti (1963) revised the classification of the Oligacanthorhynchidae, establishing the main characteristics of
the genus *Prosthenorchis* and considering their geographical distribution.

Schmidt (1972) revised the class Archiacanthocephala, focusing on Oligacanthorhynchidae and emphasizing the importance of reviewing the family due to the vague description and incorrect interpretation of the morphology of the species. Recently, Golvan (1994) revised the nomenclature of the phylum Acanthocephala and considered geographical distribution as a taxonomic criterion.

Amin et al. (2008), following Schmidt (1972), considered the genus *Prosthenorchis* as having 3 species based on morphological characteristics such as a festooned collar, ornamented proboscides, 3 different types of hooks, and tips of hooks with barbs. The 3 representative species are *Prosthenorchis elegans* (Diesing, 1851) Travassos, 1915, *Prosthenorchis lemuri* Machado, 1950, and *Prosthenorchis fraterna* (Baer, 1959) Schmidt, 1972; other species previously belonging to the genus *Prosthenorchis*, which had no collar, have been relocated to other genera from the Oligacanthorhynchidae family (Schmidt, 1972; Golvan, 1994; Amin et al., 2008). Recently, Amin (2013) considered 2 other species as belonging in the genus: *Prosthenorchis pardalis* (Southwell and Macfie, 1925) and *Prosthenorchis sinicus* Hu-Jiand, 1990.

In addition, Amin et al. (2008) described a new genus, *Paraprosthenorchis*, in the Oligacanthorhynchidae family. The genera *Paraprosthenorchis* and *Prosthenorchis* are closely related because these genera are the only ones that have festooned collars in the family. Otherwise, the genus *Paraprosthenorchis* has ornate proboscides, does not have 12 rows of 3 hooks, has hooks without barbs, does not have complex and large hook roots, has collars with about 35 festoons, and does not have subterminal gonopores (Amin et al., 2008).

Characteristics found in the new species described in the present report, including the number of hooks, number of festoons, and size of the body, were not observed in the genus *Paraprosthenorchis*, which justifies the inclusion of the new species described here in the genus *Prosthenorchis*.

The main characteristics that distinguish *Prosthenorchis cerdocyonis* n. sp. from *P. elegans*, *P. fraterna*, and *P. lemuri* are size of the body, position of the lemnisci, number of festoons, size of the egg, and host and geographical distribution (Table I). However, the lemnisci of *P. cerdocyonis* n. sp. and *P. fraterna* also reach the posterior end.

Nevertheless, *Prosthenorchis cerdocyonis* n. sp. is distinguished from *P. elegans* and *P. lemuri* by its small trunk and long lemnisci reaching the posterior end and from *P. fraterna* and *P. lemuri* by the size of the egg, which is larger than that of other species (Table I), and geographic distribution, since those species occur in Africa.

Recently, Amin (2013) included 2 new species: *Prosthenorchis pardalis* and *Prosthenorchis sinicus* in the genus *Prosthenorchis*, but their descriptions need more morphological details. Further-

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<td>Lemnisci end over body end</td>
<td>Anterior region</td>
<td>Anterior region</td>
<td>Middle region</td>
<td>Reach posterior region</td>
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<td>Eggs</td>
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<td>Length</td>
<td>0.78*</td>
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<td>Natural definitive vertebrate host</td>
<td><em>Saimiri sciurea</em></td>
<td><em>Mystax Ursus/ Challithrix chrysode</em></td>
<td><em>Lemur fulus</em></td>
<td><em>Panthera pardus</em></td>
<td><em>Cerdocyon thous</em></td>
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<td>Geographic distribution</td>
<td>Brazil/Panamá</td>
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<td>Madagascar (Africa)</td>
<td>Ituri, Congo (Africa)</td>
<td>Brazil (South America)</td>
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* Measured by the authors of the present paper based on manuscript description.

Media A+P media of measurements of anterior and posterior testis together.
more, *P. pardalis* is considered as nomen nudum (Amin, 2013), and *P. sinicus* does not show the robust collar, which is the main characteristic of the genus as shown in the original description. Consequently, *P. pardalis* and *P. sinicus* may not belong in *Prosthenorchis*, and we suggest that these species require further study.

Specimens of *Prosthenorchis* sp. (Lima, 2009) and *P. elegans* (Machado Filho, 1950), from the Helminth Collection of the Oswaldo Cruz Institute were not in good condition, and we could only measure the size of the body in *P. elegans*.

In addition, the features of *Prosthenorchis cerdocyonis* n. sp. were observed in greater detail via scanning electron microscopy, and the morphologic characteristics such as barbs on hooks, papillae, and collar were described. However, these characteristics have not previously been described by SEM in great detail for any other species of the *Prosthenorchis* genus, making comparisons of these features difficult.

In the Pantanal biome, where the samples were collected from the crab-eating fox in the present study, a significant portion of the crab-eating fox diet is composed of arthropods (87%) (Bianchi et al., 2014). This aspect suggests that these arthropods may be the intermediate host of *Prosthenorchis cerdocyonis*.

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This paper is being published in fairness to the authors since it was in the process of being accepted during the transition. However, readers are reminded that the journal no longer publishes stand-alone single species papers during the process of being accepted during the transition. However, readers are reminded that the journal no longer publishes stand-alone single species descriptions unless they are in the context of broader taxonomic, phylogenetic, or biogeographical issues that significantly advance our understanding of these aspects.

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