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First report of *Dendrorchis retrobiloba* Volonterio & Ponce de León, 2005 (Digenea, Gorgoderidae) in *Astyanax* aff. *fasciatus* (Cuvier, 1819) (Characiformes, Characidae) from southern Brazil

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Abstract
Dendrorchis retrobiloba Volonterio & Ponce de León, 2005 was first described from the swim bladder of *Astyanax fasciatus* (Cuvier, 1819) from Montevideo, Uruguay. In this study, we necropsied specimens of *A*. aff. *fasciatus* collected from Lake Guaíba, in Rio Grande do Sul, Brazil, for the analysis of their helminths. The digeneans were identified as *D*. *retrobiloba* by their elongated body, oral sucker longer than ventral sucker, and 2 posterior lobes. This is the first report of *D*. *retrobiloba* in *A*. aff. *fasciatus* from Lake Guaíba and extends the known geographic distribution of this parasite.

Key words
Characid, digenean, endoparasite, Neotropical Region, trematode.

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Introduction

The family Gorgoderidae Looss, 1899 encompasses 3 subfamilies, Anaporrhutinae Looss, 1901, Gorgoderinae Looss, 1899, and Degeneriinae Cutmore, Miller, Curran, Bennett & Cribb, 2013 (Campbell 2008, Cutmore et al. 2013). The Gorgoderinae includes digenetic trematodes with intercaecal testes, the pharynx absent or present, and the seminal receptacle and cirrus sac absent (Campbell 2008). These parasites infect primarily the urinary system of freshwater and marine fishes but are also found in amphibians.

The genus *Dendrorchis* was proposed by Travassos (1926) to accommodate *Dendrorchis neivai* Travassos, 1926, a parasite of the swim bladder of *Brycon lundii* (=*Brycon orthotaenia* Günther, 1864) from São Paulo state, Brazil. Subsequently, several other *Dendrorchis* species have been described in fish hosts, including *Dendrorchis retrobiloba* Volonterio & Ponce de León, 2005 in *Astyanax fasciatus* (Cuvier, 1819) from Montevideo, Uruguay (Volonterio and Ponce de León 2005), *Dendrorchis ritata* Soofi, Birmani & Dharejo, 2016 in *Rita rita* (Hamilton, 1822) from Jamshoro, Pakistan (Soofi et al. 2016), and *Dendrorchis pampae* Monteiro, Wendt & Zebral, 2018 in *Cynopoecilus melanotaenia* (Regan, 1912) from Rio Grande do Sul state, Brazil (Monteiro et al. 2018).

Astyanax Baird & Girard, 1854 is a relatively diverse characid genus, with 146 valid species (Froese and Pauly 2018) from a number of different geographic regions. Four *Astyanax* species—*Astyanax eigenmanniorum* (Cope, 1894), *Astyanax* aff. *fasciatus* (Cuvier, 1819), *Astyanax henseli* Melo & Buckup, 2006, and *Astyanax lacustris* (Lütken, 1875)—are known to occur in Lake Guaíba, Rio Grande do Sul (Lucena et al. 2013). The helminth fauna of these species is still very poorly known. Until now, 2 species of digenetic trematodes, 2 monogeneans, and 1 nematode have been recorded in *Astyanax* from Lake Guaíba (Fortes and Hoffmann 1999, Gallas et al. 2014, 2015, 2016). In the present study, we provide the first report of *D. retrobiloba* in *A.* aff. *fasciatus* from Lake Guaíba, which extends this parasite's known geographic distribution in the Neotropical Region to southern Brazil.

Methods

Specimens of *A. eigenmanniorum* (*n* = 20), *A.* aff. *fasciatus* (*n* = 46), *A. henseli* (*n* = 5), and *A. lacustris* (*n* = 25) from Lake Guaíba were examined for the presence of digenetic trematodes. The fish were collected with seine nets between January 2017 and October 2017 at Pintada Island, on Lake Guaíba, in the municipality of Porto Alegre, Rio Grande do Sul state, Brazil. All specimens were frozen until necropsy. The endoparasites found during the examination were fixed in A.F.A. for 48 hours (Gallas et al. 2017) and then stored in 70° GL ethanol before being stained in Delafield's hematoxylin. The specimens were clarified in cedarwood oil and mounted in Canada balsam for morphological identification. Digenean systematics follows Gibson et al. (2002) and Bray et al. (2008).

Unless otherwise indicated, measurements of the adult specimens (*n* = 3) are given in micrometers (μm), with the range of values for a given parameter being followed by the mean and the standard deviation (±). In the case of the eggs, the number of eggs measured is cited. The terms forebody and hindbody follow Yamaguti (1971). Line drawings of the specimens were obtained using a Zeiss Axiostar microscope, with CorelDraw X4® and Adobe Photoshop® CS2. The infection parameters, i.e., prevalence and the intensity and amplitude of infection, follow Bush et al. (1997). A voucher specimen of the helminth was deposited in the Helminthological Collection of the Natural Sciences Museum (Coleção Helmintológica do Museu de Ciências Naturais, CHMU) at the Lutheran University of Brazil in Canoas, Rio Grande do Sul, Brazil. The hosts were identified following Bertaco and Lucena (2010) and Lucena et al. (2013).

Results

Phylum Platyhelminthes Gegenbaur, 1859
Class Trematoda Rudolphi, 1808
Subclass Digenea Carus, 1863
Superfamily Gorgoderoidea Looss, 1899
Family Gorgoderidae Looss, 1899
Subfamily Gorgoderinae Looss, 1899

Genus *Dendrorchis* Travassos, 1926

***Dendrorchis retrobiloba* Volonterio & Ponce de León, 2005**
Figure 1

Infection parameters. Prevalence: 6.52%, mean intensity of infection: 1 helminth/host and mean abundance of infection: 0.06 helminth/host.

New records. Brazil: Rio Grande do Sul state: Municipality of Porto Alegre (30°17′11″S, 051°18′01″ W) (Fig. 2). Host: *Astyanax* aff. *fasciatus* (Cuvier, 1819). Site of infection: swim bladder. Collection of parasites: 22 February 2018: identification of parasites: 5 June 2018. Voucher specimen of helminth deposited: CHMU-290-1-1.

Identification. This description is based on 3 stained specimens mounted in Canada balsam. Body elongated, 1.9–2.35 mm (2.17 ± 0.25 mm) long, with relatively narrow forebody, 0.42–0.51 mm (0.46 ± 0.04 mm) wide, in

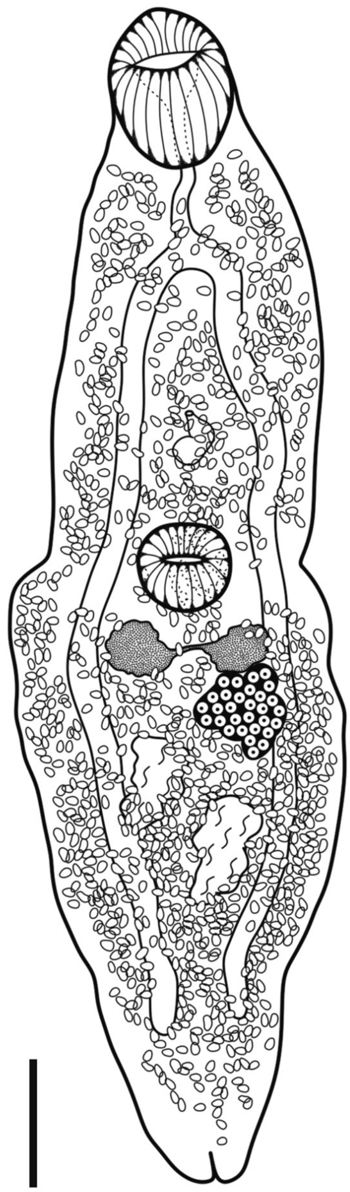

Figure 1. *Dendrorchis retrobiloba* Volonterio & Ponce de León, 2005. Ventral view of whole specimen. Scale bar = 200 μm.

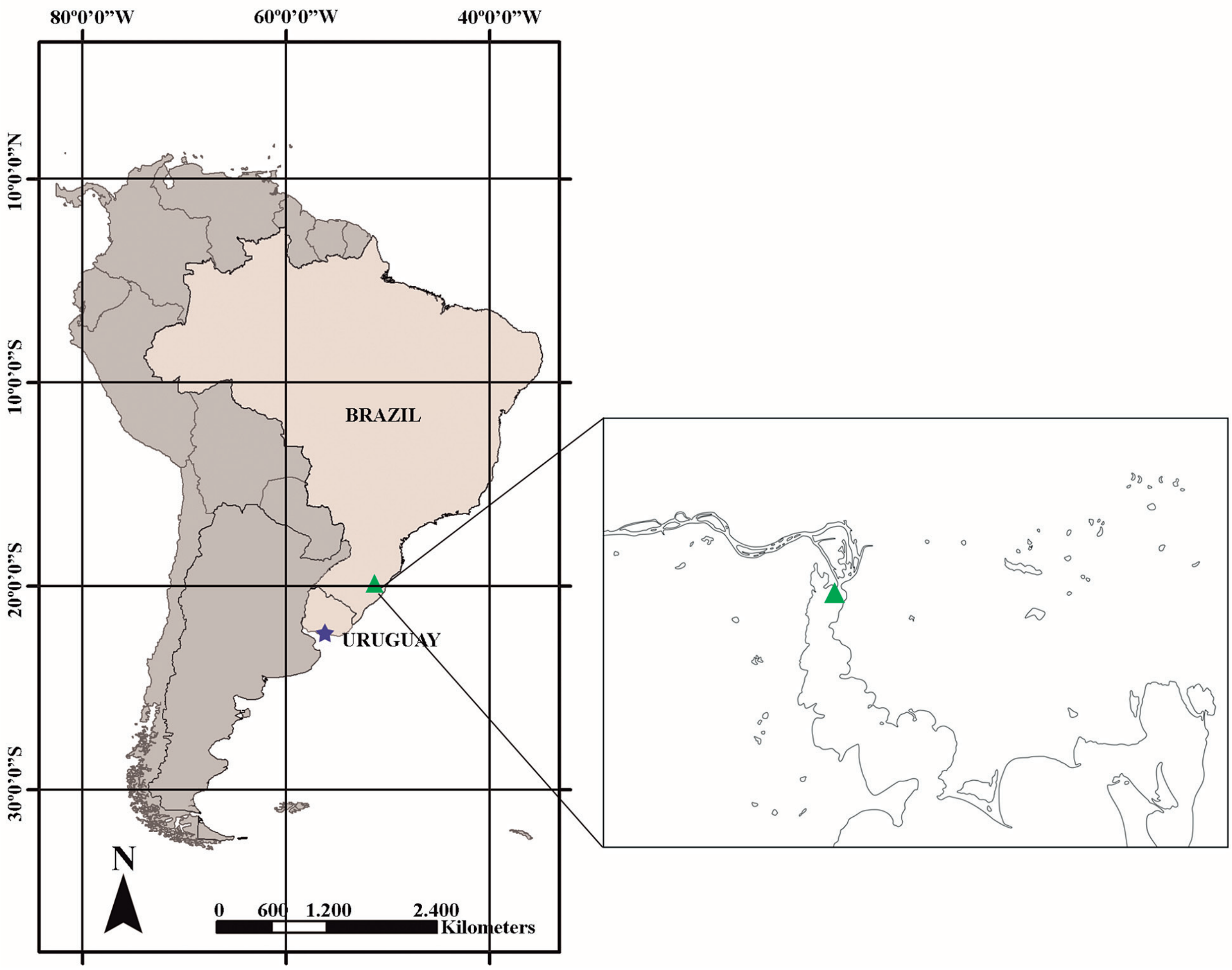

Figure 2. Map showing Cañada Del Dragón, in the Departamento de Montevideo, Uruguay (blue star) the type locality of *Dendrorchis retrobiloba* Volonterio & Ponce de León, 2005, and the new locality, Lake Guaíba, in the state of Rio Grande do Sul, Brazil (green triangle).

comparison with the hindbody, 0.64–0.66 mm (0.65 ± 0.01 mm) wide. Oral sucker subterminal, 250–310 (280 ± 30) long, 220–270 (240 ± 26) wide. Ventral sucker smaller than oral sucker, 150–180 (167 ± 15) long, 160–170 (163 ± 6) wide, and 0.81–1.1 mm (0.97 ± 0.14 mm) from anterior extremity. Total body length to oral sucker ratio: 1:7.7; total body length to ventral sucker ratio: 1:13; oral sucker to ventral sucker ratio: 1:1.7. Pharynx absent. Esophagus 90–120 (107 ± 15) long, with caecal bifurcation closer to oral sucker than ventral sucker. Caecal bifurcation 330–440 (397 ± 59) from anterior extremity. Intestinal caeca terminating close to posterior extremity of body.

Genital pore in the forebody, 0.6–0.73 mm (0.67 ± 0.07 mm) from anterior extremity. No cirrus sac or cirrus observed. Seminal vesicle present, 65–90 (75 ± 13) long and 50–55 (52 ± 3) wide. Testes lobed and irregular in shape, arranged in diagonal, intercaecal, anterior testis 110–140 (127 ± 15) long, 70–80 (77 ± 6) wide; posterior testis 100–170 (147 ± 40) long, 70–100 (90 ± 17) wide. Seminal receptacle not observed. Ovary lobed, sinistral, intercaecal, posterior to vitellaria, pretesticular, 170–190 (180 ± 10) long, 170 wide. Mehli's gland and Laurer's canal not observed. Vitellaria composed of 2 lobed masses, located between ventral sucker and ovary. Right vitellarium 100–120 (113 ± 11) long, 110 wide; left vitellarium 100–120 (110 ± 10) long, 80–130 (107 ± 25) wide. Uterus inter- and extra-caecal, in forebody and hindbody. Eggs oval, 22–32 (28 ± 3; n = 10) long, 17–20 (19 ± 1; n = 10) wide. Posterior extremity with 2 lobes, and excretory pore between them.

The specimens of *D. retrobiloba* are easily distinguished from the congeners *D. neivai*, *D. ritata*, and *D. pampae* based on morphological traits such as body shape, the size and shape of the suckers, testes, ovary and, the extension of the uterus (Travassos 1926, Volonterio and Ponce de León 2005, Soofi et al. 2016, Monteiro et al. 2018). The bodies of *D. neivai*, *D. ritata*, and *D. pampae* are pyriform, whereas *D. retrobiloba* has an elongated body. The oral sucker of *D. retrobiloba* is larger than its ventral sucker, whereas in all the other species, these 2 suckers are of similar size. In addition, *D. retrobiloba* is the only *Dendrorchis* species that has 2 posterior lobes, with a posterior cleft (Volonterio and Ponce de León 2005).

The testes of *D. neivai* are larger than the lobed ovary, while in *D. ritata* the ovary is triangular and similar in size to the testes. Irregularly shaped testes are found in *D. pampae* and *D. retrobiloba*, but in *D. retrobiloba*, the lobed ovary is a little longer than the testes. Except for *D. neivai*, which has a more extracaecal uterus with few eggs in the intercaecal area, all the other species have a uterus in the inter- or extra-caecal fields. The vitellarium in the specimens examined by Volonterio and Ponce de León (2005) was described as having two compact, pyriform masses, whereas the specimens examined in the present study presented vitellaria organized in 2 lobed masses.

Discussion

The diagnosis of the genus *Dendrorchis* provided by Travassos (1926) was amended recently by Volonterio and Ponce de León (2005), Campbell (2008), and Monteiro et al. (2018). Travassos (1926) recognized specimens with a pyriform body, small cirrus sac, branched testes, deeply lobed ovary, and vitellaria occupying all body fields, except for the intercaecal field above the vitellaria. Volonterio and Ponce de León (2005) added the following diagnostic traits: body wider posterior to the ventral sucker, testes branched or with superficial lobes, seminal receptacle present or absent, vitellaria elongate or pyriform, uterus extending to anterior portion and posterior region of the body, eggs thin-shelled without opercula.

Brooks and MacDonald (1986) examined 2 syntypes of *D. neivai* deposited in the Helminthological Collection of the Oswaldo Cruz Institute and noted the absence of a cirrus sac, which was the principal reason for Campbell's (2008) proposal for a new amendment of the diagnosis of *Dendrorchis*. This amendment included the broad and foliated hindbody, cirrus sac and seminal receptacle absent, and testes branched. Monteiro et al. (2018) further amended the diagnosis to include the presence of a cirrus sac and modify the position of the genital pore.

Given these slightly different diagnoses of the genus *Dendrorchis* (Travassos 1926, Volonterio and Ponce de León 2005, Campbell 2008, Monteiro et al. 2018), it is necessary to consider the question of the cirrus sac. If the presence of cirrus sac is considered to be a characteristic of *Dendrorchis* species (Travassos 1926, Volonterio and Ponce de León 2005, Monteiro et al. 2018), *Dendrorchis* would be the only gorgoderine genus to feature this structure (Campbell 2008). All other gorgoderine genera (except *Phyllodistomoides* Brooks, 1977 [?] and *Xystretrum* Linton, 1910) lack a cirrus sac, but present a structure (seminal vesicle) that has been misidentified as a cirrus sac by some authors. In the genus *Xystretrum*, there is a pseudosinus sac, which forms a sac-like structure together with the terminal genitalia, while the metraterm does not unite with the male duct (Campbell 2008).

In the drawings by Travassos (1926), Volonterio and Ponce de León (2005), and Monteiro et al. (2018), there is no evidence of a cirrus portion in the interior of the structure, which these authors identified as the cirrus sac. The cirrus sac identified by these authors may in fact represent a seminal vesicle, a pseudocirrus sac, or a false cirrus sac, which comprises a sac containing the male terminal genitalia, but without any differentiation of the cirrus, as defined by Yamaguti (1971). There are also considerable inconsistencies in the widths of the cirrus sac (30.7–42.9) and the internal seminal vesicle (20.4–63.0) of the specimens analyzed by Volonterio and Ponce de León (2005), which may have been the result of either an error of measurement or the misidentification of the structures.

The life cycle of the gorgoderids is still little known, and most of the studies have focused on species of the genus *Phyllodistomum* Braun, 1899 (Cribb 1987, Urabe et al. 2015). The first intermediate host of *Phyllodistomum* is typically a bivalve, with the emerging cercariae infecting arthropods (crustaceans or insects), other mollusks, or tadpoles, which act as the second intermediate host, with fish as the definitive host (Cribb 1987). The low prevalence of *D. retrobiloba* recorded in our study indicates that *A.* aff. *fasciatus* ingests small numbers of the intermediate hosts of *D. retrobiloba*, which is consistent with the composition of the contents of the stomachs and intestines of the fish specimens examined, which contained more plant matter than invertebrates. *Astyanax* species are considered to be opportunistic omnivores, ingesting the food items available in the environment (Vilella et al. 2002, Wolff et al. 2009). This may also account for the lack of *D. retrobiloba* in the other *Astyanax* species examined, given that *A. lacustris* is considered to be predominantly herbivorous, while *A. eigenmanniorum* and *A. henseli* consume mainly insects and plant matter (Vilella et al. 2002, Ferrari et al. 2015).

Prior to our study, only 2 species of digenetic trematodes (*Genarchella parva* Travassos, Artigas & Pereira, 1928 and *Zonocotyle bicaecata* Travassos, 1948) had been observed in *A.* aff. *fasciatus* from Lake Guaíba (Fortes and Hoffmann 1999). The new record represents the first report of *D. retrobiloba* in *A.* aff. *fasciatus* from southern Brazil and extends the known geographic distribution of *D. retrobiloba* from its only other known locality in Uruguay. The results of our study also provide important insights into the morphology of *D. retrobiloba* and other species of the genus.

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Authors' Contributions

MG necropsied the hosts, prepared and identified the parasites, prepared figures, and co-wrote the text; LRPU co-wrote the text.

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