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Novel host report for *Catadiscus uruguayensis* Freitas & Lent, 1939 (Trematoda, Diplodiscidae) infecting *Austrolebias* Costa, 1998 species from Uruguay

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Abstract
The genus *Catadiscus* Cohn, 1904 has a total of 16 known species that infect the intestinal tract of reptiles, amphibians, and mollusks. However, *Catadiscus* has never been found in teleosts. The annual fish *Austrolebias prognathus* (Amato, 1986) and *A. cheradophilus* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1965) were collected from temporary ponds in the southeast of Uruguay. The specimens found in the intestinal tract of these hosts were morphologically identified as *Catadiscus uruguayensis* Freitas & Lent, 1939, which until now were only known to infect amphibians. This work represents the first report of the genus *Catadiscus* infecting and developing in a fish host.

Keywords
Annual fish, new host, parasite, South America, temporary pond.

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Introduction

In South America, the genus *Catadiscus* Cohn, 1904 has a total of 16 known species which infect the intestinal tract of reptiles, amphibians and mollusks (two species from reptiles, 10 from amphibians, three known to infect both, and one reported in a mollusk) (Freitas 1943; Lent et al. 1946; Freitas and Dobbin 1956; Mañe-Garzón and Gortari 1965; Suriano 1970; Ostrowski 1978; Corrêa and Artigas 1979; Incorvaia 1983; Hamann 1992; Hamann et al. 2014). Particularly in Uruguay, three species have been reported: one from reptiles (*Catadiscus dolichocotyle* Cohn, 1913 in *Liophis miliaris* (Linnaeus, 1758) and *Chironius fuscus* (Linnaeus, 1785)), and two from amphibians (*Catadiscus corderoi* Mañé-Garzón, 1958 in *Pseudis meridionalis* Miranda-Ribeiro, 1926 and *Pseudis minutus* Günther, 1858; and *Catadiscus uruguayensis* Freitas & Lent, 1939 in *Leptodactylus latrans* (Steffen, 1815)) (Freitas and Lent 1939; Mañé-Garzón and Gortari 1965). These hosts, specifically the amphibian ones, can be found in habitats associated with freshwater ecosystems, such as wetlands and temporary ponds, where tadpoles cohabit with teleost known as annual fishes.

Annual fishes (Cyprinodontiformes, Aplocheiloidei) stand as an attractive model worldwide for several topics including evolution and genetics, as well as being

bioindicators and pest regulators (Fletcher et al. 1992; Frenkel and Goren 2000; Arenzon et al. 2003; Berois et al. 2015). They exhibit unique annual life-cycles that comprise a rainy season during which they mature and reproduce, and a dry season where their habitats (temporary ponds) become depleted of water and thus only their burrowed draught-resistant eggs remain until the next rainy season in the following year, which triggers the completion of the eggs' development (Berois et al. 2015). In Uruguay, the annual fishes that are most widely used for research are those of the genus *Austrolebias* Costa, 1998, yet their parasitic fauna is extensively unknown, with only four parasitological reports in the South American continent (Luque et al. 2011; Delgado and García 2015; Montes et al. 2017; Marcotegui et al. 2018). Aiming to expand the knowledge of parasites infecting *Austrolebias*, we report the presence of a *Catadiscus* species in two annual fishes from Uruguay.

Methods

Fish hosts were collected manually from temporary ponds in Uruguay during the rainy season of 2018 and 2019 in the Department of Rocha (33°41′26″S, 054°41′15″W) and in the Department of Lavalleja (34°11′55″S, 053°55′45″W), Uruguay (Fig. 1).

Hosts were captured, manipulated, and euthanized under the approval of the institutional Ethics Council of Animal Experimentation (CHEA, Facultad de Ciencias, Uruguay), which follows the American Veterinary Medical Association (AVMA) international guidelines for fish euthanasia (with eugenol overdose before a cervical and cephalic incision) (Leary et al. 2013). Parasites were removed from the intestine of fish, heat-killed with slight pressure under coverslip, fixed in 10% formalin and then preserved in 70% ethanol. They were then stained with Langeron's Carmine, dehydrated in ethanol series, clarified with eugenol, and mounted in *Entellan*® medium. Whole-mount specimens were observed using an Olympus BX50 microscope and digital images were taken with adapted 318CU 3.2M CMOS camera and Micrometrics SE Premium program. Illustration was done with digital tablet Genius i608X using the free license program GIMP2. The specimens were identified using the taxonomic keys proposed by Sey (1991) and Jones et al. (2005). Followingly, the description hereto was compared with the original description of the congeneric species. Distribution map was made with the program QGIS version 3.4 (QGIS Development Team 2018) and the program SimpleMappr (Shorthouse 2010). Specimens are deposited in the collection of Invertebrate Zoology of the Natural History National Museum (Museo Nacional de

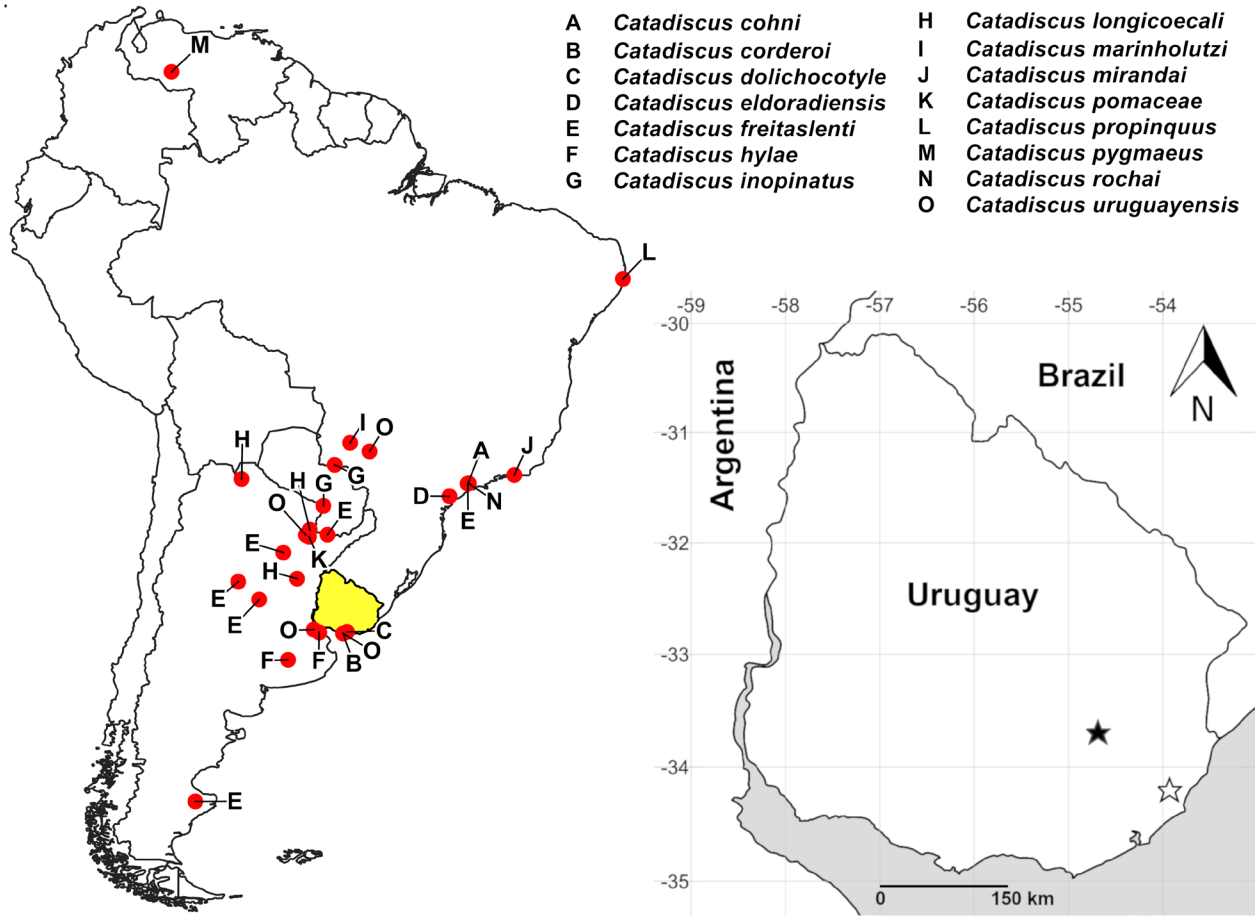

Figure 1. Known records of *Catadiscus* Cohn, 1904 in South America and the new records in Uruguay; South American reports of *Catadiscus* (red dots). On the right, a closer look to Uruguay showing the location of the temporary ponds sampled in this study (black star—Department of Rocha, 33°41′26″S, 054°41′15″W; white star—Department of Lavalleja 34°11′55″S, 053°55′45″W).

Historia Natural, MNHN), Montevideo, Uruguay.

Measurements are reported in micrometers, with the format: mean (standard deviation; minimum-maximum). Due to the small sample size, standard deviation was calculated with bootstrap (9999 repetitions) using the free software PAST4 (Hammer et al. 2001). For this same reason, no confidence intervals are reported in the parasitological indexes. The latter were calculated according to Bush et al. (1997).

Results

Superfamily Paramphistomoidea
Family Diplodiscidae
Genus *Catadiscus* Cohn, 1904

Catadiscus uruguayensis Freitas & Lent, 1939

Figure 2

Materials examined. URUGUAY • 1 specimen; Rocha Department, Ruta Vieja; 33°41′26″S, 054°41′15″W; 14 Sep. 2018; R. Vettorazzi leg.; temporary pond, manual collection, host: *Austrolebias cheradophilus* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1965); MNHN4215. • 1 specimen; Lavalleja Department, Retamosa; 34°11′55″S, 053°55′45″W; same data as for preceding, except host: *A. prognathus* (Amato, 1986); MNHN4216. • 3 specimens; same data as for preceding, except 27 Aug. 2019; MNHN4216.

Identification. Description based on three specimens stained and mounted.

Body sub-pyriform with smooth tegument, anterior surface region pigmented irregularly. Total length 1277 (218; 1100–1520), width at the equatorial level 405 (31; 370–430). Oral opening terminal, with undulated edges, diameter 133 (15; 120–150). Acetabulum sub-terminal, with medial constrain present but not well developed; 386 (23; 350–393) long, 323 (13; 310–335) wide. Pharynx strongly muscular, with extramural sacs, 211 (13; 200–225) long, 169 (4; 165–173) wide. Esophagus slim, 97 (15; 80–110) long; esophageal bulb present, 103 (20; 80–115) long, 90 (17; 70–100) wide. Intestinal ceca short, reaching equatorial level of the body, 301 (57; 250–405) long. Genital pore opens at the level of intestinal bifurcation. Cirrus sac globular, 141 (8; 134–150) long, 37 (8; 30–45) wide; distal extremity displaying seminal vesicle with sperm reservoir in living specimens. Vitelline follicles lateral, touching and surpassing posterior end of intestinal ceca. Testicle intercecal, oval; 112 (10; 100–120) long, 56 (21; 33–75) wide. Ovary post-testicular, diagonally opposite to testicle, oval; 108 (16; 90–120) long, 77 (13; 65–90) wide. Mehlis' glands visible at posterior margin of ovary. Uterus complex, forming coils which occupy entire region of body after caecal bifurcation, passing between testis and ovary. Eggs large, operculate, collapsed in fixed specimens; 81 (4; 78–86) long, 43 (2; 41–45) wide. Prevalence of 33% in *Austrolebias prognathus* (n = 6) and 3% in *Austrolebias cheradophilus* (n = 30). Mean Intensity of 2 in *A. prognathus* and 1 in *A. cheradophilus*.

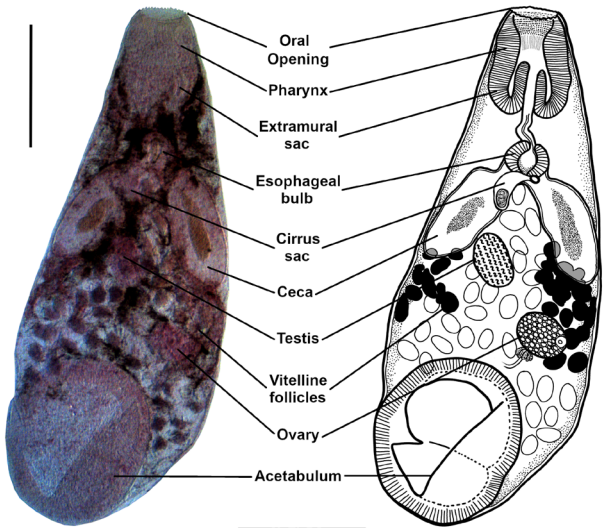

Figure 2. *Catadiscus uruguayensis* Freitas & Lent, 1939 collected from fish host; *C. uruguayensis* from first intestinal portion of *Austrolebias prognathus* (Amato, 1986) and *A. cheradophilus* (Vaz-Ferreira, Sierra, Soriano, Scaglia & Paulete, 1965) captured in temporary ponds from Southwest Atlantic basin, Southeast Uruguay. Scale bar: 300 µm.

Remarks. Given the previous description, the morphological features place the species reported now under the genus *Catadiscus* due to the lumen of the acetabulum being constricted into anterior and posterior regions, lacking a central accessory sucker or peduncle.

The specimens here described were most comparable to four of the 16 species described and currently valid for the genus, given important characters such as overall body shape, undulated edges of the oral opening, presence of extramural sacs in pharynx, presence of esophageal bulb, acetabula morphology, vitelline follicles distribution, relative position of gonads and the uterus, position of the gonopore, and egg size (Sey 1991; Jones et al. 2005). These four species are *C. uruguayensis*, *C. rochai* Correa & Artigas, 1978, *C. hylae* Incorvaia, 1983, and *C. pomaceae* Hamann, 1992 (Table 1). All the cited species are known to infect either amphibians or reptiles from Argentina, Brazil, and Uruguay (except *C. pomaceae*, which was found in the snail *Pomacea canaliculata* (Lamarck, 1801)) (Sey 1991).

In contrast to the specimens of the present work, *C. pomaceae* was described as having the uterus coils reaching between the oral opening and the cecal bifurcation, bigger pharynx, and somewhat different acetabula to body length ratio (Hamann 1992). *Catadiscus hylae* differs by not presenting undulated edges of the oral opening and having fewer vitelline follicles which do not surpass the posterior end of the intestinal ceca (Incorvaia 1983). *Catadiscus rochai* is different from the specimens of the present work, in not having uterine coils between testis and ovary (Corrêa and Artigas 1979; Sey 1991). Thus, the specimens here described shared the most similarities with *C. uruguayensis* (Sey 1991) regardless of

some variations such as vitelline follicle size, egg size, and testis size (Table 1). These differences whatsoever, have all been recorded in the literature.

Suriano (1970) and Ostrowski (1978) redescribed *C. uruguayensis*, offering insight on disagreements with the original description from Freitas and Lent (1939). For instance, Suriano (1970) and Ostrowski (1978) established the vitelline follicles as being similar in size to the eggs, as opposed to Freitas and Lent (1939) who regarded the vitelline follicles to be remarkably smaller than the eggs. Both Suriano (1970) and Ostrowski (1978) also accepted egg sizes inferior to 0.1 mm as still pertaining to the species *C. uruguayensis*, while Freitas and Lent (1939) did not. Regarding the genitalia, Ostrowski (1978) mentioned that the testis and ovary had similar size in *C. uruguayensis* collected from tadpoles of *Hyla pulchella* (Duméril & Bibron, 1841), yet the ones found in adult frogs presented considerably bigger testis. In the specimens found in this work the testis and the ovary had similar size. A possible explanation is the testicular atrophy reported in *Catadiscus*, which occurs when the uterus is filled with eggs (Freitas and Lent 1939; Ostrowski 1978).

This work represents the first report of the genus *Catadiscus* infecting and developing (adult, gravid parasites, with food content in their ceca) in a fish host.

Catadiscus uruguayensis has been reported in Uruguay by Freitas and Lent (1939) parasitizing *Leptodactylus latrans*. This definitive amphibian host is associated throughout its life history with the same type of ecosystem where *A. prognathus* and *A. cheradophilus* populations occur (Naya et al. 2003; Lavilla et al. 2010; Berois et al. 2015). Therefore, *A. prognathus* and *A. cheradophilus* could have been infected through ingestion of the tadpole of the definitive amphibian host (both *Austrolebias* species are reportedly piscivorous, thus capable of feeding on frog larvae; Costa 2009). However, this scenario would likely result in a case of concomitant predation (Johnson et al. 2010; Thieltges et al. 2013). Another possibility is infection by ingesting the metacercaria from the vegetation or substrate where they became encysted. It has not been studied if these fish have herbivore habits as juveniles, but it has been observed by Ostrowski (1978) that the substrate where the cercaria encysts is not specific, meaning they could encyst on the exterior of another prey such as an arthropod or a mollusk, which do form part of the diet of these species of *Austrolebias* (Laufer et al. 2009).

The mature specimens of *C. uruguayensis* found in this work are indicative of their ability to develop and fulfill their life cycle in freshwater fish hosts. This means that, despite the likelihood of the infection being accidental, this finding corroborates the generalist strategy of *Catadiscus*.

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Table 1. Comparison of *Catadiscus uruguayensis* Freitas & Lent, 1939 from the present work with the four most similar South American *Catadiscus* species. Data expressed in micrometers, in the form "mean ± standard deviation (minimum-maximum)", "minimum-maximum", or "mean", depending on the available data. AC: acetabula; L: length; TL: total length; W: width.

References		*C. uruguayensis*	*C. uruguayensis*	*C. hylae*	*C. pomaceae*	*C. rochai*
		Present work	**Suriano (1970); Ostrowski (1978)**	**Incorvaia (1983)**	**Hamann (1992)**	**Correa and Artigas (1979)**
Total length		1277 ± 218 (1100–1520)	990–2100	1380–3150	700–2700	1850
Medial width		405 ± 31 (370–430)	480–880	570–1560	250–1080	750
Oral opening		133 ± 15 (120–150)	100–190	80–100	110–210	90
Extramural sacs	L	211 ± 13 (200–225)	190–390	80–140	80–180	180
	W	169 ± 4 (165–173)	130–150	110–220	—	270
Esophageal bulb		97 ± 15 (80–110)	210–240	90–170	250–470	380
Pharynx	L	103 ± 20 (80–115)	72–160	110–150	190–280	—
	W	90 ± 17 (70–100)	75–140	50–90	130–170	—
Acetabula	L	368 ± 23 (350–393)	460–670	500–900	200–560	490
	W	323 ± 13 (310–335)	390–540	400–900	160–460	600
Testis	L	112 ± 10 (100–120)	140–280	—	—	170
	W	56 ± 21 (33–75)	150–280	—	—	220
Ovary	L	108 ± 16 (90–120)	65–160	—	—	100
	W	77 ± 13 (65–90)	78–130	—	—	70
Cirrus sac	L	141 ± 8 (134–150)	130	130–170	—	—
	W	37 ± 8 (30–45)	20	100	—	—
Eggs	L	81 ± 4 (78–86)	72–100	54–87	86–90	115
	W	43 ± 2 (41–45)	42–50	18–39	47–54	52
TL:AC		3.1–3.9	2.1–3.2	3.5	3.5–6.5	3.8
AC:pharynx		3.4–4.4	3.5–4.6	5.0	1.5–2.7	5.4
Locality		Uruguay	Argentina, Brazil, Uruguay	Argentina	Argentina	Brazil
Host type		Fish	Amphibian	Amphibian	Mollusk	Ophidian

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Authors' Contributions

RV collected, processed, and described the material, was the writer of the first draft, adapted it to the format of the journal, and made the major corrections throughout the peer-review process. WN coordinated and made the resources available. SM confirmed the righteousness of the identification. WN and SM worked as overseers of the research process and contributed to the correction of the draft.

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