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The South American lungfish *Lepidosiren paradoxa* as a new host for *Trichodina quelenii*

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(With 1 figure)

Abstract

Recently, the South American lungfish *Lepidosiren paradoxa* is being found inside aquaculture ponds, and even though there are a few studies on their parasite fauna, there is still much to be reported. Thus, the objective of this study is to report parasitism by trichodinids in *L. paradoxa*, as these ciliate protozoa are related to injuries and mortality in fish farming. The lungfish were collected from experimental tanks, had their tegument scraped and the resultant mucus was analyzed under an optical microscope for morphological and morphometrical analyses in Giemsa and silver nitrate stained slides. The species found was identified as *Trichodina quelleni*. This is the first report of this parasite in *L. paradoxa*, and the second report in cultivated fish in Brazil.

Keywords: aquaculture, ectoparasites, invasive species, trichodinids.

O lungfish sul-americano *Lepidosiren paradoxa* como novo hospedeiro para *Trichodina quelenii*

Resumo

Recentemente, o peixe pulmonado sul-americano *Lepidosiren paradoxa* tem sido encontrado em tanques de cultivo da aquicultura e, embora existam alguns estudos sobre a fauna de parasitas neste hospedeiro, ainda há muito a ser relatado. Assim, o objetivo deste estudo é relatar o parasitismo por tricodinídeos em *L. paradoxa*, pois esses protozoários ciliados estão relacionados a lesões e mortalidade na piscicultura. Os peixes foram coletados de tanques experimentais, tiveram seu tegumento raspado e o muco resultante foi analisado sob um microscópio óptico para análises morfológicas e morfométricas em lâminas impregnadas com Nitrato de Prata e com Giemsa. Os espécimes encontrados foram identificados como *Trichodina quelleni*. Este é o primeiro registro deste parasita em *L. paradoxa*, e a segunda ocorrência de *Trichodina quelenii* em peixes cultivados no Brasil.

Palavras-chave: aquicultura, ectoparasitas, espécies invasoras, tricodinídeos.

1. Introduction

Trichodinids are ciliate protozoa with broad geographic distribution (Maciel et al., 2017). These parasites can establish a comensal or parasitic relationship with several vertebrate and a few invertebrate hosts, such as molluscs (Xu et al., 1999; Xu et al., 2000; Hertel et al., 2004;), crustaceans (Silva et al., 2009; West et al., 2016), amphibians (Dias et al., 2009), waterfowl (Carnaccini et al., 2016) and fishs (Valladão et al., 2013). These parasites are often observed in wild and cultured freshwater marine and freshwater fish (Tantry et al., 2016), affecting the skin, gills, urinary bladder, and reproductive system of fishes (Asmat, 2001).

Among the trichodinids, the genus *Trichodina* Ehrenberg, 1838 is the most diverse, with more than 200 species affecting fish (Asmat et al., 2005). In Brazil, studies regarding this group of parasites is recent but, due to the economical losses they can cause in fish farming, interest in these ciliates is increasing (Dias et al., 2009). To date, the species of tricodinids found in native Brazilian fish have been *Trichodina heterodentata* in *Piaractus mesopotamicus* and *Prochilodus lineatus* (Pádua et al., 2012; Valladão et al., 2014), *Trichodina colisae* in *P. mesopotamicus* and *Piaractus brachypomus* (Jerônimo et al., 2012), and *Trichodina quelenii* in *Rhamdia quelen* and *Gymnotus* sp. (Hashimoto et al., 2016).

There are six species representing the lungfishs worldwide, with only one species registered in South America: *Lepidosiren paradoxa* Fitzinger, 1837 (Mesquita-Saad et al., 2002; Lainson and Ribeiro, 2006). The South American lungfish is native to the Amazon and Paraná-Paraguay basins. These animals inhabit lentic or low current environments, and support low concentrations of dissolved oxygen in water (Almeida-Val et al., 2016). There are a few records of parasitism in South American lungfish, with registers of *Haemogregarina lepidosirenis* (Jepps, 1927), *Agarella gracilis* (Dunkerly, 1915; Vita et al., 2004), and *Eimeria lepidosirenis* (Lainson and Ribeiro, 2006). Thus, the objective of this study is to report parasitism by trichodinids in this host, contributing to the knowledge of its parasite fauna.

2. Material and Methods

The studied specimens of *Lepidosiren paradoxa* came from the São Paulo State University (CAUNESP), on the Jaboticabal campus, state of São Paulo, Brazil. They were kept in 1000 L tanks at a water level of 1 m and controled temperature at 25 °C ± 1 °C at all times.

Body surface mucus smears were prepared on slides for fresh analysis under a microscope. The smears were dried at room temperature and impregnated with silver nitrate 2% to observe the adhesive disk morphology, as reported by Klein (1958). Other smears were stained with Giemsa for further study of nuclear structures, as suggested by Lom (1958).

The measurements of 100 specimens, obtained with the Moticam 2300 attached to a Nikon E200 microscope, were expressed in milimetes as arithmetic means ± standard deviation followed, in parentheses, by the minimum and maximum values and the number of measured structures. In addition, schematic drawings of the denticles, as proposed by Van As and Basson (1989), were produced by means of vectorization, with CorelDraw® X5 software.

3. Results

The population of *Trichodina* sp. parasitizing *L. paradoxa* was classified as a medium-sized trichodinids, with the morphometric data presented in Table 1. The morphology of the trichodinids found has a broad blade, exceeding the axis y + 1. Anterior margin slightly developed, almost parallel to the distal margin. Apophysis of the prominent blade. Posterior margin of the concave blade. Presence of posterior projection not well developed in some specimens. Well-developed central part with rounded end, located

Table 1. Morphometric data (μm) of the trichodinids found in *Lepidosiren paradoxa* in comparison with the morphometric data of the first report of *Trichodina quelenii* in two different hosts, *Rhamdia quelen* and *Gymnotus* sp.

Parasite species	*Trichodina quelenii*	*Trichodina quelenii*	*Trichodina quelenii*
	Present study	Hashimoto et al. (2016)	Hashimoto et al. (2016)
Host	*Lepidosiren paradoxa*	*Rhamdia quelen*	*Gymnotus* sp.
Infection site	Skin	Skin and gills	Skins and gills
Local	São Paulo, Brazil	Santa Catarina, Brazil	Mato Grosso do Sul, Brazil
Body^{D}	55.0 ± 2.5 (51.2-59.6)	54.4 ± 3.7 (49.7-61.5)	51.2 ± 4.6 (41.1-62.3)
Adhesive disc^{D}	45.2 ± 2.6 (41.8-49.5)	45.3 ± 3.8 (41-52.8)	41.2 ± 3.9 (31.8-50.2)
Border membrane^{W}	15.4 ± 1.9 (11.2-18.6)	14.6 ± 3.05 (9.8-19.7)	5.1 ± 0.5 (3.9-6.1)
Denticulate ring^{D}	26.9 ± 1.4 (24.7-29.8)	27.3 ± 2.7 (24.4-33.1)	24.4 ± 2.6 (19.2-29.8)
Number of denticles	21 ± 0.6 (20-22)	21 ± 0.6 (19-22)	19.3 ± 1.2 (18-23)
Pins per Denticle	9.0 ± 1.1 (8-11)	9.4 ± 1.5 (6-11)	8.7 ± 0.7 (8-11)
Denticle^{L}	8.7 ± 0.8 (7.2-9.9)	8.4 ± 0.5 (7.8-9.4)	8.4 ± 0.8 (6.5-10.1)
Blade^{L}	3.9 ± 0.9 (2.3-5.4)	4.3 ± 0.5 (3.9-5.5)	3.7 ± 0.5 (2.6-4.9)
Central portion^{W}	3.7 ± 0.4 (3.1-4.5)	3.8 ± 0.5 (3.1-4.7)	3.3 ± 0.3 (2.5-3.9)
Ray^{L}	6.4 ± 1.3 (3.9-8.4)	6.7 ± 1.3 (4.7-8.6)	6.5 ± 0.8 (3.9-8.3)
Span^{L}	15.6 ± 1.7 (12.4-18.5)	14.9 ± 1.7 (12.2-18.1)	13.4 ± 1.4 (9.0-15.7)
Ciliature	-	367° (364°-373°)	-
Macronucleus^{D}	50.5 ± 4.8 (39.8-67.5)	-	46.2 ± 6.3 (29.8-59.2)
MacronucleusTH	8.1 ± 1.7 (5.1-12.7)	-	7.6 ± 1.5 (4.9-11.9)
Macronucleus^{LT}	12.6 ± 5.9 (3.2-24.7)	-	11.5 ± 3.5 (6.0-20.2)
Micronucleus^{L}	3.8 ± 1.1 (2.1-6.7)	-	-
Micronucleus^{W}	5.3 ± 1.7 (3.4-10.3)	-	-
Distance from the Micronucleus to the end of the Macronucleus	20.2 ± 6.2 (6.4-31.8)	-	-

Measurement data were described as the mean ± standard deviation (minimum - maximum; number of repetitions), ^{L} = length, ^{D} = diameter, ^{W} = width, TH = thickness, ^{LT} = length between terminations of macronucleus.

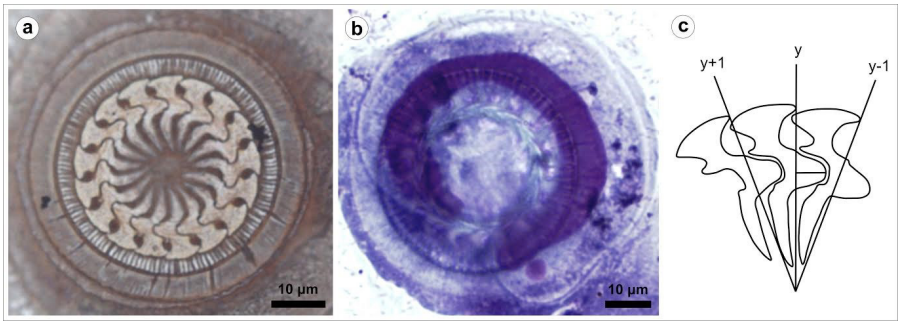


Figure 1. *Trichodina quelenii* stained with 2% silver nitrate (a); *Trichodina quelenii* stained with Giemsa with evidence of the macronucleus and micronucleus in position +y (b); Schematic drawing of denticles (c).

between the axes and + 1 and y-1. Discrete ray apophysis. Well-developed ray, thick, rounded end protruding posteriorly. The macronucleus is present in some trichodinids (51 specimens), as well as a micronucleus (21 specimens) located in the +y position, the first description of this structure being in *Trichodina quelenii* (Figure 1). According to the morphology and morphometry the trichodinids found resemble the species *Trichodina quelenii*.

4. Discussion

There are no reports of any trichodinids in *L. paradoxa* so far. There is only a recent report of the genus *Trichodina* sp. infesting the African lungfish *Protopterus annectens*, but with low prevalence, 3.25% in the dry season and 2.83% in the rainy season (Omeji et al., 2017).

This is the second report of *Trichodina quelenii*, the first description being in the studied host. The first report was made in Brazil in two different hosts, *Gymnotus* sp. of natural environment and *Rhamdia quelen* coming from natural environment and fishfarm. (Hashimoto et al., 2016). The morphometric and morphologic descriptions are similar, even though we observed the presence of micronucleus in some specimens, a characteristic not reported in the original description of this species. Trichodinids are important parasites in the production systems of several species of fish, because the accumulation of organic matter in association with high storage densities causes an environmental imbalance that facilitates the multiplication and dissemination of the parasite. The genus *Trichodina* has been reported in several species of fish, causing damage to epithelial and epidermal cells (Ovcharenko, 2015), epithelial hypertrophy and vacuolar degeneration (Abdel-Baki et al., 2011), areas of inflammatory infiltration (Valladão et al., 2013), areas of desquamation and suction in the tegument (Valladão et al., 2016) caused by the excessive spoliation that allows the consequent installation of bacterial infections (Plumb, 1997) and mortality may occur in severely parasitized fish (Xu et al., 2015).

The South American lungfish is a highly resistant fish that supports extreme environmental conditions and is therefore found to be introduced into rivers, thus increasing its range, leading to risk for native and cultured fish populations (Arantes et al., 2016). Their presence inside tanks of aquatic organisms is an important source of multiplication and dispersion of trichodinids in breeding systems, thus becoming a potential risk of contagion in this situation. The first report of this parasite under cultivation was in *Rhamdia quelen*, in the state of Santa Catarina (Hashimoto et al., 2016), but possibly other reports may occur due to the restricted contact between *L. paradoxa* and other fish grown on a larger scale in Brazil, such as Nile tilapia (*Oreochromis niloticus*) or native fish such as pacu (*Piaractus mesopotamicus*) and tambaqui (*Colossoma macropomum*). Thus, further studies should be carried out to elucidate the possible biological role of parasitic dispersant of *L. paradoxa*, in commercial fish culture environments, in order to prevent diseases, reducing financial expenses due to producion losses or mortality caused by trichodinids parasitism.

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