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Trichodina colisae (Ciliophora: Trichodinidae): novo registro de parasito para duas espécies de peixes de água doce cultivadas no Brasil

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Abstract

Family Trichodinidae comprises ciliate protozoa distributed worldwide; they are considered some of the main parasitological agents infecting cultivated fish. However, the trichodinidae parasitizing important fish species cultured in Brazil are unknown, and more taxonomic studies on this group of parasites are required. This research morphologically characterizes *Trichodina colisae* Asmat & Sultana, (2005) of pacu (*Piaractus mesopotamicus*) and patinga hybrid (*P. mesopotamicus* × *P. brachypomus*) cultivated in the central and southeast regions of the country. Fresh assemblies were made from mucus scraped from the skin, fins and gills, fixed with methanol and, subsequently, impregnated with silver nitrate and stained with Giemsa for assessment under light microscopy. This research reports not only the second occurrence of *T. colisae* in the world, but also its first occurrence in South America.

Keywords: Pacu, patinga hybrid, fish parasite, aquaculture health.

Resumo

Tricodinídeos são protozoários ciliados móveis com ampla distribuição mundial; são considerados um dos agentes parasitários que mais acometem peixes cultivados. No Brasil, a maioria dos tricodinídeos que parasitam importantes espécies de peixes cultivados são desconhecidos, o que requer mais estudos taxonômicos com esse grupo de parasitos. Este estudo caracteriza morfologicamente *Trichodina colisae* Asmat & Sultana, 2005 de pacu (*Piaractus mesopotamicus*) e do híbrido patinga (*P. mesopotamicus* × *P. brachypomus*) cultivados, respectivamente, no Centro-Oeste e Sudeste do Brasil. Foram feitas montagens a fresco do raspado de muco da pele, nadadeiras e brânquias, fixados com metanol e, posteriormente, impregnados com nitrato de prata e coradas com Giemsa para avaliação em microscopia óptica. O presente estudo relata não só a segunda ocorrência de *T. colisae* no mundo, mas também a primeira ocorrência na América do Sul.

Palavras-chave: Pacu, patinga, parasito de peixe, sanidade aquícola.

Introduction

Trichodinids are ciliate protozoa widely known as ectocommensals and/or parasites of fishes. Members of the family Trichodinidae, they present complex structures on the aboral face, composing the adhesive disk (BASSON; VAN AS, 2006), which presents fast circular movements that can cause abrasive lesion on the host when at high levels of parasitism, even being able to cause mortality (KHAN, 2004). Situations like these usually occur in fish breeding, since changes in the environmental quality directly influence the occurrence of these protozoa (HOSSAIN et al., 2008).

Knowledge on the fauna of trichodinidae infecting fish in Brazil is little known. So far, only the occurrence of *Tripartiella pseudoplatystomae* in pintado (*Pseudoplatystoma corruscans*) (PINTO et al., 2009) and *Trichodina heterodontata* in pacu (*Piaractus mesopotamicus*) (PÁDUA et al., 2012) were reported in Brazilian native fishes. Nevertheless, *Trichodina compacta* and *Trichodina magna* have been reported in Nile tilapia (GHIRALDELLI et al., 2006; MARTINS; GHIRALDELLI, 2008) and *T. heterodontata* in channel catfish (MARTINS et al., 2010); they are both exotic species farmed in Brazil.

The industrial production of native freshwater fish in Brazil is concentrated in the central and north regions, being less in the northeast, southeast and south regions of the country. Roundfish are among the species farmed in Brazil, including pacu (*Piaractus*

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mesopotamicus), tambaqui (*Colossoma macropomum*), pirapitinga (*Piaractus brachypomus*), and the hybrids tambacu (*C. macropomum* × *P. mesopotamicus*), tambatinga (*C. macropomum* × *P. brachypomus*) and patinga (*P. mesopotamicus* × *P. brachypomus*). Little information is available regarding parasite infecting these fishes in commercial production systems.

This study contributes to the description of *Trichodina colisae* Asmat and Sultana (2005) as a new parasite for pacu and patinga hybrid farmed in Brazil.

Materials and Methods

Pacu were captured in fish ponds in the southeast region of the country (20° 20' 47.1" S and 48° 11' 27.1" O) and were denominated Population A; hybrid patinga (*P. mesopotamicus* × *P. brachypomus*) farmed in fish ponds were captured in the southeast region (22° 14' 22.7" S and 48° 17' 33.4" O) and denominated Population B; whereas pacu were captured in fish ponds in the central regions (22° 19' 42.7" S and 54° 43' 55.2" O) and were denominated Population C. Fresh assemblies from mucus scraped from the skin, fins and gills were assessed using light microscopy for the searching of trichodinidae. When positive, the slides were dried; 30% were fixed with methanol and stained with Giemsa

(1 drop: 1 mL of distilled water, from 120 to 180 minutes) to highlight the nuclear apparatus. Others (70%) were subjected to impregnation with silver nitrate (2%) for observation of the adhesive disk (KLEIN, 1958).

The span of the denticle was measured from the blade tip to the ray tip, as described by Arthur and Lom (1984). All measures are in micrometers and follow the recommendations by Lom (1958) and Van As and Basson (1989), using photomicrographies taken with Nikon E200® light microscope, equipped with Moticam 2300® image capture system. The measurement of the parasite was performed with the assistance of Image-Pro Plus® software. In addition, the schematic diagram of the denticles, as proposed by Van As and Basson (1989), was built through vectorization, using CorelDRAW® X5 software.

Results

In this study, medium-sized, disk format trichodinids were found on the skin, fins and gills in 100% of pacu (Population A) and hybrid patinga (Population B) in southeastern Brazil, as well as on the skin and fins of 52% the analyzed pacu (Population C) from central region (Figure 1a-c). Measurements of taxonomic characters of the three populations are listed in Table 1. The

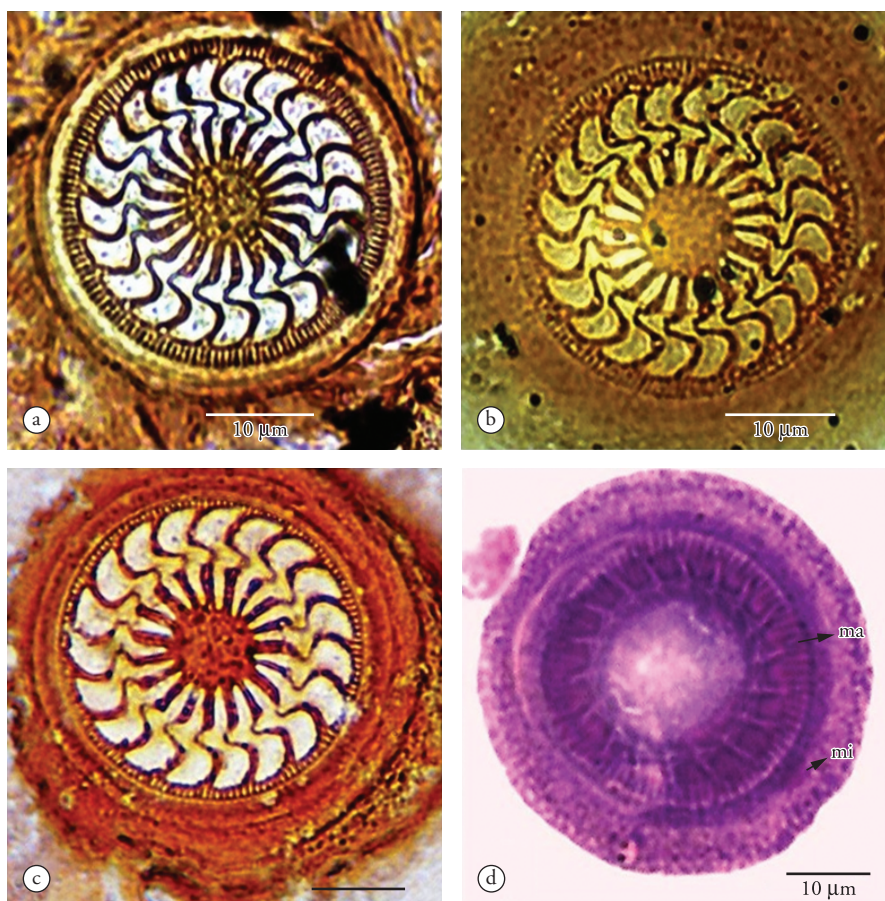


Figure 1. Klein's silver impregnated *Trichodina colisae* Asmat and Sultana (2005) from pacu (a) and hybrid patinga (b) in Southeastern Brazil, and pacu (c) from Central Brazil. Giemsa-stained nuclear apparatus (d) showing the horseshoe-shaped macronucleus (ma) and oval micronucleus (mi). Bar = 10 µm.

measurements of *Trichodinidae* populations A, B and C were set based on the assessment of 30, 45 and 28 specimens, respectively.

This trichodinid presents wide blade, forming a moderately closed angle, fitting itself in the quadrant delimited by the axes y and $y + 1$. The anterior margin of the blade is convex and its apex, in most cases, surpasses the axis $y + 1$. However, in some specimens, the blade apex touches the axis $y + 1$ without surpassing it. The distal surface of the blade is flat, sometimes round, parallel to the membrane of the border. The point of tangency is oblong in most individuals and may present acute angle in others, it is situated slightly above or at the same height of the distal point of distal surface in populations A and B, but below or at the same height from the distal point of distal surface in population C. Blade apophysis is not present. The blade fitting with the central part is thin on populations A and B, and robust in population C (Figure 2). No posterior projection was

observed in the sample. The central part is thin, triangular, with no conical part, with an oblong tip and free from indentation below the X axis. In some specimens, the presence of discrete apophysis near the fitting with the ray was noticed. The ray is moderately thick, inflated, straight, and oblong-tipped, usually accompanying the Y axis in most individuals; some may present different lengths in the same specimen, but this difference is discreet. In population A, few individuals showed discreetly posterior-directed rays, with its proximal portion surpassing the Y axis. On the other hand, in population B, in some cases, the radius is projected anteriorly, with its proximal portion surpassing the axis $y+1$. The nuclear apparatus, evidenced by the Giemsa staining, revealed the presence of horseshoe-shaped macronucleus, with oval micronucleus located in the $+y$ portion. However, the micronucleus was only detected in some specimens (Figure 1d).

Table 1. Measurements (μm) of *Trichodina colisae* Asmat and Sultana (2005) from two cultured freshwater fish in Brazil. Population A and C have the pacu as host; population B have the hybrid patinga as host.

Characters	Population A	Population B	Population C
Local	Southeastern Brazil	Southeastern Brazil	Central Brazil
Body ^D	35.6 \pm 2.6 (31.2-41.4)	40.0 \pm 3.7 (32.0-46.6)	41.9 (34.7-51.3)
Border membrane ^W	3.1 \pm 0.4 (2.2-4.2)	3.5 \pm 0.4 (2.1-4.3)	-
Adhesive disc ^D	29.5 \pm 2.4 (24.2-33.9)	32.9 \pm 3.4 (25.6-39.6)	33.3 (26.8-48.9)
Denticular ring ^D	17.7 \pm 1.8 (14.5-20.6)	19.7 \pm 2.6 (14.8-25.2)	19.7 (15.0-24.4)
Number of denticles	21.1 \pm 1.4 (19-24)	20.9 \pm 1.6 (17-25)	20.5 (18-23)
Denticle span	9.6 \pm 1.0 (7.6-11.7)	10.4 \pm 1.2 (7.8-13.9)	9.6 (5.5-11.8)
Denticle ^L	4.7 \pm 0.6 (3.7-5.7)	6.1 \pm 0.7 (4.2-7.7)	5.0 (4.0-7.1)
Blade ^L	4.0 \pm 0.3 (3.5-4.8)	3.9 \pm 0.4 (3.1-4.8)	4.0 (3.1-5.5)
Central Part ^W	1.5 \pm 0.3 (0.9-2.1)	2.0 \pm 0.3 (1.3-2.6)	1.7 (1.3-2.3)
Ray ^L	4.1 \pm 0.7 (2.8-5.7)	4.3 \pm 0.8 (2.8-6.9)	4.7 (3.1-8.6)
Pins per denticle	5.7 \pm 0.8 (5-8)	7.0 \pm 0.8 (5.0-8.0)	8.3 (7-10)

^Ddiameter, ^Wwidth, ^Llength.

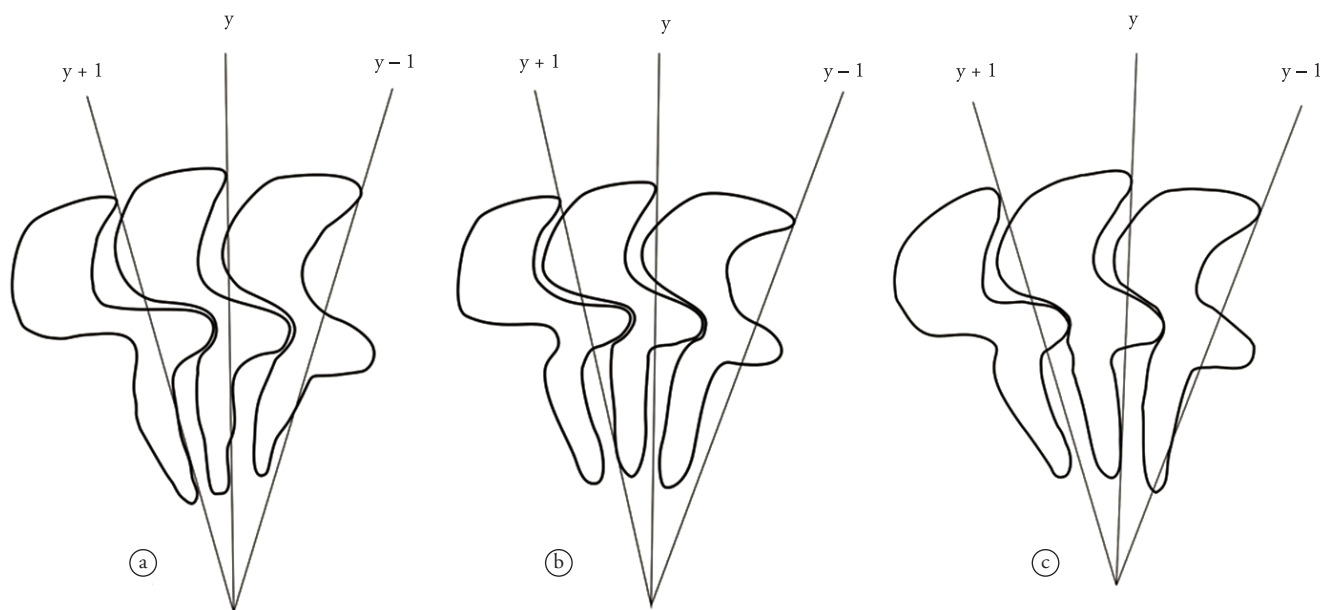


Figure 2. Schematic drawing of the denticles of *Trichodina colisae* Asmat and Sultana (2005) from pacu (a) and hybrid patinga (b) in Southeastern Brazil, and pacu (c) from Central Brazil.

Remarks

From the comparison of the 11 taxonomic characters obtained in the measurements of 10 species of trichodinidae previously described in the literature (Table 2), it was possible to observe that 45.5% of the characteristics were similar to *Trichodina nigra* Lom (1960) in the description by Basson et al. (1983) and *Trichodina pagoda* Basson and Van As (1994). *Trichodina erbilensis*

Shwani et al. (2010) and *Trichodina ngoma* Van As and Basson (1992) presented 63.6% similarity, whereas *Trichodina modesta* Lom (1970) and *Trichodina minuta* Basson et al. (1983) matched 81.8% of the measurements in this study. The trichodinids with over 90% of observed similarities were *Trichodina kazubski* Van As and Basson (1989) (90.9%), *Trichodina nkasa* Van As and Basson (1992) (90.9%), *Trichodina matsu* Basson and Van As (1994), and *Trichodina colisae* Asmat and Sultana (2005) (100% both).

Tabela 2. Measurements (μm) of the most similar species of *Trichodina* with the present material. The means are followed by standard deviation, and in parenthesis the minimum and maximum values.

Characters	<i>Trichodina modesta</i> Lom (1970)	<i>Trichodina minuta</i> Basson et al. (1983)	<i>Trichodina nigra</i> Lom (1960) in Basson et al. (1983)	<i>Trichodina kazubski</i> Van As and Basson (1989)
Local	Hungary	South Africa	South Africa and Israel	South Africa
Body ^D	44.0 (34-56)	33.3 \pm 3.2 (28.2-38.0)	45.9 \pm 3.1 (41.7-56.7)	41.2 \pm 4.5 (34.3-54.6)
Border membrane ^W	3-3.5	3.3 \pm 0.3 (2.6-4.1)	5.1 \pm 0.4 (4.0-5.9)	4.3 \pm 0.6 (3.2-5.9)
Adhesive disc ^D	33.0 (28-43)	27.2 \pm 3.0 (22.4-33.7)	35.1 \pm 2.9 (31.7-42.6)	32.9 \pm 3.7 (26.7-39.5)
Denticular ring ^D	18 (15-20)	15.5 \pm 1.7 (12.2-18.2)	20.6 \pm 2.0 (18.9-23.7)	20.2 \pm 2.4 (16.4-26.3)
Number of denticles	23 (22-25)	21 (19-22)	20 (19-22)	23 (22-26)
Denticle ^L	3-3.5	4.2 \pm 0.5 (3.2-5.7)	6.3 \pm 0.7 (5.1-7.5)	4.3 \pm 0.5 (3.6-5.7)
Blade ^L	4-4.5	3.7 \pm 0.4 (3.1-4.6)	4.6 \pm 0.7 (3.3-5.9)	4.6 \pm 0.7 (3.5-5.7)
Central Part ^W	1.5	2.2 \pm 0.2 (1.7-2.7)	2.5 \pm 0.7 (1.6-3.0)	2.0 \pm 0.4 (1.5-3.2)
Ray ^L	3.5-4.0	3.4 \pm 0.5 (2.6-4.4)	3.9 \pm 0.6 (2.5-4.9)	5.2 \pm 0.6 (3.6-6.4)
Pins per denticle	7-8	6 (6-7)	10 (8-11)	8 (7-10)
Adoral ciliary spiral	-	400-410°	380-410°	400°
Characters	<i>Trichodina nkasa</i> Van As and Basson (1992)	<i>Trichodina ngoma</i> Van As and Basson (1992)	<i>Trichodina pagoda</i> Basson and Van As (1994)	<i>Trichodina matsu</i> Basson and Van As (1994)
Local	Namibia	Namibia	Taiwan	Taiwan
Body ^D	34.1 \pm 3.0 (29.5-39.5)	43.4 \pm 6.0 (36.5-57.0)	46.5 \pm 2.4 (41.0-51.0)	40.4 \pm 2.7 (35.5-46.5)
Border membrane ^W	2.8 \pm 0.5 (2.5-4.0)	3.4 \pm 0.4 (2.5-4.0)	4.1 \pm 0.3 (3.5-5.0)	3.6 \pm 0.4 (3.0-4.0)
Adhesive disc ^D	28.5 \pm 2.8 (24.5-34.5)	36.7 \pm 5.7 (29.5-49.5)	38.2 \pm 2.7 (32.0-43.0)	33.1 \pm 3.1 (26.0-40.0)
Denticular ring ^D	16.4 \pm 1.9 (13.0-19.5)	23.1 \pm 4.0 (18.0-30.5)	22.6 \pm 2.1 (19.0-29.0)	19.0 \pm 1.8 (15.0-22.0)
Number of denticles	20 (19-22)	23 (21-27)	22 (19-24)	21 (20-27)
Denticle span	9.5 \pm 1.1 (7.5-11.5)	11.0 \pm 1.5 (8.5-15.0)	13.8 \pm 1.4 (12.0-16.0)	10.5 \pm 0.9 (8-12)
Denticle ^L	5.4 \pm 0.7 (4.5-7.0)	5.0 \pm 0.8 (3.1-6.0)	6.2 \pm 0.4 (5.5-7.0)	5.5 \pm 0.6 (5.0-7.0)
Blade ^L	4.1 \pm 0.5 (3.0-5.0)	4.4 \pm 0.7 (3.5-6.0)	5.1 \pm 0.5 (4.0-6.0)	4.2 \pm 0.4 (3.5-5.0)
Central Part ^W	1.7 \pm 0.3 (1.5-2.0)	1.7 \pm 0.2 (1.5-2.5)	2.2 \pm 0.3 (2.0-3.0)	1.9 \pm 0.2 (1.5-2.0)
Ray ^L	3.7 \pm 0.7 (2.5-5.0)	4.9 \pm 1.3 (3.0-9.0)	6.4 \pm 0.6 (5.0-7.5)	4.1 \pm 0.4 (3.0-5.0)
Pins per denticle	7 (6-8)	10 (8-10)	8 (7-9)	7 (6-8)
Adoral ciliary spiral	400-425°	408°	410-430°	415-440°
Characters	<i>Trichodina colisae</i> Asmat and Sultana (2005)		<i>Trichodina erbilensis</i> Shwani et al. (2010)	
Local	Bangladesh		Iraq	
Body ^D	36.9 \pm 3.0 (35.8-44.9)		44.4 \pm 0.4 (42-48)	
Border membrane ^W	3.7 \pm 0.4 (3.1-4.6)		3.9 \pm 0.2 (3-5)	
Adhesive disc ^D	32.2 \pm 2.6 (28.8-36.2)		37.5 \pm 0.4 (35-40)	
Denticular ring ^D	18.3 \pm 1.9 (16.3-20.9)		23.6 \pm 0.3 (22-27)	
Number of denticles	23.1 \pm 1.3 (20-24)		24.6 \pm 0.3 (23-27)	
Denticle span	10.5 \pm 0.5 (9.9-11.2)		10.9 \pm 0.2 (10-13)	
Denticle ^L	5.2 \pm 0.2 (4.9-5.6)		5.4 \pm 0.3 (4-7)	
Blade ^L	4.2 \pm 0.6 (3.1-5.1)		4.6 \pm 0.2 (4-6)	
Central Part ^W	2.0 \pm 0.1 (1.5-2.1)		2.6 \pm 0.1 (2-3)	
Ray ^L	4.1 \pm 0.2 (3.8-4.6)		3.7 \pm 0.1 (3-4)	
Pins per denticle	9.1 \pm 0.8 (8-10)		5.4 \pm 0.3 (4-8)	
Adoral ciliary spiral	-		-	

^Ddiameter, ^Wwidth, ^Llength, THthickness, ^{LT}length between terminations of macronucleus.

Trichodina pagoda (Basson and Van As, 1994) and *T. erbilensis* (Shwani et al., 2010) are similar to the material of this study regarding the number of denticles, pins per denticle, length of denticle, and ray length. *T. ngoma* (Van As and Basson, 1992) is similar concerning body measurement, border membrane, number of denticles, radial pins by denticle, denticle length, central part and ray length. On the other hand, *T. minuta* Basson et al. (1983) differs from the species found in this research in denticle length and ray length, which were smaller in this species. Similarly, *T. nkasa* (Van As and Basson, 1992) shows ray of smaller size, and this species also has a prominent apophysis in the fitting of the central part with the ray. In this research, it was possible to observe only a discrete apophysis in some specimens.

The border membrane is broad in *T. kazubski* (Van As and Basson, 1989), which can be confirmed in the description by Tang et al. (2007) for specimens collected from cyprinids in China. Additionally, this species differs from the others by presenting apophysis in the anterior blade margin and prominent apophysis of the ray (VAN AS; BASSON, 1989). In the central part, *T. kazubski* (Van As and Basson, 1989) shows discreet indentation below the X axis, opposite to the ray apophysis of the posterior denticle, which was not perceived in this research.

Two species of trichodinidae showed 100% of morphometric similarity with the specimens analyzed in this study, namely *T. matsu* (Basson and Van As, 1994) and *T. colisae* (Asmat and Sultana, 2005). Asmat and Sultana (2005) recognized the similarity between these species and have set some criteria to distinguish the two of them. Among these criteria, the following were observed in the sample specimens, namely: 1- absence of indentation in the inferior part of the X axis of the central part of *T. colisae*; 2-thick, inflated, round-tipped ray in *T. colisae*, while in *T. matsu* it is thin and sharp tipped; 3-the posterior blade margin forms a triangular curve in *T. matsu*, while in *T. colisae*, it is growing and shallow. These three main characteristics of the species based the differentiation of trichodinid species found in the Brazilian fish of this research, coinciding with *T. colisae* Asmat and Sultana (2005).

Trichodina colisae was initially reported on gills of colisa *Polyacanthus fasciatus* (synonym *Trichogaster fasciatus*), an ornamental fish from Bangladesh. This is the second record of this species of trichodinid in the world and the first one in Brazil. Pacu (*P. mesopotamicus*) and hybrid patinga (*P. mesopotamicus* × *P. brachypomus*) are new records of hosts for this parasite.

Taxonomic Summary

- Host: Colisa (*Polyacanthus fasciatus*) (synonym *Trichogaster fasciatus*);
- Location: Bangladesh;
- New hosts: Pacu (*Piaractus mesopotamicus*) and hybrid patinga (*P. mesopotamicus* × *P. brachypomus*);
- New location: Guaíra (20° 20' 47.1" S and 48° 11' 27.1" O) and Jaboticabal (22° 14' 22.7" S and 48° 17' 33.4" O), São Paulo, Southeast of Brazil; Dourados (22° 19' 42.7" S and 54° 43' 55.2" O), Mato Grosso do Sul, Central Brazil.
- Site of infestation: Skin, fins and gill.

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