

Trichodina modesta: an exotic ciliate in the Neotropical region parasitizing an unusual host

Trichodina modesta: exótico ciliado na região Neotropical parasitando hospedeiro não usual

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Abstract

In this study, an important ornamental fish, *Betta splendens* (Osphronemidae), from three different Brazilian states was examined for parasitic infestations. Smears with parasites were impregnated with silver nitrate or stained using Giemsa for taxonomic evaluation. A disc-shaped trichodinid with a body diameter of $39.7 \pm 3.3 \mu\text{m}$, adhesive disc diameter of $32.9 \pm 3.1 \mu\text{m}$ and denticulate ring diameter of $19.5 \pm 2.0 \mu\text{m}$ was found. The morphological characteristics resembled those of *Trichodina modesta* Lom, 1970, a species that shows clear host specificity for Cypriniformes. Until now, its occurrence was restricted to the Eurasian region. In the present study, a new host for *T. modesta* is reported and therefore the first occurrence of this species in the Americas. The parasite was possibly introduced into the Neotropical region through the exotic fish trade, especially of Cypriniformes used by aquarists. The distribution of this ciliate is discussed and a checklist of localities and hosts for the species is provided.

Keywords: Ornamental fish, Ciliophora, Trichodinidae.

Resumo

Neste estudo, o peixe ornamental *Betta splendens* (Osphronemidae) foi examinado a partir de três diferentes Estados brasileiros para infestações parasitárias. Esfregaços contendo parasitos foram impregnados com nitrato de prata ou corados com Giemsa para avaliação taxonômica. Foi observado o tricodínídeo em forma de disco com $39,7 \pm 3,3 \mu\text{m}$ de diâmetro do corpo, disco adesivo com $32,9 \pm 3,1 \mu\text{m}$ e anel denticulado $19,5 \pm 2,0 \mu\text{m}$ de diâmetro. As características morfológicas foram semelhantes à *Trichodina modesta* Lom, 1970, uma espécie que apresenta especificidade de hospedeiro por peixes Cypriniformes com ocorrência na Região Euro-asiática. No presente estudo, um novo hospedeiro para *T. modesta* é reportado, assim como a primeira ocorrência da espécie na América. O parasito tem sido possivelmente introduzido na região Neotropical com o comércio de peixes exóticos, principalmente Cypriniformes usados por aquaristas. A dispersão do ciliado é discutida e um “checklist” de localidades e hospedeiros apresentado.

Palavras-chave: Peixe ornamental, Ciliophora, Trichodinidae.

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Introduction

Betta splendens (Perciformes: Osphronemidae) is commonly farmed as an ornamental fish over the world. According to Monvises et al. (2009), commercialization of *Betta* spp. was valued at approximately US\$ 800,000 in Thailand alone in 2005, and also in July 2013. Ornamental fish production is of great economic importance worldwide, but there is a lack of scientific information on the diseases of these fish and dissemination of trichodinids in particular. *Trichodina nigra* Lom, 1961 (THILAKARATNE et al., 2003), *Trichodina acuta* Lom, 1961 (PIAZZA et al., 2006), *Trichodina reticulata* Hirschman and Partsch, 1955 (ALBALADEJO & ARTHUR, 1989; MAHMOUD et al., 2009; MARTINS et al., 2012; HU, 2012), *Trichodina nobilis* Chen, 1963 (MARTINS et al., 2012), *Trichodina luzhoues* Hu, 2012, and *Trichodina mutabilis* Kazubski and Migala, 1968 (HU, 2012), have been reported in ornamental fish. However, only *Trichodina* sp. (THILAKARATNE et al., 2003) and *T. acuta* (PIAZZA et al., 2006) have been reported from *Betta splendens*.

Trichodina modesta Lom, 1970, was initially described in Hungary, Slovakia and the Czech Republic by Lom (1970), with subsequent reports from Russia (STEIN, 1982; ARTHUR & LOM, 1984), Taiwan (BASSON & VAN AS, 1994), Poland (WIERZBICKA, 1997; WŁASOW et al., 2003), United Kingdom (GAZE & WOOTTEN, 1998), Turkey (ÖZER, 2007; ÖZTÜRK & ÖZER, 2007), Germany (RÜCKERT et al., 2007), China (ZHAO & TANG, 2007; LIU & ZHAO, 2010; HAN & ZHAO, 2011; TANG et al., 2013) and Bangladesh (KIBRIA et al., 2010). All of these reports were from countries in Europe and Asia. The hosts susceptible to *T. modesta* include *Crossostoma lacustre*, *Misgurnus anguillicaudatus*, *Rhinogobius brunneus* (BASSON & VAN AS, 1994) and *Aphanius danfordii* (ÖZER, 2007). All of these species are appreciated as ornamental fish, and this also includes *B. splendens*. Trichodinid dispersion is facilitated through the ornamental fish trade between countries. Albaladejo & Arthur (1989) have reported not only occurrences of *T. reticulata* and *Trichodina* sp. from ornamental fish, but also *T. acuta*, *T. nobilis*, *T. nigra*, *Trichodina heterodontata* Duncan, 1977, *Tripartiella tilapiae* Duncan, 1977, and *Trichodinella epizootica* Raabe, 1950, from farmed imported cyprinids. Consequently, exportation and importation of ornamental fish represents an important risk factor for parasite dispersion worldwide.

Trichodinid parasitism may cause lesions on its hosts under farming conditions as a result of rapid trichodinid proliferation. Pathological alterations such as hyperplasia of the secondary lamellae (YEMMEN et al., 2010, 2011), cellular desquamation, lamellar fusion (ABDEL-BAKI et al., 2011), subepithelial edema with epithelial displacement of the secondary lamellae (VALLADÃO et al., 2014) and gill necrosis (YEMMEN et al., 2011; VALLADÃO et al., 2013) are commonly associated with trichodiniasis. These ciliates are frequently associated with lesions on the body surface that lead to opportunistic bacterial infection and outbreaks of mortality (KHAN, 2009; VALLADÃO et al., 2013, 2014). Since few studies have been done on fish lesions caused by trichodinids, epidemiological studies need to be encouraged in order to establish prophylactic measures for avoiding dispersion of these fish pathogens worldwide.

This study evaluated the trichodinid fauna of *B. splendens* and registers *T. modesta* in the Americas. The distribution of this ciliate is discussed and a checklist of hosts and localities for the parasite is presented.

Materials and Methods

Study area and fish

Adult males of *Betta splendens* from Muriaé, Minas Gerais state (21° 7' 49" S; 42° 22' 3" W) (n=42), from Ribeirão Preto, São Paulo state (21° 10' 40" S; 47° 48' 36" W) (n=28) and from Cascavel, Paraná state (24° 57' 20" S; 53° 27' 19" W) (n=23) were examined. The fish had standard lengths of approximately 3.0 cm and only adult males were used in this assay for sampling. Each fish was kept separately in a two liter aquarium, avoiding contamination with others, and fed twice a day with Tetra® Color Tropical Granules™. Partial water changes of about 50% was done each day. Water parameters were kept at a pH 6.2 and a temperature of 27 °C.

Parasitic diagnosis

The body surface and gills of fish were scraped for parasitological analysis and when the parasites were present the smears were impregnated with silver nitrate using Klein's method (KLEIN, 1958) or stained with Giemsa to observe the nuclear apparatus (LOM, 1958). The span was the measurement from the extremity of the blade to the extremity of the ray as described by Arthur & Lom (1984). All measurements are in micrometers and followed the recommendations of Lom (1958) and Van As & Basson (1989) and analyzed using a Nikon E200® photomicroscope equipped with the Moticam 2300® image capture system. The parasite measurements were made using ImagePro Plus® 4.1 software. Minimum and maximum values are provided, followed in parentheses by arithmetic mean, standard deviation and number of specimens or structures measured. Schematic drawings of the denticles, as proposed by Van As & Basson (1989), were produced by means of vectorization using CorelDraw® X6 software.

Results

Parasite diagnosis

The fish examined showed trichodinid prevalence of 55.9% and *Piscinoodinium pillulare* Lom, 1981 prevalence of 21.5%, only in the fish from the states of São Paulo and Minas Gerais. No parasites were found in the fish from the state of Paraná.

Trichodinid description

The trichodinids were disc-shaped, with the following characteristics: body diameter 29.5 - 46.3 (39.7 ± 3.3; 50); border membrane width 2.3 - 4.1 (3.4 ± 0.4; 50); adhesive disc diameter 24.4 - 39.0 (32.9 ± 3.1; 50); denticulate ring diameter 14.5 - 23.4 (19.5 ± 2.0; 50); number of denticles

19.0 - 24.0 (22.0 ± 1.0 ; 50); denticle length 3.7 - 6.2 (4.6 ± 0.6 ; 50); blade length 3.3 - 5.6 (4.4 ± 0.6 ; 50); central part width 1.5 - 3.0 (2.3 ± 0.3 ; 50); ray length 2.7 - 4.6 (3.5 ± 0.4 ; 50); denticle span 8.0 - 12.5 (10.2 ± 1.0 ; 50); pins per denticle 5 - 6 (5.6 ± 0.5 ; 49); horseshoe-shaped macronucleus diameter 25.6 - 38.3 (31.6 ± 2.9 ; 40); thickness 5.0 - 10.9 (7.9 ± 1.4 ; 40); and distance between macronucleus extremities 4.6 - 10.3 (6.5 ± 1.3 ; 40).

In silver nitrate impregnated material (Figure 1a), the center of the adhesive disc is of similar appearance to the adhesive disc. The blade is sickle-shaped with a tangent point that is slightly flat and not totally parallel to the y+1 axis. The blade has a rounded apex, almost touching the y+1 axis (Figure 1b-c). The blade apophysis is rarely observed but some specimens have a discrete apophysis

situated between the blade and the central part (Figure 1c). The central part is triangular and rounded, filling half of the space between the Y and y-1 axes. An indentation below the X axis in the central part is present in some specimens. The connection between the central part and the ray is short. The ray is slender and straight with a rounded point, parallel to the Y axes. Ray apophysis is present. A few specimens showed discreetly anterior or posterior-directed rays, with tips surpassing the Y axes. The nuclear apparatus has a horseshoe-shaped macronucleus and oval micronucleus situated in the y+1 position.

A checklist of the hosts for *T. modesta* is provided in Table 1, followed by a distribution map and the chronological order of the parasite reports (Figure 2).

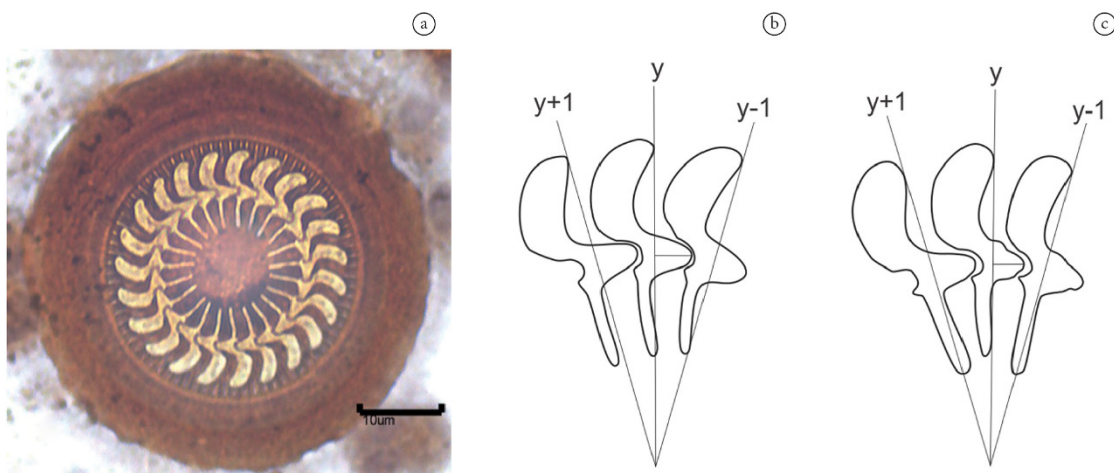


Figure 1. Adhesive disc of *Trichodina modesta* Lom, 1970, parasite of *Betta splendens*, impregnated with silver nitrate (a). Schematic drawing of the denticles of the present study (b-c).

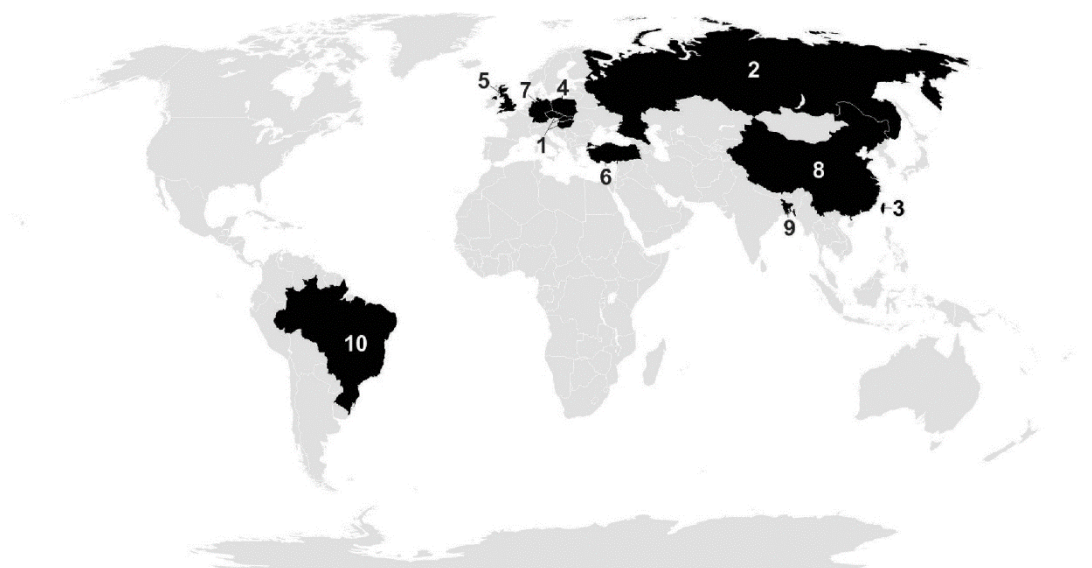


Figure 2. Worldwide dispersion of *Trichodina modesta* Lom, 1970. (1) Hungary, Slovakia and Czech Republic (Lom, 1970), (2) Russia (Stein, 1982; Arthur & Lom, 1984), (3) Taiwan (Basson & Van As, 1994), (4) Poland (Wierzbicka, 1997; Własow et al., 2003), (5) United Kingdom (Gaze & Wootten, 1998), (6) Turkey (Özer 2007; Öztürk & Özer 2007), (7) Germany (Rückert et al., 2007), (8) China (Zhao & Tang, 2007; Liu & Zhao, 2010; Han & Zhao, 2011; Tang et al., 2013), (9) Bangladesh (Kibria et al., 2010) and (10) Brazil (present study).

Table 1. Checklist of the hosts and localities of *Trichodina modesta* Lom, 1970.

Order	Family	Host	Country	Author
Cypriniformes	Cyprinidae	<i>Abramis brama</i>	Hungary	Lom (1970)
Cypriniformes	Cyprinidae	<i>Vimba vimba</i>	Slovakia and Czech Republic	Lom (1970)
Cypriniformes	Cyprinidae	<i>Abramis brama</i>	Russia	Stein (1982)
Cypriniformes	Cyprinidae	<i>Blicca bjoerkna</i>	Russia	Arthur & Lom (1984)
Cypriniformes	Balitoridae	<i>Crossostoma lacustre</i>	Taiwan	Basson & Van As (1994)
Cypriniformes	Cobitidae	<i>Misgurnus anguillicaudatus</i>	Taiwan	Basson & Van As (1994)
Perciformes	Gobiidae	<i>Rhinogobius brunneus</i>	Taiwan	Basson & Van As (1994)
Cypriniformes	Cyprinidae	<i>Abramis brama</i>	Poland	Wierzbicka (1997)
Cypriniformes	Cyprinidae	<i>Blicca bjoerkna</i>	Poland	Wierzbicka (1997)
Cypriniformes	Cyprinidae	<i>Abramis brama</i>	United Kingdom	Gaze & Wootten (1998)
Cypriniformes	Cyprinidae	<i>Tinca tinca</i>	Poland	Własow et al. (2003)
Cyprinodontiformes	Cyprinodontidae	<i>Aphanius danfordii</i>	Turkey	Özer (2007)
Cyprinodontiformes	Cyprinodontidae	<i>Aphanius danfordii</i>	Turkey	Öztürk & Özer (2007)
Cypriniformes	Cyprinidae	<i>Abramis brama</i>	Germany	Rückert et al. (2007)
Cypriniformes	Cyprinidae	<i>Rutilus rutilus</i>	Germany	Rückert et al. (2007)
Cypriniformes	Cobitidae	<i>Misgurnus anguillicaudatus</i>	China	Zhao & Tang (2007)
Siluriformes	Schilbeidae	<i>Clupisoma garua</i>	Bangladesh	Kibria et al. (2010)
Siluriformes	Bagridae	<i>Pelteobagrus bleeker</i>	China	Liu & Zhao (2010)
Cypriniformes	Cobitidae	<i>Misgurnus anguillicaudatus</i>	China	Han & Zhao (2011)
Cypriniformes	Cobitidae	<i>Misgurnus anguillicaudatus</i>	China	Tang et al. (2013)
Perciformes	Osphronemidae	<i>Betta splendens</i>	Brazil	Present Study

Discussion

The present study reveals the presence of *T. modesta*, a parasite originally reported from the Eurasian region and now found in the Americas with a new and unusual host (*B. splendens*).

Since the first report of *T. modesta* in Hungary, Slovakia and the Czech Republic by Lom (1970), it has now also been found in several other Eurasian countries (Table 1). The morphological and morphometric characteristics of *T. modesta* described in the present study resemble the original description (LOM, 1970) and other records (WIERZBICKA, 1997; GAZE & WOOTTEN, 1998; ÖZER, 2007). Despite the variation in the body diameter, *T. modesta* is classified as a small-sized (BASSON & VAN AS, 1994; GAZE & WOOTTEN, 1998; ZHAO & TANG, 2007) or medium-sized trichodinid (WIERZBICKA, 1997; ÖZER, 2007; ÖZTÜRK & ÖZER, 2007; present study). Morphometric variation may occur due to environmental and ecological factors in this ciliate group (KAZUBSKI, 1971). Previous studies found the parasite exclusively on the gills (LOM, 1970; ARTHUR & LOM, 1984; WIERZBICKA, 1997). In contrast, in the present study, we have reported the presence of this parasite both on the gills and on the body surface, in a similar way to what was found by Basson & Van As (1994), Özer (2007) and Öztürk & Özer (2007).

Gaze & Wootten (1998) commented that *T. modesta* shows a high degree of host specificity, in comparison with other cosmopolitan trichodinids, for example *T. heterodentata*. From analyzing the reports in the literature, it seems that *T. modesta* shows specificity for the order Cypriniformes. Nevertheless, in the present study, the parasite was reported in an unusual fish, similar

to that found by Basson & Van As (1994), who also reported *T. modesta* in Perciformes fish. Two Siluriformes hosts found by Kibria et al. (2010) and Liu & Zhao (2010) are further examples of its unusual occurrence.

Betta spp. is originally from Asia and it may have been responsible for the introduction of *T. modesta* into South America. On the other hand, because there are no reports of this trichodinid in the native area of this fish, this hypothesis could be challenged. *Misgurnus anguillicaudatus* has recently been found in natural Brazilian environments (GOMES et al., 2011), while *Aphanius danfordii*, *Crossostoma lacustre* and *Rhinogobius brunneus* are frequently found commercially in Brazilian pet shops. These are native fish in the endemic area for *T. modesta* and several studies have reported parasitism by this ciliate on these ornamental fish (BASSON & VAN AS, 1994; ÖZER, 2007; ÖZTÜRK & ÖZER, 2007; ZHAO & TANG, 2007; HAN & ZHAO, 2011; TANG et al., 2013).

From the above statements, it can be suggested that the introduction of *T. modesta* into the Neotropical region might be associated with large-scale commercial introductions of fish. It was also suggested by Van As & Basson (1989) that *T. heterodentata* has become dispersed worldwide as a result of cichlid introductions into new localities. In addition, cyprinid introductions have also been responsible for dissemination of *Lernaea cyprinacea* in Brazil (PORTZ et al., 2013). Dispersion of fish and their parasites into new localities may increase the host diversity for parasitic infestations, thus supporting the recent findings of *T. modesta* in non-Cypriniformes fish such as those described by Kibria et al. (2010), Liu & Zhao (2010) and in the present study.

This study shows that most of the trichodinid parasites in South America need to be evaluated. It contributes towards improving the knowledge of the parasitic fauna of an important freshwater fish (*B. splendens*). Nevertheless, this is the first record of *T. modesta* in the Neotropical region and *B. splendens* is a new host for this ciliate. These data may infer the possibility of parasite introduction into the Americas via introductions of ornamental cyprinids.

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