Trichodina nobilis Chen, 1963 and *Trichodina reticulata* Hirschmann et Partsch, 1955 from ornamental freshwater fishes in Brazil

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Abstract

In the present work *Trichodina reticulata* and *T. nobilis* (Ciliophora: Trichodinidae) are morphologically characterised from ornamental freshwater fish culture in the State of Santa Catarina, Brazil. The prevalence of infection and a list of comparative measurements are discussed. We examined "southern platyfish" *Xiphophorus maculatus* (n = 35), "goldfish" *Carassius auratus* (n = 31), "guppy" *Poecilia reticulata* (n = 20), "sailfin molly" *Poecilia latipinna* (n = 6), "beta" *Betta splendens* (n = 2) and "spotted headstander" *Chilodus punctatus* (n = 1). After being anesthetised in a benzocaine solution, fishes were examined for parasitological evaluation. A total of 51.57% fishes were parasitised by *Trichodina* spp. *Carassius auratus* was the most parasitised species, followed by *X. maculatus* and *P. reticulata*. *Beta splendens, C. punctatus* and *P. latipinna* were not parasitised by any trichodinid species. Two species of *Trichodina* were collected from the skin of fish: *T. nobilis* was found in *C. auratus*, *P. reticulata* and *X. maculatus* and *T. reticulata* was only observed in *C. auratus*. The importance of adequate handling in ornamental fish culture are also discussed.

*Keywords***:** ornamental fish, parasites, *Trichodina nobilis*, *Trichodina reticulate.*

Trichodina nobilis Chen, 1963 e *Trichodina reticulata* Hirschmann et Partsch, 1955 de peixes ornamentais de água doce no Brasil

Resumo

No presente trabalho *Trichodina reticulata* e *T. nobilis* (Ciliophora: Trichodinidae) de peixes ornamentais de água doce cultivados no estado de Santa Catarina, Brasil são caracterizadas morfologicamente. A prevalência de infecção e uma lista comparative de medidas são discutidas. Foram examinados "platis" *Xiphophorus maculatus* (n = 35), "kinguios" *Carassius auratus* (n = 31), "guppys" *Poecilia reticulata* (n = 20), "molinésias" *Poecilia latipinna* (n = 6), "betas" *Betta splendens* (n = 2) e "espada" *Chilodus punctatus* (n = 1). Após a anestesia com solução de benzocaína, os peixes foram submetidos à avaliação parasitológca. Um total de 51,57% peixes estavam parasitados por *Trichodina* spp. *Carassius auratus* foi a espécie mais parasitada, seguida por *X. maculatus* e *P. reticulata*. *Beta splendens, C. punctatus* e *P. latipinna* não estavam parasitados por tricodinídeos. Duas species de *Trichodina* foram coletadas da superfície corporal dos peixes: *T. nobilis* foi encontrada em *C. auratus*, *P. reticulata* e *X. maculatus* e *T. reticulata* foi observada apenas em *C. auratus*. A importância do manejo adequado em cultivos de peixes ornamentais também foi discutida.

Palavras-chave: peixes ornamentais, parasitos, *Trichodina nobilis*, *Trichodina reticulata*.

1. Introduction

Ornamental fish culture in Brazil has shown a rapid development in the last years (Piazza et al., 2006). As a function of the intensive culture, many parasitic and infectious diseases have emerged thereby causing serious damage to fish production (Kim et al., 2002). Under inadequate handling such as high content of organic matter, depletion of dissolved oxygen, water temperature alterations, nutritional deficiency and the lack of quarantine of introduced fish in fish farms, trichodinids find adequate substrate to reproduce causing damage to fish and consequently economic losses (Moraes and Martins, 2004).

Trichodinid ciliates are one of the most common ectoparasites in both wild and cultivated fish (Basson and Van As, 1994, Martins and Ghiraldelli, 2008). However, in Brazil, studies concerning their identification have received little attention and require further investigation. Up to now, *Trichodina* spp. from ornamental fish were reported in *Paracheirodon axelrodi* (Ferraz, 1999), *Xiphophorus helleri* and *Xiphophorus maculatus* (Garcia et al., 2009). Piazza et al. (2006) registered *Trichodina acuta* Lom, 1961 in *X. helleri, X. maculatus, Poecilia sphenops*, *Beta splendens* and *Carassius auratus*.

This study characterises morphologically *Trichodina reticulata* Hirschmann et Partsch, 1955 and *Trichodina nobilis* Chen, 1963 collected from ornamental freshwater fishes in the state of Santa Catarina, Brazil. Prevalence and a list of comparative measurements are also reported.

2. Material and Methods

Ninety five ornamental freshwater fishes were caught from a fish store at Florianópolis, Santa Catarina state, Brazil, between August and October 2009. Fish species examined in this study were: "southern platyfish" *Xiphophorus maculatus* (n = 35), "goldfish" *Carassius auratus* (n = 31), "guppy" *Poecilia reticulata* (n = 20), "sailfin molly" *Poecilia latipinna* (n = 6), "beta" *Betta splendens* (n = 2) and "spotted headstander" *Chilodus punctatus* (n = 1). Before necropsies, fishes were maintained in a 30 L aquarium for approximately five days. Fishes were sacrificed (ethics committee-CEUA nº 23080.029976/2009-63/Federal University of Santa Catarina) and analysed under a stereomicroscope for the presence of trichodinids.

Wet smears of the skin and gills were air-dried at room temperature and impregnated with silver nitrate by Klein's method for posterior examination of the adhesive disc structures and denticles under an optic microscope, as suggested by Lom (1958). Specimens are deposited in the National Institute of Amazonian Research (INPA), Manaus, AM, Brazil. All measurements (in micrometers) were made in a camera lucida. Arithmetic means ± standard deviation are followed, in parentheses, by the minimum, maximum values and the number of measured structures. Denticles description follows the recommendation of Van As and Basson (1989).

3. Results

A total of 51.57% of examined fish were parasitised by *Trichodina* spp. *Carassius auratus* was the most parasitised species, followed by *X. maculatus* and *P. reticulata* (Table 1). *Beta splendens, C. punctatus* and *P. latipinna* were not parasitised by any trichodinid species (Table 1). Two species of *Trichodina* were recovered from the skin of ornamental freshwater fishes: *Trichodina nobilis* was found in *C. auratus*, *P. reticulata* and *X. maculatus* and *T. reticulata* was only observed in *C. auratus*.

Trichodina reticulata Hirschmann and Partsch, 1955 (Figures 1a and 2a).

Description based on 50 specimens: Characterised as medium to large trichodinid (Table 2). Denticle blade fills the space between y and y-1 axes, very angular on outside margin with an angular apex not clearly demarcated. Distal blade margin rounded, leaning considerably down towards tangent point. Posterior margin of the blade shows a semilunar curve. Central part with a narrow retreat on its anterior margin (only seen in some specimens) and rounded posterior tip. Central part reaches halfway of the space between y and y + 1 axes (Figure 2a). Ray thick throughout its length, with a round tip slightly trespassing the axis y. Ray apophysis not clearly visible. Prominent

Table 1. Ornamental fish species from Florianópolis, Santa Catarina, Brazil, their common names, total length and prevalence of *Trichodina* spp.

Species	Common name	Total length	PF/EF	P (%)[a]	P (%)[b]
Beta splendens	Betta	4.6	0/2	0	0
Chilodus punctatus	Spotted headstander	4.5	0/1	0	0
Poecilia reticulata	Guppy	3.06 ± 0.68	3/20	15	3.15
Carassius auratus	Goldfish	6.39 ± 1.87	23/31	74.19	24.21
Poecilia latipinna	Sailfin molly	3.93 ± 0.66	0/6	0	0
Xiphophorus maculatus	Southern platyfish	3.73 ± 0.64	23/35	65.71	24.21
Total	-	-	49/95	-	51.57

PF: parasitised fish; EF: examined fish; P[a]: prevalence in relation to total number of each examined fish species; P[b]: prevalence in relation to total number of examined fish.

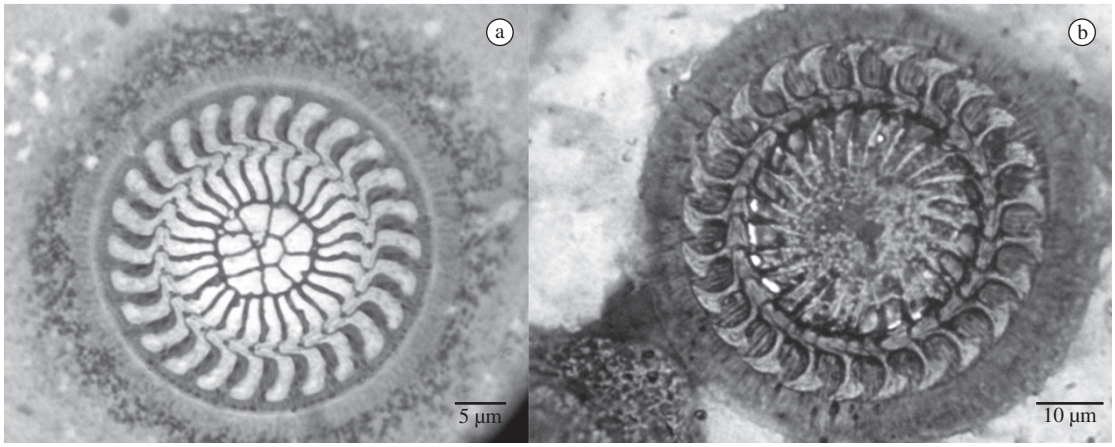

Figure 1. Photomicrographs of (a) *Trichodina reticulata,* scale: 5 µm and (b) *Trichodina nobilis*, scale: 10 µm from ornamental freshwater fishes cultured in the state of Santa Catarina, Brazil.

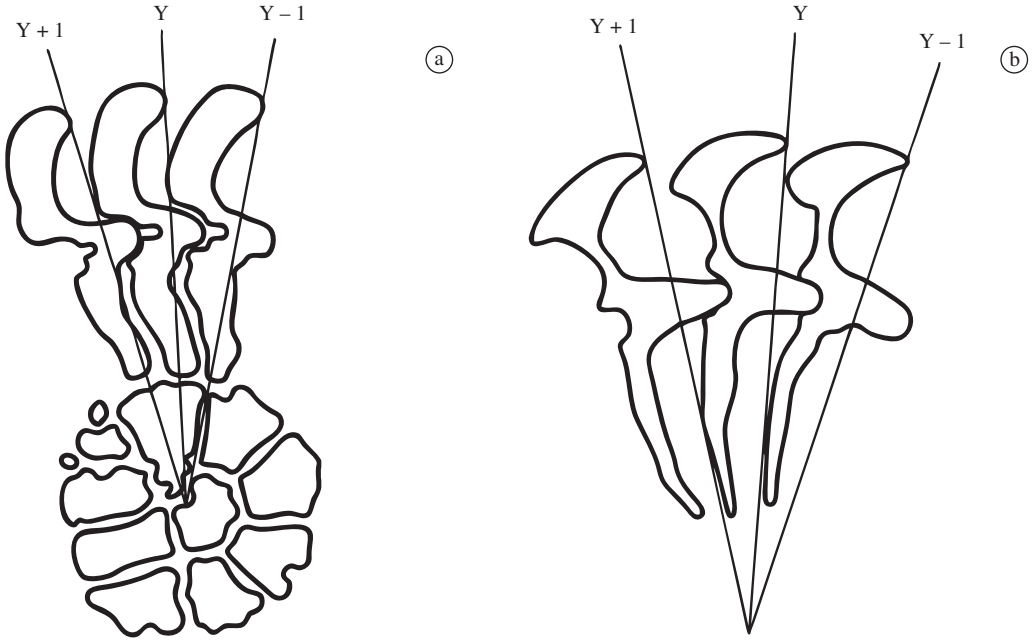

Figure 2. Schematic drawing of the denticles of (a) *Trichodina reticulata* and (b) *Trichodina nobilis*.

radial pins. Centre of adhesive disc with circular cell-like structures varying in size and number (6 to 16).

Taxonomix summary

Host: *Carassius auratus*

New Locality: Florianópolis, Santa Catarina State, Brazil

Site of infection: skin

Synonyms: *Trichodina domerguei* f. *megamicronucleus* Dogiel, 1940; *Trichodina megamicronucleata* Dogiel, 1950 (see Wellborn, 1967).

3.1. Remarks

According to Dove and O´Donoghue (2005), *T. reticulata* is a highly distinctive species well-known as a pathogen of *Carassius* spp. and other cyprinids kept for aquaculture and aquaria. It lives in the mucus of the body surface (rarely on the gills) (Lom, 1960) and presents a very distinct feature which is the presence of some circular cell-like structures in the centre of the adhesive disc. The species has a wide distribution, having been reported widely from the Czech Republic (Lom, 1960), China (Chen, 1963), Japan (Ahmed, 1976, 1977), Philippines (Albaladejo and Arthur, 1989), North America (Hirschmann and Partch, 1955; Wellborn,

1967), Israel (Basson et al., 1983), South Africa (Basson and Van As, 1993), India (Mishra and Das, 1993), Germany (Dobberstein and Palm, 2000), Australia (Dove and O´Donoghue, 2005) and Iran (Moghaddam et al., 2010).

With regard to host specificity, Wellborn (1967) registered a strong case of *T. reticulata* in the cyprinid *Abrama abrama*. In the same way, in this study the species was only found in *C. auratus*, which suggests that it may be host-specific for certain cyprinid fish.

The measurements from the present studied population agree with previous descriptions, showing some variation with the populations studied by Wellborn (1967) and Lom (1960), both presenting greater body length than the current one (Table 2). This is the first register of *T. reticulata* in Brazil and South America.

Trichodina nobilis Chen, 1963 (Figures 1b and 2b).

Description based on 32 specimens: characterised as a large trichodinid (Table 3), disc-shaped. Adoral spiral of about 440°. Anterior blade margin slightly curved. Distal margin of the blade rounded filling almost all space between the axes y and y + 1, leaning down towards the tangent point and also towards the blade apex. Blade suffers a remarkable narrowing through its middle section, slightly broadening at blade connection. Posterior margin of the blade shows a deep semi lunar curve. Detachable blade apophysis, with acuminate point. Central part with rounded tip, fitting tightly into preceding denticle and do not pass the half of the space between the axes y and y + 1. Long ray, straight to slightly curved in posterior direction situated between the axes y and y + 1. Ray apophysis discrete and small. Tip of ray sharp. Micro and macronucleus not visualised.

Taxonomix summary

Host: *Carassius auratus*

New hosts: *Poecilia reticulata*, *Xiphophorus maculatus*

New Locality: Florianópolis, Santa Catarina State, Brazil

Site of infection: skin

Table 2. Comparative measurements of *Trichodina reticulata*. The averages ± standard deviation are followed by minimum and maximum values in parenthesis.

Characters	*T. reticulata*	*T. reticulata*2	*T. reticulata3*	*T. reticulata*4
	Present study[1]	**Hirschmann and Partsch, 1955 in Albaladejo and Arthur (1989)**	**Hirschmann and Partsch, 1955 in Wellborn (1967)**	**Hirschmann and Partsch, 1955 in Basson, Van As and Paperna (1983)**
Body[D]	55.0 ± 7.6 (44.2-68.6)	60.8 (57.0-8.5)	80 (71-95)	54.4 (45.2-58.6)
Adhesive disc[D]	45.5 ± 5.6 (36.3-55.2)	46.0 (46.0-57.0)	59 (48-66)	45.0 (35.8-49.5)
Denticulare ring[D]	28.7 ± 5.2 (19.7-41.0)	34.2 (31.0-37.0)	38 (31-41)	29.3 (22.0-33.4)
Denticle number	26.3 ± 1.1 (25-28)	23.7 (22-26)	28 (23-33)	28.7 (22-29)
Pins per denticle	9.7 ± 0.7 (9-10)	10-12	10	9.7 (8.0-9.0)
Denticle Length[L]	5.1 ± 0.9 (3.9-7.8)	7.7 (7.5-8.5)	10 (8-12)	6.2 (5.0-7.2)
Blade[L]	5.5 ± 0.8 (2.3-7.8)	7.0 (6.0-7.5)	8 (6.5-9)	4.8 (3.8-5.3)
Central part[w]	2.2 ± 0.3 (1.5-3.1)	2.0 (1.5-2.0)	2.9 (2.0-3.0)	2.3 (1.3-3.4)
Ray[L]	5.2 ± 0.8 (3.9-7.8)	5.0 (5.0-7.5)	7-8	6.0 (4.3-7.0)
Span	13.3 ± 1.3 (11.8-16.5)	15.2 (14.5-16.5)	-	-
Characters	***T. reticulata*1**	***T. reticulata*2**	***T. reticulata*3**	***T. reticulata*3**
	Hirschmann and Partsch, 1955 in Dobberstein and Palm (2000)	**Hirschmann and Partsch, 1955 in Dove and O´Donoghue(2005)**	**Hirschmann and Partsch, 1955 in Basson and Van As (1993)**	**Hirschmann and Partsch, 1955 in Lom, 1960**
Body[D]	61 (57-69)	-	47.9 (40.0-55.0)	74 (55-88)
Adhesive disc[D]	46 (46-57)	48.6 (40.9-54.2)	39.3 (31.0-46.0)	52 (44-57)
Denticulare ring[D]	34 (31-37)	-	24.7 (20.5-29.0)	32 (25-36)
Denticle number	24 (22-26)	24 (23-25)	24 (22-27)	24,25 (21-34)
Pins per Denticle	10-12	10 (9-11)	9 (8-10)	10
Denticle Length[L]	-	-	5.2 (4.0-6.0)	-
Blade[L]	-	5.6 (4.8-6.7)	5.2 (4.5-6.5)	-
Central Part[w]	-	2.5 (2.2-2.9)	1.8 (1.0-2.0)	-
Ray[L]	-	5.2 (4.2-5.8)	4.9 (3.5-6.0)	-
Span	-	13.8 (11.8-14.9)	12.1 (10.0-14.5)	-

Hosts. [1,2,3]*Carassius auratus*, [4]*C. auratus*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*. [D]Diameter, [L]length, [w]width. Hosts. [1]Non provided, [2]*C. auratus* and *C. carpio*, [3]*C. auratus*. [D]Diameter, [L]length, [w]width.

Table 3. Comparative measurements of *Trichodina nobilis*. The averages ± standard deviation are followed by minimum and maximum values in parenthesis.

Characters	*T. nobilis*1	*T. nobilis*2	*T. nobilis*3	*T. nobilis*4
	Present study	Chen, 1963 in Basson and Van As (1994)	Chen, 1963 in Dobberstein and Palm (2000)	Chen, 1963 in Albaladejo and Arthur (1989)
BodyD	77.7 ± 5.7 (67.8-86.8; 10)	69.2 (63-76)	79 (70-90)	78.9 (70.0-90.0)
Adhesive disc[D]	65.1 ± 7.9 (50.0-76.5; 10)	57.3 (50.5-63)	65 (58-77)	65.5 (58.0-77.0)
Denticulare ring[D]	44.8 ± 5.9 (37.1-56.8; 10)	35.9 (30.0-40.0)	44 (39-53)	44.3 (38.5-52.5)
Denticle number	26.5 ± 0.8 (25-28; 10)	22 (21-27)	25 (23-28)	24.8 (23.0-28.0)
Pins per Denticle	12 ± 0.7 (11-13; 8)	12 (11-13)	12-14	12-14
Denticle Length[L]	9.2 ± 1.2 (7.8-13.4; 30)	9.6 (8-11)	-	11.0 (10.5-12.5)
Blade[L]	7.7 ± 0.5 (6.3-8.6; 30)	7.5 (6.5-9.0)	-	9.1 (7.5-10.5)
Central Part[w]	2.66 ± 0.5 (2.3-3.9; 30)	3.0 (2.5-3.5)	-	2.4 (2.0-3.0)
Ray[L]	10.3 ± 1.3 (7.8-12.6; 31)	9.9 (9.0-11.5)	-	10.7 (8.0-13.0)
Span	21.9 ± 2.2 (15.0-27.3; 31)	20.4 (19.0-24.0)	-	22.2 (19-25)

Hosts. [1]*C. auratus*, *Poecilia reticulata* (new host), *Xiphophorus maculatus* (new host), [2]*C. auratus*, *Capoeta semifasciolata*, C. *idella*, *H. molitrix*, *Poecilia velifera*, *P. reticulata* and tilapia, [3]Non provided, [4]*C. idella*, *Aristichthys nobilis* and *C. carpio*. [D]Diameter, [L]length, [w]width.

3.2. Remarks

Trichodina nobilis was first described by Chen (1963) parasitising cyprinids. Subsequently, it has been reported from several localities including China (Chen, 1963), Amur river (Stein, 1968; Yukhimenko, 1972), Ural (Kashkovsky, 1974), Bulgaria (Golemansky and Grupcheva, 1975), Philippines (Albaladejo and Arthur, 1989), Taiwan (Basson and Van As, 1994), Republic of Serbia (Nikolic et al., 2003) and Iraq (Al-Marjan and Abdullah, 2007). Hertel et al. (2004) reported a possible occurrence of *T. nobilis* parasitising *Biomphalaria glabrata* from Brazil through molecular techniques but since the same was not observed directly, the authors named it as *Trichodina* sp. This study can therefore be considered the first register of *T. nobilis* in Brazil and South America.

As for the site of infection, the species is reported on both skin and gills (rarely). Nikolic et al. (2003) studied the microhabitat preference of *T. nobilis* on its host and concluded its preference for fish skin. In this study, the parasite was only found in the skin of the hosts.

The population of *T. nobilis* found in this study agrees in shape and size with previous descriptions (Albaladejo and Arthur, 1989; Basson and Van As, 1994; Dobberstein and Palm, 2000) for the species (Table 3), leaving no doubt that it is *T. nobilis*. *Poecilia reticulata* and *X. maculatus* are considered new hosts for the species. The indentation in the anterior side of the blade reported by Basson and Van As (1994) was also observed in our specimens.

Data from Piazza et al. (2006) for *T. acuta* from ornamental freshwater fish collected at the same locality of the present study showed total prevalence of 4.7% including some host species also analysed in the present study such as *X. maculatus* and *C. auratus*. The present study, however, showed higher prevalence values than the former one, being *C. auratus* the most parasitised host species**.**

According to Piazza et al. (2006), diseases have caused economical losses of about 10 to 20% in cultured fish. Since this ciliated protozoan is able to cause considerable damage in cultured fish (Ghiraldelli et al., 2006), sanitary handling should be severaly applied in order to prevent massive mortalities under culture conditions.

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