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Article

Trichodina heterodentata (Ciliophora: Trichodinidae): a new parasite for *Piaractus mesopotamicus* (Pisces: Characidae)

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Abstract

Trichodinids are mobile peritrichous ciliated protozoa, and widely known as ectocommensals and/or parasites of fish and other aquatic organisms. Little is known about the trichodinid fauna in Brazilian fish. This study reports *Trichodina heterodentata* Duncan, 1977 as a new parasite for freshwater fish *Piaractus mesopotamicus* Holmberg, 1887. This is the first record of this trichodinid in southeastern Brazil. Fifty specimens impregnated with 2% silver nitrate and another fifty stained with Giemsa were used for morphometry on the taxonomic characteristics. *T. heterodentata* in this study is medium size, with a disc-shaped body measuring 49.0 to 61.0 µm, parasitizing the skin, fins and gills of pacu. Measurement comparisons between the present material and other records from different countries are presented.

Key words: Trichodinids, fish ectoparasite, aquaculture health

Introduction

Trichodinids are mobile peritrichous protozoa that present widespread distribution around the world. They often act as ectocommensals and are capable of establishing ecological relationships with mollusks (Pinto *et al.* 2006), crustaceans (Silva *et al.* 2009), fish (Martins *et al.* 2010) and amphibians (Dias *et al.* 2009). Some trichodinids can cause harmful effects on their hosts, and they act as parasites of importance in aquaculture that are capable of causing losses through mortality (Khan 2009) and diminished productive performance (Ekanem & Obiekezie 1996), as well as making the hosts susceptible to other infectious diseases like streptococcosis (Evans *et al.* 2007) and influencing the efficacy of vaccines (Martins *et al.* 2011).

Epithelial proliferation on the skin and gills is the main histopathological change caused by trichodinid infestation (Huh *et al.* 2005; Yemmen *et al.* 2011), and this may be associated with fusion of the secondary lamellae of the gill filaments and accumulation of mucus (Yemmen *et al.* 2011), along with destruction of the secondary lamellae (Schalch *et al.* 2006). Together, these structural disorders in the gill tissue cause respiratory deficit because of the obstruction that comes with tissue death, and this may cause asphyxia and culminate in mortality.

Among the trichodinids, *Trichodina heterodentata* is a cosmopolitan species and, since it was first described by Duncan (1977), more than 35 species of fishes in 14 families have now been recognized as hosts for this parasite (Martins *et al.* 2010). In South America, this parasite was first recorded in Venezuela (Van As & Basson 1989), and it has recently been recorded in Brazil (Dias *et al.* 2009; Martins *et al.* 2010) and Peru (Miranda *et al.* 2012). However, it possibly originated from the African continent and has been dispersed to other countries together with shipments of cichlids destined for aquaculture (Van As & Basson 1989).

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The pacu (*Piaractus mesopotamicus*) is a native fish species of the Paraná-Paraguay river basin that presents rapid growth, good meat quality and good acceptance in the consumer market. These characteristics make this fish a competitive species in the consumer market. However, few studies have evaluated the parasitic infections that affect this species of importance for Brazilian aquaculture. Until now, it was only known that *Trichodina* sp. occurred on pacu (Tavares-Dias *et al.* 2001; Schalch & Moraes 2005), and thus there is a need to identify the trichodinid species that affect Brazilian fish.

The present study reports *Trichodina heterodentata* Duncan, 1977 as a new parasite for freshwater fish *P. mesopotamicus* and this is the first record of this trichodinid in southeastern Brazil.

Material and methods

The fish (*Piaractus mesopotamicus* Holmberg, 1887) came from the UNESP Aquaculture Center (CAUNESP), on the Jaboticabal campus, state of São Paulo, Brazil. They were firstly kept in 400-liter tanks for quarantine purposes, in order to observe whether there was any sign of infection or infestation. After this period of acclimatization, the fish (of average weight 35 g) were put together at a greater density (0.75 fish/liter) and were kept under poor water quality conditions for 30 days, with low concentrations of dissolved oxygen. They were fed every two days with an excess quantity of feed, such that a layer of organic matter formed at the bottom of the tank. Fish were caught every two days (Carraschi *et al.* 2011), in order to examine them for manifestations of parasitic infection due to trichodinids. The water quality parameters were measured, with the following findings: dissolved oxygen 0.67 $mg.L^{-1}$; water temperature 23.1°C, conductivity 231 $\mu S.cm^{-1}$; pH 7.2; and total dissolved solids 0.150 $g.L^{-1}$.

The fish were caught with the aid of a fish net and were mechanically restrained so that mucus could be scraped from the body, fins and gills, using slides, for examination under an optical microscope. The material was dried out under dim light, and some slides were fixed with methanol and then stained using Giemsa (one drop per 1 mL of distilled water, for 120 to 180 min) in order to show the nuclear apparatus. Other slides were impregnated with 2% silver nitrate to observe the adhesive disc, as described by Lom (1958). The measurements were obtained from 50 specimens impregnated with 2% silver nitrate and another 50 specimens stained with Giemsa, to observe the nuclear apparatus and description of micronucleus position (Lom 1958).

The span is the measurement from the extremity of the blade to the extremity of the ray, as described by Arthur and Lom (1984). All the measurements were made in micrometers and followed the recommendations of Lom (1958) and Van As and Basson (1989). They were made on photomicrographs that had been obtained using a Nikon E200® photomicroscope equipped with the Moticam 2300® image capture system. The measurements on the parasites were made with the aid of the Image-Pro Plus® 4.1 software. In addition, schematic drawings of the denticles, as proposed by Van As and Basson (1989), were produced by means of vectorization, with the aid of the CorelDraw® X5 software.

Results

Phylum: Ciliophora Doflein, 1901
Class: Oligohymenophorea de Puytorac *et al.*, 1974
Order: Peritrichida Stein, 1859
Family: Trichodinidae Claus, 1874
Genus: *Trichodina* Ehrenberg, 1874
Specie: *Trichodina heterodentata* Duncan, 1977

New host: Pacu (*Piaractus mesopotamicus* Holmberg, 1887)

New location: Jaboticabal (22º 14’22.7” S; 48º 17’33.4” W), São Paulo, Brazil.

Site of infestation: skin, fins and gills.

Synonyms: *Trichodina equatorales* Kazubski, 1986 (see Bondad-Reantaso & Arthur 1989).

Description: Trichodinid of medium size with a disc-shaped body measuring 49.0 to 61.0 µm (see Figure 1 and Table 1). They have a broad sickle-shaped blade that fit into the quadrant delimited by the axes y and y +1 (see

Figure 2a–c). The anterior margin blade is convex, and its apex mostly goes beyond the y +1 axis, although in a few specimens the blade apex only touch the y +1 axis, without going beyond it. The distal surface of the blade form a shallow curve, parallel to the border membrane. The tangential point is sharp in the majority of the individuals, or slightly rounded in the others; it is located slightly below or at the same level as the distal tip of the distal blade margin. The apophysis of the blade is prominent. The blade shows moderate fit with the central part. Posterior projection is observed in some specimens. The central part is thick, triangular, free from conical parts, with an oblong tip fitting between the axes y+1 and y-1, and is free from indentation below the x axis. In some specimens, a slight apophysis was observed on the ray. The rays are moderately thick and straight, and their tips are generally sharp. Furthermore, they show different sizes: they could be short or long, and thin or thick; these characteristics are often present in the same specimen. The ray orientation shows considerable variation: sets of rays are observed projecting posteriorly (see Figure 2a); they could go beyond the y+1 axis, parallel to the y axis (see Figure 2b); and/or they could project anteriorly (see Figure 2c), without going beyond the y-1 axis. The adoral ciliature is in a spiral of between 380 and 405 (see Figure 3a). A horseshoe-shaped macronucleus is observed, with external diameter of 43.1±5.2 µm, thickness of 10.2±1.9 µm and distance between the ends of 14.9±3.8 µm. In addition, a circular micronucleus is also observed, located in the + y portion (see Figure 3b).

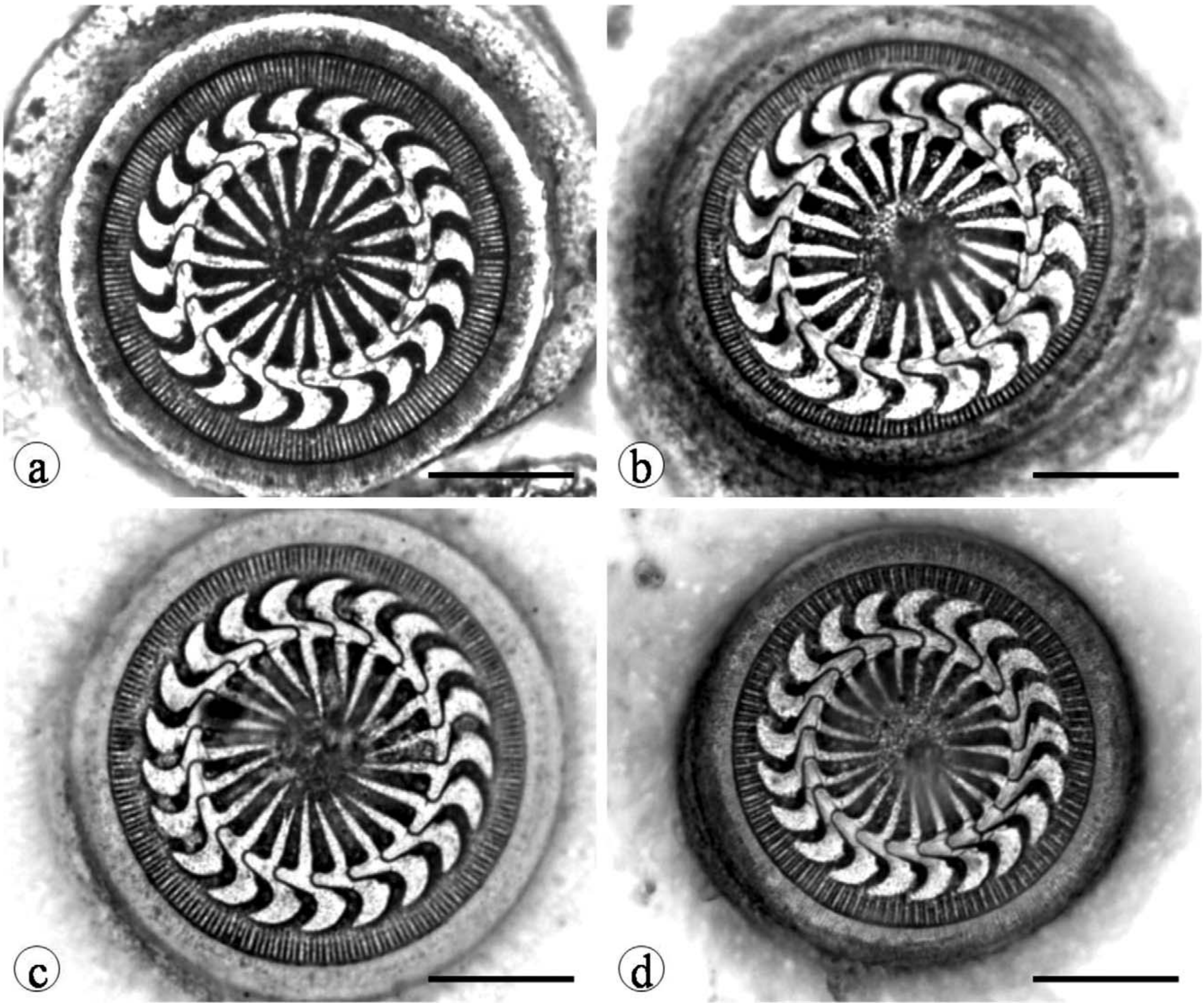

FIGURE 1. Klein's silver impregnated *Trichodina heterodentata* Duncan, (1977) (a-d) from pacu (*P. mesopotamicus*). Bar= 15µm.

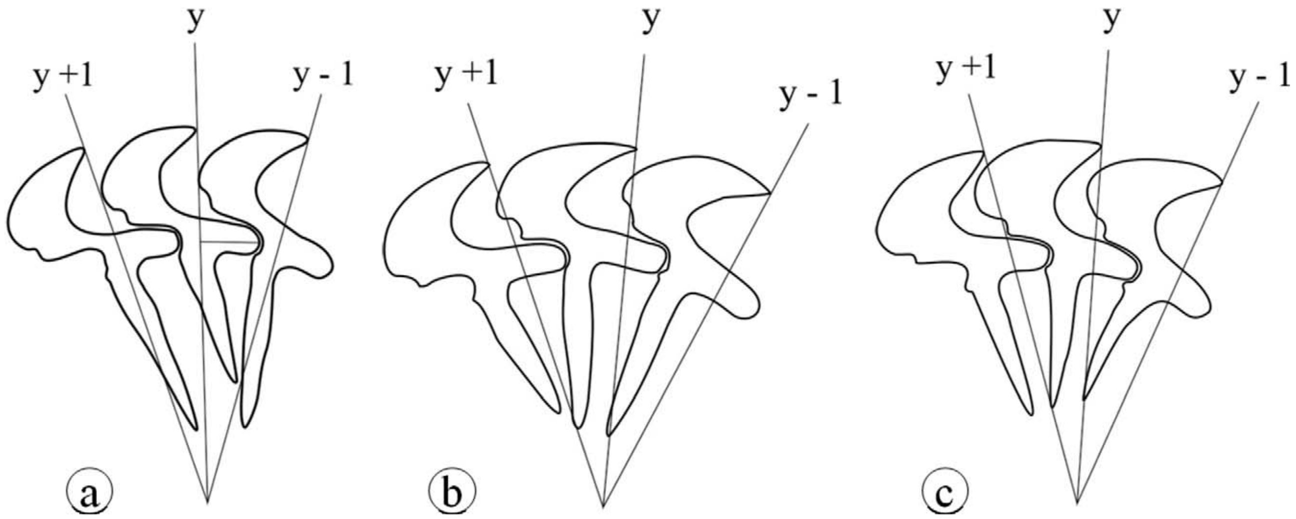

FIGURE 2. Schematic drawing of the denticles of *Trichodina heterodentata* Duncan, (1977) from pacu (*P. mesopotamicus*) showing morphological variations (a–c).

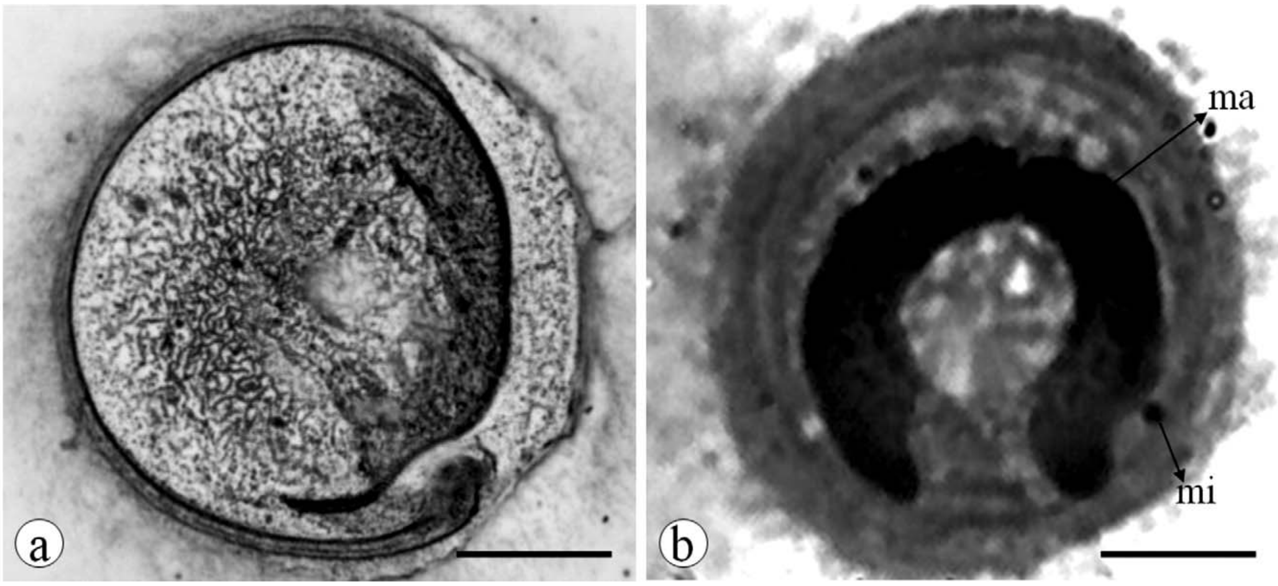

FIGURE 3. Klein's silver impregnated adoral ciliature in spiral of *Trichodina heterodentata* Duncan, (1977) (a) and Giemsa's stain nuclear apparatus (b) showing macronucleus (ma) in horseshoe shaped and circular micronucleus (mi). Bar= 15µm.

Discussion

In comparing the present material with the records of *T. heterodentata* (see Table 1), it can be seen that the body measurements of our specimens were similar to that described by Van As and Basson (1992), Basson and Van As (1994), Al-Rasheid *et al.* (2000) and Asmat (2004), from different countries. On the other hand, in the original description of the species by Duncan (1977), values greater than those of the present study are reported, as were also observed by other authors (Basson *et al.* 1983; Albaladejo & Arthur 1989; Martins *et al*. 2010). The border membrane measurements of the present study are similar to those of the populations B and C described by Duncan (1977) and greater than that found in population A. These values are also similar to those reported for *T. heterodentata* by Basson *et al.* (1983) in South Africa and Israel, Basson and Van As (1994) in Taiwan, Asmat (2004) in India and Tang and Zhao (2007) in China.

TABLE 1. Measurements data of *Trichodina heterodentata* Duncan (1977) from different countries. Minimum - maximum values (Arithmetic mean ± standard deviation).

Characters	Present study	Duncan (1977)			Van As and Basson (1983)	Albaladejo and Arthur (1989)
Local	Brazil	Philippines - A	Philippines - B	Philippines - C	Souht Africa and Israel	Philippines
Body[D]	45.0–58.8 (50.4 ± 3.7)	71–106 (85)	58–108 (80)	70–122 (93)	47.5–69.1 (55.3 ± 3.8)	50.0–65.0 (57.9 ± 3.7)
Border membrane[W]	3.5–5.5 (4.3 ± 0.4)	2.7	3.4–5.5 (4.7)	4.1	3.2–6.2 (4.2 ± 0.6)	4.0–6.0 (4.9 ± 0.4)
Adhesive disc[D]	35.1–49.7 (41.2 ± 3.3)	47–63 (56)	45–74 (57)	54–81 (67)	39.5–59.8 (46.9 ± 4.1)	45.0–55.0 (48.8 ± 3.5)
Denticular ring[D]	19.9–30.3 (24.4 ± 2.4)	26–37 (32)	29–45 (36)	30–52 (41)	23.2–37.8 (29.3 ± 2.9)	28.0–38.0 (32.5 ± 2.5)
Number of denticles	16–22 (20)	20–27 (23)	29–31 (26)	18–31 (27)	22–29 (25)	21–25 (23.5 ± 1.7)
Denticle span	11.6–15.5 (13.7 ± 0.9)	-	-	-	-	13.0–18.0 (15.3 ± 1.3)
Denticle[L]	6.1–9.1 (7.7 ± 0.7)	8.0	7.5–11 (9.2)	6.3	5.1–8.6 (6.6 ± 0.8)	7.5–9.0 (8.1 ± 0.5)
Blade[L]	3.7–5.5 (4.6 ± 0.4)	4.1	4.7–7.1 (5.7)	5.5	3.4–5.5 (4.3 ± 0.4)	5.0–6.5 (5.6 ± 0.5)
Central Part[W]	1.2–2.7 (1.9 ± 0.4)	3.4	1.4–3.4 (2.6)	4.1	1.6–3.3 (2.7 ± 0.3)	1.0–2.0 (1.9 ± 0.3)
Ray[L]	5.1–9.2 (7.2 ± 0.8)	6.9	6.9–10.3 (8.1)	8.2	4.6–8.1 (6.3 ± 0.9)	5.5–10.5 (7.7 ± 1.3)
Pins per denticle	8–12 (10)	11	6–14 (10)	11	9–13 (10)	12–14
Adoral ciliary spiral	380–405° (388.9 ± 6.9)	400º	400º	400º	400°	-

Continued.

Characters	Van As and Basson (1992)	Basson and Van As (1994)	Al-Rasheid *et al.* (2000)	Asmat (2004)	Martins *et al.* (2010)
Local	Botswana	Taiwan	Egypt	India	Brazil
Body[D]	45.5–52.5 (48.8 ± 2.5)	49.0–61.0 (53.9 ± 3.7)	51.2–60.0 (54.6)	46.1–61.2 (54.6 ± 3.3)	27.0–77.0 (59.4 ± 8.5)
Border membrane[W]	3.0–4.5 (3.4 ± 0.6)	4.0–5.0 (4.8 ± 0.3)	4.0–5.0 (3.5)	3.1–5.6 (4.5 ± 0.6)	3.0–7.0 (5.1 ± 1.7)
Adhesive disc[D]	40.0–44.0 (41.8 ± 1.7)	40.0–52.0 (44.4 ± 3.5)	44.0–52.0 (46.2)	41.8–52.0 (45.6 ± 2.8)	40.0–72.0 (60.2 ± 6.7)
Denticular ring[D]	23.0–25.5 (24.5 ± 1.0)	24.5–32.5 (27.9 ± 2.6)	28.0–36.0 (31.6)	26.0–33.6 (30.4 ± 1.7)	27.0–47.0 (38.5 ± 4.5)
Number of denticles	20–22 (21)	21–24 (22)	21.0–24.0 (23.0)	21–26 (23.1 ± 1.2)	23.0–28.0 (24.4 ± 1.6)
Denticle span	11.5–14.5 (12.0 ± 0.8)	12.0–16.0 (13.8 ± 0.8)	12.8–16.0 (14.6)	13.7–17.9 (15.0 ± 1.0)	12.0–22.0 (18.4 ± 2.2)
Denticle[L]	6.0–8.0 (7.1)	6.0–9.0 (7.4 ± 0.7)	8.0–10.4 (9.2)	7.6–9.2 (8.3 ± 0.6)	7.0–13.0 (10.3 ± 1.2)
Blade[L]	2.0–5.0 (4.1 ± 0.9)	4.5–5.0 (4.8 ± 0.3)	5.6–7.2 (6.3)	4.1–7.1 (5.3 ± 0.6)	4.0–8.0 (6.2 ± 0.8)
Central Part[W]	1.5–4.0 (2.5 ± 0.7)	2.0–3.0 (2.3 ± 0.3)	1.6–2.4 (2.2)	2.0–3.1 (2.8 ± 0.4)	2.0–6.0 (3.8 ± 0.7)
Ray[L]	5.5–7.5 (6.3 ± 0.9)	5.5–7.0 (6.4 ± 0.4)	6.4–8.8 (7.5)	5.9–8.2 (6.9 ± 0.7)	3.0–12.0 (8.5 ± 1.7)
Pins per denticle	10–12 (11)	8–10 (10)	10.0–13.0 (12.0)	-	5.0–15.0 (11.8 ± 2.1)
Adoral ciliary spiral	-	430°	-	395–400°	292–325 (307.0° ± 12.6)

[D]diameter, [W]width; [L]length

The measurements for the adhesive disc and denticulate ring in the present study are smaller than those described by Duncan (1977), Basson *et al.* (1983), Albaladejo and Arthur (1989), Al-Rasheid (2000), Asmat (2004) and Martins *et al.* (2010). On the other hand, they coincide with those of Van As and Basson (1992) and Basson (1994). The span of the denticle is similar to the majority of the descriptions (Van As & Basson 1992; Basson & Van As 1994; Al-Rasheid *et al.* 2000), but less than that found by Martins *et al.* (2010).

Trichodina heterodentata displays great variation in the dimensions of the denticles and their structures, such as the blade, central part and ray (Basson *et al.* 1983; Albaladejo & Arthur 1989; Van As & Basson 1992; Basson & Van As 1994; Al-Rasheid *et al.* 2000; Asmat 2004; Martins *et al.* 2010; Miranda *et al.* 2012). Thus, the detailed description of the morphology and arrangement of these parts on the schematic drawing, as proposed by Van As and Basson (1989), are of great value in differentiating between trichodinid species. Duncan (1977) described some of these particular features in showing differences within and between species in three populations of *T. heterodentata* in the Philippines. Ray orientation in the anterior or posterior direction is one of the main characteristics of this species, as supported by Van As and Basson (1989) and Van As and Basson (1992). In addition, this trichodinid has a prominent apophysis at the point where the blade fits into the central part, and a slight apophysis on the rays at the point where they fit into the central part (Martins *et al.* 2010). As observed in the present study, Martins *et al.* (2010) also described rays of different sizes and formats in specimens of *T. heterodentata* collected from channel catfish, *Ictalurus punctatus*.

The number of radial pins per denticle seems not to vary much in *T. heterodentata* (Duncan 1977; Basson *et al.* 1983, Albaladejo & Arthur 1989; Van As & Basson 1992; Basson & Van As 1994; Al-Rasheid *et al.* 2000) although a number greater than in the present study is reported by Martins *et al.* (2010). The angular dimension of the adoral ciliature observed in the present study coincided with the descriptions of Basson *et al.* (1983), Van As and Basson (1992) and Asmat (2004), while it is smaller than thst obtained by Basson and Van As (1992) and greater than that found by Martins *et al.* (2010). On the other hand, the measurements on the nuclear apparatus are similar to that described for the populations A and C in the report by Duncan (1977), and similar to the description by Martins *et al.* (2010).

Our specimens present morphological variations in measurements and in denticle morphology that coincide with *T. heterodentata*. Although the present material do not show similarity with all the values presented in the original description of the species (Duncan, 1977), it coincides with several other records of the specie from different countries (Basson *et al.* 1983; Albaladejo & Arthur 1989; Van As & Basson 1992; Basson & Van As 1994; Al-Rasheid *et al.* 2000; Asmat 2004; Martins *et al.* 2010; Miranda *et al.* 2012). Therefore, despite these variations in measurements, these specimens collected from Brazilian freshwater fish have the characteristics of *T. heterodentata*, and this is the first record of this parasite on pacu and the first record in southeastern Brazil.

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