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Short communication

Paratrichodina africana (Ciliophora): A pathogenic gill parasite in farmed Nile tilapia

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ABSTRACT

Trichodinids are ciliated protozoa that are widely known as one of the main groups of fish parasites. The genus *Trichodina* presents the greatest species diversity. However, records of *Paratrichodina* species are scarce, and little is known about their pathogenicity in hosts. The present study provides new records of *Paratrichodina africana* Kazubski and El-Tantawy (1986) in Nile tilapia from South America and descriptions of pathological changes and seasonality. A total of 304 farmed fish were examined. From gill scraping, parasites were identified using Klein's nitrate impregnation method. Gill samples were fixed for histopathological analysis. Small trichodinid found in this study have a prominent blade apophysis and narrow central part and blade shape that corresponds to the characteristics of *P. africana* Kazubski and El-Tantawy (1986). Gill lesions were proportional to parasite intensity, in which the gill tissue was compromised in heavy infestation. Proliferative disturbances were found, including epithelial hyperplasia, desquamation, and mononuclear and eosinophilic infiltrate that culminated in necrosis. We did not observe a seasonality effect on the occurrence of *P. africana*. This ciliated protozoan causes compromised respiratory capacity that leads to severe gill lesions and currently is an important pathogen that afflicts intensive tilapia cultures in Brazil.

1. Introduction

Trichodinids are ciliated protozoan parasites in vertebrates (Dias et al., 2009; Pádua et al., 2012) and invertebrates (Pinto et al., 2006; Silva et al., 2009) that inhabit mainly aquatic ecosystems. Species of the genus *Trichodina* are the most numerous, but records of *Paratrichodina* in fish are scarce, composed of approximately 10 described species (Tang et al., 2012). The genus *Paratrichodina* was proposed by Lom (1963) and since recorded in Europe (Lom and Haldar, 1976), Africa (Kazubski and El-Tantawy, 1986), Asia (Tang et al., 2012), North America (Lom and Haldar, 1976), and South America (Pantoja et al., 2012). They have been associated with gill and urinary infections in fish (Lom and Dyková, 1992), but little is known about their pathogenicity in hosts.

Hyperplasia with epithelium displacement and secondary lamellar fusion were found in *Solea aegyptiaca* parasitized by *Trichodina gobii* (Yemmen et al., 2011a). Additionally, *Trichodina puytoraci* caused tissue inflammation, hypertrophy, degeneration, aneurysms, and necrosis in the gills of *Mugil cephalus* (Yemmen et al., 2011b),

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whereas proliferative and degenerative alterations and desquamation were reported in *Tetradon fahaka* parasitized by *Trichodina fahaka* (Abdel-Baki et al., 2011). These lesions are not specific to trichodiniasis and can be caused by other fish parasites (Grano-Maldonado et al., 2011; Pádua et al., 2013). Consequently, gill lesions cause a respiratory deficit (Pádua et al., 2012), leading to outbreak (Khan, 2009).

Nile tilapia (*Oreochromis niloticus*) is a fish that is cultured worldwide and distributed throughout the entire Brazilian territory. Its productive characteristic is responsible for it being the most important fish cultured in cages (Brasil, 2012). Among the parasites that affect tilapia, Dinoflagellida, Trichodinidae and Monogenea are indicated as the main etiological agents (Jerônimo et al., 2011). In fact, *Trichodina compacta* Van As and Basson (1989), *Trichodina magna* Van As and Basson (1989), and *Paratrichodina africana* Kazubski and El-Tantawy (1986) were recorded in tilapia cultured in Brazil (Ghiraldelli et al., 2006; Martins and Ghiraldelli, 2008; Pantoja et al., 2012). Nevertheless, the specific pathological aspects of *P. africana* are unknown.

The present study presents new records of *P. africana* in South America and provides descriptions of pathological changes and the influence of seasonality in cage-reared Nile tilapia in Brazil.

2. Materials and methods

2.1. Study area and fish

A total of 304 farmed Nile tilapia was analyzed in fish farms that showed historic of chronic mortality. The fish were cage-reared in reservoirs at the hydroelectric power station in southeastern Brazil, which comprises Rio das Velhas (19° 08′31.8″ S; 47° 41′05.6″ W), Nova Ponte, Minas Gerais and the Paraná River reservoir (20° 16′39.8″ S; 51° 03′38.2″ W), Santa Fé do Sul, São Paulo. In northeastern Brazil, the fish were cage-reared in a small reservoir in the Vale do Juliana River (13° 43′57.56″ S; 39° 09′00.79″ W) and earth pond hatchery in the city of Ituberá, Bahia.

2.2. Gross pathology, parasitic assessment, and seasonality

Apparently healthy and moribund fish were observed for pathological and parasitological diagnosis *in loco*. Body surface mucus, fins, and gills were scraped for light microscopic observation. When parasitized the smears were air-dried at room temperature for laboratory examination.

Positive samples were transported to the Laboratory of Aquatic Organism Pathology (LAPOA) at the Aquaculture Center of UNESP (CAUNESP). The smears were impregnated with silver nitrate using Klein's method (Klein, 1958) for the posterior examination of adhesive disk structures and denticles under a light microscope as suggested by Lom (1958). The span was the measurement from the extremity of the blade to the extremity of the ray as described by Arthur and Lom (1984). All of the measurements were made in micrometers and followed the recommendations of Lom (1958) and Van As and Basson (1989). Measurements were made on photomicrographs that were obtained using a Nikon E200® photomicroscope equipped with the Moticam 2300® image capture system. The parasite measurements were made using ImagePro Plus® 4.1 software. Schematic drawings of the denticles, as proposed by Van As and Basson (1989), were produced by means of vectorization using CorelDraw® X5 software.

During a 1 year period, eight samples were collected in a facility situated in southeastern Brazil (20° 16′39.8″ S; 51° 03′38.2″ W). This region is characterized by a tropical climate and seasons are not well defined. Because of this peculiarity, we compared dates between the dry (April to September) and rainy (October to March) periods (Alves et al., 2005).

2.3. Histopathological analysis

Gills with discrete, moderate and severe infestation were fixed in a buffered 10% formalin solution, processed according to usual histopathological techniques, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin–eosin. The slides were analyzed, and photomicrographs were obtained using a Nikon E200® photomicroscope equipped with the Moticam 2300® image capture system.

2.4. Statistical analysis

The values of prevalence from the dry and rainy periods were subjected to analysis of variance (ANOVA). Significant results were followed by Student's *t*-test. Additionally, a morphometric comparison among *Paratrichodina* populations was made using ANOVA and Tukey's test at a probability of 5%.

3. Results

3.1. Parasitic diagnosis

The examined fish showed trichodinid infestation on the body surface, fins and gills and mixed infection by Monogenea and *Epistylis* sp. Fish reared in cages in the states of São Paulo and Bahia showed discrete to moderate parasitism by *Chilodonella hexasticha* in 23.4% of the examined fish. In all of the facilities, moribund fish were parasitized by small trichodinids on the gills. From a total of 304 fish examined in the northeast and southeast, 100% were parasitized by trichodinids. However, fish from São Paulo, Bahia and Minas Gerais showed 65.9%, 87.5%, and 100% prevalence rates of small trichodinid parasitism on the gills, respectively. Healthy fish with no clinical signs sometimes showed discrete to moderate infestation by small trichodinids on the gills.

3.2. Paratrichodina description

Small trichodinids that were found on the gills of tilapia reared in different regions in the Brazilian territory showed the same characteristics as the genus *Paratrichodina* Lom (1963). For morphometry, the parasites were divided into three populations according to the sampling site (Table 1). After comparison, the different populations were

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Table 1
Measurements of *Paratrichodina africana* Kazubski and El-Tantawy (1986) in Nile tilapia reared in cages in Brazil. The data are presented as minimum–maximum values (arithmetic mean ± standard deviation; number of individuals measured).

Characters	Population A	Population B	Population C
Locality	Nova Ponte, MG, Brazil	Santa Fé do Sul, SP, Brazil	Ituberá, BA, Brazil
Body^{D}	18.7–28.9 (22.8 ± 2.4; 34)	17.4–27.2 (22.9 ± 3.0; 21)	18.8–28.6 (23.3 ± 2.3; 50)
Border membrane^{W}	1.3–2.3 (1.9 ± 0.2; 34)	1.5–3.2 (1.9 ± 0.4; 21)	1.6–2.3 (1.9 ± 0.2; 50)
Adhesive disc^{D}	14.8–24.9 (19.2 ± 2.5; 34)	14.4–23.7 (19.1 ± 2.9; 21)	15.3–24.7 (19.6 ± 2.4; 50)
Denticular ring^{D}	10.2–15.4 (12.3 ± 1.3; 34)	8.8–15.2 (12.3 ± 2.1; 21)	9.3–16.6 (12.4 ± 1.8; 50)
Number of denticles	19.0–27.0 (23.8 ± 1.5; 31)	20.0–26.0 (23.3 ± 2.0; 21)	19.0–27.0 (22.9 ± 2.3; 50)
Denticle span	4.1–6.4 (5.3 ± 0.6; 34)	4.1–6.9 (5.6 ± 0.8; 21)	4.4–6.4 (5.5 ± 0.5; 50)
Denticle^{L}	2.0–3.3 (2.6 ± 0.4; 34)^{a}	2.3–3.4 (2.8 ± 0.3; 21)^{b}	2.0–4.0 (3.1 ± 0.4; 50)^{c}
Blade^{L}	2.2–3.0 (2.7 ± 0.2; 16)	2.6–3.4 (2.9 ± 0.3; 6)	2.4–4.0 (3.0 ± 0.3; 50)
Central Part^{W}	0.7–1.0 (0.9 ± 0.1; 16)	0.8–1.0 (0.9 ± 0.1; 6)	0.6–1.1 (0.8 ± 0.1; 50)
Ray^{L}	1.4–2.1 (1.7 ± 0.2; 16)	1.4–1.9 (1.7 ± 0.2; 6)	1.3–2.1 (1.7 ± 0.2; 50)
Pins per denticle	3.0–5.0 (4.3 ± 0.6; 21)	4.0–5.0 (4.4 ± 0.5; 8)	4.0–5.0 (4.3 ± 0.5; 10)

D, diameter; W, width; L, length; morphometric data with letter superscripts are significantly different ($p < 0.05$).

determined to belong to the same parasite species, *P. africana* Kazubski and El-Tantawy (1986).

Disk-shaped ciliate (Fig. 1a and b) were observed, with a body diameter that varied from 17.4 to 28.9 μm and adoral ciliature of 195.8 ± 16.3° (Fig. 1b). Wide, spatulate-shaped blades were also found that filled the space between the *y* and *y* + 1 axes (Fig. 1c–e). The anterior margin of the blade convex had an apex that neither trespassed nor touched the *y* + 1 axis. The distal surface of the blade was flat in the majority of the specimens with a discrete curve. The tangent point was rounded and situated below the distal point of the blade margin. The tip of the rounded blade apophysis trespassed the *y* + 1 axis. The connection between the blade and central part was elongated and slender, with a posterior projection in the majority of the specimens. The central part was delicate, triangular, and oblong-pointed and filled the *y* + 1 and *y* − 1 axes. The centripetal ray was well-developed, with no apophysis, and discretely curved

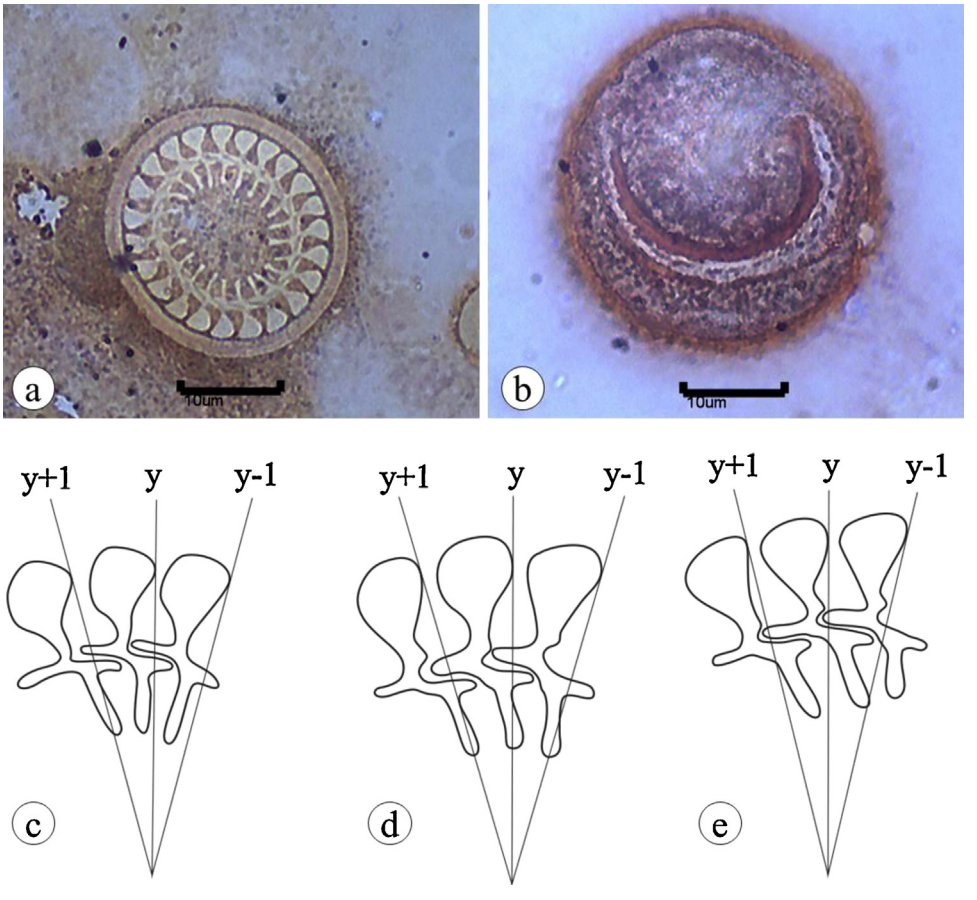

Fig. 1. Klein's silver-impregnated *Paratrichodina africana* Kazubski and El-Tantawy (1986) in farmed Nile tilapia that show adhesive discs (a) and adoral ciliature (b). Scale bar = 10 μm. Schematic drawing of the denticles of *P. africana* in farmed Nile tilapia that show morphological variations (c–e).

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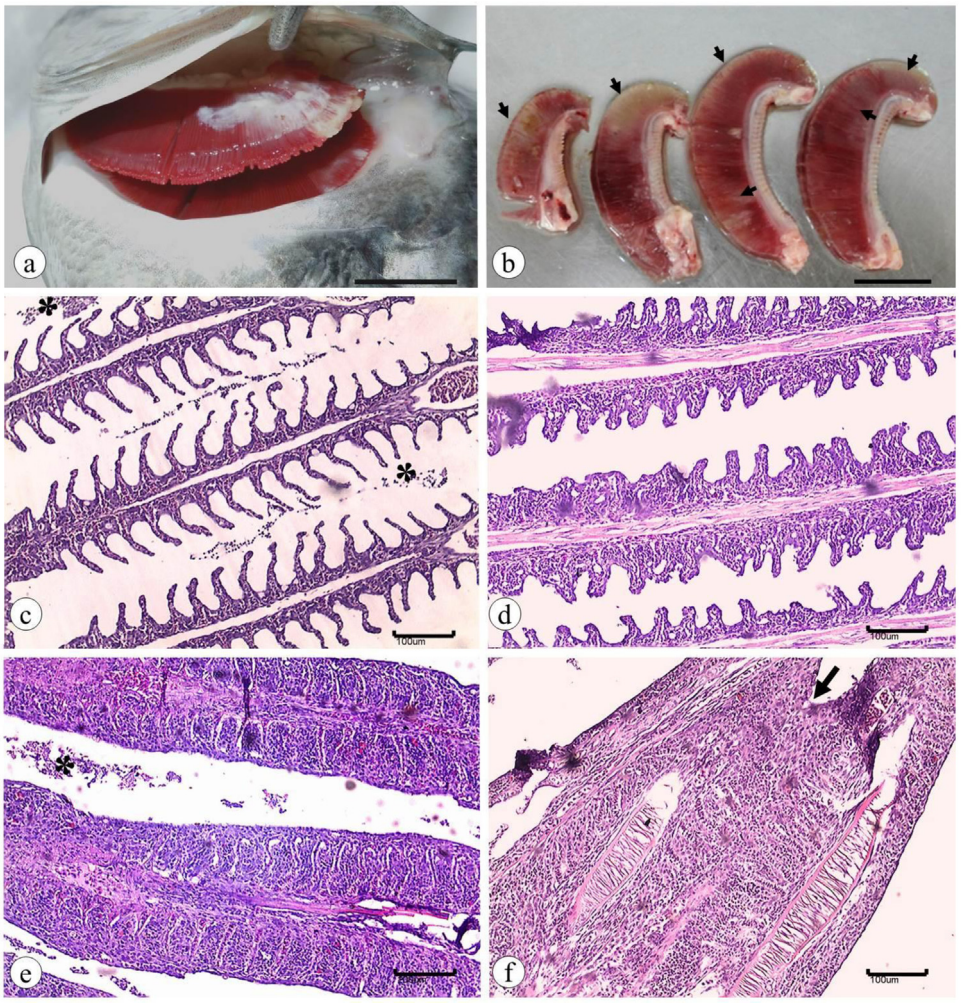

Fig. 2. Gills of farmed Nile tilapia parasitized by *Paratrichodina africana* Kazubski and El-Tantawy (1986) from southeastern Brazilian that show a milky-like aspect (a). Scale bar = 3 cm. The gill arches of farmed Nile tilapia from northeastern Brazil show whitish multifocal areas that suggest necrosis (arrow in b). Scale bar = 2 cm. Histological sections of the gills of Nile tilapia with different levels of infestation by *P. africana*: Fish with discrete infestation with discrete hyperplasia, mononuclear infiltrate, and desquamated cells (asterisk in c). Fish with moderate infestation with moderate hyperplasia with secondary lamellar fusion, mononuclear and eosinophilic inflammatory infiltrate, and few multifocal areas of necrosis (d). Fish with severe infestation with complete fusion of the secondary lamellae, proliferation of mucus cells, mononuclear and eosinophilic inflammatory infiltrate, multifocal areas of necrosis, congestion, and desquamated cells (asterisk in e). Fusion of gill filaments (arrow in f). Scale bar = 100 μm.

in some specimens with a rounded point. The ray showed variations in its orientation that were parallel to the $y-1$ axis (Fig. 1c), on the $y-1$ axis (Fig. 1d), or posteriorly projecting to the y-axis (Fig. 1e) and did not pass the $y-1$ axis. Adoral ciliature spirals varied from 170° to 215°. Specimens are deposited in the National Institute of Amazonian Research (INPA), Manaus, AM, Brazil under the number 010 and 011.

3.3. Gross pathology and seasonality

Fish with discrete and moderate infestation by *P. africana* did not exhibit macroscopic changes. Nevertheless, highly parasitized fish showed increased mucus production, paleness in the gills, and multifocal whitish areas, suggesting necrosis. Additionally, a milky-like aspect was found on the gills of fish from southeastern Brazil (Fig. 2a and b).

A seasonal effect on the occurrence of this trichodinid was not found ($p > 0.5$). The prevalence of infestation was 60.5% (37.0–74.4%) during the dry period and 70.1% (54.8–80.6%) during the rainy period.

3.4. Histopathological analysis

The gill lesions were proportional to parasite intensity (discrete, moderate, or severe), compromising organ function. Different levels of hyperplasia in parasitized fish led to partial or total secondary lamellar fusion (Fig. 2c–e). In some cases, fusion of the gill filament was found (Fig. 2f). We observed hyperplasia of mucus cells and mononuclear and eosinophilic inflammatory infiltrate beyond

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desquamation in the spaces between the gill filaments. Congestion and interstitial hemorrhages were also observed in the gills. Multifocal to coalescent areas of gill necrosis were observed in severely parasitized fish. The presence of small trichodinids on the gills was confirmed by the histopathological analysis.

4. Discussion

The present study presented new records of the occurrence of *P. africana* in South America and revealed a wide distribution throughout the Brazilian territory. This species has been sparsely reported in the literature. It was initially described in Egypt and Kenya (Kazubski and El-Tantawy, 1986) and later in India (Mitra and Bandyopadhyay, 2006), Bangladesh (Kibria et al., 2009) and Brazil (Pantoja et al., 2012). Comparisons of these findings showed that the body of our specimens was shorter than that observed in the original description by Kazubski and El-Tantawy (1986) but coincided with the descriptions of Mitra and Bandyopadhyay (2006) and Kibria et al. (2009). Adhesive disk and denticulate ring measurements and the number of denticles were consistent with specimens from Africa (Kazubski and El-Tantawy, 1986) and Bangladesh (Kibria et al., 2009) and slightly lower than the description of Mitra and Bandyopadhyay (2006).

Our specimens that were collected from different hydrographic basins showed similarities among the populations. The few differences included a denticle length that was higher in population C than in the other populations but coincided with the description of Mitra and Bandyopadhyay (2006). The border membrane in population B had a maximum value of 3.2 μm, but this was similar to the arithmetic mean of populations A and C and the studies by Mitra and Bandyopadhyay (2006) and Kibria et al. (2009).

Our specimens showed the same denticle features as the findings of Kazubski and El-Tantawy (1986), Mitra and Bandyopadhyay (2006), and Kibria et al. (2009), including the presence of a posterior projection in the blade. This characteristic can be absent in *Paratrichodina yangtzeus* (Hu, 2009) or discrete in *Paratrichodina rotundiformis* (Tang et al., 2012). The present study contributed additional information on the ray position relative to the *y*-axes.

Little is known about the pathogenicity of *Paratrichodina* species in hosts. However, the pathological changes revealed in this study showed the pathogenic potential of *P. africana* in Nile tilapia in intensive culture systems in cages. However, the alterations described herein are not specific and also found in other parasitic (Grano-Maldonado et al., 2011; Pádua et al., 2013) and bacterial (Starliper and Schill, 2011) infections. Additionally, the aggravation of gill lesions observed in the present study possibly occurred from opportunistic bacterial infection, in which the abrasive action exerted by *P. africana* on the gills of the host may favor infection by bacterial agents. In fact, ciliate parasites increase the susceptibility of fish to bacterial infection (Xu et al., 2012a,b).

In the present study, no seasonal influence was found on *P. africana* infestation in tilapia, similar to studies of *T. magna* and *T. compacta* in southern Brazil (Jerônimo et al., 2011). These latter studies, however, found a relationship between parasitism and the culture system. In Turkey, no influence was observed in the prevalence of *Trichodina modesta*, but higher parasitism intensity was observed in the warmest months (Özer, 2007). A water temperature of 29 °C inhibited the proliferation of *T. puytoraci* in fish from Tunisia, in which the greatest prevalence and intensity were observed at 18 °C (Yemmen et al., 2011b). These variations indicate that the seasonality effect on trichodinid infestation might be related to environmental conditions, the culture system, and the ecology of the parasite.

P. africana is an exotic parasite that was possibly introduced into Brazil by imports of cichlid fish for aquaculture. This might be true when analyzing *Trichodina heterodentata*, *T. magna*, and *T. compacta*. The parasite is widely distributed throughout Brazilian territories, including the northern region (Pantoja et al., 2012) and northeastern and southeastern regions (present study). This ciliate found favorable conditions in the cage-reared tilapia system and is currently an important etiologic agent that afflicts tilapia cultures in Brazil.

Conflict of interest

The authors declare no conflicts of interest.

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