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Short communication

First record of *Chilodonella hexasticha* (Ciliophora: Chilodonellidae) in Brazilian cultured fish: A morphological and pathological assessment

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ABSTRACT

Chilodonelids are small ciliated protozoans found worldwide and can be dangerous in culture conditions. This study presents morphometric data on the ciliate *Chilodonella* that is found in cultured Nile tilapia (*Oreochromis niloticus*), native bait fish tuvira (*Gymnotus* aff. *inaequilabiatus*) and native pacu (*Piaractus mesopotamicus*) and includes a histopathological assessment of the changes that occur in the pacu. For parasitic diagnosis, skin and gill samples were scraped onto slides, dried at room temperature, stained with Giemsa or impregnated with silver nitrate, and the measurements were obtained from photomicrographs. In the diseased pacu, the first gill arch was collected and fixed in a 10% buffered formalin solution for histopathological analysis. Parasite specimens from the different collection sites were identified morphologically as *C. hexasticha* Kiernik (1909). Diseased fish exhibited depigmentation, skin ulceration, scale loss, excessive mucus production and gill lesions. Histopathological analysis of pacu gills displayed epithelial proliferation with mononuclear inflammatory infiltrate, hemorrhages, and scattering necrosis. In Brazilian-farmed fish this is the first record of *C. hexasticha*, which has great pathogenic potential in cultured freshwater species. In addition, two new hosts are presented.

1. Introduction

Chilodonellids are small ciliated protozoans found worldwide as free-living species (Jee et al., 1996) on both invertebrate (Das, 2003) and vertebrate hosts (El-Tantawy and El-Sherbiny, 2010). *Chilodonella piscicola* (Zacharias, 1894) Jankowski, 1980 (syn. *C. cyprini* Moroff, 1902) and *Chilodonella hexasticha* (Kiernik, 1909) are two important species that have been identified to parasitize fish and found on the body surface, gills, and fins of the hosts. For identification of these ciliates, the characteristics of Giemsa's stained specimens allied to Klein's silver impregnation for kineties observation has been used (Klein, 1958; El-Tantawy and El-Sherbiny, 2010). However, the number of kineties constitutes the most important taxonomic characteristic that distinguishes *C. hexasticha* from *C. piscicola* (Kazubski and Migala, 1974).

These parasites do not present host specificity, have a monoxenic life cycle, and can be observed causing severe host lesions. Channel catfish (*Ictalurus punctatus*) and goldfish (*Carassius auratus*) parasitized by *C. hexasticha* presented epithelial hyperplasia, gill lamellae fusion, inflammatory infiltrate, hemorrhages, edema and necrosis (Hoffman et al., 1979). Infestation by *C. piscola* leads to reduced growth and chronic mortalities associated

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with degeneration, loss of blood cells, and erosion in the gill lamellae were found in juvenile masou salmon (*Oncorhynchus masou*) (Urawa and Yamao, 1992). The large presence of *C. hexasticha*, which was a result of low temperatures, overcrowding, and poor feeding, was responsible for the farmed cichlid fish mortalities (Paperna and Van As, 1983).

Protozoan diversity in aquaculture is wide and represents an important cause of fish diseases and/or may provide a portal of entry for secondary infections (Martins et al., 2011). In Brazil, there are few taxonomic studies on protozoan parasites of farmed fish. Mobile peritrichid ciliates have been associated with the exotic Nile tilapia and channel catfish (Ghiraldelli et al., 2006; Martins and Ghiraldelli, 2008; Martins et al., 2010) and in the native pacu (Pádua et al., 2012). The dinoflagellate *Piscinoodinium pillulare* (Martins et al., 2001) and the ciliate *Ichthyophthirius multifiliis* (Tavares-Dias et al., 2001) were reported in farmed freshwater fish. In addition, Fernandes et al. (2011) have reported *Chilodonella* sp. on *Odontesthes bonariensis* (Atherinopsidae) in southern Brazil and Silva et al. (2011) have reported *Chilodonella* sp. on an amazon wild fish *Oxydoras niger* (Doradidae), but no reports were found on the description of *Chilodonella* species on Brazilian farmed fish (Eiras et al., 2012).

The continental finfish aquaculture is an agribusiness in expansion in Brazil. The Nile tilapia is an exotic species mostly produced between farmed fish, followed by exotic cyprinids and native fish that include the roundfish as the tambaqui (*Colossoma macropomum*), pacu (*Piaractus mesopotamicus*) and an intergeneric hybrid tambacu (*C. macropomum* female x *P. mesopotamicus* male) (Brasil, 2012). The tuvira is a knifefish species native to the Pantanal basin not farmed in commercial scale, only in laboratorial condition as an experimental model. This species has economic importance because of its use as live bait for sport fishing, but constant exploitation has caused its numbers to diminish in its natural environment (Moraes and Espinosa, 2001).

Little is known about the fauna of protozoa parasite that affects fish in Brazil. The present study presents morphometric data for *C. hexasticha* that are found in the Nile tilapia and bait fish tuvira (*Gymnotus* aff. *inaequilabiatus*) in Central Brazil and Nile tilapia and native pacu (*Piaractus mesopotamicus*) in Southeastern Brazil and includes an assessment of histopathological changes in pacu gills.

2. Materials and methods

Nile tilapia from the GIFT lineage were acquired from a fish farm located in Palotina municipality, Paraná, South Brazil (24°12′S; 53°50′30″W) and transported to the Fish-farm Laboratory at Embrapa Agropecuária Oeste, Dourados, MS, Central Brazil. The fish were distributed in cages that were made from plastic net (8 mm between knots) and had a 60-L total capacity, placed into fiber tanks with a 1000-L total capacity, constant flow of water (10 L min^{-1}), and a temperature of approximately 26 °C. The same fiber tank was utilized to harvest tuvira that were acquired from a fish farm that produces bait fish (as described by Pádua et al., 2011). The two fish species were kept in the same tank for seven days.

Five diseased tuvira (70–100 g) and five Nile tilapia (150–300 g) with skin lesions were sampled by scraping their skin and gills for microscopical analysis in spring 2010. In case of presence of ciliates, the smears were fixed with methylic alcohol and stained with Giemsa or silver nitrate 2% (Klein, 1958) (Synth®, Diadema-SP, Brazil) for taxonomic evaluation.

Seven juvenile diseased pacu (100–250 g weight) that were cultured in ponds in Guaíra municipality, São Paulo State (20°20′47.1″S; 48°11′27.1″W) (water temperature approximately 25 °C) and ten Nile tilapia (50–150 g weight) from the GIFT lineage that were collected in cages located at Tiete River, Arealva municipality, São Paulo State (22°05′15.6″S; 48°51′44.6″W) (water temperature approximately 23 °C) were examined in summer 2011. For a parasitic diagnosis, skin and gill samples were scraped onto slides, dried at room temperature and stained with Giemsa, or impregnated with silver nitrate 2%. In the diseased pacu, the first gill arch was collected and fixed in a 10% buffered formalin solution for histopathological analysis. The gills were embedded in paraffin; the embedded tissues were sliced into 5-μm thick sections and stained with hematoxylin and eosin (HE) at the Histology Laboratory of Animal Physiology and Morphology Department, UNESP, Jaboticabal, SP.

Chilodonellid measurements were obtained from photomicrographs (Nikon E200®) that were obtained with an image capture system Moticam 2.300®. Parasite measurements were performed using Image-Pro Plus® 4.1 software according to El-Tantawy and El-Sherbiny (2010). A morphometric comparison among chilodonellids from the Nile tilapia and tuvira that were kept in the same tank (Central Brasil) was performed with variance analysis and Student's *t*-test at a 5% probability. In addition, a morphometric comparison among chilodonellids from Central Brazil and other two populations from Southeastern Brazil was performed with variance analysis and Tukey's test at a 5% probability.

3. Results

3.1. Gross pathology and diagnosis of parasitic co-infestation

After seven days of maintenance in tanks one tuvira was found dead, and in the subsequent five days 10 other fish died. No mortality was observed in tilapia. All diseased fish exhibited depigmentation, skin ulceration, scale losses, excessive mucus production and gill lesions that resembled necrosis. Microscopical examination revealed a large presence of *Chilodonella* sp. in all of the diseased tuvira. We also observed a discrete *Chilodonella* sp. infestation that was accompanied by a moderate *Epistylis* sp. infestation and the presence of Monogenea (Platyhelminthes) in tilapia from Central Brazil.

The examined tilapia from Southeastern Brazil showed severe gill lesions, and clear epithelium suggesting necrosis. In addition, skin lesions and depigmentation associated with scale loss and fin erosion were also observed. On

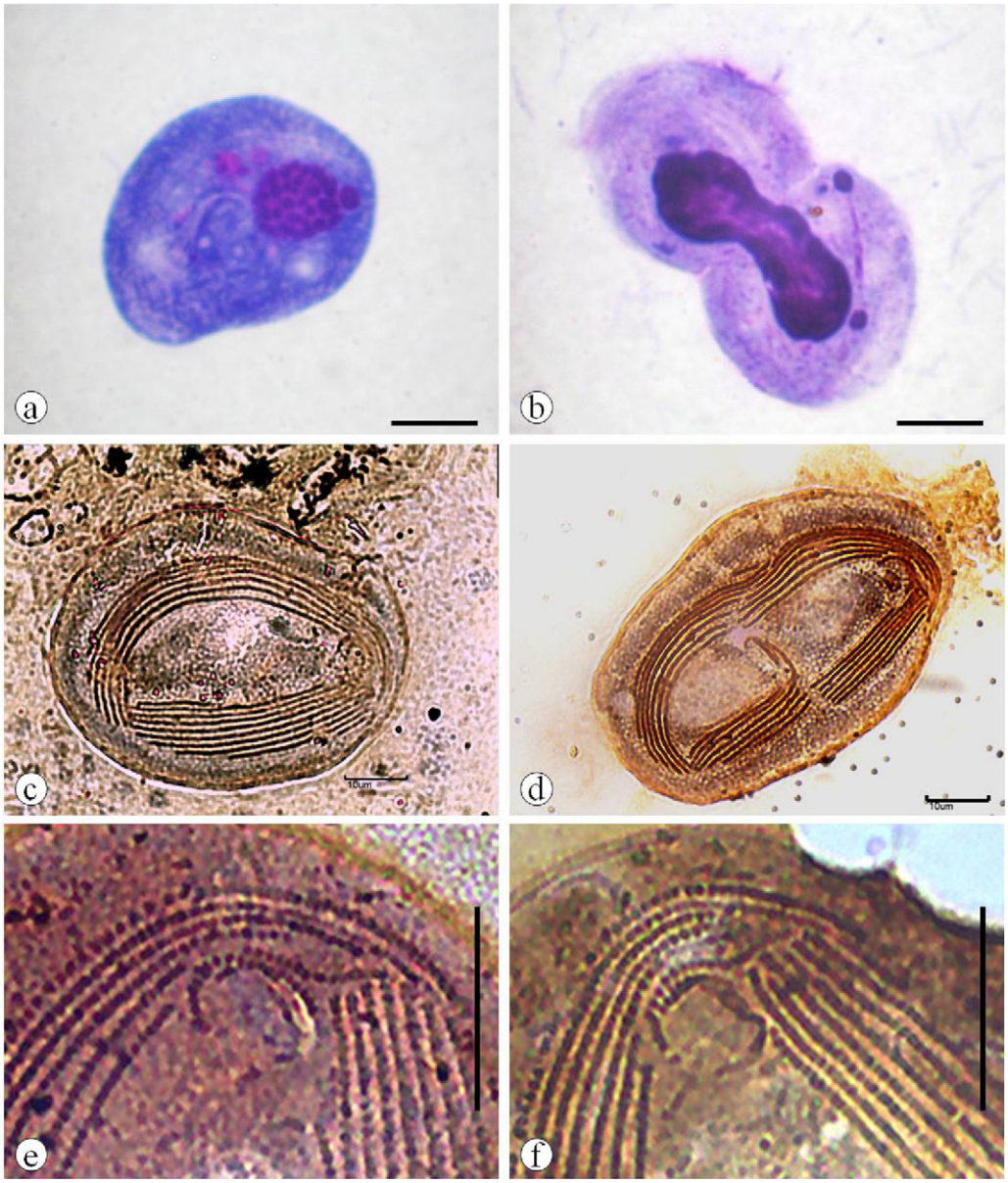

Fig. 1. Giemsa-stained *Chilodonella hexasticha* reveals a nuclear apparatus (a–b) and binary fission (b). The kineties (c–f) and binary fission (d) can be observed in the silver nitrate-impregnated specimens. High magnification photomicrography of the oral ciliature (e–f). Bar: 10 μm.

these fish, a severe *Chilodonella* sp. infestation, a discrete presence of trichodinids, and a moderate presence of Monogenea and *Epistylis* sp. on the skin and gills were observed.

In contrast, in the diseased pacu from ponds, macroscopical lesions were found only in the gills in which were characterized by a multifocal distribution and clear color also suggesting necrosis. These fish presented a mixed infestation by trichodinids, *Apiosoma* sp., and Monogenea that was primarily on the body surface. *Chilodonella* sp. severely infested the gills, which were followed by a moderate infestation on the body surface.

3.2. Chilodonellid description

The *Chilodonella* specimens in this study were dorsal-ventrally flattened, slightly asymmetric and presented with an oval-shaped body. The slightly oval macro- and micronuclei were located on the posterior region. The chromatin of the macronucleus had a shriveled aspect, whereas the micronucleus was smooth and compact (Fig. 1). The difference between the chilodonellids found on tuvira and tilapia (Central Brazil) was not statistically significant ($p > 0.05$), therefore were designated as population A. Specimens from Southeastern Brazil exhibited some differences in the body dimensions and number of ciliary kineties and

Table 1
Morphometric data of three populations of *C. hexastica* from fishes in Brazil. Data presented are means ± standard deviation, followed in parentheses by minimum, maximum, and number of individuals measured.

Characteristics	Population A	Population B	Population C
Host	*O. niloticus* and *G.* aff. *inaequilabiatus*	*Piaractus mesopotamicus*	*O. niloticus*
Site of infection	Gills and skin	Gills and skin	Gills and skin
Location	Dourados, MS, Brazil	Guaíra, SP, Brazil	Arealva, SP, Brazil
Body length	34.1 ± 2.8 (28.6–41.0; 63)[a]	49.8 ± 7.5 (34.1–66.5; 45)[b]	47.7 ± 6.2 (37.6–71.8; 45)[b]
Body width	25.4 ± 2.6 (18.9–32.3; 63)[a]	36.7 ± 6.1 (21.0–47.5; 45)[b]	40.0 ± 5.6 (26.8–57.9; 45)[c]
Macronucleus length	10.9 ± 1.2 (8.4–14.7; 60)[a]	16.0 ± 1.9 (12.7–21.1; 45)[c]	14.8 ± 4.0 (9.6–20.3; 34)[b]
Macronucleus width	10.4 ± 1.1 (8.5–14.5; 60)[a]	14.1 ± 2.0 (9.4–20.8; 45)[c]	11.9 ± 1.9 (9.1–17.0; 34)[b]
Micronucleus length	2.8 ± 0.4 (1.7–3.7; 55)[a]	2.8 ± 0.6 (1.9–4.5; 27)[a]	3.5 ± 0.8 (1.9–5.4; 28)[b]
Micronucleus width	3.4 ± 0.5 (1.9–4.4; 55)[a]	3.4 ± 0.6 (1.7–4.5; 27)[a]	3.9 ± 0.7 (2.2–5.2; 28)[b]
Nr. kineties right	5.9 ± 1.1 (4–9; 48)[b]	6.5 ± 0.9 (5–10; 45)[a]	6.8 ± 0.8 (6–7; 45)[a]
Nr. kineties left	4.4 ± 0.8 (3–6; 48)[a]	7.5 ± 0.7 (5–9; 45)[b]	8.3 ± 1.1 (5–11; 45)[c]

Morphometric data with the same alphabetic superscripts are not significantly different ($p > 0.05$).

were classified as population B (pacu), and population C (tilapia) (Table 1 and Fig. 2). After a comparison, it was concluded that the different populations belonged to the same parasite species *C. hexasticha* Kiernik (1909) (Table 2).

3.3. Histopathology

Histopathological analysis of the pacu gills displayed different levels of scattering hyperplasia (i.e., discrete, moderate and severe), lamellar fusion, scattering necrosis, discrete subepithelial edema, and mononuclear inflammatory infiltrate, which was composed of lymphocytes. Moreover, congestion, aneurisms, and extensive areas of interstitial hemorrhages were also found (Fig. 3).

4. Discussion

The specimens from Central Brazil (population A) in the present study were slightly smaller than those described by Hoffman et al. (1979), Paperna and Van As (1983), Langdon et al. (1985), Kazubski and Migala (1974), Ahmed et al. (2000), and Mitra and Haldar (2004), but similar to those found by Imai et al. (1985), Jee et al. (1996), and El-Tantawy and El-Sherbiny (2010) (Table 2). The macronucleus length of the *Chilodonella* specimens from tilapia and tuvira was

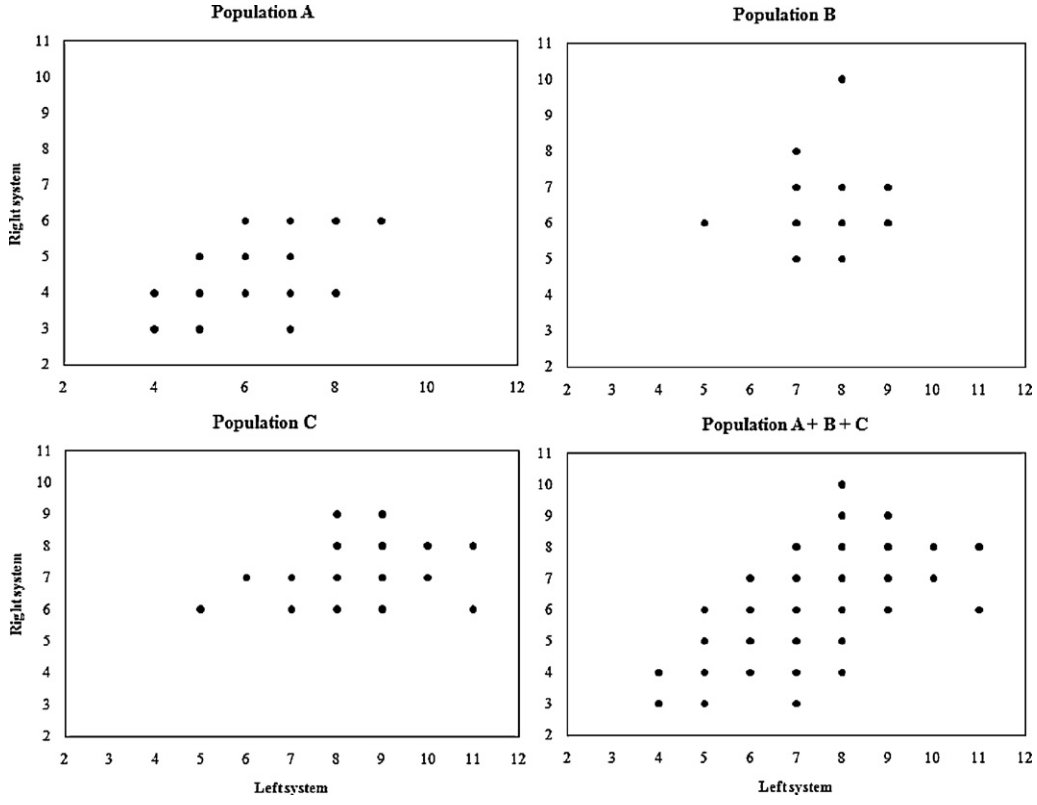

Fig. 2. The relationship between the number of kineties on the right and left systems of *Chilodonella hexasticha* from different populations of cultured Brazilian fish. Population A: Central Brazil; populations B and C: Southeastern Brazil.

Table 2
Measurements of *C. hexastica* from different countries. Data presented are means ± standard deviation, followed in parentheses by minimum, maximum values.

Characteristics	Kazubski and Migala (1974)	Hoffman et al. (1979)	Hoffman et al. (1979)	Paperna and Van As (1983)	Imai et al. (1985)	Jee et al. (1996)
Host	Eight species of fish[a]	*Carassius auratus*	*Ictalurus punctatus*	Four Tilapia species	*Symphysodon discus*	*Carassius carassius*
Infection site	Skin and gills	Gills	Gills	Gills and skin	Gills	Gills and skin
Location	Poland	USA	USA	Israel and South Africa	Japan	Korea
Body length	58.0 (48–70)	42.3 (29.6–55.7)	45.8 (37.5–60.0)	45 ± 6 (31–67)	30–40	36.5 ± 4.2 (30–45)
Body width	46.0 (35–61)	30.3 (22.0–49.0)	33.0 (22.5–42.5)	38 ± 5 (29–51)	20–30	23.5 ± 3.8 (15–30)
Macronucleus length	–	–	–	–	–	9.8 ± 1.7 (8–15)
Macronucleus width	–	–	–	–	–	–
Micronucleus length	–	–	–	–	–	2.9 ± 0.5 (2–4)
Micronucleus width	–	–	–	–	–	–
Nr. kineties right	6.1 (5–8)	6.6 (5–8)	6.4 (6–7)	6–7	1–3	3.7 ± 0.5 (3–5)
Nr. kineties left	8.4 (6–10)	8.4 (6–10)	6.6 (6–7)	6–7	5–7	4.1 ± 0.5 (3–5)

Characteristics	Ahmed et al. (2000)	Mitra and Haldar (2004)	El-Tantawy and El-Sherbiny (2010)
Host	*Tilapia zillii*	*Nandus nandus*	*Clarias gariepinus*
Infection site	Skin	Gills	Gills and skin
Location	Egypt	India	Egypt
Body length	50.2 (49.2–50.6)	48.3 ± 6.3 (38.8–59.2)	37.9 ± 6.5 (29.7–50.6)
Body width	33.2 (31.8–34.6)	43.2 ± 5.0 (35.7–53.0)	27.7 ± 7.9 (22.0–39.6)
Macronucleus length	19.5 (18.5–20.6)	–	13.4 ± 2.4 (11.0–17.1)
Macronucleus width	16.9 (16.6–17.2)	–	10.1 ± 1.6 (7.7–11.0)
Micronucleus length	2.3 (2.1–2.5)	–	3.9 ± 0.5 (3.3–4.4)
Micronucleus width	2.3 (2.1–2.5)	–	2.7 ± 0.5 (2.2–3.3)
Nr. kineties right	6–7	6.7 ± 0.6 (5–7)	8 (7–8)
Nr. kineties left		6.9 ± 0.4 (6–8)	8 (8–10)

[a] *Coregonus peled*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Lucioperca lucioperca*, *Salvelinus fontinalis*, and *Tinca tinca*.

smaller than that observed by Ahmed et al. (2000), whereas the size of micronucleus did not vary among the populations compared in the present study. Our specimens from Central Brazil exhibited a higher number of kineties on the right side when compared to *C. hexasticha* that was reported by Imai et al. (1985) and Jee et al. (1996). However, the number of kineties was similar to that found in the majority of the studies (Kazubski and Migala, 1974; Hoffman et al., 1979; Paperna and Van As, 1983; Langdon et al., 1985; Ahmed et al., 2000; Mitra and Haldar, 2004; El-Tantawy and El-Sherbiny, 2010).

The specimens from the Southeastern Brazil (populations B and C) exhibited larger body dimensions in comparison to those in population A. Nevertheless, these measurements are in agreement with those found in channel catfish (*I. punctatus*) (Hoffman et al., 1979), tilapia (Paperna and Van As, 1983), Australian river gizzard shad (*Nematolosa erebi*) (Langdon et al., 1985), and Gangetic leaffish (*Nandus nandus*) (Mitra and Haldar, 2004). Both the body dimensions and macronucleous sizes of the specimens from the populations B and C were larger than those found in population A. In contrast, these values were lower than those observed by Ahmed et al. (2000). The macronucleus size in population C was similar to that reported by El-Tantawy and El-Sherbiny (2010). In contrast, the size of the micronucleus of population B was similar to that found in population A and to that recorded by Jee et al. (1996). The micronucleus dimension of the specimens found on tilapia that were collected in Southeastern Brazil (population C) was smaller than that found by Jee et al. (1996) but similar to the specimens of El-Tantawy and El-Sherbiny (2010).

The number of ciliary kineties constitutes the most important taxonomic characteristic that separates *C. hexasticha* from *C. piscicola*. *Chidonella piscicola* has more numerous and less spaced ciliary kineties (Kazubski and Migala, 1974; Rintamäki et al., 1994; Mitra and Haldar, 2004; El-Tantawy and El-Sherbiny, 2010). Our specimens from Southeastern Brazil (populations B and C) have a similar number and disposition of ciliary kineties and are different from that observed in population A but similar to the measurements reported by Kazubski and Migala (1974), Hoffman et al. (1979), and Mitra and Haldar (2004). Although we observed slight differences in measurements among the three investigated populations, our results suggest that the specimens found in Brazilian freshwater fish are *C. hexasticha* Kiernik (1909).

The alteration in fish behavior and gross pathology in this study are in agreement with the findings of Paperna and Van As (1983) and Urawa and Yamao (1992) that reported darkened body color, lethargy, abraded skin, and depreciated gill morphology. Epithelial proliferation is one of the most observed alterations in the host gills, which was observed in pacu and also reported by Hoffman et al. (1979), Paperna and Van As (1983), Langdon et al. (1985), Urawa and Yamao (1992). Mononuclear inflammatory infiltrate, aneurisms, and hemorrhages were also found by Hoffman et al. (1979) and Paperna and Van As (1983). However, the occurrence of necrosis and desquamation in the gill epithelium, which can cause mortality, were the most severe lesions that were found in our fish. These results agree with Paperna and Van As (1983)

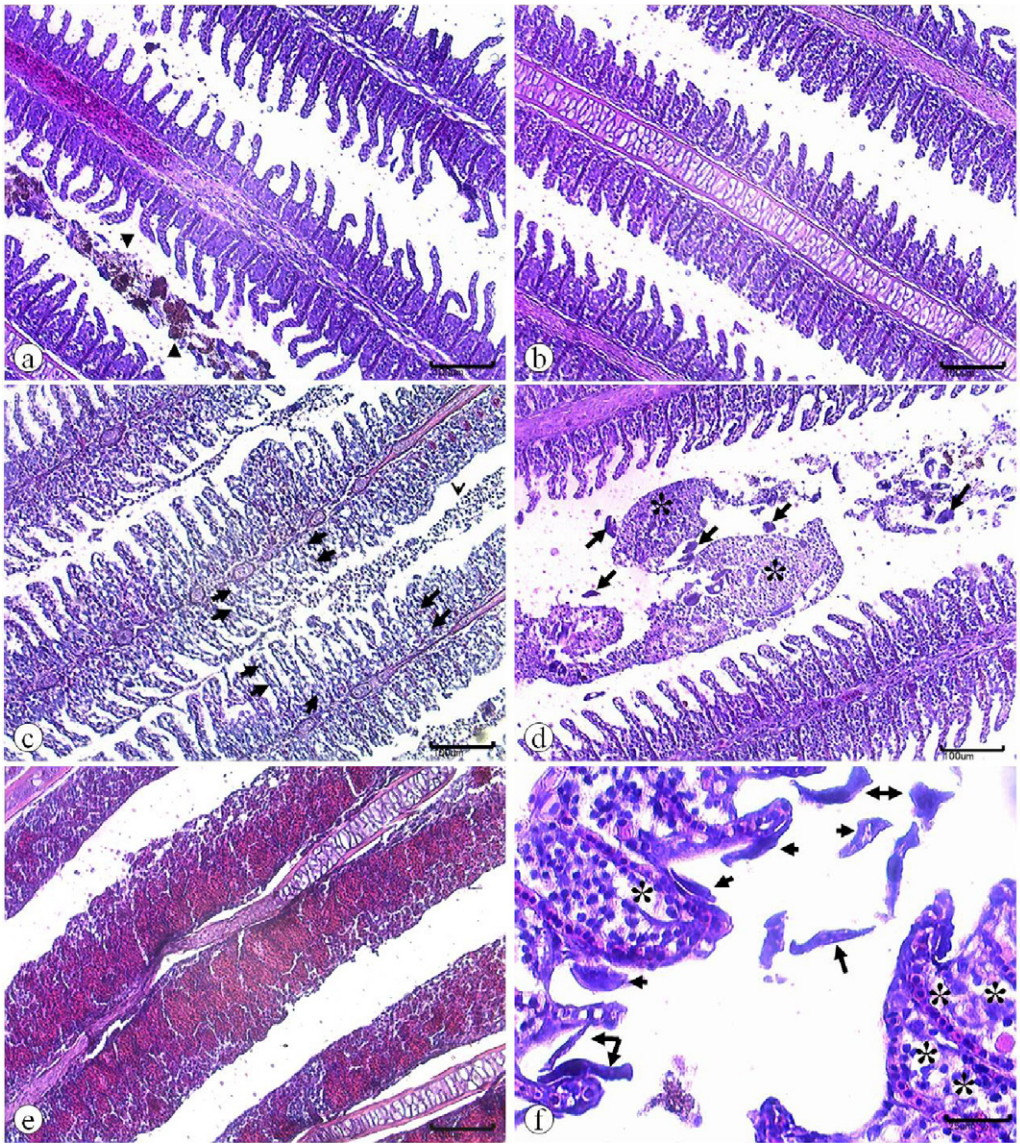

Fig. 3. The histopathology of pacu (*Piaractus mesopotamicus*) gills that are parasitized by *Chilodonella hexasticha*. Epithelial hyperplasia (a–f), lamellar fusion (b–f), necrosis (c – arrows; f – asterisk), cellular desquamation (d – asterisk), interstitial hemorrhage (e), and chilodonellids (d and f – arrows). Hematoxylin–eosin stained. Bar = 100 μm (a–e) and 25 μm (f).

who highlighted the nonspecific characteristic of these lesions.

In Brazil, this is the first report of *C. hexasticha* in cultured fish, in which tuvira and pacu are considered a new host for this parasite.

Conflict of interest

The authors declare no conflicts of interest.

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