



Short communication

First record of *Chilodonella hexasticha* (Ciliophora: Chilodonellidae) in Brazilian cultured fish: A morphological and pathological assessmentS.B. Pádua^a, M.L. Martins^{b,*}, J.R. Carrijo-Mauad^c, M.M. Ishikawa^d, G.T. Jerônimo^b, J. Dias-Neto^a, F. Pilarski^a^a Aquaculture Center of São Paulo State University (CAUNESP), Jaboticabal, Brazil^b AQUOS-Aquatic Organism Health Laboratory, Aquaculture Department, Federal University of Santa Catarina (UFSC), Brazil^c School of Biological and Environmental Sciences, Federal University of Grande Dourados (UFGD), Brazil^d Embrapa Western Agriculture, Brazil

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ABSTRACT

Chilodonellids 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.

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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. piscicola* 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 Brazil) 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

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