Host-parasite relationship during *Epistylis* sp. (Ciliophora: Epistylididae) infestation in farmed cichlid and pimelodid fish

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Abstract – The objective of this work was to describe the host-*Epistylis* sp. relationship during infestation on farmed fish. Five Nile tilapia (*Oreochromis niloticus*) and ten hybrid surubim catfish (*Pseudoplatystoma reticulatum* x *P. corruscans*), all diseased, were used for in vivo morphological analysis of sessile peritrichs by contrast microscopy. Fragments of infected tissues were subjected to histological processing and scanning electron microscopy. *Epistylis* sp. caused hemorrhagic ulcer disease, and cichlids were more prone to develop infestations throughout the body surface due to the attachment of the colonies to the scales, which did not occur with pimelodids. Multifocal granulomatous dermatitis was observed, associated with the hydropic degeneration of the epithelium and to ulcerative areas of necrosis. Microscopic examination showed the presence of bacterial microflora associated to *Epistylis* sp. peduncles. Therefore, this species can be considered a portal of entry for opportunistic bacteria.

Index terms: *Oreochromis niloticus*, *Pseudoplatystoma reticulatum*, *Pseudoplatystoma corruscans*, histopathology, pathogenesis, sessile peritrich.

Relação hospedeiro-parasita durante infestação por *Epistylis* sp. (Ciliophora: Epistylididae) em peixes ciclídeos e pimelodídeos cultivados

Resumo – O objetivo deste trabalho foi descrever a relação hospedeiro-*Epistylis* sp. durante infestação em peixes cultivados. Foram utilizados cinco tilápias-do-nilo (*Oreochromis niloticus*) e dez híbridos de surubim (*Pseudoplatystoma reticulatum* x *P. corruscans*), todos parasitados, para análise morfológica in vivo de peritríquios sésseis por microscopia de contraste de fase. Fragmentos de tecidos infectados foram processados para histologia e microscopia eletrônica de varredura. *Epistylis* sp. causou doença ulcerativa hemorrágica, e os ciclídeos foram mais susceptíveis ao parasitismo na superfície do corpo em razão da fixação das colônias nas escamas, o que não ocorreu nos pimelodídeos. Foi observada ocorrência de dermatite granulomatosa multifocal, associada à degeneração hidrópica do epitélio e às áreas ulcerativas de necrose. O estudo microscópico mostrou a presença de bactérias associadas aos pedúnculos de *Epistylis* sp. Portanto, esta espécie pode ser considerada porta de entrada para bactérias oportunistas.

Termos para indexação: *Oreochromis niloticus*, *Pseudoplatystoma reticulatum*, *Pseudoplatystoma corruscans*, histopatologia, patogênese, peritriquio séssil.

Introduction

Several ciliated protozoans establish ecological relationships with wild or farmed fish, acting as ectocommensals, epibionts, or parasites (Lom & Dyková, 1992). Environmental factors, such as physicochemical characteristics of water and organic matter in suspension, affect the occurrence of these agents (Pritchett & Sanders, 2007; Hossain et al., 2008). Under intensive fish rearing conditions, there is an increased discharge of nutrients into the aquatic environment, which favors the proliferation

of microorganisms that can act as pathogens. Ciliates, including *Trichodina heterodentata* (Martins et al., 2010; Pádua et al., 2012b; Valladão et al., 2014), *Paratrichodina africana* (Valladão et al., 2013), *Chilodonella hexasticha* (Pádua et al., 2013b), and *Epistylis* sp. (Fernández, 2012; Pádua et al., 2013a; Valladão et al., 2015), have recently been reported to cause important diseases in farmed fish in South America, being considered emerging pathogens in aquaculture.

However, little is known about the ecological relationship between farmed fish and *Epistylis* sp. This sessile peritrich is often described as an epibiont on aquatic organisms, although studies have shown deleterious effects by the ciliate on its hosts (Hüseyin & Selcuk, 2005; Visse, 2007; Pádua et al., 2012a). In addition, the bacterial colonization of the peduncle of these protozoans contributes to aggravate the injuries on host tissues, characterizing *Epistylis* sp. as an agent of diseases of mixed etiology, considered important infirmities for farmed fish (Hazen et al., 1978; Martins et al., 2015).

In Brazil, the exotic Nile tilapia (*Oreochromis niloticus*) is the most cultivated species, presenting economic relevance worldwide, and, in South America, catfishes from the *Pseudoplatystoma* genus are one of the main native fish reared in captivity (Boletim estatístico da pesca e aquicultura, 2011). The intensification of production of both species increases the infections by parasites and bacteria. Parasites, such as Protozoa, Metazoa, and Myxozoa, were identified in Nile tilapia (Pantoja et al., 2012) and *Pseudoplatystoma* spp. (Campos et al., 2008; Jerônimo et al., 2013); however, data on the host-pathogen relationship are still scarce. The present study shows new information on the pathogenesis of *Epistylis* sp. infestation, featuring the pathological process that occurs in two freshwater fish of economic importance.

The objective of this work was to describe the host-*Epistylis* sp. relationship during infestation on farmed fish.

Materials and Methods

The following fish were examined: five diseased Nile tilapia juveniles from the GIFT lineage, weighing 30 to 50 g, collected in 2012 from cages located in the Tiete River, in the municipality of Arealva, in the state of São Paulo, Brazil (22º05'16"S, 48º51'45"W); and ten diseased hybrid surubim catfish (*Pseudoplatystoma reticulatum* x *P. corruscans* juveniles), weighing 10 to 20 g, collected in 2010 from a raceway located in the municipality of Terenos, in the state of Mato Grosso do Sul, Brazil (20º25'58"S, 55º17'09"W).

Moribund fish collected by net were observed for pathological and parasitological diagnosis in loco. All body surface, fins, and gills of each fish were scraped separately (using glass slides) for light microscope observation, according to Eiras et al. (2006). Diseased fish were transported to the laboratory of invertebrate morphology of Universidade Estadual de São Paulo Júlio de Mesquita Filho for scraping observations of the living organisms with differential interference contrast microscopy, using an Axio Imager Z2 microscope (Carl Zeiss, Oberkochen, Germany). Parasite measurements were performed on living parasites, following methods normally used for this parasite (Ma & Overstreet, 2006; Kühner et al., 2016) and using the software Image Pro Plus (Media Cybernetics Inc., Rockville, MD, USA). Measurements are presented as mean±standard deviation (minimum-maximum, and number of structures measured). This was only possible for parasites of Nile tilapia, since catfish analysis was carried out far from the laboratory and the facility did not have an available photomicroscope.

Skin and fin fragments with multifocal *Epistylis* sp. colonies visible to the naked eye, considered as score 4 according to Pádua et al. (2013a), were fixed in 10% buffered formalin solution, processed according to usual techniques for histopathology, embedded in paraplast block, sectioned at 5.0 µm, and stained with hematoxylin and eosin. The slides were analyzed, and photomicrographs were obtained using a photomicroscope (model Eclipse E200, Nikon Imaging Sales Co., Ltd., Shanghai, China) equipped with the Moticam 2300 image capture system (Quimis, Diadema, SP, Brazil).

Scale and fin fragments with *Epistylis* sp. colonies were fixed in 2.5% glutaraldehyde and postfixed in 1% OsO_4 (osmium tetroxide), both in 0.1 mol L^{-1} cacodylate buffer (pH 7.4), according to Valladão et al. (2014). Afterwards, the fragments were dehydrated with serial concentrations of ethanol and critical-point dried. The biological material was

mounted on aluminum stubs, sputter coated with gold, and examined using a scanning electron microscope at an accelerating voltage of 15 Kv (model JSM-5410, JEOL, Tokyo, Japan).

Results and Discussion

All sampled Nile tilapia showed mixed parasitic infestation by trichodinids and occasionally by *Gyrodactylus cichlidarum* on the tegument and gills. *Epistylis* sp. was the most predominant parasite of all fish, affecting mainly the body surface and rarely gill tissues, with a wide distribution on scales and fins. Hybrid surubim presented mixed infestation by *Trichodina* sp. and *Epistylis* sp., in which the colonies affected only the rigid structures of the fish surface, such as fin rays, stingers, head, operculum, and lips; however, no infestation was observed in the gill tissue. In Nile tilapia, the scales facilitated the development of parasitism, affecting large extensions of the tegument, which did not occur with pimelodids. Similar findings were described in the *Astacus leptodactylus* crayfish (Hüseyin & Selcuk, 2005), which have extensive carapace, allowing the colonization by these ciliates. Although pimelodids are less susceptible to skin infestations, recent studies have shown a high incidence of the parasite *Epistylis* sp. on South American catfish, especially on intergeneric and interspecific hybrids, which are more susceptible when compared to pure species (Pádua et al., 2013a).

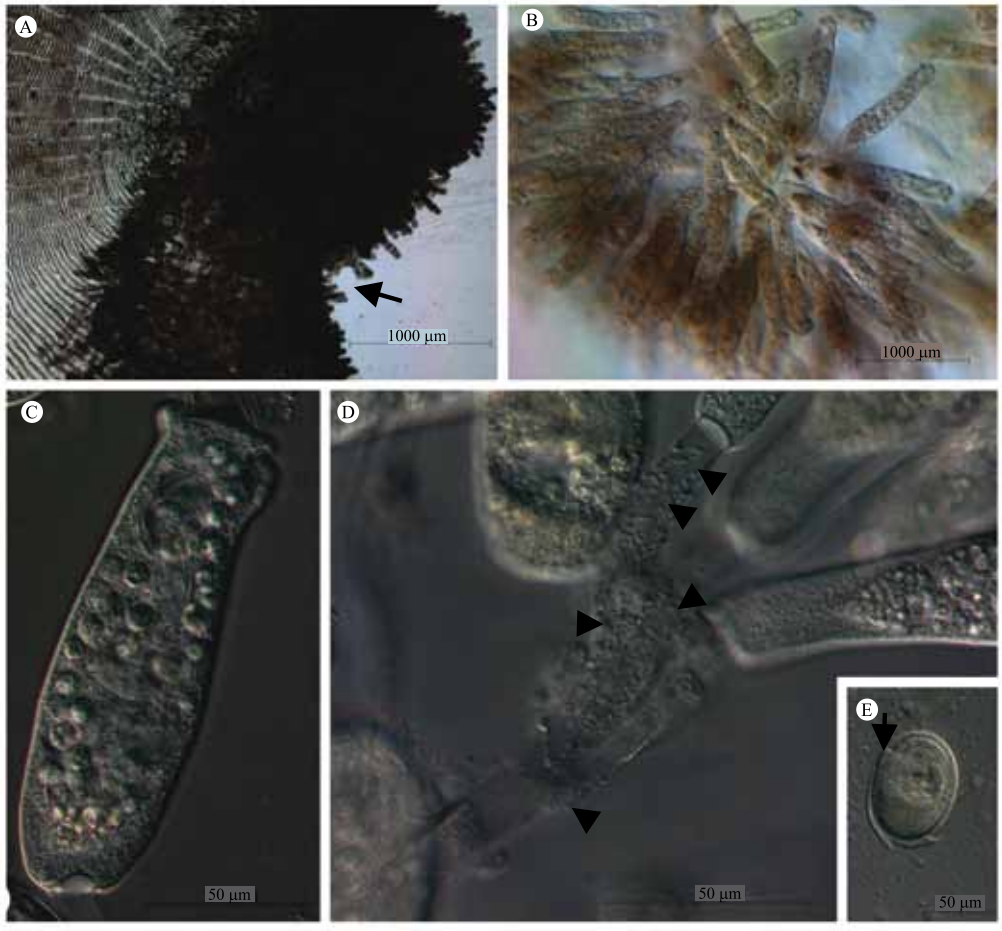

Figure 1. *Epistylis* sp. from fish farmed in the Tiete River, in the municipality of Arealva, in the state of São Paulo, Brazil, observed in fresh examination by differential interference contrast microscopy. Colonies attached on scale of Nile tilapia (*Oreochromis niloticus*) showing erosion (A, arrow); colony with set of zooids (B) and individual zooid (C); peduncle colonized by bacilli (D, arrowheads); and telotroch (E, arrow).

Epistylis sp. presented 8 to 58 zooids showing conical-elongated format (Figure 1 A, B, and C). The morphology was characteristic of the *Epistylis* genus: the peduncle was dichotomously branched and noncontractile; there was one contractile vacuole located in the zooid apical portion; the macronucleus was transverse C-shaped; and the cytoplasm was full of food vacuole (Ma & Overstreet, 2006). The peduncle of this protozoan was colonized by bacilli bacteria and occasionally by filamentous bacteria, arranged in cords (Figure 1 D). Free telotroch were observed, showing ciliature formed in the equatorial region of the cell wall and C-shaped macronucleus (Figure 1 E). All morphometric data for *Epistylis* sp. collected from Nile tilapia are described in Table 1. However, species identification remains difficult. Currently, molecular techniques have been developed to support the systematics of these Protozoa (Utz & Eizirik, 2007; Jiang et al., 2016).

Nile tilapia with high infestation levels, corresponding to score 4 (Pádua et al., 2013a), presented petechial hemorrhage in the area where the colony was attached to, which resulted in corrosion and loss of scales, and in the progression of the most severe lesions to multifocal ulcers. Similarly, hybrid surubim catfish showed ulcerative and bleeding lesions associated with colonies attached to the tegument in severely infested fish.

Table 1. Morphometric data and number of structures measured (n) on the body of *Epistylis* sp. obtained from scales of Nile tilapia (*Oreochromis niloticus*), in the municipality of Arealva, in the state of São Paulo.

Traits	Morphometric data (μm)	n
Body length	135.5±31.4 (95.1–208.8)	25
Length from peristomial lip	124.1±31.3 (81.8–194.6)	25
Width	34.7±4.4 (28.0–46.1)	25
Width at midpoint	39.8±4.9 (31.9–54.3)	25
Width at peristomial lip	39.0±3.5 (33.3–47.8)	25
Height of peristomial lip	12.6±2.1 (9.5–16.6)	25
Width of epistomial disc	26.3±4.5 (18.8–35.6)	25
Width of scopula	16.4±3.5 (10.4–26.3)	25
Length of basal peduncle	539.2±152.7 (282.4–806.4)	18
Width of basal peduncle	17.1±3.0 (12.8–24.4)	18
Length of lateral peduncle	163.3±44.2 (85.4–243.8)	25
Number of zooids	32.3±13.0 (8.0–58)	18

Histological sections showed focal hyperplasia in the epithelium injured by the parasite, as well as hydropic degeneration with multifocal necrosis adjacent to the colony, proliferation of claviform and mucous cells, and moderate mononuclear and granulocytic inflammatory infiltrate with the presence of giant cells, featuring multifocal granulomatous dermatitis (Figure 2 A and B). Moreover, parasites were observed adjacent to the ulceration area without penetrating the host skin, and several bacteria were associated to the colony (Figure 2 C and D). However, the development of colonies may result in local irritation, since multifocal granulomatous dermatitis was found in infested fish, characteristic of a chronic pathological process (Ulrich et al., 2007). Mononuclear infiltration and giant cell formation have also been reported in the evolution of chronic inflammatory response in teleost fish (Belo et al., 2005, 2012; Sakabe et al., 2013), including peritrich infestation (Valladão et al., 2015).

The scanning electron microscopy analysis showed fixed colonies on scales and fins, with partially contracted zooids, which rarely showed slightly protuberant epistomial disc (Figure 3 A, B, and C). Abundant bacterial microflora – with circular shape, in streaks, and without penetrating the host skin – was observed on the peduncle and on the attachment surface (Figure 3 D and E). The bacterial association with *Epistylis* sp. colonies in fish was first described by Hazen et al. (1978), who registered *Aeromonas hydrophila* as the primary causative agent of red-sore disease in North American cichlid. In the present study, abundant bacterial microflora was associated to the peduncle surface of *Epistylis* sp., and most of the pathological changes may result from bacterial proteolytic enzymes (Martins et al., 2015). Recently, Valladão et al. (2015) have also proposed that tissue damage may have occurred due to the action of immune cells at the site of parasitism, because these cells are provided with hydrolytic molecules capable of causing cell damage.

In order to avoid parasite dissemination and host susceptibility, the best management practices allied to regular fish health knowledge should be the most important measures adopted in rearing facilities.

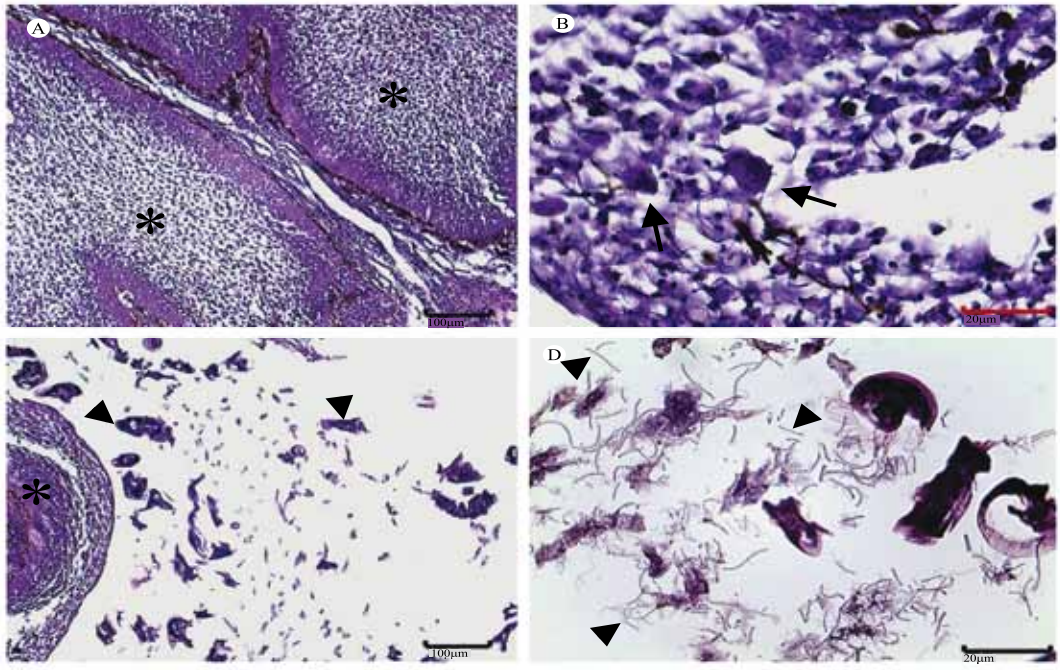

Figure 2. Histopathological sections of fins from farmed hybrid surubim catfish (*Pseudoplatystoma reticulatum* x *P. corruscans*) collected in the municipality of Terenos, in the state of Mato Grosso do Sul, Brazil, infested with *Epistylis* sp. Hydropic degeneration of the epithelium (A, asterisks), showing multinucleated macrophages (B, arrows) under higher magnification; panoramic view of bacterial colony showing zooids (C, arrowheads) and host tissue without penetration (C, asterisk); and bacterial colonies (D, arrowheads). Stained with hematoxylin and eosin. Bars = 100 µm (A and C) and 20 µm (B and D).

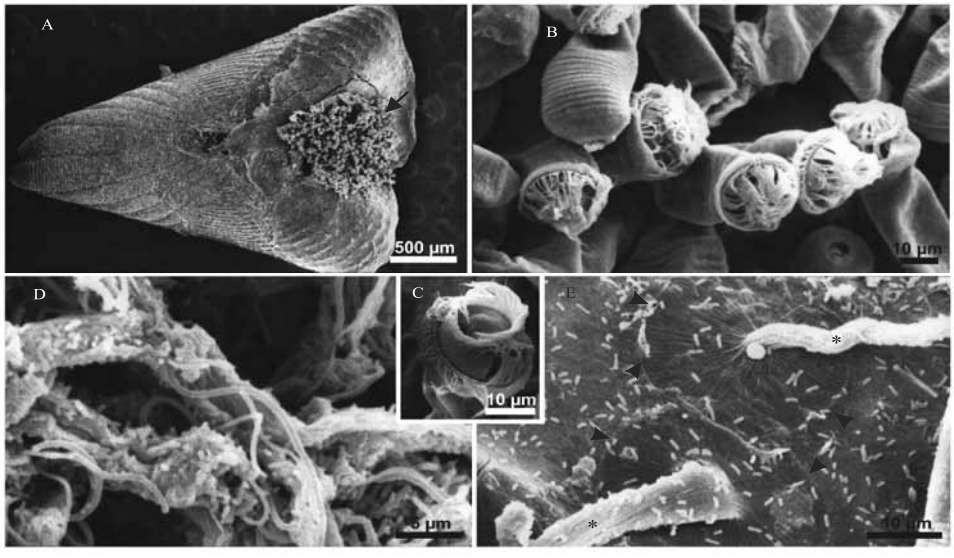

Figure 3. Scanning electron microscopy of *Epistylis* sp. from fish farmed in the Tiete River, in the municipality of Arealva, in the state of São Paulo, Brazil. Colonies attached on scale of Nile tilapia (A, arrow); partially contracted zooids showing oral ciliature (B) and epistomial disc with clear ciliature (C); peduncle colonized by bacillary and filamentous bacteria (D, arrowheads); peduncles attached to the host epithelium (E, asterisks) and its trajectory changes shown in rays; and large amount of bacilli bacteria associated to the attachment site on the host (E, arrowheads).

Conclusions

1. *Epistylis* sp. infestation in cichlid and pimelodid fish may cause mixed diseases by opportunistic bacteria with pathological lesions on the hosts and, therefore, can be considered a portal of entry for these bacteria.

2. Severe histological changes were observed on injured tissue, such as focal hyperplasia and hydropic degeneration with multifocal necrosis adjacent to the colony, characterizing multifocal granulomatous dermatitis.

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