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Short notes

An outbreak of *Ichthyophthirius multifiliis* (Ciliophora: Ichthyophthiriidae) in wild endemic fish fauna *Steindachneridium parahybae* (Siluriformes: Pimelodiae) in Brazil

Lincoln Lima Corrêa[1], Paulo Sérgio Ceccarelli[2], Marcos Tavares-Dias[3]

[1]Universidade Federal do Oeste do Pará – UFOPA., Av. Mendonça Furtado, Nº 2946, Fátima, CEP 68040-470, Santarém, Pará, Brasil
[2]PS Ceccarelli and Cia Lta. Rua Gregorio Pedroso de Lima, Nº 73, Jardim Limoeiro, CEP 13641-051, Pirassununga, estado de São Paulo, Brasil
[3]Embrapa Amapá, estado do Amapá, Rodovia Juscelino Kubitschek, Km 5, Nº 2600, Universidade, CEP: 68903-419, Macapá, Amapá, Brasil

Corresponding Author: Lincolin L. Corrêa; e-mail: lincorre@gmail.com

ABSTRACT. The ciliate *Ichthyophthirius multifiliis* is an important pathogen of freshwater fish that occurs in both temperate and tropical regions around the world. The purpose of present study was to report an outbreak of *I. multifiliis* in *Steindachneridium parahybae* from the Paraiba do Sul River in state of São Paulo (Brazil). The fins, tegument and gills of *S. parahybae* were examined for the presence of *I. multifiliis*, and blood parameters (Red Blood Cell, thrombocytes and leukocytes) were evaluated. All the examined fish (100%) were parasitized with *I. multifiliis* and the mean infection intensity was 15.5 parasites per fish. All the fish died 72 hours as a consequence of ichthyophthiriasis with alterations to the tegument coloration and open opercula. Red Blood Cell counts and hemoglobin concentration were low, indicating an anemic process.

Keywords: *Ichthyophthirius multifiliis*, ichthyophthiriasis, infection, anemia, Brazil

Introduction

Ichthyophthirius multifiliis Fouquet, 1876 is a ciliate that causes the ichthyophthiriasis, a serious worldwide problem for farmed freshwater fish. All species of freshwater fish are considered susceptible to *I. multifiliis*, and the parasite has been found in both farmed and wild fish. This ciliate is often found on the skin and fins as well as in the gills of infected fish. The disease is highly contagious due to its direct life cycle, spreads rapidly from one fish to another without the need for additional hosts [1–6]. Consequently, it can cause severe infections, particularly when fishes are crowded together. Unlike other parasites which frequently result in subclinical infections. Ichthyophthiriasis usually results in high morbidity and mortality, leading to economic losses for the aquaculture industry [3,5–7]. However, the epizootic occurrence of *I. multifiliis* in wild fish has been rarely reported, although this ciliated protozoan has been recorded in wild fish without causing disease several times. Fish introductions are believed to have spread this protozoan almost everywhere in the world from its origins in Asia [7], as it has been introduced in number regions through the transportation of fish.

Physical and chemical alterations in cultivation water, such as changes in pH, water hardness, ion strength, ionic concentrations and temperature, directly influence the life cycle of *I. multifiliis* during the nonparasitic stage [5]. The tissue morphology of the skin and gills of fish changes significantly after infection by *I. multifiliis* [6,8]. In general, the theronts invade and penetrate the epidermis of the fish and form cavities enclosed by epithelial cells where they settle and feed. This

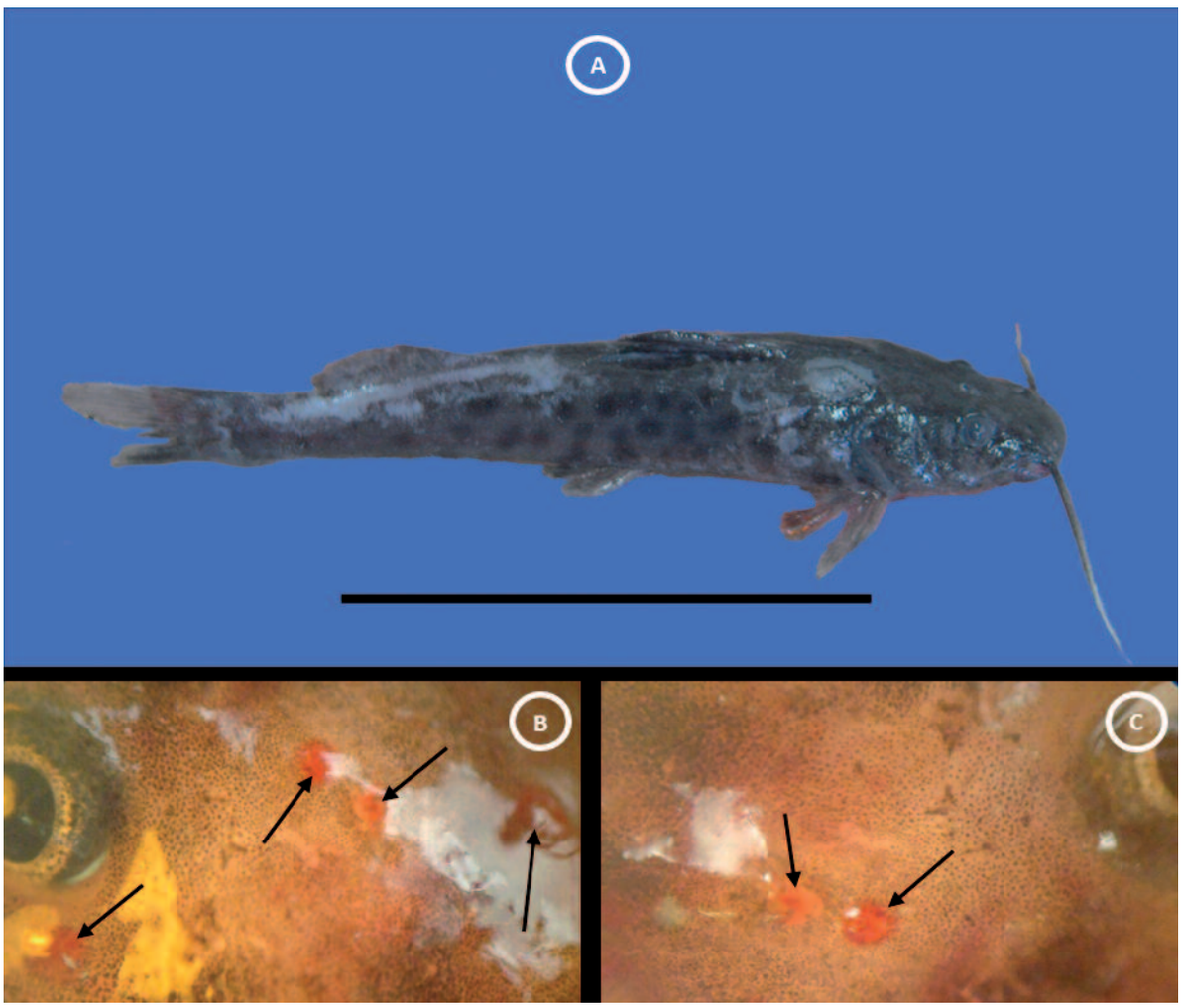

Fig. 1A–C. *Steindachneridion parahybae* in dorsal view (A) and ventral view (B). Hemorrhagic focus on the head region of *Steindachneridion parahybae* infected by *Ichthyophthirius multifiliis* (C).

process is mediated by a combination of mechanical and enzymatic actions [6].

Steindachneridion parahybae, is a Pimelodidae fish popularly known as surubim-do-paraíba that can reach about 60 cm in standard length [9]. It is endemic to the Paraíba do Sul River basin, and while its biology is little known, it is known to have the characteristics of a migratory species and nocturnal habits [9,10]. It is, however, threatened by environmental disturbances caused by anthropic pressure and overfishing in the Paraíba do Sul River, although it is a species of potential interest for aquaculture. This species is listed in the red book in the category of risk of extinction and Criteria in Danger B2ab (i, ii, iii, v). The aim of the present study was therefore to report an outbreak of *Ichthyophthirius multifiliis* in *S. parahybae* collected from the Paraiba do Sul River in the state of São Paulo (Brazil) and to describe the blood parameters of the infected fish.

Materials and Methods

One hundred adults of *S. parahybae* were collected from the Paraiba do Sul River and kept in a tank at the National Specialized Continental Fish Research and Conservation Center of the Chico Mendes Institute (CEPTA), in Pirassununga, São Paulo (21º55'55"S and 47º22'37"W), for production of fingerlings, and in future, to repopulate the Paraiba do Sul River.

All fingerlings of this work are F1 wild fish from the Paraiba do Sul River from induced spawning at the Hydrobiology and Aquaculture Station of Companhia Energética de São Paulo (CESP) of Paraibuna, State of São Paulo. Fingerlings (6.7 ± 0.3 cm and 1.6 ± 0.09 g) produced were kept in a 1000 L tank with constant water, under controlled pH

Table 1. Hematological parameters of *Steindachneridion parahybae* (N=20) infected with *Ichthyophthirius multifiliis*

Parameters	Mean ± SD	Range
Red blood cells ($\times 10^6$ μL^{-1})	1.01 ± 0.14	0.70–1.23
Leukocytes (μL^{-1})	16,244 ± 4419	11,050–25,300
Thrombocytes (μL^{-1})	3633 ± 1178	1400–5820
Hematocrit (%)	17.9 ± 3.4	10.0–24.0
Hemoglobin (g dL^{-1})	2.3 ± 0.07	2.2–2.4
VCM (fL^{-1})	179.5 ± 42.1	129.0–273.9
CHCM (g dL^{-1})	13.4 ± 3.2	9.2–23.1

MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration

(6.1), temperature (20.2°C), dissolved oxygen (6.0 mg L^{-1}), alkalinity (10.0 mg L^{-1}), water hardness 9.0 mg L^{-1}) and electrical conductivity (14.0 µS cm^{-1}) conditions, and then presented signs of diseases.. In Mach 2012, 30 *S. parahybae* fingerlings in the process of domestication for aquaculture died and exhibited changes in body surface coloration and open opercula. Thus, other 20 fingerlings were then collected for analysis of the fins, skin and gills, as well as for the collection of blood. A scalpel was used to scrape mucus from the skin and fins of fish and smears were prepared on slides in a drop of water under a cover slip and then examined to confirm the identification of *I. multifiliis* at 100× magnification in microscope. In addition, smears of gills was performed to examination the identification of *I. multifiliis* at 100x magnification in microscope. The total number of *I. multifiliis* present in fields of smears was counted in a microscope for estimation of parasite intensity.

The ecological terms used were those proposed by [11]. The weight and total length of each examined fish were measured. A blood sample was collected from each fish by puncturing the caudal vessel using syringes (1.5 mL^{-1}) containing 25,000 U mL^{-1} of sodium heparin. Blood was used to determine the hematocrit through the microhematocrit method, total erythrocyte count in the Neubauer chamber was calculated, and the hemoglobin concentration was identified using the cyanmethemoglobin method. With this data, the Wintrobe hematimetric indices were ca (MCHC). Blood smears panchromatically stained with rapid panoptic were prepared and used for the total leukocyte and total thrombocyte counts [12].

Results

All the specimens of *S. parahybae* examined exhibited hemorrhagic foci on the surface of their bodies (Fig. 1A–C).The infection rate of the analyzed fish by *I. multifiliis* was 100% and the mean infection intensity was 15.5 parasites per fish, with the highest frequency in the gills (Fig. 2A–C).

The hematological parameters of fish of the species *S. parahybae* infected by *I. multifiliis* are described in Table 1.

Discussion

Outbreaks of *I. multifiliis* have been reported wild and farmed fish (3,5–7,13,14]), but are less frequent in wild fish. In current study, *S. parahybae* fingerlings presented severe ichthyophthiriasis that led to mortality. Epizootic outbreaks of *I. multifiliis* are common event in farmed fish and have caused economic losses for fish farming from the Brazil [14,15]. These outbreaks are more frequent in the regions South, Southeast and Center-West from Brazil, which have seasonal variations of temperature. However, susceptibility to *I. multifiliis* differs among fish species. Treatments with increased water flow in tanks of cultivation, formalin, potassium permanganate, sodium chloride and copper sulfate have been used for controlling or for eliminating *I. multifiliis* in farmed fish in Brazil. Such products must be applied strategically whenever occur high infection levels for control of diseases. However, when these recommended chemicals are over-used or misused can lead to parasite's potential drug resistance [14].

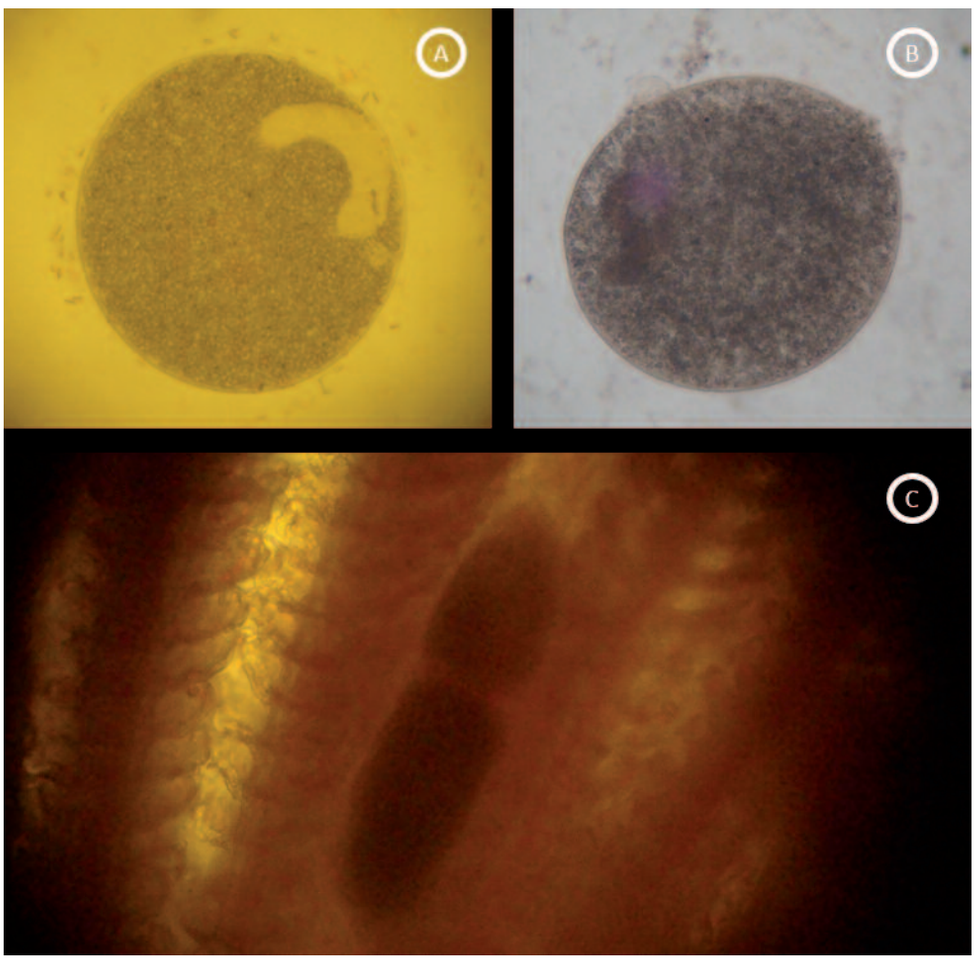

Fig. 2A–C. Tomont of *Ichthyophthirius multifiliis* of tegument of *Steindachneridion parahybae* (A). Tomite of *Ichthyophthirius multifiliis* of tegument of *Steindachneridion parahybae* (B). Theront of *Ichthyophthirius multifiliis* on gills of *Steindachneridion parahybae* (C). Light microscopy with magnification ×100.

In fingerling of *S. parahybae* in phase of domestication an outbreak of *I. multifiliis* was observed, and fish exhibited changes in body surface coloration and opercula. *Ichthyophthirius multifiliis* control in *S. parahybae* fingerlings is a key point to reach a high production; hence, efforts to avoid diseases must be concentrated in this phase of the cultivation. Hines and Spira [16], studying the dynamics of *I. multifiliis* infection, observed that younger fish are more susceptible to this disease as their immune system is still developing. Another factor that may have favored *I. multifiliis* infection in *S. parahybae* was the low water temperature (20.2°C) in the culture tank. Environmental temperatures from 20 to 25°C favor the proliferation of the infective stages of *I. multifiliis* and may cause disease outbreaks in a fish population [5,14]. In *Orestias* spp. from Lake Titicaca, the outbreak of *I. multifiliis* also seemed linked to the low temperature in the environment [13]. However, an exception have been reported for *Colossoma macropomum* at Central and North regions of Brazil, because mortalities of up to 50% in fingerlings infected with *I. multifiliis* occurred when the water temperatures ranged from 29.5 to 31.5°C [14].

Piscine blood parameters provide an insight into various processes of an organism. For example, erythrocyte parameters (i.e. Red Blood Cells counts/RBC, hematocrit and hemoglobin) are especially recommended for monitoring the health of a fish population, as they can indicate anemiant processes [12,16]. The hematological parameters of *S. parahybae* infected with *I. multifiliis* are described in Table 1. The RBC number and hemoglobin concentrations in *S. parahybae* with the gills and integument infected by *I. multifiliis* were low when compared to other species of Pimelodidae [17]. These low RBC number and hemoglobin levels indicate signs of anemia, due to the hemorrhagic foci on the body surface of *S.*

parahybae caused by infection by *I. multifiliis*. These ciliate parasites may cause significant tissue damage, which results in an impaired osmotic and hematological balance [1,3,4,6,16].

In summary, in order to control infection of *I. multifiliis* in *S. parahybae* fingerling, as well as in other fish, is fundamental first characterize the hygienic-sanitary status in cultivation tanks. Furthermore, the use of chemotherapeutics should not be used only when ichthyophthiriasis occurs, in which treatment is employed as a measure to reduce the mortality of fish.

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