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Host–parasite–environment relationship, morphology and molecular analyses of *Henneguya eirasi* n. sp. parasite of two wild *Pseudoplatystoma* spp. in Pantanal Wetland, Brazil☆

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ABSTRACT

A new myxosporean species, *Henneguya eirasi* n. sp., is described parasitizing the gill filaments of *Pseudoplatystoma corruscans* and *Pseudoplatystoma fasciatum* (Siluriformes: Pimelodidae) caught in the Patanal Wetland of the state of Mato Grosso, Brazil. The parasite formed white, elongated plasmodia measuring up to 3 mm. Mature spores were ellipsoidal in the frontal view, measuring 37.1 ± 1.8 μm in total length, 12.9 ± 0.8 μm in body length, 3.4 ± 0.3 μm in width, 3.1 ± 0.1 μm in thickness and 24.6 ± 2.2 μm in the caudal process. Polar capsules were elongated and equal in size, measuring 5.4 ± 0.5 μm in length and 0.7 ± 0.1 μm in width. Polar filaments had 12–13 coils. Histopathological analysis revealed that the parasite developed in the sub-epithelial connective tissue of the gill filaments and the plasmodia were surrounded by a capsule of host connective tissue. The plasmodia caused slight compression of the adjacent tissues, but no inflammatory response was observed in the infection site. Ultrastructure analysis revealed a single plasmodial wall connected to the ectoplasmic zone through numerous pinocytotic canals. The plasmodial wall exhibited numerous projections and slightly electron-dense material was found in the ectoplasm next to the plasmodial wall, forming a line just below the wall. Partial sequencing of the 18S rDNA gene of *H. eirasi* n. sp. obtained from *P. fasciatum* resulted in a total of 1066 bp and this sequence did not match any of the Myxozoa available in the GenBank. Phylogenetic analysis revealed the *Henneguya* species clustering into clades following the order and family of the host fishes. *H. eirasi* n. sp. clustered alone in one clade, which was the basal unit for the clade composed of *Henneguya* species parasites of siluriform ictalurids. The prevalence of the parasite was 17.1% in both fish species examined. Parasite prevalence was not influenced by season, host sex or host size.

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1. Introduction

The pimelodids *Pseudoplatystoma corruscans* Spix and Agassiz, 1829, popularly known in Brazil as "pintado" or "surubim", and *Pseudoplatystoma fasciatum* Linnaeus, 1766, known as "cachara", are carnivorous, migratory fish that play an important role in the fishing economy where they occur. These fish attain large sizes (up to 100 kg for *P. corruscans* and up to 20 kg for *P. fasciatum*) and are among the most important freshwater fish in Brazil (Campos, 2005). *P. corruscans* occurs in the São Francisco and Prata river basins, while *P. fasciatum* occurs in the Amazon, Corantijn, Essequibo, Orinoco and Prata river basins (Froese and Pauly, 2009). Due to the quality of the meat and high market value (Campos, 2005), fisheries targeting these species attained approximately 3,570,000 kg (2,597,000 kg for *P. corruscans* and 973,000 kg for *P. fasciatum*) in 2006 (Ibama, 2008). The rapid growth of these species has also caught the interest of fish farmers (Campos, 2005). The production of these species in fish farms reached approximately 1,094,000 kg in 2006 (Ibama, 2008), representing an important option for Brazilian fish farmers.

Regarding infection by Myxosporeans, *Henneguya corruscans* Eiras et al., 2009 and *Henneguya pseudoplatystoma* Naldoni et al., 2009, have been reported infecting pimelodids of the genus *Pseudoplatystoma* (Eiras et al., 2009; Naldoni et al., 2009). The genus *Henneguya* includes 204 known species (Lom and Dyková, 2006), 37 of which have been reported to infect South American fish (Eiras et al., 2008, 2009; Azevedo et al., 2009; Naldoni et al., 2009).

As part of ongoing research on the characteristics of myxosporean parasites of freshwater fish in Brazil, a new species of *Henneguya* found infecting wild specimens of pintado and cachara in the Pantanal wetland (Brazil) is described in the present study, using prevalence, morphological, ultrastructural, molecular and histological analyses.

2. Materials and methods

Eighty-two wild young and adults specimens of *P. corruscans* (size ranging from 34 to 131 cm in length) and seventy of *P. fasciatum* (size ranging from 42 to 114 cm in length) were collected in the Pantanal wetland: Aquidauna River (20°29′19″S/55°46′49″W), Miranda River (20°11′27″S/56°30′19″W), Paraguay River (17°54′58″S/57°28′01″W) and Cuiabá River (17°50′32″S/57°23′46W). Exams were performed in the rainy season (spring 2001, 2002, 2003, 2004 and 2009) and dry season (autumn 2003, 2004, 2005 and 2008).

Immediately after capture, the specimens were transported alive to the field laboratory mounted nearby, where they were killed by transection of the spinal cord, measured, weighed and submitted to necropsy. Plasmodia with mature spores were examined on fresh mounts with a light microscope. The morphological studies of the spores were based on mature spores obtained from different specimens (38 from *P. corruscans* and 41 from *P. fasciatum*). Measurements were performed on a computer equipped with an Axivision 4.1 image capture software coupled to an Axioplan 2 Zeiss Microscope. The dimensions of the spores were expressed in μm as the mean ± standard deviation (SD). Smears containing free spores were stained with Giemsa solution and mounted in a low-viscosity mounting medium (Cytoseal™) as permanent slides. For the histological analysis, fragments of infected organs were fixed in 10% buffered formalin and embedded in paraffin. Serial sections of 4 μm in thickness were stained with Sirius Red, a stain developed by Montes and Junqueira (1991) to study the distribution of collagen fibers. For the transmission electron microscopy, plasmodia were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 12 h, washed in a glucose–saline solution for 2 h and post-fixed in OsO_4, all done at 4 °C. After dehydration in an acetone series, the material was embedded in EMbed 812 resin. Semithin sections were stained with toluidine blue and examined in a light microscope. Ultrathin sections, double stained with uranyl acetate and lead citrate were examined in an LEO 906 electron microscope operated at 60 kV.

For the molecular study, plasmodia were removed from the host tissue and fixed in ethanol PA. The plasmodium content was collected in a 1.5-ml microcentrifuge tube and the DNA was extracted using the Wizard® Genomic DNA Purification kit (Promega, USA), following the manufacturer's instructions. DNA content was determined using the NanoDrop 2000 spectrophotometer (Thermo Scientific) at 260 nm. Polymerase chain reaction (PCR) was carried out in a final volume of 25 μl, which contained 10–50 ng of extracted DNA, 1× Taq DNA polymerase buffer (Invitrogen), 0.2 mmol of dNTP (Invitrogen), 1.5 mmol of $MgCl_2$, 0.2 pmol of each primer (Invitrogen), 0.25 μl (1.25 U) of Taq DNA polymerase (Invitrogen) and ultrapure (MilliQ) water. The Eppendorf AG 22331 Hamburg Thermocycler was used. An ~1600 bp 5′ fragment of the SSU rDNA gene was amplified using the primers MX5–MX3 (Andree et al., 1999) in the following manner: initial denaturation step at 95 °C for 5 min followed by 35 cycles of denaturation (95° C for 60 s), annealing (62° C for 60 s) and extension (72° C for 120 s), finished with an extended elongation step at 72° C for 5 min. PCR products were submitted to electrophoresis in 1.0% agarose gel (BioAmerica) in a TBE buffer (0.045 M Tris–borate, 0.001 M EDTA pH 8.0), stained with ethidium bromide and analyzed in a FLA-3000 (Fugi) scanner. Size of the amplified fragments was estimated by comparisons with the 1 kb DNA Ladder (Invitrogen). Purified PCR products were sequenced using the same primer pair used in the amplification and another primer pair (MC5–MC3 (Eszterbauer, 2004)) with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) in a ABI 3730 DNA sequencing analyzer (Applied Biosystems™).

A standard nucleotide–nucleotide BLAST (blastn) search was conducted (Altschul et al., 1997). The sequence of the *H. eirasi* n. sp. was aligned for comparisons with sequences in the GenBank using DAMBE (Xia and Xie, 2001). Phylogenetic analyses, using *Ceratomyxa robertsthomsoni* as the outgroup, were conduced with the MEGA 4.0 program (Tamura et al., 2007) using the neighbor-joining (NJ) phylogenetic method. The Kimura two-parameter (K2P) evolution sequence model was used in the analysis. Bootstrap analysis (1000 replicates) was employed to assess the