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Morphostructural data and phylogenetic relationships of a new cnidarian myxosporean infecting spleen of an economic and ecological important bryconid fish from Brazil

Tiago Milanin<sup>a</sup>, Patrick D. Mathews<sup>b,c,\*</sup>, André C. Morandini<sup>b,e</sup>, Omar Mertins<sup>d</sup>, Fabienne Audebert<sup>c</sup>, Jose O.L. Pereira<sup>a</sup>, Antonio A.M. Maia<sup>a</sup>

<sup>a</sup> Department of Basic Sciences, Faculty of Animal Science and Food Technology, University of São Paulo, 13635-900 São Paulo, Brazil

<sup>b</sup> Department of Zoology, Institute of Biosciences, University of São Paulo, 05508-090 São Paulo, Brazil

<sup>c</sup> Laboratoire de Biologie des Organismes et Écosystèmes Aquatiques, Museum National de d'Histoire Naturelle, Sorbonne Université, CNRS, IRD, UCA, CP 26, 75005 Paris, France

<sup>d</sup> Department of Biophysics, Paulista Medical Scholl, Federal University of São Paulo, 04023-062 São Paulo, Brazil

<sup>e</sup> Centro de Biologia Marinha, University of São Paulo, 11612-109 São Sebastião, Brazil

\*Corresponding author at: Department of Zoology, Institute of Biosciences, University of São Paulo - USP, Rua do Matão, travessa 14, nº. 10, 05508-090 Cidade Universitária São Paulo, SP, Brazil. E-mail address: [patrickmathews83@gmail.com](mailto:patrickmathews83@gmail.com) (Patrick D. Mathews).

## ABSTRACT

A new cnidarian myxosporean infecting the spleen of an economic and ecological important bryconid fish (*Salminus franciscanus*) is described based on integrative taxonomic approach including morphological, ultrastructural, biological traits,

geography, molecular data and phylogenetic analysis. In a total of thirty specimens examined, nineteen (63.3%) were infected by an undescribed parasite species belonging to the genus *Myxobolus*. Plasmodial development was asynchronous, with young development in the periphery and mature myxospores in the central area and without projections and microvilli in the plasmodial wall. Mature myxospores were ovoid in shape and measured  $7.9\pm 0.2\ \mu\text{m}$  (7.6–8.1  $\mu\text{m}$ ) in length and  $5.4\pm 0.1\ \mu\text{m}$  (5.0–5.6  $\mu\text{m}$ ) in width. The two polar capsules were equal in size, occupying a little more than half of the myxospore body, measuring  $4.0\pm 0.2\ \mu\text{m}$  (3.9–4.1  $\mu\text{m}$ ) in length and  $1.7\pm 0.1\ \mu\text{m}$  (1.5–1.8  $\mu\text{m}$ ) in width. The polar tubules coiled in six turns, perpendicular to the long axis of polar capsule. Phylogenetic analysis placed the new species within a clade containing **nine** myxobolid species from South American characiforms fish and appears as a close species of *Myxobolus pantanalis*. Nevertheless, the sequences of the new species and *M. pantanalis* have a large genetic divergence of 13.5% in their SSU rDNA. In light of the differences observed from the integrative taxonomy, we confidently considered that this isolate is a new species of cnidarian myxosporean, *M. douradae* n. sp., increasing the knowledge of diversity of this enigmatic group of cnidarians.

*Keywords:* Cnidaria, *Myxobolus*, Ultrastructure, Phylogeny, Brazil

## 1. Introduction

Myxozoans are a group of obligate parasitic cnidarians, which represent about 20% of presently known cnidarian biodiversity [1]. They have complex life cycles that exploit invertebrate as well as vertebrate hosts [2]. For myxozoans, fishes represent the majority of intermediate host, although amphibians, reptiles, birds and mammals are also parasitized. Thus, these parasites exhibit a high degree of host diversity [3,4]. Among myxozoans, freshwater histozoic *Myxobolus* species are recognized for their strict or high host-organ/tissue specificity [5], infecting a large variety of fishes within a wide

geographical range [6-9]. Regarding pathogenecity, previous studies conducted in South American freshwater fishes reported *Myxobolus* species to cause considerable pathological alterations such as lymphocytic meningoencephalomyelitis, myocarditis of the heart, perivascular edema in the interlamellar area and deformation and compression of capillaries of the gill lamellae [10-13].

The genus *Salminus* Agassiz, 1829 includes predatory and migratory characid fish, which are of great ecological and economical importance [14]. *Salminus franciscanus* Lima & Britski, 2007, vernacularly called “dourado”, is endemic to the São Francisco River basin from Brazil and can reach up to one meter in length and 30 kg of total weight [14]. It is among the most economically important fish in the region with great acceptance in the market for human nutrition. At present, the conservation status of *S. franciscanus* is unknown due the fact that it was never properly identified. Nevertheless, the wild populations of *S. franciscanus* currently faces serious threats from aquatic pollution by domestic sewage, mining, factory waste, tanneries and agriculture [15]. In addition, a recent disaster, the rupture of the Brumadinho mining tailings dam in Brazil, has had negative biological effects on the water and sediments of the Paraopeba River, which is one of the main tributaries of the São Francisco River [16]. Despite, *S. franciscanus* is a valued commercial fish and it plays a key ecological role as top predator. Its parasitic fauna is currently understudied, particularly concerning myxozoan parasites.

In the present study, we describe a new host-specific histozoic myxozoan, *Myxobolus douradae* n. sp. that infects the spleen of *S. franciscanus* from Brazil, an economically and ecologically important fish whose population faces serious threats due to habitat deterioration.

## 2. Materials and methods

A total of thirty specimens of *S. franciscanus* were collected from the São Francisco River (17°20' 06.05'' S, 44°50'16.37'' W) near of municipality Pirapora, state of Minas Gerais, Brazil, in November 2018. The access to the genetic data and fish collecting were authorized by the Brazilian Ministry of the Environment (SISGEN A33CB83 and SISBIO n° 27964 –1). The fish were transported live to a field laboratory, where they were euthanized by pithing and examined using a light microscope to identify parasites. The methodology of the present study was approved by the Ethics Committee on Animal Research of the Department of Veterinary Medicine, University of São Paulo (Proc. FZEA-USP n° 14.1.391.74.9, Federal Law No. 11794, dated 8 October 2008 and Federal Decree N° 6899, date 15 July 2009), in accordance to international procedures.

For morphological and morphometric characterization, the analyses were performed based on the criteria outlined by Lom and Arthur [17]. Mature myxospores were fixed in 10% formalin and transported to the laboratory of Parasitology and Parasite Immunology of the Department of Veterinary at University of São Paulo, Brazil. Smears containing free myxospores were air dried, fixed with methanol and stained with Giemsa solution to mount on permanent slides that were deposited in the cnidarian collections of the Museum of Zoology of the University of São Paulo-USP, São Paulo, Brazil. Photographs and measurements were taken from 30 myxospores using a computer equipped with Axiovision 4.1 image capture software coupled to an Axioplan 2 Zeiss microscope (Carl Zeiss AG, Oberkochen, Germany).

For ultrastructural analysis, plasmodia were fixed in 2.5% glutaraldehyde with 0.1 M buffered cacodylate (pH 7.4) for 24 h, washed in a glucose-saline solution for 2 h, and post-fixed with osmium tetroxide (OsO<sub>4</sub>). All procedures were performed at 4 °C. After dehydration in an ascending ethanol series, the samples were embedded in EMbed 812 resin (Electron Microscopy Sciences, Hatfield, PA, USA) [18]. Ultrathin sections were

double stained with uranyl acetate and lead citrate and examined in a LEO 906 electron microscope operated at 60 kV in the Institute of Biology at University of Campinas, Brazil.

For molecular analysis, plasmodia were dissected from the host tissue and fixed in absolute ethanol. The genomic DNA was extracted using DNeasy® Blood & Tissue Kit (Qiagen, Valencia, USA), in accordance with the manufacturer's instructions. The DNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). Polymerase chain reactions (PCRs) were performed in accordance with Mathews et al. [19] and conducted in a final volume reaction of 25 µL, which comprised 10–50 ng of extracted DNA, 0.2 pmol for each primer, 12.5 µL of Dream Taq Green PCR Master Mix (Thermo Scientific) and nuclease-free water. Partial 18S rDNA sequence was amplified using the primer pairs ERIB1 (ACCTGGTTGATCCTGCCAG; [20] with ACT1r (AATTTACCTCTCGCTGCCA; [21] and Myxgen4F (GTGCCTTGAATAAATCAGAG; [22] with ERIB10 (CTTCCGCAGGTTACCTACGG; [20]), which amplified two fragments containing approximately 1,000 and 1,200 bp respectively. PCR amplification was done by initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 60 s, 64°C (ERIB1-ACT1r) or 58°C (Myxgen4F-ERIB10) for 60 s, 72°C for 120 s, and then final elongation at 72°C for 5 min. PCRs were performed in an AG22331 Hamburg Thermocycler (Eppendorf, Hamburg, Germany). PCR products were subjected to electrophoresis in 2.0% agarose gel (BioAmerica, Irvine, USA) in a TAE buffer (Tris-Acetate EDTA: Tris 40 mM, acetic acid 20 mM, EDTA 1 mM), stained with Sybr Safe DNA gel stain (Invitrogen by Life Technologies, Carlsbad, USA), and then analyzed with a Stratagene 2020E trans illuminator (Stratagene California, San Diego, USA). The size of the amplicons was estimated by comparison with the 1 Kb Plus DNA Ladder

(Invitrogen by Life Technologies). PCR products were purified using USB® ExoSap-IT® (Thermo Fisher Scientific) in accordance to the manufacturer's instructions and sequenced using the same PCR primers plus two additionally MC5 and MC3 (CCTGAGAAACGGCTACCACATCCA, GATTAGCCTGACAGATCACTCCACGA respectively) primers used to overlap the obtained amplification fragments [23]. Sequencing was performed using a BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems Inc., California, USA) in an ABI 3130 automatic DNA analyzer (Applied Biosystems Inc.™) at the Human Genome Research Center (HGRC), at the University of São Paulo. The obtained sequence was visualized, assembled and edited using BioEdit 7.1.3.0 software [24]. A standard nucleotide BLAST search was conducted to verify the similarity of the sequence obtained in this study with other sequences available in GenBank [25]. Phylogenetic analysis was conducted using the most closely related myxozoans sequences with similarity >80%. The sequences were aligned with ClustalW within BioEdit version 7.1.3.0 [24]. Phylogenetic analysis was performed using Maximum Likelihood (ML) method with a Kimura 2-parameter (K2P) evolution sequence model in MEGA 6.0 [26]. Bootstrap analysis (1,000 replicates) was employed to assess the relative robustness of the branches in ML tree. *Ceratomyxa amazonensis* Mathews, Naldoni, Maia, Adriano, 2016 and *Ceratomyxa vermiformis* Adriano and Okamura, 2017 sequences were used as outgroups. To evaluate the genetic distance between the myxozoan species clustering together with the new sequence obtained, the pairwise method with the p-distance model in MEGA 6.0 was performed [26].

### 3. Results

Of a total of thirty specimens of *S. franciscanus* examined, nineteen (63.3%) were infected in the spleen by an unknown cnidarian myxosporean species belonging the

genus *Myxobolus*. The same were not found in any other organ and no signs of diseases were observed in the infected individuals.

### 3.1. Taxonomy summary

Phylum: Cnidaria Verrill, 1865.

Class: Myxosporea Bütschli, 1881.

Order: Bivalvulida Shulman, 1959.

Family: Myxobolidae Thélohan, 1892.

Genus: *Myxobolus* Thélohan, 1892.

*Myxobolus douradae* n. sp.

Type host: *Salminus franciscanus* Lima and Britski, 2007

Type locality: São Francisco River (17°20'06.05''S, 44°50'16.37''W) near of municipality of Pirapora, state of Minas Gerais, Brazil.

Site of infection: Serous membrane of the spleen.

Type material: Syntypes-air-dried slide stained with Giemsa deposited in the cnidarian collection of Zoology Museum of the University of São Paulo-USP, São Paulo, Brazil (MZUSP 8509). Partial 18S rDNA sequence (2,031bp) was deposited in GenBank under accession number MT644269.

Etymology: The specific name (*M. douradae*) is based on host species common name.

### 3.2. Morphological data

Plasmodia were rounded and small, measuring up to 25 µm in diameter. In the wall of the plasmodia there are no projections and no microvilli, and the periphery of the



ectoplasm had several pinocytic channels (Fig. 1A-C). The development of the plasmodia was asynchronous, with young developmental in the periphery and mature myxospores in the central area (Fig. 1A-B). Mature myxospores ovoid in shape from the frontal view and measured  $7.9\pm 0.2\ \mu\text{m}$  (7.6–8.1  $\mu\text{m}$ ) in length and  $5.4\pm 0.1\ \mu\text{m}$  (5.0–5.6  $\mu\text{m}$ ) in width (Fig. 2A-B). The two polar capsules were equal in size, occupying a little more than half of the myxospore body (Fig. 2A-B). They measured  $4.0\pm 0.2\ \mu\text{m}$  (3.9–4.1  $\mu\text{m}$ ) in length and  $1.7\pm 0.1\ \mu\text{m}$  (1.5–1.8  $\mu\text{m}$ ) in width. The polar tubules coiled in six turns, perpendicular to the long axis of the polar capsule (Figs. 1D and 2B).

### 3.3 Molecular data and phylogenetic analysis

The sequencing of the SSU rDNA from the myxospores of *M. douradae* n. sp., resulted in a sequence of 2,031bp with a GC content of 49.7%. BLAST search revealed that this sequence did not match any of myxozoans available in GenBank database. Sequences with the highest similarity identified by BLAST search were from *Myxobolus pantanalis* Carriero, Adriano, Silva, Ceccarelli and Maia, 2013 (query coverage 89%, maximum identities 85.7%), reported in the gill filaments of *Salminus brasiliensis*, from the Pantanal National Park, state of Mato Grosso, Brazil [27]. Phylogenetic relationship analysis based on the 58 most closely related myxozoan sequences placed *M. douradae* n. sp. sequence within a subclade containing **nine** myxobolid parasite species of South American characiform fish (Fig. 3). In this same subclade, *M. douradae* n. sp. appears as a closely related species of *M. pantanalis* (GenBank accession number KF296349). A genetic distance analysis of the SSU rDNA sequences of the myxobolids that cluster in the same clade with the new *M. douradae* n. sp. sequence showed the largest genetic divergence among these species, varying between 13.5% for *M. pantanalis* and 20.4% for *Myxobolus oliveirai* Milanin, Eiras, Arana, Maia, Alves, Silva, Carriero and Adriano, 2010 (Table 1).

#### 4. Discussion

With approximately 2,400 species described globally, myxozoans display a broad diversity [1]. However, myxozoan diversity in Central and South America is poorly known [28]. Due to extensive morphological plasticity of myxozoan spores, it is highly recommended to integrate multiple characters to enable a robust identification and description of new myxosporean taxa [29]. Following these criteria, in our study, integrating morphological, ultrastructural, biological traits (host/organ-tissue specificity), geography, molecular data and phylogenetic analysis, we described a new species of freshwater histozoic myxobolid, *Myxobolus douradae* n. sp., that infects the spleen of *S. franciscanus*, an ecological and economical important bryconid fish.

The morphometrical comparison of *M. douradae* n. sp. with all the other *Myxobolus* species described to infect bryconid fishes from South America, revealed a number of remarkable differences, as evidenced in Table 2. Indeed, in the BLAST search, the nucleotide similarity of *M. douradae* n. sp. to other bryconid myxobolid species is very low, with 86.5% as the highest percentage of identity. Comparing with previously described *Myxobolus* species, the species of highest morphological similarities to *M. douradae* n. sp. was *Myxobolus colossomatis* Molnár and Békési, 1993 described to infect the characid fish *Colossoma macropomum* Cuvier, 1816 [30]. Nevertheless, it has been noticed that *M. douradae* n. sp. was readily distinguishable in myxospore body length ( $10.4 \pm 0.5 \mu\text{m}$  in length for *M. colossomatis* and  $7.9 \pm 0.2 \mu\text{m}$  to the new species), body width ( $7.7 \pm 0.3 \mu\text{m}$  in *M. colossomatis* and  $5.4 \pm 0.1 \mu\text{m}$  to the new species) and number of coils of the polar tubule (seven to eight in *M. colossomatis* and six in the new species). Plasmodia of *M. douradae* n. sp. were located in the serous membrane of the spleen, while *M. colossomatis* was described infecting several organs (fins, gills, heart and intestine). On the same vein, host species isolation plays an important role in

myxosporean population divergence [31]. Such isolation concerns particularly parasites of freshwater fishes which usually inhabit in isolate watersheds [32]. Thus, locality of collection is an indispensable trait for accurately distinguishing new freshwater histozoic myxobolids. Accordingly, differences in the locality of these species were noted, with *M. douradae* n. sp. found in the São Francisco River basin and *M. colossomatis* found in the Amazon River basin, both watersheds not connected to each other, which is sufficient to allow speciation of their myxosporean species.

Phylogenetic analyses, showed an association between *M. douradae* n. sp. and *M. pantanalis* reported to infect *S. brasiliensis* [27]. Morphometrically, mature myxospores of *M. pantanalis* are different than those *M. douradae* n. sp. (Table 2) in myxospore body length ( $9.5 \pm 0.3 \mu\text{m}$  in length for *M. pantanalis* and  $7.9 \pm 0.2 \mu\text{m}$  for *M. douradae* n. sp.), the number of coils of the polar tubule (four–five in *M. pantanalis*, and six in *M. douradae* n. sp.) and in the width of the body, which is substantially smaller in the new species ( $6.5 \pm 0.4 \mu\text{m}$  in *M. pantanalis* and  $5.4 \pm 0.1 \mu\text{m}$  in *M. douradae* n. sp.). Differences were also observed in the organ/tissue infected by *M. douradae* n. sp. plasmodia located in the serous membrane of the spleen, while *M. pantanalis* was reported to infect in the distal region of the gill filaments [27]. Indeed, it has been recognized that freshwater histozoic *Myxobolus* species display a strict or high organ and/or tissue specificity, thus it is considered a taxonomic key for the identification of these parasites [23,29]. Moreover, the two myxozoan parasites were found in different localities, with *M. pantanalis* in the Pantanal biome inside La Plata River basin and *M. douradae* n. sp. in São Francisco River basin, both separated by around 1,350 km from each other. In addition, studies suggested that the separation of the La Plata River and São Francisco River basins occurred approximately at 1.8 Ma [33], which is enough time for allopatric speciation to occur between these two myxozoan parasites. Indeed, in

the pairwise similarity analysis, these two species have 255 different nucleotides out of a total of 1,901 evidencing a large genetic divergence of 13.5% in their SSU rDNA. Taken together, we confidently consider that these data are sufficient to identify this isolate as a new cnidarian myxozoan species, *M. douradae* n. sp., adding to the already extensive diversity of this enigmatic group of parasitic cnidarians.

Aquatic pollutants have been evidenced to cause fish hormonal disturbances and histological changes in the skin, gonads, gills, liver, spleen, and kidneys, consequently debilitating overall the health of fishes [15,34,35]. In fact, Savassi et al. [15] described in wild specimens of *S. franciscanus* histopathological changes in important organs such as spleen and liver and in some reproductive parameters caused by heavy metal accumulation. Moreover, it has been reported that pollution of the aquatic environment can affect fish susceptibility on myxozoan infections, hence increasing pathogenicity and host mortality [36-38], due to increased host stress and impaired immune response [39]. For instance, El-Matbouli and Hoffmann [36] evidenced the effect of sewage effluents on the prevalence of proliferative kidney disease-PKD in farmed and wild trout, where PKD prevalence decreased dramatically after the effluents were diverted. In the same vein, sewage effluents or polluted waters can enhance aquatic oligochaetes populations due to nutrient enrichment, with a consequent effect on the development of infectious spores [36,40]. Indeed, aquatic oligochaetes involved as definitive hosts in the life cycles of myxosporean parasites may release thousands of infective actinospores, which can remain viable for up to four weeks in the water [41]. The climate in the region of our study is tropical humid with annual average temperature of 27°C, which favors acceleration of the life cycles, favoring that oligochaetes release actinospores for shorter periods and at higher intensity [42], and facilitating the contact

of these fishes with infective actinospores, especially during the dry season when the water level is low and fishes are more concentrated [43].

Along the course of São Francisco river there have been reported more than ten species of aquatic oligochaetes [44], with some species, e.g. *Pristina americana*, being involved in the life cycles of myxozoan parasites of characiform and siluriform fishes from South America [45]. In all this context, the high occurrence of *M. douradae* n. sp. observed in the spleen of wild specimens of *S. franciscanus* suggests that the infection by this parasite is frequent and that the same can become a potentially important pathogen in wild *S. franciscanus* populations, considering that the infected organ (spleen) plays an important role in fish metabolism and physiology [46]. Therefore, the prevalence, dispersion and the impact of infection by the new myxosporean parasite described here need to be constantly monitored in order to better prevent future disease outbreaks, which can seriously impact in susceptible free ranging fish populations.

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### **References**

[1] S.D. Atkinson, J.L. Bartholomew, T. Lotan, Myxozoans: ancient metazoan parasites find a home in phylum Cnidaria, *Zoology* 129 (2018) 66–68, <https://doi.org/10.1016/j.zool.2018.06.005>.

- [2] B. Okamura, A. Gruhl, J.L. Bartholomew, An introduction to Myxozoan evolution, ecology and development. In: B. Okamura, A. Gruhl, J.L. Bartholomew (Eds.), *Myxozoan Evolution, Ecology and Development*. Springer, Cham, 2015, pp. 1–20
- [3] I. Fiala, P. Bartošová-Sojková, B. Okamura, H. Hartikainen, Adaptive radiation and evolution within the Myxozoa. In: B. Okamura, A. Gruhl, J.L. Bartholomew (Eds.), *Myxozoan evolution, ecology and development*. Springer, Cham, 2015, p. 69–84.
- [4] A.S. Holzer, P. Bartošová-Sojková, A. Born-Torrijos, A. Lövy, A. Hartigan, I. Fiala, The joint evolution of the Myxozoa and their alternate hosts: A cnidarian recipe for success and vast biodiversity, *Mol. Ecol.* 27 (2018) 1651–1666, <https://doi.org/10.1111/mec.14558>
- [5] K. Molnár, E. Eszterbauer, Specificity of infection sites in vertebrate hosts, in: B. Okamura, A. Gruhl, J.L. Bartholomew (Eds.), *Myxozoan Evolution, Ecology and Development*, Springer, Switzerland, 2015, pp. 295–313.
- [6] P.D. Mathews, A.A.M. Maia, E.A. Adriano, Morphological and ultrastructural aspects of *Myxobolus niger* n. sp. (Myxozoa) gill parasite of *Corydoras melini* (Siluriformes: Callichthyidae) from Brazilian Amazon, *Acta Trop.* 158 (2016) 214–219, <https://doi.org/10.1016/j.actatropica.2016.03.016>
- [7] P.D. Mathews, O. Mertins, T. Milanin, L.L. Espinoza, A.P. Flores-Gonzales, F. Audebert, A.C. Morandini, Molecular Phylogeny and taxonomy of a new *Myxobolus* species from the endangered ornamental fish, *Otocinclus cocama* endemic to Peru: A host parasite coextinction approach, *Acta Trop.* 210 (2020) 105545, <https://doi.org/10.1016/j.actatropica.2020.105545>
- [8] A. Gupta, H. Kaur, 18S and 28S rDNA identity and phylogeny of two novel myxosporeans infecting gills of cyprinid carps inhabiting a cold-water wetland in

northern India, *Microb. Pathog.* 120 (2018) 97–108, <https://doi.org/10.1016/j.micpath.2018.04.042>.

[9] A. Gupta, H. Kaur, Two new species of *Myxobolus* (Cnidaria: Myxosporaea) infecting freshwater fishes of Ranjit Sagar Wetland, Punjab, India, *Microb. Pathog.* 147 (2020) 104421, <https://doi.org/10.1016/j.micpath.2020.104421>

[10] E.A. Adriano, S. Arana, A.L. Alves, M.R.M. Silva, P.S. Ceccarelli, F. Henrique-Silva, A.A.M. Maia, *Myxobolus cordeiroi* n. sp., a parasite of *Zungaro jahu* (Siluriformes Pimelodidae) from Brazilian Pantanal: morphology, phylogeny and histopathology, *Vet. Parasitol.* 162 (2009) 221–229, <https://doi.org/10.1016/j.vetpar.2009.03.030>

[11] T. Milanin, J.C. Eiras, S. Arana, A.A.M. Maia, A.L. Alves, M.R.M. Silva, M.M. Carriero, P.S. Ceccarelli, E.A. Adriano, Phylogeny, ultrastructure, histopathology and prevalence of *Myxobolus oliveirai* sp. nov. a parasite of *Brycon hilarii* (Characidae) in the Pantanal wetland, Brazil, *Mem. Inst. Oswaldo Cruz* 105 (2010) 762–769.

[12] E. Matos, M. Videira, M. Velasco, O. Sanches, C.S. Carmona, P. Matos, Infection of the heart of *Pimelodus ornatus* (Teleostei, Pimelodidae), by *Myxobolus* sp. (Myxozoa, Myxobolidae). *Rev. Bras. Parasitol. Vet.* 23 (2014) 543–546, <https://doi.org/10.1590/s1984-29612014083>

[13] J.I. Sindeaux-Neto, M. Velasco, J.M.V. Da Silva, P.F.S. Dos Santos, O. Sanches, P. Matos, E. Matos, Lymphocytic meningoencephalomyelitis associated with *Myxobolus* sp. (Bivalvulidae: Myxozoa) infection in the Amazonian fish *Eigenmannia* sp. (Sternopygidae: Gymnotiformes). *Ver. Bras. Parasitol. Vet.* 25 (2016) 158–162, <https://doi.org/10.1590/S1984-29612016023>

- [14] F.C.T. Lima, H.A. Britski, *Salminus franciscanus*, a new species from the rio São Francisco basin, Brazil (Ostariophysi: Characiformes: Characidae), *Neotrop. Ichthyol.* 5 (2007) 237–244, <https://doi.org/10.1590/S1679-62252007000300001>
- [15] L.A. Savassi, F.P. Arantes, M.V. Gomes, N. Bazzoli, Heavy Metals and Histopathological Alterations in *Salminus franciscanus* (Lima & Britski, 2007) (Pisces: Characiformes) in the Paraopeba River, Minas Gerais, Brazil, *Bull Environ Contam Toxicol.* 96 (2016) 478-83, <https://doi.org/10.1007/s00128-016-1732-8>
- [16] C.D.S. Vergilio, D. Lacerda, B.C.V. Oliveira, E. Sartori, G.M. Campos, I. Souza-Pereira, D.B. Aguiar, T.S. Souza, M.G. Almeida, F. Thompson, C.E. Rezende CE, Metal concentrations and biological effects from one of the largest mining disasters in the world (Brumadinho, Minas Gerais, Brazil), *Sci. Rep.* 10 (2020) 5936, <https://doi.org/10.1038/s41598-020-62700-w>
- [17] J. Lom, J.R. Arthur, A guideline for the preparation of species descriptions in Myxosporea, *J. Fish. Dis.* 12 (1989) 151–156.
- [18] P.D. Mathews, R.R.M. Madrid, O. Mertins, V.L.S. Rigoni, A.C. Morandini, A new *Myxobolus* (Cnidaria: Myxosporea) infecting the ornamental catfish *Corydoras schwartzi* from the Purus River in Brazil, *Eur. J. Taxon.* 620 (2020) 1–14, <https://doi.org/10.5852/ejt.2020.620>.
- [19] P.D. Mathews, O. Mertins, T. Milanin, L.L. Espinoza, G. Alama-Bermejo, F. Audebert, A.C. Morandini, Taxonomy and 18S rDNA-based phylogeny of *Henneguya multiradiatus* n. sp. (Cnidaria: Myxobolidae) a parasite of *Brochis multiradiatus* from Peruvian Amazon, *Microb. Pathog.* 147 (2020) 104372, <https://doi.org/10.1016/j.micpath.2020.104372>



- [20] J.R. Barta, D.S. Martin, P.A. Liberator, M. Dashkevich, J.W. Anderson, S. D. Feighner, A. Elbrecht, A. Perkins-Barrow, M.C. Jenkins, H.D. Danforth, M. D. Ruff, H. Profous-Juchelka, Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences, *J. Parasitol.* 83 (1997) 262–271.
- [21] S.L. Hallett, A. Diamant, Ultrastructure and small-subunit ribosomal DNA sequence of *Henneguya lesteri* n. sp. (Myxosporea), a parasite of sand whiting *Sillago analis* (Sillaginidae) from the coast of Queensland, Australia, *Dis. Aquat. Org.* 46 (2001) 197–212.
- [22] A. Diamant, C.M. Whipps, M.L. Kent, A new species of *Sphaeromyxa* (Myxosporea: Sphaeromyxina: Sphaeromyxidae) in devil firefish, *Pterois miles* (Scorpaenidae), from the northern Red Sea: morphology, ultrastructure, and phylogeny, *J. Parasitol.* 90 (2004) 1434–1442.
- [23] K. Molnár, E. Eszterbauer, C. Székely, A. Dan, B. Harrach, Morphological and molecular biological studies on intramuscular *Myxobolus* spp. of cyprinid fish, *J. Fish. Dis.* 25 (2002) 643–652.
- [24] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucleic Acids Symp. Ser.* 41 (1999) 95–98.
- [25] Altschul, T.L. Madden, A.A. Schaffer, J.H. Zhang, Z. Zhang, W. Miller, D. J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acids Res.* 25 (1997) 3389–3402.
- [26] K. Tamura, G. Stecher, D. Peterson, A. Filipowski, S. Kumar, MEGA 6: molecular evolutionary genetics analysis version 6.0, *Mol. Biol. Evol.* 30 (2013) 2725–2729.

- [27] M.M. Carriero, E.A. Adriano, M.R.M. Silva, P.S. Ceccarelli, A.A.M. Maia, Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American species, *PloS One* 8 (9) (2013) 73713, <https://doi.org/10.1371/journal.pone.0073713>.
- [28] B. Okamura, A. Hartigan, J. Naldoni, Extensive uncharted biodiversity: the parasite dimension, *Integr. Comp. Biol* 58 (2018) 1132–1145, <https://doi.org/10.1093/icb/icy039>
- [29] S.D. Atkinson, P. Bartošová-Sojková, C.M. Whipps, J.L. Bartholomew, Approaches for characterizing myxozoan species, in: B. Okamura, A. Gruhl, J.L. Bartholomew (Eds.), *Myxozoan Evolution. Ecology and Development*, Springer, Cham, 2015, pp. 111–123.
- [30] K. Molnár, L. Békési, Description of a new *Myxobolus* species: *M. colossomatis* n. sp. from the teleost *Colossoma macropomum* of the Amazon river basin, *J. Appl. Ichthyol.* 9 (1993) 57–63.
- [31] M. Wang, Y. Zhao, C. Yang, The impacts of geographic and host species isolation on population divergence of *Myxobolus lentisuturalis*, *Parasitol. Res.* 1189 (2019) 1061–1066, <https://doi.org/10.1007/s00436-019-06234-9>.
- [32] G.S.A. Moreira, E.A. Adriano, M.R.M. Silva, P.S. Ceccarelli, A.A.M. Maia, Morphology and 18S rDNA sequencing identifies *Henneguya visibilis* n. sp., a parasite of *Leporinus obtusidens* from Mogi Guaçu River, Brazil, *Parasitol. Res.* (2014) 113:81–90, <https://doi.org/10.1007/s00436-013-3629-3>
- [33] J.I. Montoya-Burgos, Historical biogeography of the catfish genus *Hypostomus* (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna, *Mol. Ecol.* 12 (2003) 1855–1867.

- [34] P.S. Prado, C.C. Souza, N. Bazzoli, E. Rizzo, Reproductive disruption in lambari *Astyanax fasciatus* from a Southeastern Brazilian reservoir, *Ecotoxicol. Environ. Safe.* 74 (2011) 1879–1887, <https://doi.org/10.1016/j.ecoenv.2011.07.017>
- [35] S.K. Gupta, M. Chabukdhara, P. Kumar, J. Singh, F. Bux, Evaluation of ecological risk of metal contamination in river Gomti, India: a biomonitoring approach, *Ecotoxicol Environ Safe* 110 (2014) 49–55, <https://doi.org/10.1016/j.ecoenv.2014.08.008>
- [36] M. El-Matbouli, R.W. Hoffmann, Influence of water quality on the outbreak of proliferative kidney disease-field studies and exposure experiments, *J. Fish Dis.* 25 (2002) 459–467.
- [37] D.J. Marcogliese, D.K. Cone, Myxozoan communities parasitizing *Notropis hudsonius* (Cyprinidae) at selected localities on the St. Lawrence River, Quebec: possible effects of urban effluents, *J. Parasitol.* 87 (2001) 951–956.
- [38] F. Bucher, R. Hofer, M. El-Matbouli, Prevalence and pathology of *Zschokkella nova* (Myxosporea) in the liver of bullhead *Cottus gobio* from a polluted river. *Dis Aquat Org* 14 (1992) 137–143.
- [39] A. Sitjà-Bobadilla, H. Schmidt-Posthaus, T. Wahli, J.W. Holland, C.J. Secombes, Fish immune responses to Myxozoa, in: B. Okamura, A. Gruhl, J.L. Bartholomew (Eds.), *Myxozoan Evolution. Ecology and Development*, Springer, Cham, 2015, pp. 253-280.
- [40] H. Hartikainen, B. Okamura, Castrating parasites and colonial hosts, *Parasitology* 139 (2012) 547–556, <https://doi.org/10.1017/S0031182011002216>
- [41] E. Eszterbauer, S. Atkinson, A. Diamant, D. Morris, M. El-Matbouli, H. Hartikainen, Myxozoan life cycles: practical approaches and insights. In: B. Okamura,

A. Gruhl, J.L. Bartholomew, (Eds.), *Myxozoan Evolution, Ecology and Development*. Springer, Cham, 2015, pp. 175–198.

[42] O.Z. Rácz, Experimental Study of intrapiscin and intraoligochaete Developmental Stages of Fish-parasitic Myxozoans. Ph.D. Dissertation, 2004, Szent István University.

[43] P.D. Mathews, O. Mertins, J.O.L. Pereira, A.A.M. Maia, E.A. Adriano, Morphology and 18S rDNA sequencing of *Henneguya peruviansis* n. sp. (Cnidaria: myxosporea), a parasite of the Amazonian ornamental fish *Hyphessobrycon loretoensis* from Peru: a myxosporean dispersal approach, *Acta Trop.* 187 (2018) 207–213, <https://doi.org/10.1016/j.actatropica.2018.08.012>.

[44] A.L. Suriani-Affonso, R.S. França, M. Marchese, O. Rocha, Environmental factors and benthic Oligochaeta (Annelida, Clitellata) assemblages in a stretch of the Upper São Francisco River (Minas Gerais State, Brazil), *Braz. J. Biol.* 71(2011) 437-446, <http://dx.doi.org/10.1590/S1519-69842011000300013>.

[45] T. Milanin, S.D. Atkinson, M.R.M. Silva, R.G. Alves, L.E.R. Tavares, A.M. Ribeiro, A.A.M. Maia, Occurrence of two novel actinospore types (Cnidaria: Myxozoa) in fish farms in Mato Grosso do Sul state, Brazil, *Parasitol. Res.* 117 (2018)1757–1764, <https://doi.org/10.1007/s00436-018-5856-0>

[46] D. Mendil, Determination of trace metal levels in seven fish species in lakes in Tokat, Turkey, *Food Chem.* 90 (2005) 175–179.

## Legends

Fig. 1. Electron micrograph of a plasmodium of *Myxobolus douradae* n. sp. parasite of spleen of the bryconid *Salminus franciscanus*. A- Host–parasite interface showing the membrane (black arrows) of the plasmodium (P) in contact with host spleen (H). Note an asynchronous development plasmodium with numerous young sporoblasts developmental in the periphery (\*) and with mature myxospores in the central area (ms). Scale bar: 10  $\mu$ m. B- Amplification of selected region in A (white rectangle), showing young sporoblasts (ysb) with the polar tubule inside of the polar capsule (pc), abundant valve-forming material (large white arrows) and sporoplasm (spl) with its nucleus (Nu) and sporoplasmosomes (thin white arrows). Scale bar: 2  $\mu$ m. C- Region of ectoplasm (ec) showing the presence of numerous pinocytotic channels (black arrows). Scale bar: 500 nm. D- Polar capsule (pc) showing polar tubule with six turns (pt). Scale bar: 500 nm.

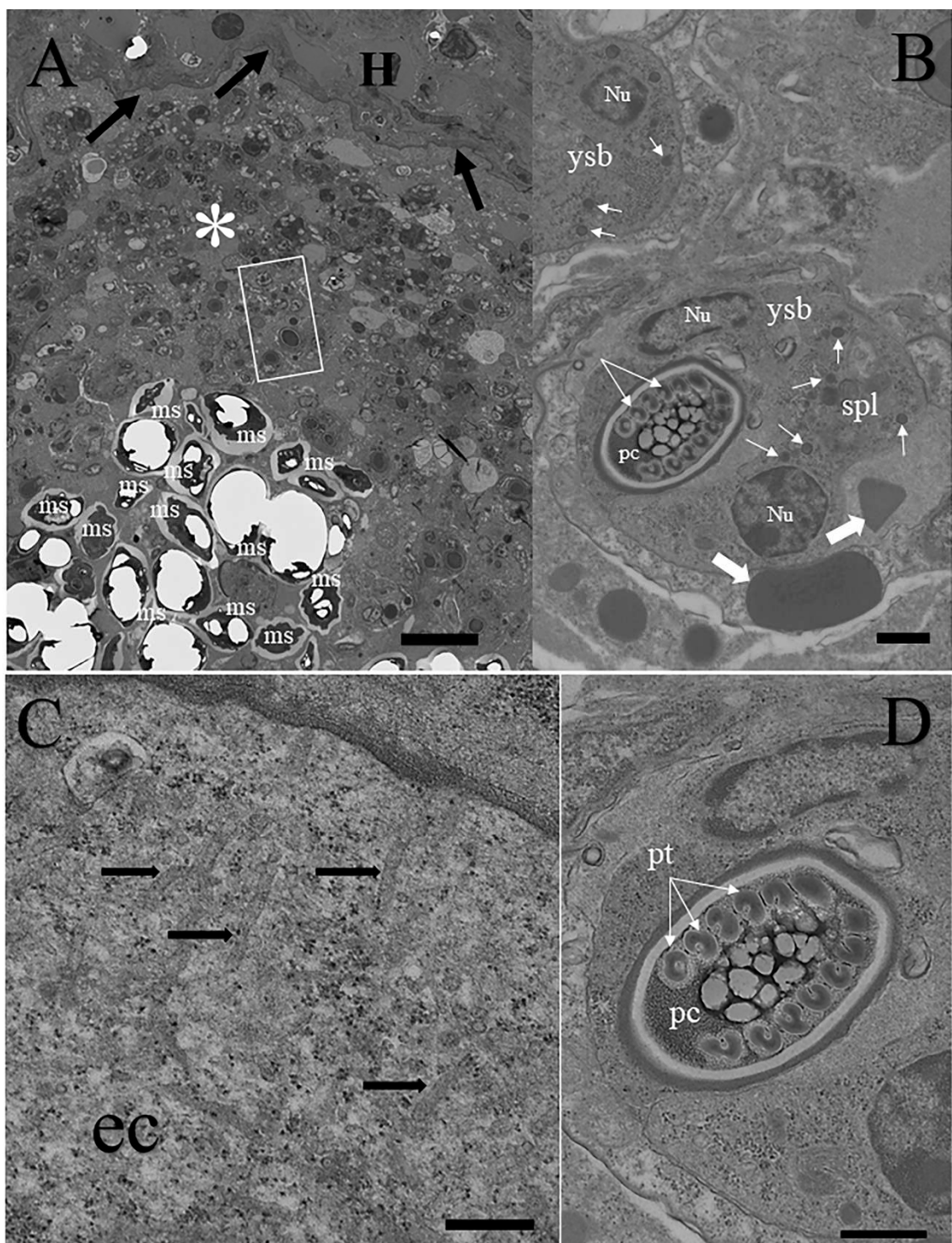
Fig. 2. *Myxobolus douradae* n. sp. parasite of spleen of *Salminus franciscanus*. A- Micrographs of mature myxospores. B- Line drawing of myxospore. Scale bars = 5  $\mu$ m.

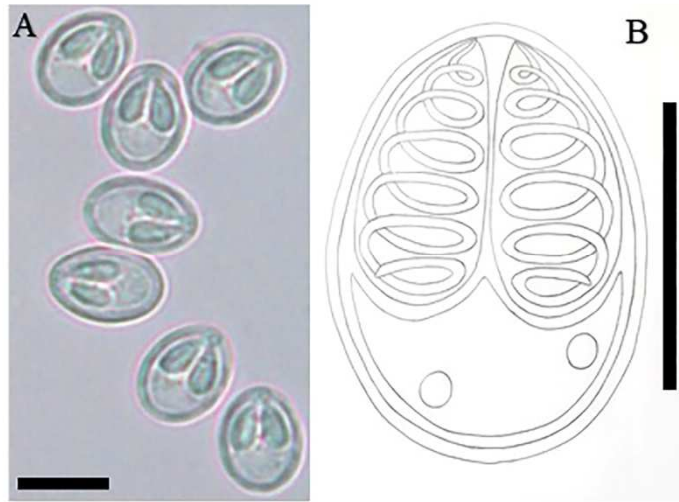
Fig. 3. Maximum likelihood tree, based on partial SSU-rDNA sequencing, showing the relationship between *Myxobolus douradae* n. sp., parasite of spleen of the bryconid *Salminus franciscanus* and others myxobolid species. The numbers above the nodes indicate bootstrap confidence levels.

Table 1. Pairwise identities of SSU-rDNA sequences from myxobolids species clustering together with *Myxobolus douradae* n. sp., adjusted for missing data. The

upper triangular matrix shows the number of differences of nucleotides and the lower triangular matrix shows the differences in terms of percentage of nucleotides.

Table 2. Comparative data of *Myxobolus douradae* n. sp. with all *Myxobolus* species parasites of bryconid fishes. Spore dimensions, infection sites, and collection sites are given. PCL: length of polar capsules; PCW: width of polar capsules; NCPT: number of coils of polar tubules; -: no data. All measurements are range in  $\mu\text{m}$  and/or means  $\pm$  SD.





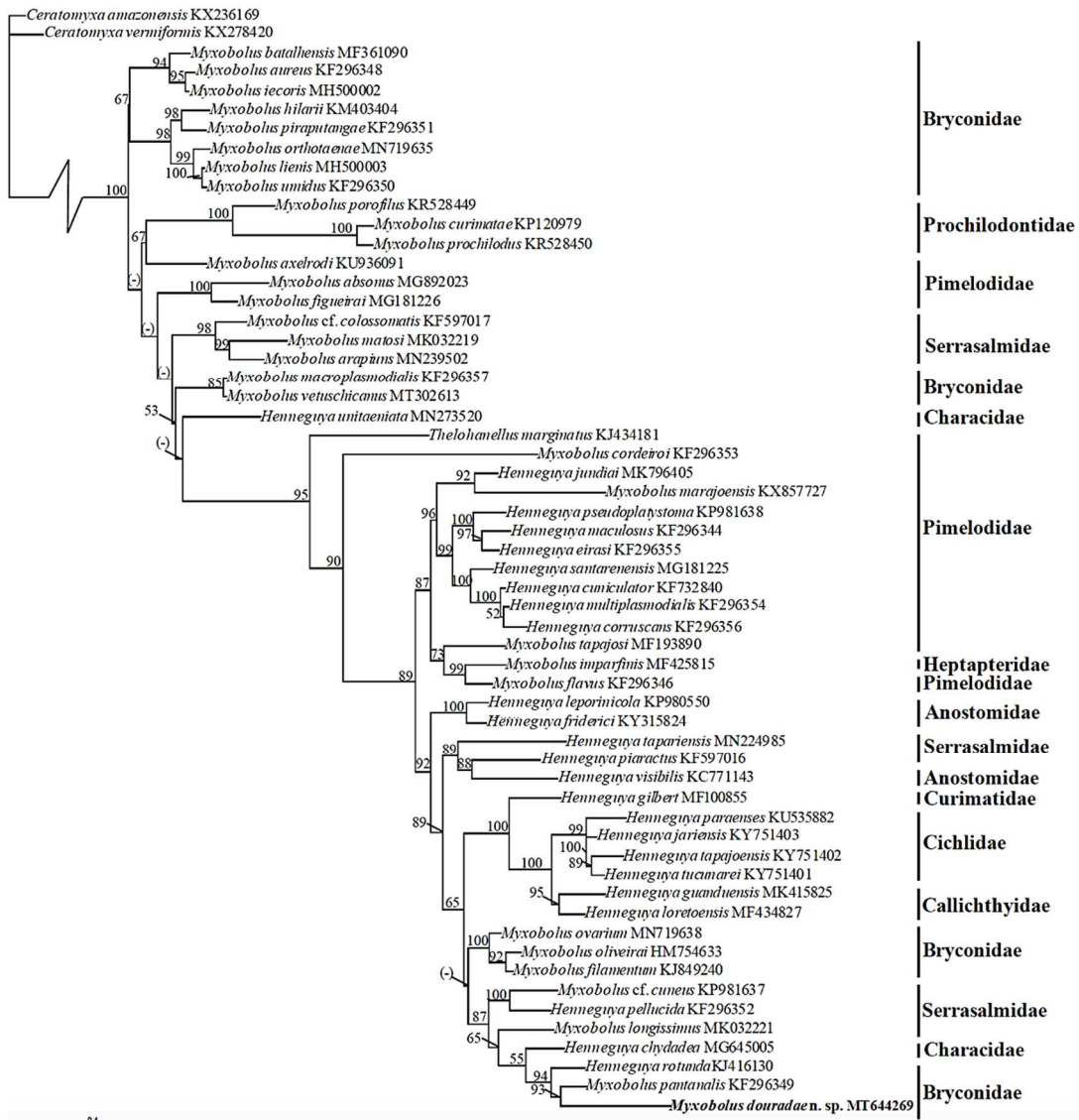




Table 1. Pairwise identities of SSU-rDNA sequences from myxobolids species clustering together with *Myxobolus douradae* n. sp., adjusted for missing data. The upper triangular matrix shows the number of differences of nucleotides and the lower triangular matrix shows the differences in terms of percentage of nucleotides.

Species	1	2	3	4	5	6	7	8	9	10
<b>1- <i>M. douradae</i> n. sp. MT644269</b>	-	313	310	306	325	298	335	212	261	255
2- <i>M. ovarium</i> MN719638	17	-	131	112	253	234	240	194	225	258
3- <i>M. oliveirai</i> HM754633	20.4	8.6	-	68	240	235	212	15.5	201	241
4- <i>M. filamentum</i> KJ849240	16.5	6.2	4.5	-	255	243	249	189	217	237
5- <i>M. cf. cuneus</i> KP981637	17.8	14	15.9	13.8	-	222	276	215	286	264
6- <i>H. pellucida</i> KF296352	19.4	15	15.6	15.5	14.2	-	247	187	259	244
7- <i>M. longissimus</i> MK032221	17.6	13	13.9	13.2	14.9	15.8	-	218	261	271
8- <i>H. chydadea</i> MG645005	15.2	14.4	171	13.5	15.5	16.2	15.5	-	206	217
9- <i>H. rotunda</i> KJ416130	14.3	12.5	13.3	11.7	15.6	16.6	14	14.8	-	189
10- <i>M. pantanalis</i> KF296349	13.5	14.3	16	12.8	14.5	15.8	14.4	15.5	10.3	-

Species	Spore length	Spore width	Diameter	PCL	PCW	NCPT	Site of infection	Host	Locality
<i>Myxobolus douradoe</i> n. sp	7.6-8.1 (7.9±0.2)	5.0-5.6 (5.4±0.1)	-	3.9-4.1 (4.0±0.2)	1.5-1.8 (1.7±0.1)	6	Spleen	<i>Salminus franciscanus</i>	São Francisco River, Brazil
<i>Myxobolus ovarium</i>	9.2-11.0 (9.8 ± 0.4)	5.9-6.9 (6.5 ± 0.3)	4.6-5 (4.9 ± 0.1)	3.9-6.2 (4.7 ± 0.5)	1.8-2.4 (2.1 ± 0.2)	9	Ovary	<i>Brycon orthotaenia</i>	São Francisco River, Brazil
<i>Myxobolus orthotaeniae</i>	10.0-11.4 (10.7 ± 0.5)	7.3-8.6 (8.1 ± 0.4)	5.3-7.0 (6.8 ± 0.4)	4.2-5.4 (4.9 ± 0.3)	1.9-2.9 (2.7 ± 0.3)	8	Liver	<i>Brycon orthotaenia</i>	São Francisco River, Brazil
<i>Myxobolus iecoris</i>	11.4-14.2 (12.8 ± 0.8)	7.7-9.9 (8.7 ± 0.6)	6.5-7.5 (6.9 ± 0.4)	4.9-7.4 (5.9 ± 0.5)	2.3-3.5 (3.0 ± 0.2)	8-9	liver	<i>Salminus franciscanus</i>	São Francisco River, Brazil
<i>Myxobolus lienis</i>	10.3-13.8 (12.0 ± 0.6)	6.8-9.3 (8.3 ± 0.5)	6.9-7.0 (7.0 ± 0.6)	3.9-5.8 (4.6 ± 0.5)	2.0-3.5 (2.8 ± 0.3)	5-6	spleen	<i>Brycon orthotaenia</i>	São Francisco River, Brazil
<i>Myxobolus batalhensis</i>	14.0-15.4 (15.2 ± 0.8)	8.0-8.7 (8.5 ± 0.5)	5.0-5.2 (5.2 ± 0.3)	5.0-5.5 (5.2 ± 0.3)	2.6-3.0 (2.8 ± 0.2)	6-9	Ovary/Liver	<i>Salminus hilarii</i>	Batalha River, Brazil
<i>Myxobolus hilarii</i>	9.8 - 3.41 (1.5 ± 0.8)	9.7 - 12.4 (11.0 ± 0.7)	6.7 - 9.0 (7.6 ± 1.0)	6.0 - 7.2 (6.5 ± 0.4)	3.6 - 5.3 (4.0 ± 0.2)	5 - 7	Kidney	<i>Brycon hilarii</i>	Fish farm, São Paulo, Brazil
<i>Myxobolus filamentum</i>	7.4 - 9.7 (9.0 ± 0.3)	5.1-7.3 (6.2 ± 0.4)	4.8-5.7 (5.3 ± 0.3)	3.7-5.4 (4.7 ± 0.3)	1.2-2.2 (1.7 ± 0.1)	10	Gill filaments	<i>Brycon orthotaenia</i>	São Francisco River, Brazil
<i>Myxobolus aureus</i>	12.6 ± 0.5	8.3 ± 0.3	5.5 ± 0.3	5.7 ± 0.3	2.9 ± 0.2	7-8	Liver	<i>Salminus brasiliensis</i>	Pantanal wetland, Brazil
<i>Myxobolus pantanalis</i>	9.3 ± 0.4	6.5 ± 0.4	-	4.2 ± 0.5	2.0 ± 0.1	4-5	Gill filaments	<i>Salminus brasiliensis</i>	Pantanal wetland, Brazil
<i>Myxobolus umidus</i>	13.5 ± 0.7	7.8 ± 0.4	7.7 ± 0.1	5.1 ± 0.4	2.7 ± 0.3	4-5	Spleen	<i>Brycon hilarii</i>	Pantanalwetland, Brazil
<i>Myxobolus piraputangae</i>	10.1 ± 0.5	8.7 ± 0.5	6.7 ± 0.3	5.2 ± 0.4	3.0 ± 0.3	4-5	Kidney	<i>Brycon hilarii</i>	Pantanal wetland, Brazil
<i>Myxobolus brycon</i>	6.5 - 7.2 (6.9)	3.9-4.8 (4.2)	1.9-2.8 (2.5)	3.8-4.7 (4.2)	1.7 - 2.5 (1.9)	8-9	Gill filaments	<i>Brycon hilarii</i>	Pantanalwetland, Brazil
<i>Myxobolus oliveirai</i>	11.2 ± 0.4	7.4 ± 0.5	4.6 ± 0.6	5.6 ± 0.2	2.3 ± 0.2	6-8	Gill filaments	<i>Brycon hilarii</i>	Pantanal wetland, Brazil

<i>Myxobolus salminus</i>	10.1 ± 0.4	6.1 ± 0.4	5.0 ± 0.6	4.6 ± 0.2	1.7 ± 0.1	7-8	Gillfilaments	<i>Salminus brasiliensis</i>	Pantanalwetland, Brazil
<i>Myxobolus macropasmodialis</i>	10.5-12 (11)	8-9 (8.5)	5-5.5 (5.2)	4-5 (4.5)	2-3 (2.8)	6	Spleen	<i>Brycon hilarii</i>	Pantanal wetland, Brazil
<i>Myxobolus paranensis</i>	12-15	7-8	--	6-7	2.5	--	Abdominal cavity	<i>Salminus maxillosus</i> = <i>S.</i> <i>brasiliensis</i>	Mogi Guaçu River, Brazil

**Table 2.** Comparative data of *Myxobolus douradae* n. sp. with all *Myxobolus* species parasites of bryconid fish. Spore dimensions, infection sites, and collection sites are given. PCL: length of polar capsules; PCW: width of polar capsules; NCPT: number of coils of polar tubules; --: no data. All measurements are range in  $\mu\text{m}$  and/or means  $\pm$  SD,

