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ORIGINAL PAPER

Morphological and molecular description of *Myxobolus batalhensis* n. sp. (Myxozoa, Myxosporea), a liver and ovary parasite of *Salminus hilarii* in Brazil

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Abstract Plasmodia containing myxospores belonging to the genus *Myxobolus* Bütschli, 1882 were found in the ovaries and liver of *Salminus hilarii*. Despite its economic value, this fish host has no previous reports of myxozoan infections. Herein, we describe *Myxobolus batalhensis* n. sp. using morphological and ultrastructural data, as well as histological and SSU rDNA molecular data. The mature myxospores were elongated, measuring in average 15.2 ± 0.8 μm in length, 8.4 ± 0.4 μm in width, and 5.1 ± 0.2 μm in thickness. Polar capsules were elongated and measured 5.3 ± 0.3 μm in length and 2.8 ± 0.3 μm in width. Polar filaments had 6–9 coils. Histopathological analysis showed coagulation necrosis associated with cell lysis as a response of the host cell to the parasite in the ovaries. No inflammatory reaction was observed in the liver, although the presence of the plasmodia caused changes in tissue structure. The phylogenetic analysis of South American myxobolid species showed *M. batalhensis* n. sp. as sister species of *Myxobolus aureus*. This is the first report of a myxozoan species parasitizing *S. hilarii* and the first myxozoan species described in the Batalha river.

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Introduction

Myxozoans are microscopic parasitic cnidarians of fish (Okamura et al. 2015). The occurrence of myxozoan parasites has been extensively studied in aquaculture due to their pathogenic potential (Feist and Longshaw 2006). Myxozoans can induce significant mortalities in wild and farmed fishes, leading to major economic losses (Naldoni et al. 2009; Morsy et al. 2012; Gómez et al. 2014). Among the problems related to myxozoan infections are reduced respiratory capacity (Adriano et al. 2005), longitudinal compression of the body (Longshaw et al. 2003), degenerative cardiomyopathy (Yokoyama et al. 2005), and intestinal necrosis (Alvarez-Pellitero et al. 2008).

Among myxozoans, the class *Myxosporea Bütschli*, 1881 represents more than 2200 species, with approximately 910 species belonging to the genus *Myxobolus Bütschli*, 1882 (Lom and Dykova 2006). Of the known *Myxobolus* species, approximately 60 have been described from Brazil (Eiras et al. 2014). Species have been described from various tissues and organs, including gills (Adriano et al. 2009; Azevedo et al. 2014), liver (Carriero et al. 2013), spine (Longshaw et al. 2003), and heart (Ye et al. 2014). Reports of myxozoans in Brazil parasitizing the gonads include *Henneguya amazonica* Rocha et al., 1992 from the testes of *Hoplosternum littorale* Hancock, 1828 (Torres et al. 1994), *Myxobolus lutzi* Aragão, 1919 parasitizing the testes of *Poecilia vivipara* Bloch and Schneider, 1801 (Aragão 1919), and *Myxobolus testicularis* Tajdari et al., 2005 parasitizing the testes of *Hemiodopsis microlepis* Kner, 1859 (Tajdari et al. 2005).

The species *Salminus hilarii* Valenciennes, 1850 is of great importance for commercial and sport fishing (Andrade et al. 2004). The tabarana or dourado-branco is a characid fish of the subfamily Salminae with a wide distribution in Brazilian river basins (Godoy 1975). To date, there is no record of myxozoans parasitizing *S. hilarii*, but three myxozoan species have been described from other members of the genus, *Salminus brasiliensis* (Cuvier, 1816), namely *Myxobolus salminus* Adriano et al., 2009 and *Myxobolus pantanalis* Carriero et al., 2013 both in gills, and *Myxobolus aureus* Carriero et al., 2013 in liver (Adriano et al. 2009; Carriero et al. 2013).

During a parasitological survey of *S. hilarii*, plasmodia containing myxospores morphologically consistent with *Myxobolus* were observed in ovaries and liver. The main goal of this study was to identify and compare the myxobolid species using morphological and molecular data as well as to evaluate their phylogenetic relationship with other representatives of the *Myxobolidae* family. In addition, we provided ultrastructural and histological details of the plasmodia, comparing the pathologies caused by the parasite in different organs.

Materials and methods

Fish collection

From March 2014 to June 2015, specimens of *S. hilarii* ($n = 8$; 27–30.2 cm total length, 491.55–698.40 g; 6 females/2 males) were collected in Reginópolis, Batalha river (21° 52′ 33.0″ S, 49° 14′ 19.9″ W), São Paulo, Brazil. Fish were collected using gill nets and euthanized by neural pithing immediately after capture. All fish captured had a minimum size of 20 cm, which is the first stage of sexual maturation (Andrade et al. 2004). Sex was determined by external morphology of the fish and confirmed by observation of the gonads after dissection. Fish were examined within 24 h of capture, and fresh smears of gills, stomach, intestine, heart, liver, gonad, bone marrow, kidney, swim bladder, and gall bladder were evaluated under a differential interference contrast microscope (Leica DMLB 5000, Leica Microsystems, Wetzlar, Germany) at ×1000 magnification. Samples of liver and ovaries infected with myxozoans were collected for further molecular and morphological analysis.

Morphological examination

Morphological measurements of fresh spores followed the recommendations of Lom and Arthur (1989): spore length, spore width, spore thickness, polar capsule length, polar capsule width, and number of turns of the polar filament. Measurements of 30 spores from livers and 30 spores from ovaries from the five infected fish were taken fresh from digital images at ×1000 magnification, under light microscopy with Leica software application suite LAS V3.8 (Leica Microsystems).

For transmission electron microscopy (TEM), one oocyte from ovaries and one small piece of liver containing plasmodia were fixed in Karnovsky's solution at 4 °C for 10 h. After being rinsed overnight in the fixative at 4 °C, tissue was post-fixed in 2% osmium tetroxide for 3 h at 4 °C. Subsequently, the tissue was immersed in 0.5% uranyl acetate in distilled water for 2 h. Tissue fragments were dehydrated through an ascending ethanol series followed by propylene oxide and embedded in Epon resin. Semi-thin sections were stained with methylene blue-Azure II and observed under differential interference contrast microscope. Ultra-thin sections were double-stained with aqueous uranyl acetate and lead citrate and observed under a JEOL 100CXII (TEM) operated at 60 kV.

For histological analysis, both infected and uninfected oocytes were fixed with Karnovsky's solution, and dehydration was initiated afterwards in increasing concentrations of alcohol (3× into 70% for 2 h and 95% for 4 h). The material underwent a resin-alcohol mixture for 12 h. Finally, the tissue was embedded in resin (HistoResin, Leica, Germany). The 3 μm sections were stained with hematoxylin-eosin, then examined under light microscope.

Molecular analysis

Two myxozoan plasmodia, one from ovaries and one from liver, were preserved in absolute ethanol. DNA isolation was carried out in accordance with the animal tissue protocol of the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Partial SSU rDNA gene was amplified using a combination of PCR and nested PCR with general eukaryotic and myxozoan primers (Table 1). Amplifications were performed on a Peltier 200 Thermocycler (MJ Research, Watertown, MA), with initial denaturation at 94 °C for 3 min, followed by 28 or 35 cycles for primary PCR, and 35 cycles for nested PCR of 94 °C for 40 s, 55 °C for 40 s, 72 °C for 2 min, and a final extension at 72 °C for 10 min. PCR reactions were performed in 10 μL total volume, containing 0.625 U GoTaq® Flexi PCR polymerase (Promega, San Luis Obispo, California, USA), 2 μL of 5× Flexi PCR Buffer, 0.75 mM of $MgCl_2$ (Promega), 0.5 μL of Rediload Dye (Invitrogen, Carlsbad, CA), 0.2 mM from each dNTP, 0.5 μM from each primer, and 10 ng of template DNA. Nested PCR was performed in a 25-μL volume and with 150 ng of PCR product as template. PCR products were electrophoresed through 1% agarose gel in TAE buffer stained with 1% SYBRsafe (Invitrogen) alongside a 1-kb DNA ladder (Invitrogen) and visualized under UV-light. After checking for the presence of the expected size DNA amplicons, PCR products were cleaned up using ExoSAP-IT® (Affymetrix, Santa

Table 1 SSU rDNA primers used in this study, sequence, amplicon size, PCR rounds, and reference

Primer	Sequence 5′–3′	Amplicon size (bp)	PCR round	Author
ERIB1	ACCTGGTTGATCCTGCCAG	≈ 1900	1st	Barta et al. (1997)
ERIB10	CTTCCGCAGGTTCACCTACGG			Barta et al. (1997)
MyxGP2F	WTGGATAACCGTGGGAAA	≈ 800	Nested	Kent et al. (1998)
MyxospecR	GGTTCNCDGRGGGMCCAAC			Fiala (2006)
Act1R	AATTTCACCTCTCGCTGCCA	≈ 900	1st (paired with ERIB1)	Hallet and Diamant (2001)
MyxGen4F	GTGCCTTGAATAAATCAGAG	≈ 900	1st (paired with ERIB10)	Diamant et al. (2004)

Clara, CA, USA). The PCR products were sequenced using PCR and nested PCR primers on a ABI 3730 capillary sequence machine (Applied Biosystems, Foster City, CA, USA) at the Oregon State University Center for Genome Research and Biocomputing.

The sequences obtained for each sample were assembled and edited using BioEdit v. 7.0.9 (Hall 2011) to obtain longer consensus sequences of around 1700–1900 bp. The consensus sequences were aligned to all known sequences from South American myxobolids reported from freshwater fish using the ClustalW algorithm (Larkin et al. 2007) and default settings in Geneious version 7.1.3 (Kearse et al. 2012). The malacosporean, *Buddenbrockia allmani* (KJ150260), was used as outgroup, for being of an ancestral clade related to Myxosporea. The nucleotide substitution model was selected from 82 potential evolutionary models based on the Akaike information criterion using jModel Test 2.1 (Posada 2008), which identified the general time reversible model with gamma distribution and invariable rates (GTR + I + G) as the model of best fit. The nucleotide frequencies were A = 0.2369, C = 0.1935, G = 0.2930, and T = 0.2766 with estimated nucleotide substitution rates of AC = 1.3171, AG = 4.0876, AT = 1.7304, CG = 0.7000, CT = 4.5812, and GT = 1.0000. The gamma distribution was 0.3190.

Bayesian inference (BI) and maximum likelihood (ML) methods were used for phylogenetic analysis. MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) was used to perform the BI using Markov Chain Monte Carlo (MCMC) tree searches as implemented. Two parallel runs of four simultaneous MCMC searches for 5 million generations each were performed. The trees were used by MrBayes to estimate the posterior probability of each node in our phylogenetic reconstruction. Tracer v1.6 (Rambaut et al. 2014) was used to check the stationarity of all parameters sampled by the chains. PhyML 3.1 (Guindon et al. 2010) software was used to perform ML analysis, using bootstrap confidence calculated with 1000 replications and GTR + I + G evolutionary model. The trees obtained were visualized using Figtree 1.4.3 (Rambaut 2012) and edited using Adobe PhotoShop (Adobe Systems Inc., CA, USA). An alignment containing only trimmed sequences of *Myxobolus* spp. parasites of bryconid fishes from South America was used to produce a distance matrix.

Results

The morphology of the myxospores found in the ovaries and liver of *S. hilarii* corresponded with the genus *Myxobolus* according to Fiala et al. (2015). Myxozoan parasites were recovered from five of six females examined, and each positive fish had simultaneous infections in both the ovaries and liver. No myxozoans were present in either of the two male fish collected. As a consensus of morphological, ultrastructural, and molecular data, a new *Myxobolus* species is described.

Molecular and phylogenetic analysis

A 1901-bp fragment of the SSU rDNA gene was amplified from spores infecting the ovaries and a 1768-bp fragment of the SSU rDNA gene from spores infecting the liver of *S. hilarii*. Both sequences were identical. The BLAST search using our newly generated SSU rDNA sequences did not match with other myxozoan sequences available in GenBank. The closest species was *M. aureus*, which showed a 93.6% similarity with a difference of 53 nucleotides (Table 2).

Phylogenetic analysis demonstrated the South American myxozoans that parasitize bryconid fishes clustered into two polyphyletic groups, one falling within a clade of gill infecting myxozoans and another that falls within a cluster of other viscera infecting myxozoans. Based on this data, *M. batalhensis* n. sp. appears as a sister species of *M. aureus* (Fig. 1).

Description of *Myxobolus batalhensis* n. sp.
Phylum Cnidaria Hatschek, 1888
Unranked subphylum Myxozoa Grassé, 1970
Class Myxosporea Bütschli, 1881
Order Bivalvulida Shulman, 1959
Family Myxobolidae Thélohan, 1892
Genus Myxobolus Bütschli, 1882
Myxobolus batalhensis n. sp.

Table 2 Similarity in SSU rDNA sequences of *Myxobolus* species parasites of Bryconid fishes from South America. Lower triangle shows actual nucleotide difference, while the upper triangle shows the percentage of nucleotide difference

	Host isolate	1	2	3	4	5	6	7	8	9	10
1	*Myxobolus batalhensis* n. sp. (Gonad)		0.01	6.4	26.4	25	25	13.7	13.3	14.2	14.6
2	*Myxobolus batalhensis* n. sp. (Liver)	1		6.4	26.4	25	25	13.7	13.3	14.2	14.6
3	*Myxobolus aureus*	53	53		25.7	25.4	25.2	15.1	14.1	14.6	15.9
4	*Myxobolus pantanalis*	224	223	218		13.7	13.3	27.5	26.9	28.1	27.3
5	*Myxobolus oliveirai*	213	212	216	115		3	25.9	25	26.1	24.8
6	*Myxobolus filamentum*	213	212	214	112	25		26	25.1	26.1	24.7
7	*Myxobolus piraputangae*	114	113	125	233	220	221		10.2	8.2	6.3
8	*Myxobolus umidus*	110	110	116	228	212	213	84		10	16.7
9	*Myxobolus hilarii*	118	117	120	238	222	222	67	82		16.9
10	*Myxobolus macroplasmodialis*	122	121	132	231	211	210	135	139	140	

Myxospores Myxospores: (n = 30 fresh spores from ovaries and n = 30 fresh spores from liver). Elongate and ellipsoidal spores (Fig. 2a, b). Measurements for spores found in ovary were 15.2 ± 0.8 (14.0–15.4) μm spore length, 8.5 ± 0.5 (8.0–8.7) μm spore width, and 5.2 ± 0.3 (5.0–5.2) μm spore thickness. Polar capsules elongate and pyriform, 5.2 ± 0.3 (5.0–5.5) μm in length and 2.8 ± 0.2 (2.6–3.0) μm in width. Polar filament allocated in 6–9 coils. Measurements for spores found in liver were 15.3 ± 0.9 (14.0–15.5) μm spore length, 8.3 ± 0.3 (8.1–8.5) μm spore width, and 5.0 ± 0.1 (4.9–5.0) μm spore thickness. Polar capsules elongate and pyriform, 5.4 ± 0.1 (5.3–5.5) μm in length and 2.8 ± 0.1 (2.8–2.9) μm

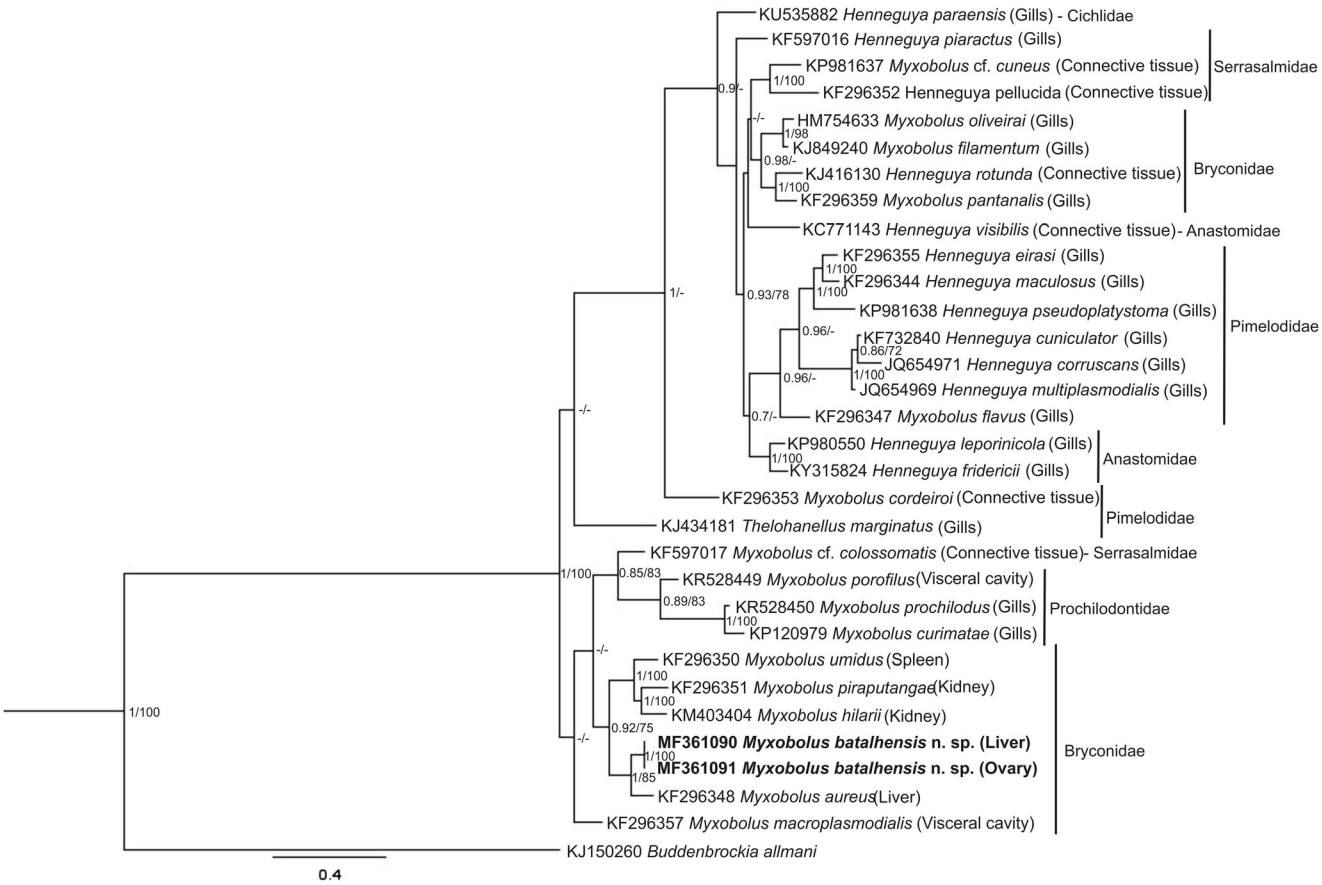

Fig. 1 Maximum likelihood phylogenetic tree based on partial SSU rDNA sequences showing the position of *Myxobolus batalhensis* n. sp. among other myxobolids from South America. Numbers at the nodes represent Bayesian posterior probability and bootstrap values (BI/ML) gaining more than 0.7 posterior probability (BI) and 70% support (ML). Values lower than 0.7/70 are represented by dashes, and in parenthesis, the infected organ. Scale bar is given under the tree

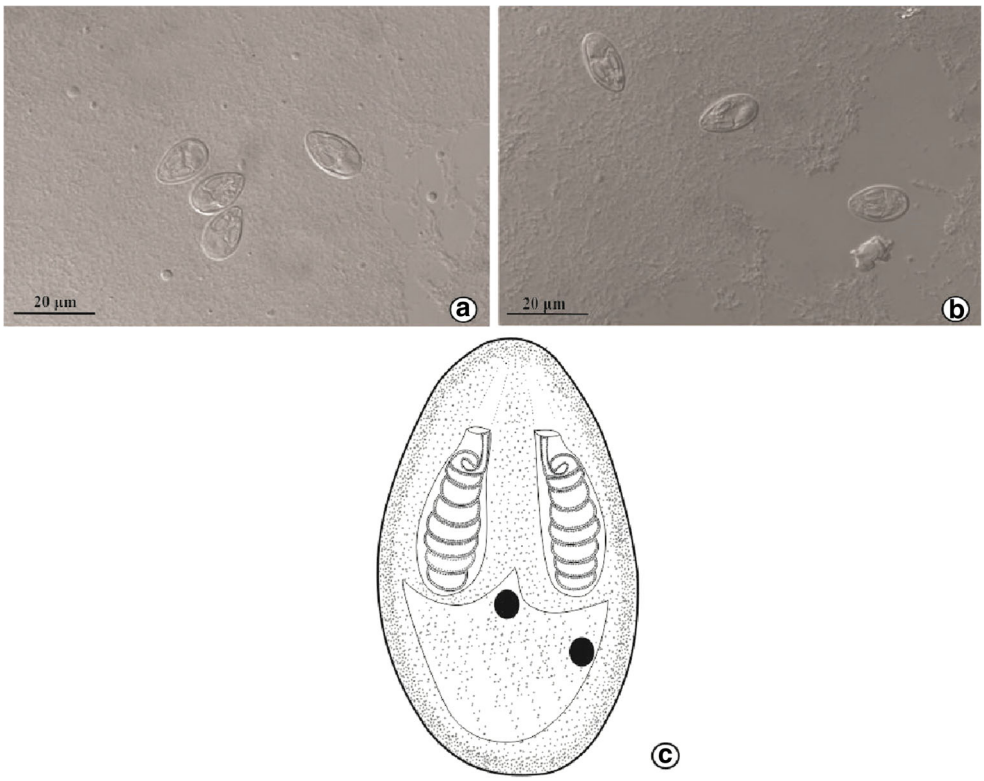

Fig. 2 **a**–**c** Images of *Myxobolus batalhensis* n. sp. spores in *Salminus hilarii*. **a** Mature spores found in ovary. **b** Mature spores found in liver. **c** Schematic illustration of spore

in width. Polar filament allocated in 6–9 coils. Binucleate sporoplasm. The presence of two valves, meeting at sutural line. Polar capsules had anterior ends close to each other (Fig. 2c).

Plasmodia Plasmodia is whitish and round, approximately 0.25 cm in the liver (Fig. 3a) and approximately 0.5 cm diameter in ovaries (Fig. 3b, c).

Taxonomic summary

Type host *Salminus hilarii* Valenciennes, 1850

Type locality Batalha river (22° 38′ 36.6″ S, 49° 11′ 49.4″ W), Reginópolis, São Paulo, Brazil

Site of infection Ovaries and liver

Prevalence 62.5% (5/8) in *S. hilarii* ovaries and liver

Etymology The species name is derived from the name of the river where the hosts were collected.

Material deposited Glycerogelatin glass slides containing myxospores were deposited in the collection of the Museum of Zoology, "Adão José Cardoso," Institute of Biology, University of Campinas (UNICAMP), São Paulo, Brazil (ZUEC-MYX 66 and ZUEC-MYX 67).

Sequence data The 1901- and 1768-bp of SSU rDNA sequences obtained from infected *S. hilarii* ovaries (GenBank Acc. Num. MF361090) and liver (GenBank Acc. Num. MF361091) respectively.

Remarks

The morphology of *M. batalhensis* n. sp. was compared to other *Myxobolus* spp. reported from bryconid fishes (Table 3). Among the 11 species included in the comparison, only *Myxobolus paranensis* Bonetto and Pignalberi, 1965 has been reported from gonads, although from a different host (*S. maxillosus*). Moreover, the ranges of spore body length and number of turns in the polar filament coil were considerably more variable than the values reported here for *M. batalhensis* n. sp., while the polar capsules differed in length.

Among *Myxobolus* species that infect fish of the family Bryconidae, the most similar to *M. batalhensis* n. sp. was *Myxobolus umidus* Carriero et al., 2013. However, they differed in spore thickness, number of polar filament coils, and site of infection (spleen vs. ovary/liver). Comparably, *M. aureus* infects the liver of another *Salminus* sp., *S. brasiliensis*, although *M. batalhensis* n. sp. spores are considerably larger. The minimum and maximum number of coils of the polar filament also differed between these two species,

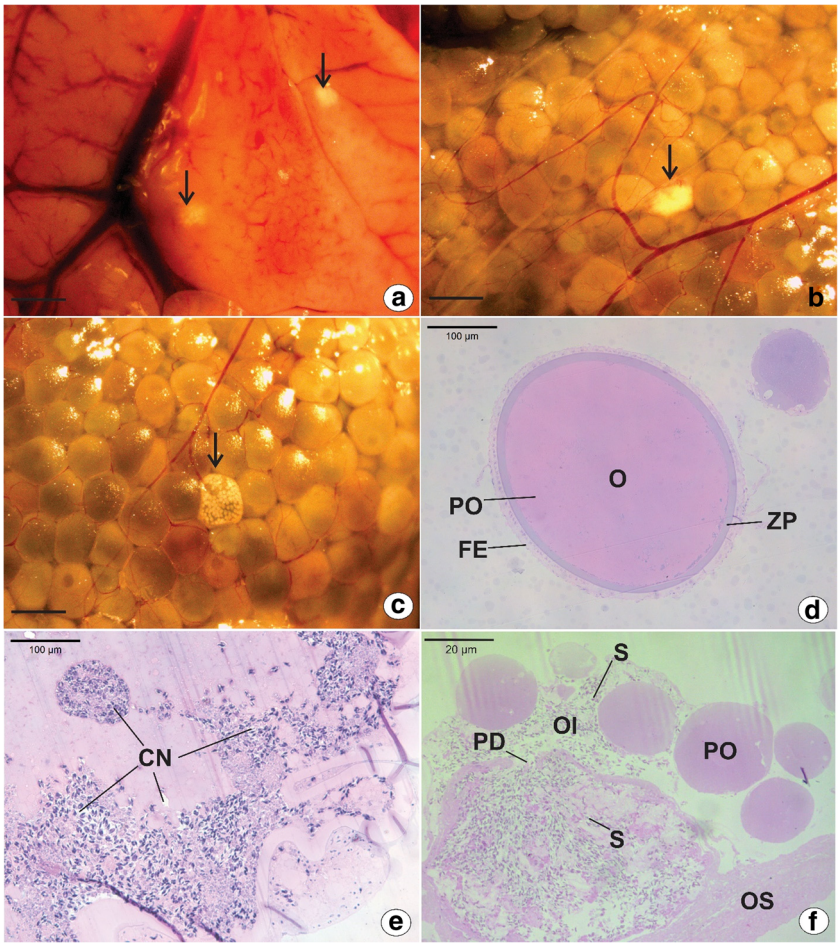

Fig. 3 **a**–**f** Images of infected organs and histological analysis of uninfected and infected oocytes by *Myxobolus batalhensis* n. sp. **a** The presence of plasmodia (arrows) in the liver of *Salminus hilarii*. Bar 1 cm. **b** Whitish infected oocyte (arrow). Bar 1 cm. **c** Oocyte infected (arrow), with the presence of numerous plasmodia inside. Bar 1 cm. **d** Example of uninfected primary oocyte (PO) with the ooplasm (O), follicular epithelium (FE), and zona pellucida (ZP) intact (staining: HE). **e** Mass of spores filling an infected oocyte and probably impacting on their reproductive function by the coagulation necrosis associated with cell lysis (CN) (staining: HE). **f** Histological comparison of mature oocyte in the ovarian stroma (OS) infected and healthy primary oocytes (PO). Infected oocyte often showed disruption of the zona pellucida (PD) and released a large number of spores (S) in ovarian interstitium (OI). The absence of follicular epithelium associated with cell lysis (staining: HE)

being 6–9 for *M. batalhensis* n. sp. and 7–8 for *M. aureus* (Table 3).

When compared with *Myxobolus* species that parasitize gonads of fish from Brazil, *M. batalhensis* n. sp. showed to be larger than *M. lutzi* (10 µm) and *M. testicularis* (8.6 ± 0.5 µm). Both species parasitize the testes and have not been reported from ovaries.

Histological analysis

The plasmodia found in the liver were in average 0.25 cm in diameter and contained numerous mature spores (Fig. 3a). Macroscopically, the presence of plasmodia in the liver disrupted hepatic tissue structure but did not illicit a marked immune response.

The plasmodia found in the ovaries could completely fill an oocyte (Fig. 3b, c). When infected and healthy primary oocytes were compared, external and internal structural differences were observed. In the uninfected primary oocytes, it was possible to observe the follicular epithelium, zona pellucida, and ooplasm (Fig. 3d). However, in infected oocytes, it was not possible to observe any of the normal architecture, such as teca cells, zona radiata, and even the nucleus, due to the degradation caused by the parasite. Coagulation necrosis associated with cell lysis as a response to the developing parasite resulted in reduction of the functional area of the oocyte (Fig. 3e). Spores were observed occupying the entire oocyte cytoplasm, displacing the nucleus of the oocyte. It was also observed that the zona pellucida of follicles ruptured and the absence of the follicular epithelium, releasing spores into the ovarian interstitium (Fig. 3f).

Ultrastructural analysis

Ultrastructural observations were based on spores found in oocytes and liver of *S. hilarii*. Spores consisted of two polar capsules, two enveloping valve cells, and one binucleate sporoplasm with numerous sporoplasmosomes (Fig. 4a, b). The sporoplasm contained abundant endoplasmic reticulum and numerous mitochondria, and the polar capsules consisted of an inner lucent layer and a thin outer dense layer (Fig. 4c). Inside the fully developed polar capsule, six to nine turns of the polar filament were observed. The polar filament was arranged in a granular matrix (Fig. 4c). Along the suture line, spore valves (Fig. 4d) formed deep invaginations (Fig. 4e, f). The opening for polar filament discharge was located in the

Table 3 Comparison of *Myxobolus batalhensis* n. sp. to other *Myxobolus* species parasites of Bryconid fishes from South America

Species	Spore length	Spore width	Thickness	LPC	WPC	CPF	Site of infection	Host	Study
M. batalhensis n. sp.	15.2 ± 0.8 (14.0–15.4)	8.5 ± 0.5 (8.0–8.7)	5.2 ± 0.3 (5.0–5.2)	5.2 ± 0.3 (5.0–5.5)	2.8 ± 0.2 (2.6–3.0)	6–9	Ovary	*Salminus hilarii*	This study
	15.3 ± 0.9 (14.0–15.5)	8.3 ± 0.3 (8.1–8.5)	5.0 ± 0.1 (4.9–5.0)	5.4 ± 0.1 (5.3–5.5)	2.8 ± 0.1 (2.8–2.9)	6–9	Liver	*Salminus hilarii*	This study
M. aureus	12.6 ± 0.5	8.3 ± 0.3	5.5 ± 0.3	5.7 ± 0.3	2.9 ± 0.2	7–8	Liver	*Salminus brasiliensis*	Carriero et al. (2013)
M. paranensis	12–15	7–8	-	6–7	2.5	8–11	Testes, ovary	*Salminus maxillosus*	Bonetto and Pignalberi (1965)
M. hilarii	11.5 ± 0.8	11.0 ± 0.7	7.6 ± 1.0	6.5 ± 0.4	4.0 ± 0.2	5–7	Kidney	*Brycon hilarii*	Capodifoglio et al. (2016)
M. brycon	6.9 (6.5–7.2)	4.2 (3.9–4.8)	2.5 (1.9–2.8)	4.2 (3.8–4.7)	1.9 (1.7–2.5)	8–9	Gill	*Brycon hilarii*	Azevedo et al. (2011)
M. oliveirai	11.2 ± 0.4	7.4 ± 0.5	4.6 ± 0.6	5.6 ± 0.2	2.3 ± 0.2	6–8	Gill	*Brycon hilarii*	Milanin et al. (2010)
M. piraputangae	10.1 ± 0.5	8.7 ± 0.5	6.7 ± 0.3	5.2 ± 0.4	3.0 ± 0.3	4–5	Kidney	*Brycon hilarii*	Carriero et al. (2013)
M. umidus	13.5 ± 0.7	7.8 ± 0.4	7.7 ± 0.1	5.1 ± 0.4	2.7 ± 0.3	4–5	Spleen	*Brycon hilarii*	Carriero et al. (2013)
M. filamentum	9.0 ± 0.3	6.2 ± 0.4	5.3 ± 0.3	4.7 ± 0.3	1.7 ± 0.1	10–11	Gill	*Brycon orthotaenia*	Naldoni et al. (2015)
M. macroplasmodialis	11 (10.5–12)	8.5 (8–9)	5.2 (5–5.5)	4.5 (4–5)	2.8 (2–3)	6	Abdominal cavity	*Salminus maxillosus*	Molnár et al. (1998)
M. pantanalis	9.3 ± 0.4	6.5 ± 0.4	-	4.2 ± 0.5	2.0 ± 0.1	4–5	Gill	*Salminus brasiliensis*	Carriero et al. (2013)
M. salminus	10.1 ± 0.4	6.1 ± 0.4	5.0 ± 0.6	4.6 ± 0.2	1.7 ± 0.1	7–8	Gill	*Salminus brasiliensis*	Adriano et al. (2009)

All measurements are in μm

LPC length of polar capsule, *WPC* width of polar capsule, *CPF* number of coils of polar filament, - no data

shell valve next to the apical end of spore where the polar filament was invaginated (Fig. 4f).

Discussion

Spore morphology combined with molecular data, host organ, and specificity is essential data for the determination of a new myxozoan species (Kato et al. 2017). In accordance with this taxonomic strategy, we described a new species of *Myxobolus*, a liver and ovary parasite of a commercially important fish species in Brazil.

Phylogenetic analysis performed here supports previous work that suggests host family and tissue tropism which are strong phylogenetic signals for myxobolid species parasitizing families of South American fishes (Eszterbauer 2004; Fiala 2006; Carriero et al. 2013; Capodifoglio et al. 2016). We obtained a well-supported phylogenetic tree demonstrating two polyphyletic groups, one composed of species of *Myxobolus* and another composed primarily of species of *Henneguya*. In the *Myxobolus* group, *M. aureus*, a liver parasite, appeared as a sister group of *M. batalhensis* n. sp. The sequence divergence between both species (Table 2) exceeded values typically associated with interspecific variability (Cech et al. 2012), supporting the claim that *M. batalhensis* n. sp. and *M. aureus* are not conspecific. *Myxobolus* spp. that parasitize fish of the Bryconidae family, such as *M. umidus* in spleen of *Brycon hilarii* Valenciennes, 1850, *Myxobolus hilarii* Capodifoglio et al. 2016 in kidney of *B. hilarii* and *Myxobolus piraputangae* Carriero et al., 2013 in kidney of *B. hilarii* also clustered in the myxobolid clade. A subclade formed by *Myxobolus macroplasmodialis* Molnár et al., 1998, a parasite of the visceral cavity of a bryconid fish, appeared as the clade basal species.

Similar host and tissue tropism was observed in the clade composed primarily of *Henneguya* spp. The species were divided into two major groups, with one group consisting mainly of *Henneguya* spp. parasitizing fish of the family Pimelodidae Eigenmann, 1918 and another group composed mainly of *Henneguya* spp. infecting various species of the

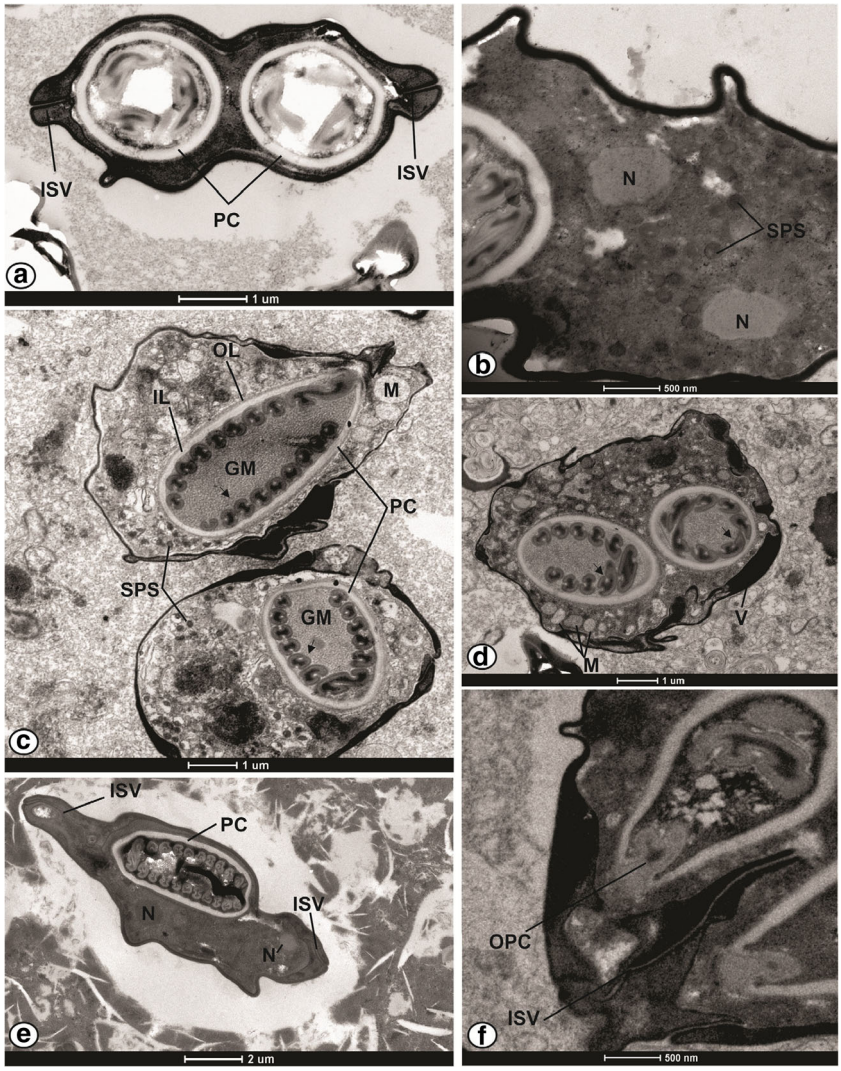

Fig. 4 **a**–**f** Transmission electron microscopy images of spores of *Myxobolus batalhensis* n. sp. in the ovary and liver of *Salminus hilarii*. **a** A transverse section showing two polar capsules (PC). Notice the deep invagination of the shell valve suture (ISV). **b** Section through a spore with a binucleate (N) sporoplasm containing numerous sporoplasmosomes (SPS). **c** Section of two spores showing sporoplasmosomes (SPS) in the sporoplasm, mitochondria (M), and large polar capsules (PC) containing coiled polar filament (arrow, 6–9 coils). Notice the outer electron-dense layer (OL), an inner electron-lucent layer (IL), and the granular matrix (GM) of the polar capsules. **d** Section of spore showing numerous mitochondria (M) and two polar capsules with the polar filament (arrows); valve (V). **e** Notice the deep invagination of the shell valve suture (ISV), the binucleate (N) sporoplasm, and the large polar capsule (PC) in this section. **f** Notice the invagination of the shell valve (ISV) and the opening of the polar capsule (OPC)

families Bryconidae and Serrasalmidae Bleeker, 1859. Carriero et al. (2013) and Capodifoglio et al. (2016) suggested myxobolids parasitizing members of the Bryconidae had a polyphyletic origin, with phylogenetic placement largely influenced by host species, consistent with trends reported here. Comparatively, Molnár (1994) suggested tissue affinity is an important character for specific assignment. In the current study, we noted that myxozoan parasites of fish from South America tend to form two main groups: one of them that infects gills and their connective tissue, while the other group are parasites of the body and visceral organs.

While the number of males analyzed in this study was low, *Myxobolus batalhensis* n. sp. were only observed in female fish. This may indicate a preference of the parasite for ovaries and that parasites found in the liver could be an aberrant occurrence, or that physiological and behavioral factors related to host sex may be involved in the dynamics of infection.

According to the latest reviews of *Myxobolus* spp. (Eiras et al. 2005, 2014), this genus demonstrates a wide range of tissue tropism and host specificity. Data suggest that myxozoans have serious pathological potential when encysted on reproductive tissues (Swearer and Robertson 1999; Sitjà-Bobadilla and Alvarez-Pellitero 1993) both in wild and cultured fish (Moran et al. 1999; Palenzuela 2006). Sitjà-Bobadilla (2009) emphasized the importance of controlling myxozoans in aquaculture, especially those species that cause damage to host gonads, as these can affect the host reproductive capacity by means of parasitic castration through disintegration of the infected oocyte membrane (Grankoto et al. 2001). In general, studies on ovarian infections of myxozoans focused on the ultrastructural features (Okaeme et al. 1988; Obiekezie and Okaeme 1990), but a combination of ultrastructural and histological analyses are needed for the elucidation of their pathological effects. In our study, we observed that *M. batalhensis* n. sp. infection lead to membrane destruction of affected oocytes and vacuolar degeneration. The structure of these infected oocytes was altered by the

massive presence and proliferation of spores, most likely affecting their normal development and fertilization.

Several *Myxobolus* spp. have been described infecting liver of South American fishes (Molnár and Békési 1993; Adriano et al. 2006) including *M. aureus* infecting the liver of *S. brasiliensis* (Carriero et al. 2013). In none of these cases did the parasites cause serious damage to the host. Although *M. batalhensis* n. sp. caused histopathological changes in the liver, the host response in the fish examined was minimal. This may reflect when they were sampled and thus should be further studied to fully understand the pathologic potential of this parasite.

Given the spore morphology, the morphometric data and SSU rDNA gene sequence obtained in the present study, we reported the new species *Myxobolus batalhensis* n. sp. This finding contributes to our knowledge of the biodiversity of myxozoans in Brazil. To the best of our knowledge, *M. batalhensis* n. sp. is the first record of a myxozoan species parasitizing *S. hilarii* and the first myxozoan species described in the Batalha river.

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Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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