


Sphaerospora festivus n. sp., a parasite of the flag cichlid, *Mesonauta festivus* (Teleostei: Cichlidae) from eastern Amazon, Brazil

Sphaerospora festivus n. sp., um parasito do ciclídeo bandeira, *Mesonauta festivus* (Teleostei: Cichlidae) da Amazônia oriental, Brasil

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Abstract

The present study describes a new species of the genus *Sphaerospora* found in the urinary bladder of the flag cichlid, *Mesonauta festivus* collected in Corre Água district of the municipality of Macapá, Amapá State (Brazil). The study includes morphological and phylogenetic analyses of the new parasite, to determine the relationship of the new species with related myxosporean species. The new species has polysporous plasmodia, which vary in size and shape. The mature myxospores are subspherical shape in valvar view. In the sutural view, the myxospores are 5.3 ± 0.2 (5.2-5.6) μm in length and 7.0 ± 0.7 (6.3-7.7) μm in width, with two piriform polar capsules equal size, 2.5 ± 0.2 (2.3-2.8) μm in length and 1.8 ± 0.2 (1.6-2.0) μm in width. The phylogenetic analyses of a partial sequence of the 18S rRNA gene confirmed the status of the new species and determined the relationship of the new species and related myxosporean species. The sum of the evidence indicates that, *Sphaerospora festivus* n. sp. belongs to the family Sphaerosporidae, and is the first record of the genus *Sphaerospora* from Brazil.

Keywords: Amazonia, morphometry, myxozoa, *Sphaerospora festivus*, 18S rRNA.

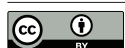
Resumo

O presente estudo tem como objetivo descrever uma nova espécie de *Sphaerospora* encontrado na bexiga urinária de *Mesonauta festivus*, coletado no distrito Corre Água, no município de Macapá, estado do Amapá (Brasil). Foram realizadas análises morfométricas e filogenéticas, nas quais se avaliou a relação entre as espécies de mixosporídeos já descritas. A nova espécie possui plasmódio poliespórico, que varia em tamanho e forma. Os esporos maduros são subsféricos. Na visão sutural, apresentam $5,3 \pm 0,2$ (5,2-5,6) μm de comprimento e $7,0 \pm 0,7$ (6,3-7,7) μm de largura, com duas cápsulas polares piriformes de tamanhos iguais, $2,5 \pm 0,2$ (2,3-2,8) μm de comprimento e $1,8 \pm 0,2$ (1,6-2,0) μm de largura. As análises filogenéticas das sequências parciais do gene 18S rDNA confirmam ser uma nova espécie e determinou a relação desta com outros myxozoários já relatados. Conclui-se que a espécie em estudo pertence à família Sphaerosporidae, gênero *Sphaerospora*, e nova espécie, *Sphaerospora festivus* n. sp. e primeira ocorrência de parasitos desse gênero no Brasil.

Palavras-chave: Amazônia, morfometria, myxozoa, *Sphaerospora festivus*, 18S rRNA.

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Introduction

Myxozoans are microscopic metazoan parasites of the phylum Cnidaria (Jiménez-Guri et al., 2007) which parasitise many fish species of economic interest, and may also infect other vertebrates, such as birds, amphibians, reptiles, and mammals (Lom & Dyková, 2006; Bartholomew et al., 2008; Bartošová et al., 2013; Aguiar et al., 2017; Matos et al., 2018). These parasites can spoil the produce of fish farms by rendering the meat unfit for human consumption (Naldoni et al., 2009; Feist & Longshaw, 2006; Velasco et al., 2016). Myxozoans parasitise fish as myxospores, which may present a variety of shapes and sizes, with one or more amoebic sporoplasms, and typically two, but sometimes more polar capsules (Pavanelli et al., 2013).

The genus *Sphaerospora* Thélohan, 1982 includes approximately 102 valid species (Patra et al., 2018), although molecular data are available for a reduced number of taxa. These species are spherical in shape, with subspherical myxospores that have two valves and two polar capsules, usually with binucleated sporoplasm (Eszterbauer et al., 2013). The species of this genus are described mainly from the myxospore morphology, including the size and configuration of the body and the polar capsules (Molnár, 2011).

The majority of myxozoans are host-specific, although a single fish species may host a number of different myxosporean species. These parasites have a strong coevolutionary relationship with their hosts, in addition to a high rate of molecular evolution, reflecting their rapid adaptive radiation (Bartošová-Sojtková et al., 2018; Silva et al., 2020). *Mesonauta festivus* (Heckel, 1840), is a bentopelagic cichlid, abundant in the basins of the Amazon, Paraná and Paraguay in South America, where it is widely targeted by collectors for the ornamental fish trade (Kullander, 1998). Despite the wide distribution of these species, which reflects their dispersion capacity related to specific biological and behavioral characteristics (Pires et al., 2015), there are no reports on the establishment of parasitic relationships between myxosporean and this species of fish in the tropical region.

The parasitology of natural fish populations provides important insights into parasite-host-environment relationships, as well as the different strategies adopted by the parasites and their ecological characteristics (Azevedo et al., 2011; Silva et al., 2011). The present study describes the morphological traits, and the molecular and phylogenetic characteristics of a new myxozoan species of the genus *Sphaerospora*, found parasitising of *M. festivus* specimens collected in the Corre Água district of the municipality of Macapá, Amapá State (Brazil).

Materials and Methods

Twenty specimens of *Mesonauta festivus* were collected between November 2018 and March 2019 in Piririm river from the district of Corre Água in the municipality of Macapá, in the state of Amapá Brazil (0°45'7.44" N, 50°49'57.20" W). The present study was approved by the UFRA Ethics Committee for the Experimental Use of Animals (CEUA/UFRA n. 013/2014), and was registered in the IBAMA Biodiversity Authorisation and Information System (SISBIO/ICMBIO licence number 50376-1).

The fish were captured using gillnets and cast nets, with an average body length of 8.53 ± 2.24 cm and an average weight of 18.02 ± 2.81 g. The specimens were placed alive in coolers containing water from the natural habitat, which was aerated continuously, for transportation to the Laboratory of Animal Morphophysiology and Health at Amapá State University (LABMORSA/UEAP) in Macapá, where they were kept in 60-L glass aquariums. Prior to necropsy, each fish was either anaesthetised with tricainamethanesulfonate (MS222 SIGMA) at a concentration of 50 mg L^{-1} (Topic Popovic et al., 2012) or euthanised by neural myelotomy. The specimens are examined under a stereomicroscope to determine the presence of cysts or isolated parasites. Small samples were extracted from the urinary bladder for examination under a light microscope, with small fragments being placed on microscope slides and covered with a coverslip to determine the presence of parasitic myxospores. These samples were used to produce photomicrographs using a Zeiss AxioScope A1 microscope with Nomarski differential interference contrast attached to a Zeiss AxioCam 512 camera equipped with Zen 2.3 colour software, for the measurement of the fresh samples, in the Carlos Azevedo Research Laboratory at the Federal Rural University of Amazonia (ISPA/UFRA) in Belém. This procedure was used to determine the length (L) and width (W) of the myxospore body, and the length (PL) and width (PW) of the polar capsules.

Fragments of the urinary bladder were processed using standard histological techniques for embedding in paraffin, and were stained with hematoxylin and eosin (H&E) and Zielh-Neelsen. Fragments containing myxospores of eukaryote microparasites were collected for molecular analyses, for which they were stored in a freezer in 80% ethanol, following the protocol of Matos et al. (2018) and Silva et al. (2018).

Extraction and sequencing of the DNA

The DNA of the parasites was extracted using the commercial PureLink® Genomic DNA kit (Invitrogen, USA), following the maker's specifications. A partial sequence of the 18S rDNA was amplified by PCR using universal eukaryote primers ERIB1 and ERIB10 (Barta et al., 1997), for the first amplification, and the primers MC5 and MC3 (Molnár, 2002) for the second round of amplification. The PCR was run in a final volume of 25 µl using Master Mix Taq polymerase (PROMEGA, Madison, USA), with the parameters of the first amplification cycle being adjusted as follows: initial denaturation at 94°C for 5 minutes, followed by 36 cycles at 94°C for 30 seconds, 60°C for 40 seconds, and 72°C for 45 seconds, with a final extension at 72 °C for 5 min. The second amplification cycle was adjusted as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 60 seconds, polymerisation at 52°C for 60 seconds, and extension at 72°C for 2 minutes, with a final extension at 72 °C for 5 min.

The PCR products were visualised by electrophoresis in agarose gel and purified using the ExoSAP-IT® PCR product cleanup kit (GE Life Sciences), to remove the excess primers and dNTPs, prior to sequencing with Big Dye Terminator v3.1 (Applied Biosystems, Foster City, CA) in an ABI 3100sequencer (Applied Biosystems).

Phylogenetic analyses

The partial sequences were assembled in the Codon Code Aligner software (CodonCode Corporation, Dedham, Massachusetts) and compared with the myxozoan sequences deposited in GenBank using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI). The multiple 18S rDNA sequences were aligned in BioEdit (Hall, 1999), with ambiguous and non-amplified regions being omitted (Holzer et al., 2007; Gunter et al., 2009).

The Bayesian Inference (BI) was based on the nucleotide substitution models selected using the Bayesian Information Criterion (BIC), which was run in jModeltest 2.1.10 (Posada, 2008). The GTR + G model was identified as the best nucleotide substitution model for the 18S rDNA datasets. The Markov Chain Monte Carlo (MCMC) algorithm was run in BEAST v.1.8.4 (Drummond et al., 2012) with 10,000,000 generations sampled every 10,000 steps (Brooks et al., 2011). The results of the BI were used to reconstruct the phylogenetic tree based on 40 myxozoan sequence. The 18S rDNA sequence of *Tetracapsuloides bryosalmonae* was included as the outgroup. The matrix of *p* distances for the *Sphaerospora* species was compiled in Geneious v8.0.5, based on the BI alignment.

Results

The presence of polysporic plasmodiums in the urinary bladder of *M. festivus* was observed in two of the twenty samples examined, representing a prevalence of 10%. Histological analysis of the gallbladder showed the histozoic behavior of myxozoans, with their association with the internal epithelium of the urinary bladder (Figure 1).

The myxospores are slightly flattened anteroposterior, straight and prominent, parallel to the polar capsules, with binucleated sporoplasm and the posterior end of the serrated valves, resulting in longitudinal grooves on the surface, which extend outwards. No caudal filament was observed, and the two polar capsules were equal in size, ranging in shape from piriform to ovoid, located at the posterior end of the valves (Figure 2). The myxospore morphology was compatible with the description of the genus *Sphaerospora* (Eszterbauer et al., 2013; Patra et al., 2018)

The histozoic myxospores had an average length of 5.3 ± 0.2 µm ($n = 30$) and a width of 7.0 ± 0.7 µm ($n = 25$). The capsules are identical and have an average length of 2.5 ± 0.2 µm and a width of 1.8 ± 0.2 µm ($n = 20$). The polar filament is helically wound, with 4-5 spirals, converging with the apex of the myxospores (Table 1).

Taxonomic summary

- Phylum Cnidaria Hatschek, 1888
- Class Myxosporia Bütschli, 1881
- Order Bivalvulida Schulman, 1959
- Suborder Variisporina Lom and Noble, 1984
- Family Sphaerosporidae Davis, 1917
- Genus *Sphaerospora* Thélohan, 1892

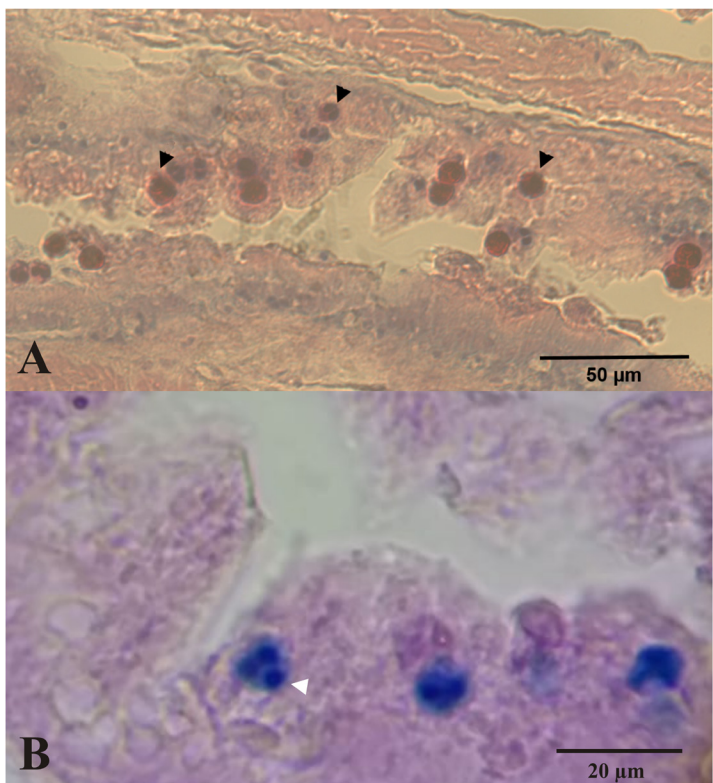


Figure 1. Photomicrograph of *Sphaerospora festivus* n. sp. in histological sections of the urinary bladder of *Mesonauta festivus* from Piririm River in the municipality of Macapá, Amapá State (Brazil). (A) Hematoxylin-Eosin staining, black arrows = myxospores; (B) Ziehl-Neelsen staining, white arrows = polar capsules.

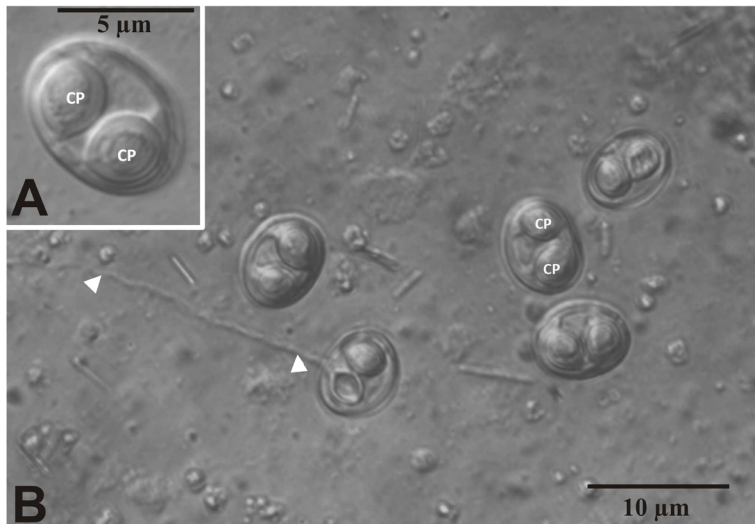


Figure 2. Differential interference microscopy of the myxospores of *Sphaerospora festivus* n. sp. observed in the urinary bladder of *Mesonauta festivus*. (A) Detail of the myxospore and polar capsules (PC); (B) View of myxospore with one of the polar filaments extruded (white arrow).

Species *Sphaerospora festivus* n. sp. (Figure 3)

Host: *Mesonauta festivus* (Heckel, 1840)

Infection site: internal epithelium of the urinary bladder.

Type locality: Piririm River, district of Corre Água, municipality of Macapá, State of Amapá, Amazon, Brazil (0°45'7.44" N, 50°49'57.20" W).

Prevalence: Two out of twenty fish analyzed (10%).

Table 1. Morphometric characteristics the *Sphaerospora* species parasites of urinary trate. Information is provided on the host species, locality, infection site, and the dimensions of the myxospores and polar capsules (in µm).

<i>Sphaerospora</i> species	Host	Locality	Infection site	Parameters					References
				L	W	CL	CW	Coils	
<i>Sphaerospora festivus</i> n. sp.	<i>Mesonauta festivus</i>	Brazil	UB	5.3 ± 0.2 (5.2-5.6)	7.0 ± 0.7 (6.3-7.7)	2.5 ± 0.2 (2.3-2.8)	1.8 ± 0.2 (1.6-2.0)	4-5	Present study
<i>S. bliccae</i>	<i>Blicca bjoerkna</i>	Hungary	KT	10.4 (9.7-11.1)	9.6 (9.3-10.1)	4.3 (3.9-4.7)	3.9 (3.8-4.2)	4-5	Patra et al. (2018)
<i>S. dentata</i>	<i>Scardinius erythrophthalmus</i>	Czech Republic	KT	9.7 (9.4-9.8)	9.4 (9.4-9.6)	4.0 (3.9-4.2)	3.5 (3.2-3.6)	4-5	Patra et al. (2018)
<i>S. gutta</i>	<i>Scardinius erythrophthalmus</i>	Czech Republic	KT	6.4 (6.2-6.6)	6.5 (6.3-6.7)	2.5 (2.3-2.6)	2.3 (2.2-2.5)	4-5	Patra et al. (2018)
<i>S. diversa</i>	<i>Squalius cephalus</i>	Czech Republic	KT	6.5 (6.3-6.7)	7.5 (7.0-7.9)	2.5 (2.3-2.7)	2.4 (2.3-2.5)	3-4	Patra et al. (2018)
<i>S. squalii</i>	<i>Squalius cephalus</i>	Czech Republic	KT	6.5 (6.1-7.1)	6.5 (6.2-7.0)	3.2 (3.0-3.4)	2.8 (2.7-3.0)	3-4	Patra et al. (2018)
<i>S. abrami</i>	<i>Abramis brama</i>	Czech Republic	KT	13.4 (12.2-14.1)	14.6 (14.0-15.3)	7.1 (6.6-7.6)	5.9 (5.4-7.2)	2-3	Patra et al. (2018)
<i>S. rutili</i>	<i>Rutilus rutilus</i>	Czech Republic	KT	8.8 (8.3-9.8)	8.9 (8.8-9.3)	3.7 (3.2-4.2)	3.2 (2.7-3.5)	3-4	Patra et al. (2018)
<i>S. olsoni</i>	<i>Atherinops offinis</i>	USA	KT	6.0 ± 0.5 (5.2-7.1)	5.8 ± 0.1 (5.7-6.0)	2.0 ± 0.1 (1.8-2.4)	2.0 ± 0.1 (1.8-2.4)	3-4	Sanders et al. (2015)
<i>S. sparidarum</i>	<i>Dentex dentex</i> and <i>Sparus aurata</i>	Western mediterranean	KT	5.08 ± 0.17 (5.88-6.18)	6.02 ± 0.42 (5.0-7.1)	2.01 ± 0.18 (2.6-3.5)	2.75 ± 0.27 (2.4-3.2)	6-7	Sitjà-Bobadilla & Alvarez-Pellitero, (2001)
<i>S. epinephelli</i>	<i>Epinephelus malabaricus</i>	Indian Ocean	KT	8.7 ± 0.4 (7.8-10.0)	8.2 ± 0.5 (7.0-9.5)	3.7 ± 0.3 (2.9-4.4)	3.7 ± 0.3 (2.9-4.4)	6-7	Supamattaya et al. (1991)
<i>S. angulata</i>	<i>Carassius auratus</i>	Czech Republic	KT	6-53 (5-85-7-5)	6-39 (5-54-7-12)	3-14 (2-8-3-5)	2-41 (2-4-2-7)	4-5	Holzer et al. (2013)
<i>S. renicola</i>	<i>Scomber scombrus</i>	Czech Republic	KT	7.3 (6.0-8.0)	7.2 (6.4-8.3)	1.7-2.3	1.3-1.6	4-5	Dyková & Lom (1982)

Abbreviations: L = length of the myxospore; W = width of the myxospore; CL = Capsule Length; CW = Capsule Width; UB = urinary bladder; KT = kidney tubules.

Specimens: Two slides of the histological section (5 µm thick) stained with H&E and Zielh-Neelsen, containing myxospores of the new myxosporean species were deposited in the Zoology Museum of the Brazilian National Amazonian Research Institute (INPA) in Manaus, Amazonas, Brazil, under the catalogue number INPA 067/20.

GenBank accession number: MW370523

Etymology: The species epithet refers to that of the host species.

Phylogenetic analysis

The analysis of the 18S-rRNA of *Sphaerospora festivus* n. sp. rendered a partial sequence of 2,032 base pairs (bps). The BLASTn search did not identify any identical or near-identical 18S-rRNA sequences in GenBank. The phylogenetic tree generated by the Bayesian Inference (BI) was composed of two major clades (Figure 4). Clade I include species of the genera belonging to several families of myxozoans, including some species of *Sphaerospora*, while clade II, which had strong support, was composed exclusively of species of the genus *Sphaerospora*. The BI also identified clusters of *Sphaerospora* organized by the group of hosts and, in part, with the type of habitat occupied by the hosts. The genetic distances between the partial sequence of the 18S rDNA of *Sphaerospora festivus* and other sequences of representatives of the genus *Sphaerospora* registered in the NCBI database was greater than 24% (Table S1).

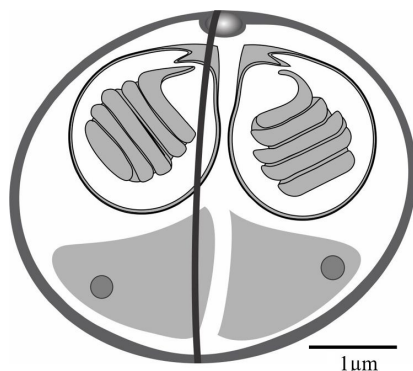


Figure 3. Diagram of the subspherical myxospore with prominent sutures (in the transverse section) showing the two subspherical capsules and sporoplasm.

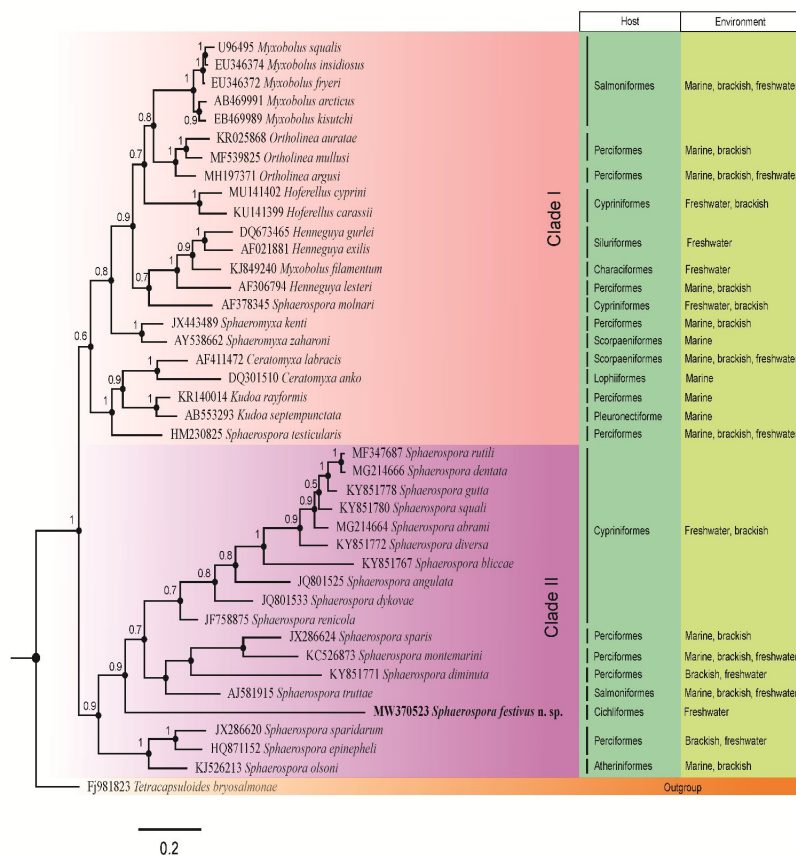


Figure 4. Bayesian Inference tree derived from the 18S-rDNA sequences of species of seven myxozoan genera, all parasites of marine, estuarine or freshwater fish. The nodal support is based on the posterior probabilities of the BI.

Discussion

The genus *Sphaerospora* currently includes approximately 102 valid species, which have been found parasitising amphibians and fish in different regions around the world (Jirků et al., 2007; Patra et al., 2018). These myxozoans are parasites of the urinary bladder and its ducts, as well as the gills, tegument, urethra, gonads, and intestine (Patra et al., 2018). The *Sphaerospora* species found in fish are typically present in hosts that occur in marine or estuarine environments (Jirků et al., 2007; Bartošová et al., 2011; Holzer et al., 2013; Patra et al., 2018; Casal et al., 2019).

Despite the clustering of most species of *Sphaerospora* analyzed in a monophyletic clade. The inclusion of *S. molnari* and *S. testicularisin* in the clade formed by different families of myxozoa, reflects the fact that the phylogeny of the genus *Sphaerospora* has a paraphyletic factor (Holzer et al., 2007; Fiala, 2006). Some species of *Sphaerospora* have also grouped according to their host species, due to the phylogenetic proximity of these hosts and the strict coevolutionary relationships of the parasites with their hosts (Patra et al., 2018; Holzer et al., 2018; Zhang et al., 2018).

The BI placed *Sphaerospora festivus* n. sp. in clade II, which confirms the hypothesis that the new species belongs to the genus *Sphaerospora*, as indicated by its morphological traits. The genetic distances between *Sphaerospora festivus* n. sp. and its counterparts indicates a high level of divergence, which is consistent with the marginal position of the new species within clade II, which suggests the formation of a lineage of *Sphaerospora* species associated with cichlid hosts, since the species of this genus tend to have a strong coevolutionary relationship with their host strains, as shown here and in previous studies (Patra et al., 2018; Zhang et al., 2018).

The fact that *Sphaerospora festivus* n. sp. having a freshwater host would also imply a degree of similarity with the species of *Sphaerospora* that infect freshwater and estuarine cypriniforms, although this was not supported by the analysis, with *Sphaerospora festivus* n. sp. in a relatively basal position compared to the more derived freshwater group. This may indicate that this species diverged long before the species of *Sphaerospora* that parasitize cypriniforms in Europe and Asia and would be more consistent with a long evolutionary history of the *Sphaerospora* lineage in South America.

The majority of *Sphaerospora* species are parasites of marine or estuarine fish hosts, while some others are associated with fish that migrate between the marine and freshwater environments (Jirků et al., 2007; Patra et al., 2018). The phylogenetic analysis presented here indicate the divergence of an apparently unique freshwater lineage which includes only *Sphaerospora festivus* n. sp. This would be consistent with the hypothesis that the radiation of the freshwater *Sphaerospora* lineage in South America was initiated by marine incursions. The Cichlidae is an essentially marine family, although it also has many freshwater representatives, such as *M. festivus* in the Amazon basin (Friedman et al., 2013). In this case, the ancestral *Sphaerospora* may have been introduced to the freshwater environment during marine incursions by its ancestral fish host, as observed in *Ceratomyxa* and monogenean parasites and their respective fish hosts, that is cichlids and *Plagioscion* spp. (Boeger & Kritsky, 2003; Friedman et al., 2013; Zatti et al., 2018), subsequently adapting to the freshwater conditions.

The typical co-evolutionary relationship between *Sphaerospora* species and their hosts may have been decisive to the success of this process, given that the diversification of this myxozoan is driven primarily by that of its hosts (Patra et al., 2018). It is not possible, at the present time, to provide divergence times for the new species, given the sparse myxozoan fossil record, which would be necessary for the calibration of the molecular clock.

Conclusions

This study presents the description of a new species of *Sphaerospora* found parasitising the urinary bladder of the festive cichlid, *M. festivus*. This is the first record of the occurrence of a *Sphaerospora* species in the Amazon region, and the study also contributes to the understanding of the biodiversity of the Neotropical region, and in particular, that of the Brazilian Amazon basin. The results of the present study indicate the existence of a *Sphaerospora* lineage adapted to the freshwater environment of the Amazon region through its association with a specific cichlid host.

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Supplementary Material

Supplementary material accompanies this paper.

Supplement table 1 - Genetic distances in percentages between of *Sphaerospora* species analysed in the present study.

This material is available as part of the online article from <http://www.scielo.br/RBPV>

Supplement 1. Genetic distances in percentages between of *Sphaerospora* species analysed in the present study.

<i>Sphaerospora</i> species	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	
[1] <i>Sphaerospora festivus</i> n. sp.	-																					
[2] <i>S. olsoni</i>	30.0	-																				
[3] <i>S. spardarum</i>	29.2	69.0	-																			
[4] <i>S. epinepheli</i>	28.6	68.9	72.2	-																		
[5] <i>S. testicularis</i>	29.5	50.6	52.0	47.7	-																	
[6] <i>S. molnari</i>	29.6	49.4	51.0	48.9	56.4	-																
[7] <i>S. kenti</i>	31.6	53.7	55.6	52.6	62.3	62.3	-															
[8] <i>S. zaharoni</i>	30.7	54.4	56.0	52.5	61.6	61.4	78.3	-														
[9] <i>S. diminuta</i>	27.1	32.2	39.0	37.6	37.4	36.3	39.0	39.5	-													
[10] <i>S. truttae</i>	26.6	47.1	49.5	47.0	44.3	44.6	47.8	48.2	41.5	-												
[11] <i>S. sparis</i>	26.8	36.6	40.4	39.9	37.5	37.9	37.5	38.2	39.9	48.3	-											
[12] <i>S. motemarini</i>	26.1	35.9	39.9	38.2	37.4	37.5	37.9	38.5	37.4	46.5	61.2	-										
[13] <i>S. bliccae</i>	24.9	37.3	36.1	36.9	34.9	32.8	35.1	34.6	34.2	41.8	41.9	40.6	-									
[14] <i>S. diversa</i>	25.6	36.8	39.4	39.0	35.2	35.4	35.7	36.3	37.8	45.2	48.9	49.9	46.0	-								
[15] <i>S. abrami</i>	24.4	35.3	35.6	35.1	28.9	32.0	31.2	31.6	30.6	41.0	44.4	43.9	44.3	75.1	-							
[16] <i>S. squalii</i>	25.5	38.5	39.6	39.1	34.7	35.0	34.8	35.7	38.4	45.9	49.9	50.5	47.9	78.0	89.0	-						
[17] <i>S. gutta</i>	24.5	37.8	39.0	39.2	34.3	34.7	34.5	35.4	37.6	45.6	49.3	48.3	46.2	77.0	89.1	89.5	-					
[18] <i>S. rutili</i>	24.2	37.8	39.1	39.1	34.4	35.2	34.9	35.6	37.9	45.5	48.5	47.7	46.1	76.3	88.2	88.3	93.4	-				
[19] <i>S. dentata</i>	25.0	37.6	38.5	38.5	33.7	35.3	35.3	35.7	37.1	44.9	46.8	47.2	45.8	75.1	86.7	87.5	93.1	97.7	-			
[20] <i>S. angulata</i>	26.9	39.6	39.4	38.6	38.1	37.7	38.8	39.5	39.6	47.2	45.7	48.1	45.4	55.9	53.0	59.5	57.4	56.1	55.5	-		
[21] <i>S. dykoveae</i>	28.3	40.2	40.6	40.7	40.3	38.6	40.2	41.0	40.7	48.9	48.7	48.5	47.2	56.8	51.8	59.0	56.2	55.6	54.3	63.5	-	