Phylogeny, ultrastructure, histopathology and prevalence of *Myxobolus oliveirai* sp. nov., a parasite of *Brycon hilarii* (Characidae) in the Pantanal wetland, Brazil

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This paper presents the morphological, histological and ultrastructural characteristics of Myxobolus oliveirai *sp. nov., a parasite of the gill filaments in* Brycon hilarii *from the Brazilian Pantanal. Out of 216* B. hilarii *specimens examined (126 wild and 90 cultivated), 38.1% of wild specimens (n = 48) were infected. The parasites form elongated plasmodia primarily in the tip of gill filaments, reaching about 3 mm in length. A thorough comparison with all the* Myxobolus *species described from South American hosts, as well as nearly all the* Myxobolus *species described so far is provided. Partial sequencing of the 18S rDNA gene revealed a total of 1,527 bp. The* Myxobolus *species parasite of* B. hilarii *did not match any of the Myxozoa available in GenBank. In the phylogenetic analysis,* M. oliveirai *sp. nov. composed a monophyletic group with eight other species: five species of* Myxobolus *parasites of mugilid fishes, two parasites of pangasiid and one of centrarchid. Infection prevalence values of the parasite revealed no significant differences between wet and dry seasons or between males and females. The importance of the infection to the farming of the host species is emphasized.*

Key words: Myxozoa - Myxosporea - histology - molecular analysis - fish - piraputanga

Brycon hilarii (Valenciennes, 1950) (Syn. *Brycon microlepis* Perugia, 1897) is an endemic characid species of the upper Paraguay Basin (Resende 2003), popularly known as piraputanga. It is highly appreciated in central Brazil (especially in the states of Mato Grosso and Mato Grosso do Sul) (Resende 2003) and is a very valuable species, fetching high market prices. *B. hilarii* has recently been introduced into aquaculture in several regions of Brazil, with a production of 633,000 kg on fish farms in 2006 (IBAMA 2008), demonstrating the considerable potential of this species to this activity.

Myxosporean parasites are among the most important fish pathogens (Schmahl et al. 1989) and more than 2,300 species have been reported to infect fish (marine and/or freshwater fish) in either their natural environment or on farms (Adriano et al. 2006, 2009a, b, Feist & Longshaw 2006, Eiras et al. 2008, Azevedo et al. 2009). The *Myxobolus* Butschli, 1882, is the genus with the greatest number of species (approximately 790 valid species) (Eiras et al. 2005, Lom & Dyková 2006) and many of these species are reported as pathogenic to fish (Kent et al. 2001, Feist & Longshaw 2006). In South America, 30 *Myxobolus* species have been reported (Azevedo et al. 2010, Eiras et al. 2010), but none have been reported to infect fish from the genus *Brycon*.

As part of ongoing research on the characteristics of myxosporea parasites of freshwater fish in Brazil, the present paper describes a new species of *Myxobolus* found to infect wild specimens of piraputanga in the Brazilian Pantanal wetland using morphological, histological, ultrastructural and molecular phylogeny analyses.

MATERIALS AND METHODS

A total of 126 specimens of wild *B. hilarii* caught in the Pantanal Mato-Grossense in central Brazil and 90 farmed specimens from three fish farms in the state of São Paulo (SP) were examined. The wild fish were examined in two seasons: spring (flood period) of 2001-2004 (n = 45) and autumn (drought period) of 2003-2005 and 2008 (n = 81). The fish were captured at three locations: in the Aquidauana, Miranda and Paraguay Rivers in the southern region of the Pantanal (n = 34), in rivers and lakes of the Pantanal National Park in the central region (n = 60) and in the Cuiabá and Manso Rivers in the municipality of Nobres in the northern region (n = 32). The fish from the Miranda, Aquidauana and Paraguay Rivers were caught in the spring of 2001 and 2002 as well as the autumn of 2003, whereas those from the

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Pantanal National Park and the municipality of Nobres were caught in the spring of 2003 and 2004 as well as the autumn of 2004, 2005 and 2008. The cultivated specimens were examined in the summer and winter of 2008. Immediately after collection, the live fish were transported to the field laboratory mounted nearby, where they were measured, weighed and necropzied. Cysts were removed from the gill filaments of different piraputanga specimens and examined under a light microscope. Spore dimensions (µm) were expressed as mean ± standard deviation. Smears containing free spores were stained with Giemsa solution and mounted in a low-viscosity mounting medium as permanent slides. For histological analysis, fragments of infected organs were fixed in 10% buffered formalin, embedded in paraffin, cut into serial sections (4 µm in thickness) and stained with haematoxylin/eosin and Sirius red. For transmission electron microscopy, plasmodia were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 12 h, washed in glucose-saline solution for 2 h and post-fixed in OsO_4, all at 4ºC. After dehydration in an acetone series, the material was embedded in EMbed 812 resin. Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined in an LEO 906 electron microscope operated at 60 kV.

For molecular analysis, plasmodia were removed from the host tissue and fixed in ethanol. After rupturing the plasmodia with the aid of a needle, the contents were collected in a 1.5 mL microcentrifuge tube. DNA was extracted using the Wizard® Genomic DNA Purification kit (Promega, USA), following the manufacturer's instructions. DNA content was determined using the NanoDrop 2000 spectrophotometer (Thermo Scientific) at 260 nm. Polymerase chain reaction (PCR) was carried out in a final volume of 25 µL, which contained 10-50 ng of extracted DNA, 1X Taq DNA polymerase buffer (Invitrogen), 0.2 mmol of dNTP (Invitrogen), 1.5 mmol of MgCl2, 0.2 pmol of each primer (Invitrogen), 0.25 µL (1.25 U) of Taq DNA polymerase (Invitrogen) and ultra pure (MilliQ) water. The Eppendorf AG 22331 Hamburg Thermocycler was used. An ~1600 bp 5' fragment of the SSU rDNA gene was amplified using the primers MX5-MX3 (Andree et al. 1999) in the following manner: an initial denaturation step at 95ºC for 5 min followed by 35 cycles of denaturation (95ºC for 60 s), annealing (62ºC for 60 s) and extension (72ºC for 120 s), finished with an extended elongation step at 72ºC for 5 min. PCR products were submitted to electrophoresis on 1% agarose gel (BioAmerica) in a Tris-Borate-EDTA buffer (0.045 MTris-borate, 0.001 M EDTA pH 8.0), stained with ethidium bromide and analyzed in a FLA-3000 (Fugi) scanner. The size of the amplified fragments was estimated by comparisons with the 1 kb DNA Ladder (Invitrogen).

Purified PCR products were cloned in pCR®4-TOPO® vectors from the TOPO-TA Cloning® kit for sequencing (Invitrogen). A single clone was sequenced using MX5-MX3 and MC5-MC3 (Eszterbauer 2004) primer pairs with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) in an ABI 3730 DNA sequencing analyzer (Applied Biosystems™).

A standard nucleotide-nucleotide Basic Local Alignment Search Tool (BLAST) (blastn) search was conducted (Altschul et al. 1997). The sequence of the *Myxobolus* species obtained from *B. hilarii* was aligned with sequences obtained in the GenBank using DAMBE (Xia & Xie 2001). To evaluate the position of the *Myxobolus* species obtained from *B. hilarii* in relation to other *Myxobolus* spp, phylogenetic analyses were conducted using the phylogenetic methods neighbor-joining (NJ) and maximum parsimony (MP) [MEGA 4.0 programme (Tamura et al. 2007)] and maximum likelihood (ML) [MEGA 5.0 (programme in test phase)]. The Kimura 2-parameter (K2P) evolution sequence model was used in the analysis. Bootstrap analysis (1,000 replicates) was employed to assess the relative robustness of the branches of the NJ, MP and ML trees using the MEGA 4.0 and 5.0 programmes. The distance analyses were performed using the K2P model conducted using the MEGA 4.0 programme (Tamura et al. 2007). The species *Ceratomyxa seriolae* and *Ceratomyxa shasta* were used as outgroups in the phylogenetic analyses.

The effects of season, study location and sex of the host on the prevalence of the parasite were assessed using the χ^2 test, with the level of significance set at $p < 0.05$.

RESULTS

Among the 126 wild and 90 cultivated specimens of piraputanga (a total of 216) examined in the present study, 22.2% (n = 48) had plasmodia of an unknown parasite from the genus *Myxobolus*. The prevalence was of 38.1% in the wild specimens and 0% in the cultivated specimens. In the wild specimens, the prevalence of the parasite varied significantly with regard to study location ($\chi^2_2 = 28.79$; $p = 0.00$), with the lowest prevalence found in southern region of the Pantanal (2.9%). No significant difference was found between the central (43.3%) and northern regions (65.6%) ($\chi^2_1 = 4.15$; $p = 0.04$). The parasite was found in both seasons studied (prevalence of 33.3% in spring and 40.7% in autumn) and the variation between seasons was not significant ($\chi^2_1 = 0.63$; $p = 0.41$). Regarding the sex of the host, the prevalence was 32.2% for female specimens and 16.2% for males; this difference was also not significant ($\chi^2_1 = 3.07$; $p = 0.07$). Sex was not defined in seven infected specimens.

Myxobolus oliveirai sp. nov.
(Figs 1-5)

Plasmodia elongated in shape and measuring up to 3 mm were found primarily in the distal extremity of the gill filaments (Fig. 1A). Histological and ultrastructural analysis revealed that the development of the plasmodia induced the expansion of the gill filaments and the compression caused thinning of the tissue of the distal extremity, which exhibited only a delicate layer of cells (Fig. 1C). In the proximal extremity, growth of the plasmodia occurred by invaginations in the filament tissue (Figs 1C, 2A). The plasmodia were surrounded by a well-defined capsule of host connective tissue, with distinct delicate and interlaced collagen fibres (Figs 2B, 3A). The plasmodium wall consisted of a single layer that was continuous with pinocytic canals extending into the granular layer of the plasmodium ectoplasm (Fig. 3A, C).

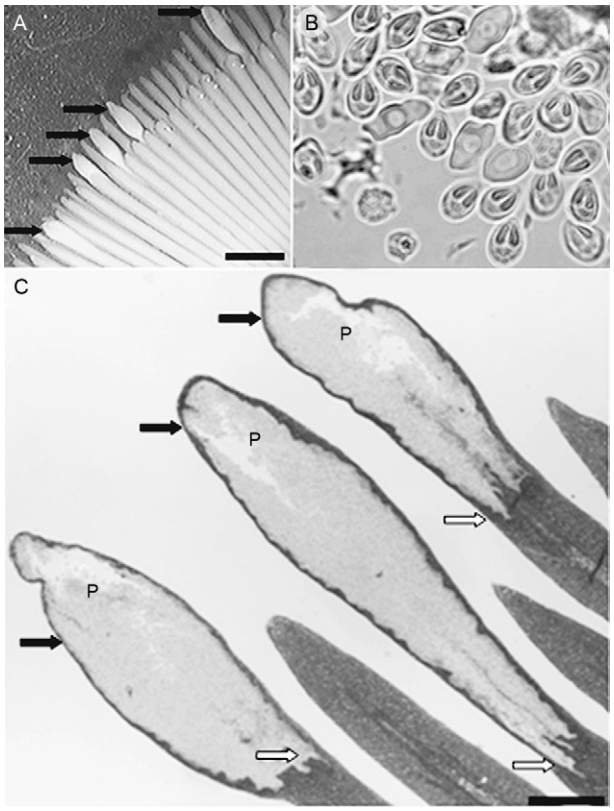

Fig. 1: light photomicrographs of *Myxobolus oliveirai* sp. nov., parasite of *Brycon hilarii*. A: formalin-fixed gill filaments showing plasmodia in the distal extremity (arrows). Bar = 3 mm; B: mature fresh spores. Bar = 10 µm; C: histological section showing plasmodia (P) in the distal extremity. Note the expansion of the gill filaments with compression and thinning of the tissue of the extremity distal (black arrows) as well as invaginations in the filament tissue in the proximal extremity (white arrows). Bar = 20 µm. Sirius red staining.

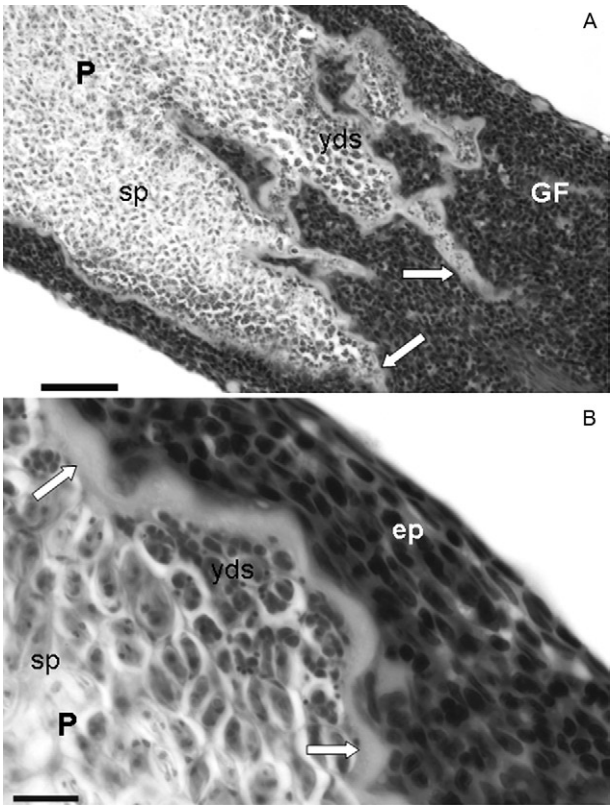

Fig. 2: light photomicrographs of histological section of gill filaments (GF) of *Brycon hilarii* parasitized by *Myxobolus oliveirai* sp. nov. (A) showing a plasmodium (P) with spores in central area (sp), young developmental stages (yds) in the plasmodial periphery and invaginations in the filament tissue in the proximal extremity (arrows). Bar = 50 µm; B: histological section showing a connective tissue capsule (arrows) surrounding P. Note yds in sp in the central area. Bar = 10 µm. Sirius red staining; ep: epithelium.

The plasmodia exhibited development with different sporogenic stages, such as generative cells and disporoblastic pansporoblasts (Fig. 3C) along the periphery of the plasmodia (occurring in a thin layer of not more than 40 µm), followed by a layer containing immature spores and mature spores occurring in the internal region (Figs 2-4). The spores were pear-shaped in the frontal view (Figs 1B, 5A), measuring 11.2 ± 0.4 µm in length, 7.4 ± 0.5 µm in width and 4.6 ± 0.6 µm in thickness. The polar capsules were elongated in shape and equal in size, measuring 5.6 ± 0.2 µm in length and 2.3 ± 0.2 µm in width. The anterior ends of polar capsules were close to one another and the polar filaments exhibited 6-8 turns arranged perpendicular to the longitudinal axis of the capsules (Figs 4C, 5A). A few, small sporoplasmosomes were found in the sporoplasm (Fig. 4A) and two nuclei were discernible when stained with Giemsa (Fig. 5A).

In the molecular analysis, the specific primer pair MX5-MX3 successfully amplified an approximately 1,600-bp fragment of the 18S rDNA gene in the spores obtained from plasmodia found infecting the gill filaments of *B. hilarii*. The BLAST search using the partial 18S rDNA sequence data (1,527 bp) of the *Myxobolus* species parasite of *B. hilarii* did not match any of the Myxozoa available in the GenBank.

In the phylogenetic analyses, the *Myxobolus* species clustered into three distinct lineages. *Myxobolus spirosulcatus* represented the basal group and *Myxobolus acanthogobii* clustered as the sister group of the remaining species of *Myxobolus* (Fig. 6). These remaining species clustered in a monophyletic group composed of numerous species divided into four smaller clades (A-D) (Fig. 6). *M. oliveirai* sp. nov. formed a monophyletic unit with eight other *Myxobolus* species in Clade C: five species parasites of hosts from the family Mugilidae; two parasites of Pangasiidae hosts and one parasite of Centrarchidae. Mean genetic divergence within Clade C (Table) was 20.4%. In this clade, the smallest distance in reference to *M. oliveirai* sp. nov. was with *Myxobolus hakyi* (16.6%) and the largest was with *Myxobolus osburni* (28.6%).

Type host - *B. hilarii* (Valenciennes, 1850) (Characidae, Bryconinae).

Etymology - The specific epithet name is in homage to Ricardo Afonso Torres de Oliveira, research assistant of the laboratory of Saúde e Bem Estar dos Peixes of the

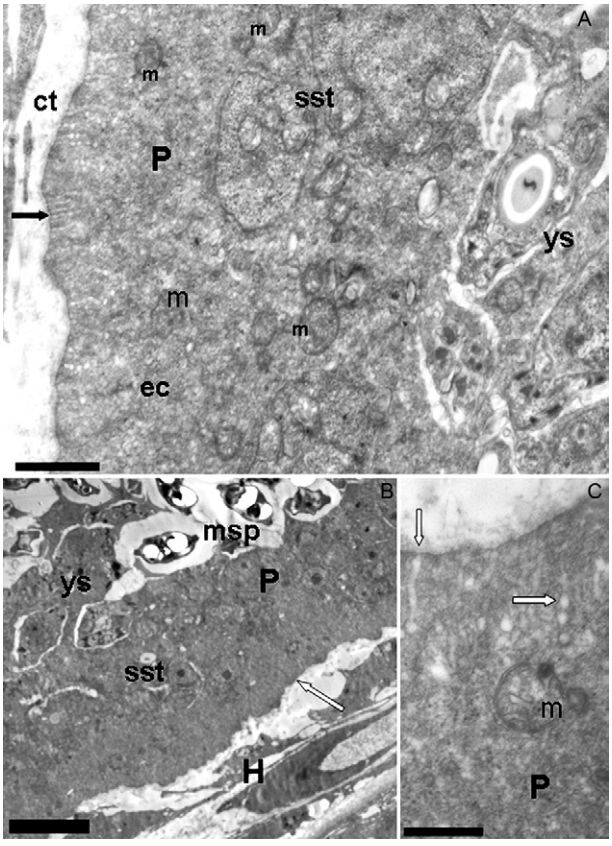

Fig. 3: electron micrographs of *Myxobolus oliveirai* sp. nov. parasite of gill filaments in *Brycon hilarii*. A-C: host-parasite interface; A: showing the capsule of connective tissue (ct) surrounding the plasmodium (P). Note the ectoplasm area (ec) showing pinocytotic canals (arrow) and mitochondria (m), sporogenic stages (sst) and an inner layer of young spores (ys). Bar = 1 µm; B: smaller magnification showing the host-parasite interface (arrow), sst, ys and transversal sections of mature spores (msp). Bar = 5 µm; C: detail of P showing pinocytotic canals terminating in pinocytotic vesicles (arrows) and m. Bar = 0.5 µm; H: host.

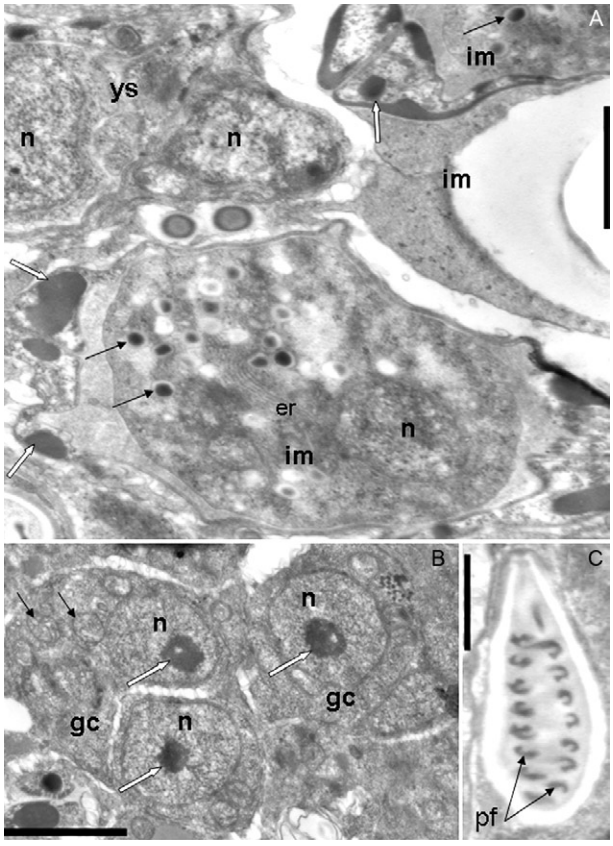

Fig. 4: electron micrographs of *Myxobolus oliveirai* sp. nov. parasite of gill filaments in *Brycon hilarii*. A: plasmodium showing transversal section of young spore (ys) with two nuclei (n) and immature spores (im) showing sporoplasmossomes (thin arrows), sporoplasm nucleus and valve-forming material (white arrows). Bar = 1 µm; B: generative cells (gc) with n containing conspicuous nucleolus (white arrows) and mitochondria (thin arrows). Bar = 2 µm; C: longitudinal section of a polar capsule with its polar filaments (pf) sections. Bar = 1 µm.

Centro Nacional de Pesquisa e Conservação de Peixes Continentais of Instituto Chico Mendes de Conservação da Biodiversidade, who, in recent years, has provided great support to the fieldstrip of our research group.

Type data and depository - Pantanal Mato-Grossense (Aquidauana, Cuiabá, Miranda and Paraguay Rivers) in the Paraguay basin, Brazil.

Specimens deposited - Slides with stained spores (syntype) have been deposited in the collection of the Museum of Natural History, Institute of Biology, State University of Campinas, SP, Brazil (accession ZUEC 28). The 18S rDNA sequence was deposited in GenBank under the accession HM754633.

Prevalence - Fourty-eight of 216 *B. hilarii* examined (22.2%) had plasmodia of an unknown parasite from the genus *Myxobolus* - 48/126 wild specimens (38.1%) and 0/90 (0%) cultivated specimens.

Site of infection - Distal extremity of the gill filaments.

DISCUSSION

M. oliveirai sp. nov. was compared with all *Myxobolus* species previously reported in fish of South America (Azevedo et al. 2010, Eiras et al. 2010) and other geographic regions (Eiras et al. 2005). Among the South American species, *M. oliveirai* sp. nov. resembles the body shape of *Myxobolus cordeiroi* (Adriano et al. 2009a), a parasite of *Zungaro jahu*; *Myxobolus chondrophilus* (Nemeczek, 1926), reported to infect *Sardinella anchovina*; *Myxobolus associatus* (Nemeczek, 1926), a parasite of *Leporinus mormyrops*; *Myxobolus cunhai* (Penido, 1927), a parasite of *Pygocentris piraya*; *Myxobolus serrasalmi* (Walliker 1969), a parasite of *Serrasalmus rhobeus*; *Myxobolus maculatus* (Casal et al. 2002), a parasite of *Metynnis maculates*; *Myxobolus* sp. (Walliker 1969), a parasite of *Colossoma bidens*, and *Myxobolus* sp. (Walliker 1969), a parasite of *Serrasalmus* sp. Among all these species, however, only *M. cordeiroi* and the *Myxobolus* sp. parasite of *Serrasalmus* have a similar size to that of *M.*

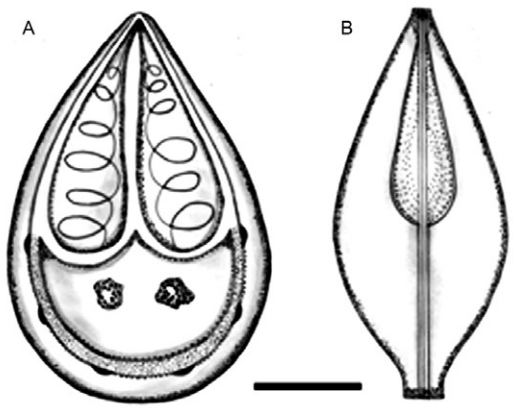

Fig. 5: schematic representation of mature spores of *Myxobolus oliveirai* sp. nov. A: frontal view; B: lateral view. Bar = 3 µm.

oliveirai sp. nov. Although the description of *Myxobolus* sp. parasite of *Serrasalmus* sp. (Walliker 1969) is poor, a superficial comparison reveals that the anterior end of the spores of *Myxobolus* sp. are more pointed than in *M. oliveirai* sp. nov. Furthermore, the infection site (kidney) and host (*Serrasalmus* sp.) of the parasite described by Walliker (1969) differ from those of *M. oliveirai* sp. nov., which infects gill filaments in *B. hilarii*. Regarding *M. cordeiroi*, the differences were in the number of polar filament turns, infection sites (connective tissue of several organs) and host (a pimelodid fish). The spore dimensions of the different species do not fit the values found in the present material. Therefore, our specimens cannot be identified with any of the aforementioned species.

When comparing *M. oliveirai* sp. nov. with species from other continents (Eiras et al. 2005), we selected those species that most resembled our material in their sets of characteristics. Thus, we included *Myxobolus bellus* infecting the integument of *Carpioides carpio* in the USA (Kudo 1934), *Myxobolus bramaeformis* described from the kidneys and gut of *Hypophthalmichthys molitrix* in the Amur Basin (Akhmerov 1960), *Myxobolus fahmii* infecting the gills of *Barbus bynni* in Egypt (Ali et al. 2002) and *Myxobolus pseudosquamae* parasitizing the gills and kidney of *Sinocyclochilus grahami tingi* in China (Ma & Zhao 1993). Despite some similarities, the features of our specimens do not match the characteristics of any of the aforementioned species. Moreover, their hosts are phylogenetically very different and their geographic locations are also quite different. In particular, only a very few number of species have as small a spore thickness (4.6 ± 0.6 µm) as that described for *M. oliveirai* sp. nov. Considering the 744 nominal species reported by Eiras et al. (2005), only 21 have a similar spore thickness. In these cases, however, the other spore features are very different. Furthermore, this is the first report of a myxosporean species infecting fish from the genus *Brycon* and the BLAST search using the partial 18S rDNA sequence of *M. oliveirai* sp. nov. did not match any of the Myxozoa available in GenBank.

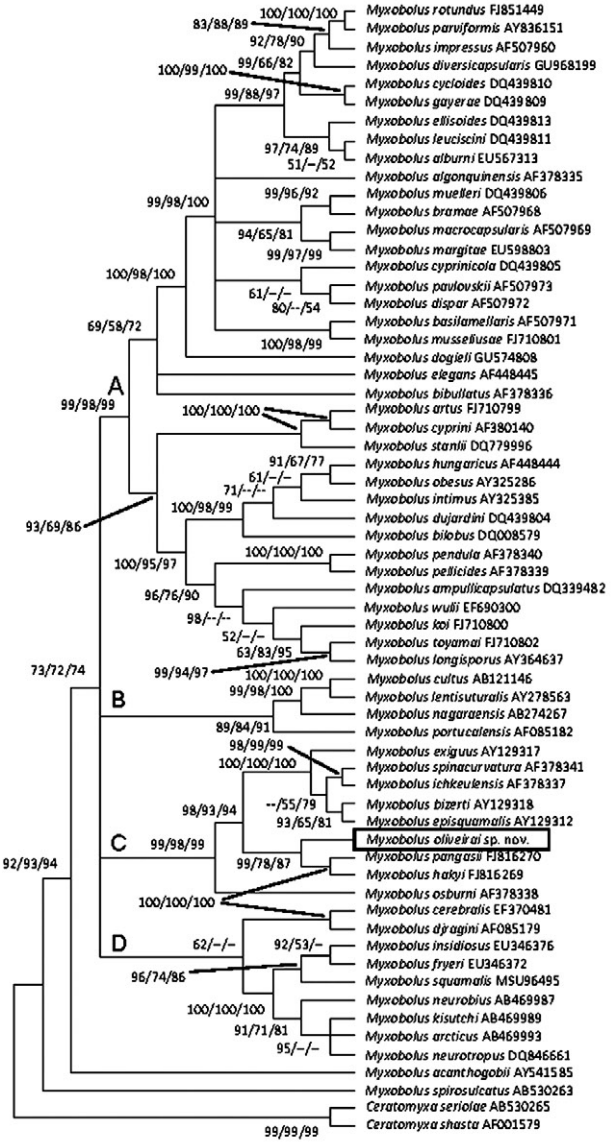

Fig. 6: condensed phylogenetic tree (bootstrap > 50) of consensus among neighbor-joining, maximum parsimony and maximum likelihood showing relationship between *Myxobolus oliveirai* sp. nov. and other *Myxobolus* spp based on partial 18S rDNA. Genbank accessions are given after species name. Numbers above nodes indicate bootstrap confidence levels.

The molecular phylogenetic analysis was based on the comparative analyses of the 18S rDNA gene in 62 species of *Myxobolus* (approximately 1,500 bp) obtained from GenBank with *M. oliveirai* sp. nov. To enhance the accuracy of the analysis, smaller species were not used. The results, with high bootstrap values, showed the *Myxobolus* species clustering as a monophyletic unit. *M. spirosulcatus* was the most divergent species of the genus in this analysis and *M. acanthogobii* appears to be the sister group of the large monophyletic clade composed by the remaining *Myxobolus* species. This large clade further divided into four monophyletic units

TABLE

Pairwise distances based on 1,527-bp fragments of the 18S rRNA gene of *Myxobolus oliveirai* sp. nov. and related *Myxobolus* species using Kimura 2-parameters model of sequence evolution

	1	2	3	4	5	6	7	8
Myxobolus oliveirai sp. nov.	-	-	-	-	-	-	-	-
Myxobolus spinacurvatura	0.183	-	-	-	-	-	-	-
Myxobolus osburni	0.286	0.290	-	-	-	-	-	-
Myxobolus ichkeulensis	0.195	0.095	0.296	-	-	-	-	-
Myxobolus pangasii	0.167	0.227	0.294	0.223	-	-	-	-
Myxobolus hakyi	0.166	0.225	0.294	0.224	0.013	-	-	-
Myxobolus bizerti	0.201	0.146	0.305	0.156	0.219	0.219	-	-
Myxobolus exiguus	0.206	0.155	0.305	0.171	0.226	0.222	0.171	-
Myxobolus episquamalis	0.188	0.143	0.292	0.133	0.222	0.215	0.124	0.142

(A-D), with unsolved groups, despite the high bootstrap values supporting the relationships.

M. oliveirai sp. nov. clustered in Clade C together with eight other species, forming a monophyletic unit, where it appears as sister group of the clade formed by *M. pangasii* and *M. hakyi*, which are parasites of *Pangasianodon hypophthalmus* (Pangasiidae: Siluriformes) and, together, these three species compose the sister group of the clade formed by five species (*Myxobolus exiguus*, *Myxobolus spinacurvatura*, *Myxobolus ichkeulensis*, *Myxobolus bizerti* and *Myxobolus episquamalis*), parasites of mugilid hosts. *M. osburni*, a parasite of *Lepomis gibbosus*, which is a Perciformes from the family Centrarchidae, appears as the basal unit of Clade C.

The mean genetic divergence estimated in Clade C was 20.4%. With regard to the new species, the smallest divergence was with *M. hakyi* (16.6%), a sister taxa of *M. oliveirai* sp. nov., whereas the largest divergence was with *M. osburni* (28.6%) (Table) which is the basal species for Clade C.

The phylogenetic tree obtained here is in accordance with results described by Ferguson et al. (2008) and Liu et al. (2010), who report *M. acanthogobii* as a species with the greatest divergence among *Myxobolus* spp as well as in relation to the clustering of *M. exiguus*, *M. spinacurvatura*, *M. ichkeulensis*, *M. bizerti*, *M. episquamalis*, *M. pangasii* and *M. hakyi* in a monophyletic unit.

The results of the present study show the phylogenetic relation of the new species with other *Myxobolus* species available in GenBank. This is the first phylogenetic study on a *Myxobolus* species parasite of a South American characid host considering Myxozoa parasites of fresh water fish. From this region, only the 18S rDNA gene sequence of *M. cordeiroi* (Adriano et al. 2009a), with around 500 bp, has been deposited in GenBank. Thus, additional molecular and phylogenetic studies of *Myxobolus* spp parasites of this fish family are needed to identify the true position of the *Myxobolus* parasites of characid hosts in relation to the *Myxobolus* species parasites of other families of fish.

There was a much lower prevalence of *M. oliveirai* sp. nov. in the southern region of the Pantanal than that found in the central and northern areas, demonstrating that environmental characteristics may influence the distribution of this myxosporean species in the Brazilian Pantanal. Environmental parameters (water temperature and water flow) have been reported to influence the prevalence of *Myxobolus cerebralis* (Baldwin et al. 2000, Hallett & Bartholomew 2008). The Brazilian Pantanal is the largest floodplain area in the world, with approximately 150,000 km^2 (Silva 1986, Ceccarelli et al. 2007), and this very large ecosystem has areas with heterogeneous features, which may have contributed toward the lower prevalence of *M. oliveirai* sp. nov. in the southern region. However, the nature of the parameters that influence the prevalence of infection is unknown.

Regarding the seasons studied, the variation in prevalence was non-significant, revealing that season had no influence over infection by *M. oliveirai* sp. nov. in the present study. This finding is in contrast to results described by Gbankoto et al. (2001), who report the occurrence of significant seasonal variations in *Myxobolus* sp. and *Myxobolus zillii* parasites of *Sarotherodon melanotheron melanotheron* and *Tilapia zillii*, respectively, and Gbankoto et al. (2003), who report seasonal differences in the infection of *S. melanotheron melanotheron* by *Myxobolus heterospora*. However, the results of the present study are in agreement with those described by Gbankoto et al. (2003), who report no seasonal variation in the infection of *T. zillii* by *M. heterospora*.

The comparison of infection between female and male hosts revealed no influence of sex on the prevalence of *M. oliveirai* sp. nov. This finding is in agreement with accounts describing some Myxozoa species (Gbankoto et al. 2001, Viozzi & Flores 2003), but contrasts with others (Muzzall 1995, Gbankoto et al. 2003) that reported a significant difference between females and males regarding the prevalence of infection.

The histological analysis of piraputanga gills infected with *M. oliveirai* sp. nov. revealed numerous large cysts primarily in the distal area of the gill filaments, but no pronounced inflammatory response was found in the infection site, which is similar to findings described for other South American myxoporean species (Barassa et al. 2003, Eiras et al. 2008, 2009, Adriano et al. 2009a, b). However, the development of the plasmodia caused ex-

pansion and compression of the gill filaments, producing a thinning of the tissue at the distal extremity, whereas the growth of the plasmodia in proximal extremity occurred through invaginations, pervading and occupying the filament tissue. These structural alterations in the gills are similar to structural changes reported for other myxosporean species (Feist & Longshaw 2006).

The ultrastructural analysis revealed that sporogenesis in *M. oliveirai* sp. nov. followed the general pattern of other *Myxobolus* species (Current et al. 1979, Casal et al. 1996, 2002, Adriano et al. 2006) and the plasmodium wall consisted of a single membrane, which was continuous with pinocytic canals extending to the ectoplasm, as seen in some *Myxobolus* species (Current et al. 1979), but differed from others delimited by a double membrane (Casal et al. 2002, 2006).

Gill infections by Myxoporea in farmed fish can cause significant tissue damage (Martins et al. 1997, 1999, Adriano et al. 2005a, b, 2006, Feist & Longshaw 2006) and occasionally death (Martins et al. 1999, Feist & Longshaw 2006). Although the histopathological changes produced by *M. oliveirai* sp. nov. impaired the function of the gill surface, the infection apparently did not significantly affect the general health of the fish. However, the histopathological analysis only addressed the wild host specimens (farmed fish were not infected) and we do not know the impact that infection by *M. oliveirai* sp. nov. may have on farmed specimens, especially in intensive farming. Thus, the presence and dispersion of *M. oliveirai* sp. nov. needs to be monitored closely by commercial fish farmers.

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