UNIVERSIDADE ESTADUAL DE CAMPINAS
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# SISTEMÁTICA E INTERAÇÃO PARASITO-HOSPEDEIRO DE MIXOSPORÍDEOS PARASITOS DE PEIXES ORNAMENTAIS DA BACIA AMAZÔNICA

# SYSTEMATICS AND HOST-PARASITE INTERACTION OF MYXOSPOREAN PARASITES OF ORNAMENTAL FISH FROM THE AMAZON BASIN

**Campinas**
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# SYSTEMATICS AND HOST-PARASITE INTERACTION OF MYXOSPOREAN PARASITES OF ORNAMENTAL FISH FROM THE AMAZON BASIN

*Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Doutor em Biologia Animal, na área de concentração em Relações Antrópicas, Meio Ambiente e Parasitologia.*

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***"Livros abrem janelas para o mundo e têm o poder de transformar vidas."***

***Ralph Lauren***

## RESUMO

Peixes, em ambiente natural ou sistemas de criação, são hospedeiros de várias espécies de parasitos, que podem causar importantes alterações patológicas e enfermidades. Entre estes parasitos, os mixosporídeos, devido a grande diversidade e ao potencial patogênico de algumas espécies, têm sido alvo de pesquisas em várias regiões do mundo. Atualmente são conhecidas mais de 2.300 espécies de mixosporídeos, a grande maioria parasitando peixes em ambiente natural ou em pisciculturas, sendo algumas espécies responsáveis por altas taxas de mortalidade em várias partes do mundo. Entretanto, apesar de vários estudos terem mostrado distribuição moderada de espécies de mixosporídeos em muitas espécies de peixes de água doce de ambiente natural e cultivados na América do Sul, informação acerca das espécies que infectam peixes ornamentais é ainda escassa. Este trabalho teve como objetivo o estudo da diversidade e da interação parasito-hospedeiro de espécies de mixosporídeos encontrados infectando peixes ornamentais da bacia Amazônica, no estado do Amazonas, Brasil e na região de Iquitos, Peru. No Amazonas os peixes foram obtidos a partir de entrepostos de peixes ornamentais, os quais são capturados em rios, igarapés, igapós e várzeas daquele estado. Em Iquitos, os peixes foram provenientes de piscicultura e de ambiente natural e foram capturados com o auxílio de redes de pesca, tarrafas ou peneiras. Estudos morfológicos, ultraestruturais e moleculares foram realizados para a análise das espécies de mixosporídeos encontradas parasitando os peixes ornamentais examinados. No total seis novas espécies foram descritas sendo três do gênero *Henneguya*, uma do gênero *Myxobolus*, uma do gênero *Ceratomyxa* e uma do gênero *Myxidium*. Nos peixes provenientes da Amazônia brasileira foram descritas *Henneguya* n. sp. 1, encontrada nos filamentos branquiais de espécimes de *Corydoras melini, Ceratomyxa* n. sp. infectando a vesícula biliar de *Symphysodon discus, Myxidium* n. sp. infectando a vesícula biliar de *Corydoras melini* e *Myxobolus* n. sp. no arco da brânquia de *Corydoras melini*. Nos exemplares coletados na Amazônia Peruana foram descritas *Henneguya* n. sp. 2 infectando os filamentos branquiais de *Corydoras leucomelas* e *Henneguya* n. sp. 3 infectando os filamentos branquiais de *Hyphessobrycon loretoensis.*



Palavras-chave: Myxozoa, Ultraestrutura, Filogenia, Peixes ornamentais, Amazônia.

## ABSTRACT

Fish, in natural environment or farmed are hosts of several species of parasites, which may cause important pathological changes and diseases. Among these parasites, because of the great diversity and the great pathogenic potential of some species, the myxosporeans have received increasing attention from researchers in several regions of the world. Currently, more than 2.300 species of myxosporeans are known, most of which infect fish, both in the natural environment and breeding systems, with some species responsible for high mortality rates in various parts of the world. However, in South America although several studies have shown a wide distribution of species of myxosporeans in many species of freshwater fish from natural environment and farmed, information about the species of myxosporeans that infect ornamental fish is still scarce. The aim of this thesis was to study the diversity and host-parasite interaction of species of myxosporeans found infecting ornamental fish from the Amazon basin (Amazonas State in Brazil and Iquitos in Peru. In Amazonas, the fish were obtained from warehouses of ornamental fish, which are caught in rivers, streams, *igapós* (blackwater floodplains) and *várzeas* (whitewater floodplains). In Iquitos, the fish were obtained in fish farms and in the natural environment and were captured with the aid of fishing nets or sieves. Morphological, ultrastructural and molecular studies were carried out to analyze the species of myxosporeans found parasitizing the examined ornamental fish. In total, six new species were described, three of the genus *Henneguya*, one of the genus *Myxobolus*, one of the genus *Ceratomyxa* and one of the genus *Myxidium*. In the fish from the Brazilian Amazon were described *Henneguya* n. sp. 1 found in the gill filaments of specimens of *Corydoras melini*, *Ceratomyxa* n. sp. infecting the gallbladder of *Symphysodon discus*, *Myxidium* n. sp. infecting the gallbladder of *Corydoras melini* and *Myxobolus* n. sp. found in the gill arch of *Corydoras melini*. From specimens collected in the Peruvian Amazon were described *Henneguya* n. sp 2 infecting the gill filaments of *Corydoras leucomelas* and *Henneguya* n. sp. 3 infecting the gill filaments of *Hyphessobrycon loretoensis*.



**Key words:** Myxozoa, Ultrastructure, Phylogeny, Ornamental fish, Amazon.

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## 1. INTRODUÇÃO

O aumento da demanda por parte dos entusiastas de peixes ornamentais na Alemanha, Estados Unidos da América, Japão, Holanda, Reino Unido, Itália e Bélgica, entre outros países, foi responsável pelo desenvolvimento da economia em torno do aquarismo, com a movimentação mundialmente de mais de 400 milhões de exemplares por ano (ANJOS et al., 2009). A grande maioria dos peixes utilizados como ornamental é oriunda de sistemas de criação. Entretanto, quando se trata de diversidade de espécies ornamentais de água doce, o maior número de espécies é coletado em ambiente natural, principalmente da bacia Amazônica (MOREAU e COOMES, 2006; JUNK et al., 2007; ANJOS et al., 2009). A comercialização de peixes ornamentais na região Amazônica tem grande importância econômica e social, sendo responsável pela subsistência de comunidades pesqueiras de pequenas cidades de países como Colômbia, Peru, Brasil, Guiana, Bolívia e Equador (AJIACO-MARTINEZ et al., 2001; MOREAU e COOMES, 2007; JUNK et al., 2007; ANJOS et al., 2009).

Devido à crescente demanda mundial por peixes ornamentais, desenvolveu-se na região amazônica uma intensa rede de atividades voltadas para a produção e comércio de peixes ornamentais, principalmente relacionada à pesca extrativista, mas também, nos últimos tempos, com o cultivo de algumas espécies. Para o comércio de peixes ornamentais, o grande desafio é a produção de grandes quantidades de peixe de boa qualidade. Neste contexto, os parasitos são importantes ameaças aos sistemas de criação nos países produtores, bem como expoentes de risco às populações nativas de peixes dos países para onde são exportados os peixes (BIOSECURITY, 2005; HALLET et al., 2015), possibilitando a introdução de patógenos que, em última instância, podem ter efeitos devastadores sobre espécies de peixes nativos suscetíveis (HALLET et al., 2015).

Na Amazônia peruana, nos últimos dez anos, o valor total das exportações de peixes ornamentais movimentou em média cerca de 2,5 milhões de dólares/ano, sendo o Peru o segundo país com maior exportação de peixes ornamentais provenientes da região Amazônica, depois da Colômbia (MOREAU e COOMES, 2006; PRANG, 2007). No Brasil, a exportação de peixes ornamentais atingiu mais de 6 milhões de dólares em 2007, sendo que o estado do Amazonas, onde a atividade é mais intensa, atingiu 3,8 milhões de dólares neste mesmo ano (IBAMA, 2007), possibilitando a geração de mais de dez mil empregos diretos e indiretos, aparecendo como

o terceiro maior produto extrativista explorado no estado, atrás somente da indústria madeireira e da castanha (ANJOS et al., 2009).

Do lado da Amazônia peruana, a cidade de Iquitos, no departamento de Loreto é um dos principais sítios envolvidos com o comércio de peixes ornamentais com 28 empresas de exportação de peixes de aquário estabelecidas na cidade, exportando para 24 países aproximadamente 361 espécies, compreendidas em 139 gêneros, 36 famílias e 13 ordens (TELLO e CANEPA, 1991; MOREAU e COOMES, 2007; PRANG, 2007). Esta região é banhada pelo rio Nanay, um dos principais afluentes do rio Amazonas em território peruano, o qual inclui muitos tipos de microhabitats que abrigam grande diversidade de peixes importantes para o consumo humano e uso ornamental, além de apresentar em áreas próximas (igapós e várzeas), um alto grau de endemismo (CORREA e ORTEGA, 2010).

Do lado da Amazônia brasileira, a região com maior envolvimento no comércio de peixes ornamentais é a do alto Rio Negro, com grande destaque para o município de Barcelos (ANJOS et al., 2009). Nesta região é permitida a extração de aproximadamente 70 espécies para exportação (CHAO, 2001; PRANG, 2007), tendo a Europa como o principal importador (PRANG, 2007). Em Manaus e Barcelos, o governo do Amazonas, em parceira com o Ministério da Pesca e Aquicultura, propuseram a implementação de Centros de Entrepostos de Pesca. Estes locais servem para adaptação, triagem e criação das espécies mais importantes no mercado de peixes ornamentais, com vista para o desenvolvimento e a modernização da atividade.

Em pisciculturas ou em ambiente natural, peixes estão expostos a vários patógenos (EIRAS, 1994; WOO, 2006). Vírus, bactérias, fungos, protozoários, metazoários podem ser agentes etiológicos ou vetores de doenças em peixes (EIRAS, 1994; WOO, 2006) afetando negativamente o desenvolvimento dos mesmos. É sabido que o comércio de peixes ornamentais representa importante mecanismo de disseminação e introdução de parasitos de peixes. Essa introdução pode ter efeitos graves, especialmente quando os parasitas entram em contato com hospedeiros não habituais suscetíveis. Vários casos de parasitos introduzidos, às vezes com devastadoras consequências, podem ser encontrados na literatura (MORAVEC et al., 1999; EVANS e LESTER, 2001; KIM et al., 2002), e o envolvimento de peixes ornamentais foi descrito num certo número de estudos (FERRAZ e SOMMERVILLE, 1998; MOUTON et al.,

2001; THILAKARATNE et al., 2003; PIAZZA et al., 2006; PRANG, 2007; GARCIA et al., 2009).

Entre os vários patógenos de peixes, o subfilo Myxozoa, que agrupa cinidários adaptados ao parasitismo, apresenta grande diversidade de espécies e algumas são importantes patógenos (FEIST e LONGSHAW, 2006; OKAMURA et al., 2015). Os mixozoários compõem um grupo de parasitos economicamente importantes (ALAMA-BERMEJO et al., 2012, KODADKOVA et al., 2015) e atualmente são conhecidas cerca de 2400 espécies, as quais são agrupadas nas classes Myxosporea Bütschli, 1881 e Malacosporea Canning, Curry, Feist, Longshaw & Okamura, 2000 (BARTOŠOVA-SOJKOVA et al., 2014).

O ciclo biológico é coplexo e envolve hospedeiros vertebrados e invertebrados. Em malacosporídoes, o ciclo alterna entre briozoários e peixes, enquanto em mixosporídeos o ciclo envolve anelídeos e peixes, anfíbios, répteis, aves aquáticas e pequenos mamíferos (Figura 1) (Eiras, 2005; OKAMURA et al., 2015).

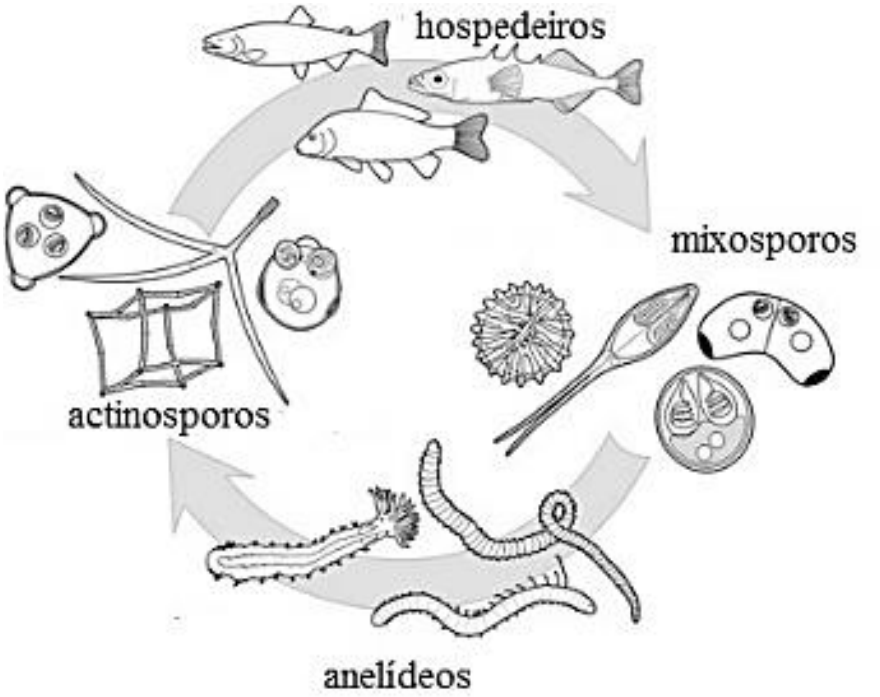


**Figura 1-** Ciclo de vida dos mixosporídeos. Fonte: Atkinson, 2011.

Os peixes parasitados por mixosporídeos podem apresentar diferentes alterações patológicas, dependendo do órgão e/ou tecido infectado, bem como da espécie de parasito envolvida: a doença proliferativa das brânquias ocorre no bagre do canal

(*Ictaluris punctatus*), deformações na cabeça e na coluna vertebral podem ser observadas em salmonídeos, liquefação muscular em *Coryphaena hippurus* e *Paralichthys adspersus*, hipertrofia dos rins de Goldfish *Carassius auratus*, reação granulomatosa e espessamento da túnica na bexiga natatória, hiperplasia do epitélio das lamelas e compressão dos capilares de *Piaractus mesopotamicus*, compressão dos capilares e do epitélio lamelar de *Astyanax altiparanae*, diminuição da área de epitélio respiratório de pintado híbrido (*Pseudoplatystoma corruscans* x *Pseudoplatystoma fasciatum*) e alteração do fluxo de sangue em infecções do endotélio de vasos sanguíneos dos filamentos branquiais de *Salminus brasiliensis* (LANGDON, 1991; LOM e DYKOVÁ, 1995; CASTRO e BURGOS, 1996; ALLEN e BERGERSEN, 2002; BARASSA et al., 2003; FEIST e LONGSHAW, 2006; ADRIANO et al., 2006; 2009; NALDONI et al., 2009; ELWELL et al., 2009).

No Peru, estudos sobre a diversidade de mixosporídeos parasitos de peixes são escassos e estão restritos a dois recentes estudos (ESPINOZA et al., 2017; MATHEWS et al., 2017). No Brasil, entretanto, estudos sobre mixosporídeos parasitos de peixes de importância econômica têm recebido grande atenção nos últimos anos (AZEVEDO e MATOS, 2002; ADRIANO et al*.,* 2002; 2005a; 2006; 2009; 2012; NALDONI et al., 2009; EIRAS et al., 2010; MILÁNIN et al., 2010; AZEVEDO et al., 2010; CARRIERO et al., 2013; MATHEWS et al., 2015; 2016a, b, c) e algumas espécies têm se mostrado importantes patógenos para seus hospedeiros em sistemas de cultivo (MARTINS et al., 1997; ADRIANO et al., 2005b; NALDONI et al., 2009).

Várias espécies de peixes da bacia Amazônica têm grande importância econômica, tanto para a pesca extrativista visando o consumo humano ou o aquarismo, como a pesca esportiva, sendo que nos últimos anos muitas destas espécies de peixes têm sido introduzidas com sucesso na piscicultura (ARAUJO-LIMA e GOULDING, 1997; ALCÁNTARA et al., 2004; MOREAU e COOMES, 2007; PRANG, 2007).

Como relatado acima, a bacia Amazônica alberga grande diversidade de espécies de peixes de água doce que se destacam no comércio mundial de ornamentais. Neste contexto, este projeto teve como objetivo avaliar a diversidade de mixosporídeos parasitos de peixes de importância econômica para o aquarismo - acará-disco (*Symphysodon discus*), Coridora (*Corydoras* spp.) e tetra (*Hyphessobrycon loretoensis*). Foram examinados peixes oriundos da pesca extrativista e da criação em piscicultura nas regiões de Iquitos, Peru e do estado do Amazonas, Brasil. Para tanto, análises

morfológicos, ultraestruturais e moleculares foram realizados visando a descrição das novas espécies, bem como avaliar aspectos da interação parasito-hospedeiro e das realções filogenéticas.

## 2. JUSTIFICATIVA

A escolha de peixes ornamentais para o desenvolvimento deste projeto teve como razão a importância que estes organismos têm para a economia de algumas regiões da bacia Amazônica, sendo responsável pela manutenção e subsistência de grande parte das comunidades pesqueiras de pequenas cidades de países como o Brasil, Colômbia, Peru, Equador e Guiana, bem como pela importância que os peixes ornamentais têm no contexto global, com mais de 400 milhões de exemplares sendo movimentados anualmente. Esta intensa movimentação global de peixes ornamentais pode ainda ser de grande importância para a disseminação e introdução de patógenos, o que pode muitas vezes ter efeitos graves em hospedeiros não habituais nos ambientes onde são introduzidos.

A escolha da região de Iquitos, no Peru, está relacionada ao fato de ser este um importante centro com intensa atividade de captura de peixes ornamentais, com 28 empresas ali estabelecidas e dedicadas à exportação de peixes ornamentais, fornecendo mais de 80% de todos os peixes comercializados para o exterior a partir do Peru. Outro fator importante é o bem estabelecido contato que o Doutorando tem com a cidade de Iquitos, onde já desenvolveu trabalhos de colaboração com pesquisadores do *Instituto de Investigaciones de la Amazonia Peruana -IIAP*, a qual está envolvida com a pesquisa de peixes. Na Amazônia brasileira, a escolha do estado do Amazonas deveu-se ao fato de ser esta região um importante polo de produção e comércio de peixes ornamentais.

No contexto dos parasitos de peixes, mixosporídeos têm recebido crescente atenção dos pesquisadores e produtores de peixes de todo o mundo, sendo que várias espécies são causadoras de grandes prejuízos em pisciculturas. Como a maioria das espécies de mixosporídeos têm desenvolvimento tissular, dependendo do órgão em que se desenvolvem e/ou da resposta a que induzem aos hospedeiros, estes parasitos podem afetar de forma decisiva o desenvolvimento dos peixes. Um importante fator a ser realçado quanto às infecções por mixosporídeos em peixes de sistemas de criação, é a ausência de métodos de tratamento eficazes, o que é um grande dificultandor do controle. Assim, são de grande importância os estudos que buscam ampliar o

conhecimento da diversidade dos mixosporídeos parasitos de peixes, principalmente aquelas espécies que infectam peixes de importância econômica da América do Sul, onde está a maior diversidade de peixes de água doce do mundo, com muitas destas espécies tendo grande potencial para a utilização em pisciculturas, sendo que algumas já foram introduzidas na cadeia produtiva, visando tanto a produção de alimentos como o aquarismo. Neste contexto, a ampliação do conhecimento sobre a diversidade de parasitos de peixes como um todo, pode ser de grande valia para o desenvolvimento de técnicas de manejo buscando prevenir e/ou controlar estes parasitos em ambiente de criação, minimizando os efeitos negativos na produção.

## 3. OBJETIVOS

### 3.1. *Objetivo Geral*

Estudo da diversidade e da interação parasito-hospedeiro de espécies de mixosporídeos parasitos de peixes ornamentais procedentes de ambiente natural e de sistemas de criação na bacia Amazônica, na região de Iquitos, Peru e no estado do Amazonas, Brasil.

### 3.2. *Objetivos Específicos*

1) Identificar e descrever espécies de mixosporídeos encontrados infectando peixes ornamentais, utilizando características morfológicas e sequenciamento de DNA da pequena subunidade ribossomal (SSU - do inglês *small subunit ribosomal*);

2) Avaliar aspectos da interação parasito-hospedeiro nas infecções de mixosporídeos em peixes ornamentais amazônicos, mediante análises em microscopia de luz e eletrônica de transmissão;

3) Avaliar a posição filogenética das espécies de mixosporídeos encontrados infectando peixes ornamentais em relação as outras espécies de mixosporídeos cujas sequências de SSU rDNA estão disponíveis no *GenBank*.

## 4. MATERIAL E MÉTODOS

Este projeto foi desenvolvido numa parceria entre o Departamento de Biologia Animal do Instituto de Biologia da UNICAMP, o laboratório de Genética Evolutiva do Departamento de Ciências Biológicas do Instituto de Ciências Ambientais, Químicas e Farmacêuticas da Universidade Federal de São Paulo-UNIFESP, Campus Diadema, SP.

No período entre o segundo semestre de 2014 e o primeiro semestre de 2016 foram realizadas 6 coletas, sendo três em cada uma das regiões escolhidas para o estudo. Na região de Manaus os peixes foram capturados por pescadores e transportados para os entrepostos da região. Na região da cidade de Iquitos, peixes provenientes de piscicultura e de ambiente natural foram capturados com o auxílio dos técnicos do IIAP, utilizando redes de pesca, tarrafas, peneiras ou covos, dependendo das características das espécies e do ambiente de captura. Os peixes foram transportados vivos para o Laboratório de Parasitologia de Peixes do IIAP, na cidade de Iquitos e no caso das coletas realizadas no Estado do Amazonas, foram levadas até um laboratório de campo montado num hotel na cidade de Manaus. Em alguns casos, os peixes foram enviados, via transporte aéreo, para o Laboratório de Genetica Evolutiva da UNIFESP. As capturas foram autorizadas pelo Ministério do Meio Ambiente (SISBIO nº 44268-4) e nossa metodologia foi aprovada pelo Comitê de Ética em Uso Animal da Universidade de Federal de São Paulo (CEUA/UNIFESP nº 9209080214).

Após a eutanásia e a mensuração dos peixes, foi realizada a análise externa, seguida pela necrópsia com exposição das brânquias e da cavidade visceral, com a finalidade de detectar eventuais alterações nas características dos órgãos e plasmódios e/ou cistos de mixosporídeos. Órgãos ou tecidos infectados por mixosporídeos foram coletados e fixados seguindo o protocolo das técnicas que foram empregadas para as análises subsequentes (microscopia ótica e eletrônica e análise molecular). No total, foram examinados no mínimo 30 espécimes de cada espécie de peixe ornamental envolvido no estudo (*Corydoras melini*, *Corydoras leucomelas*, *Symphysodon discus*, *Hyphessobrycon loretoensis*) (Figura 2).

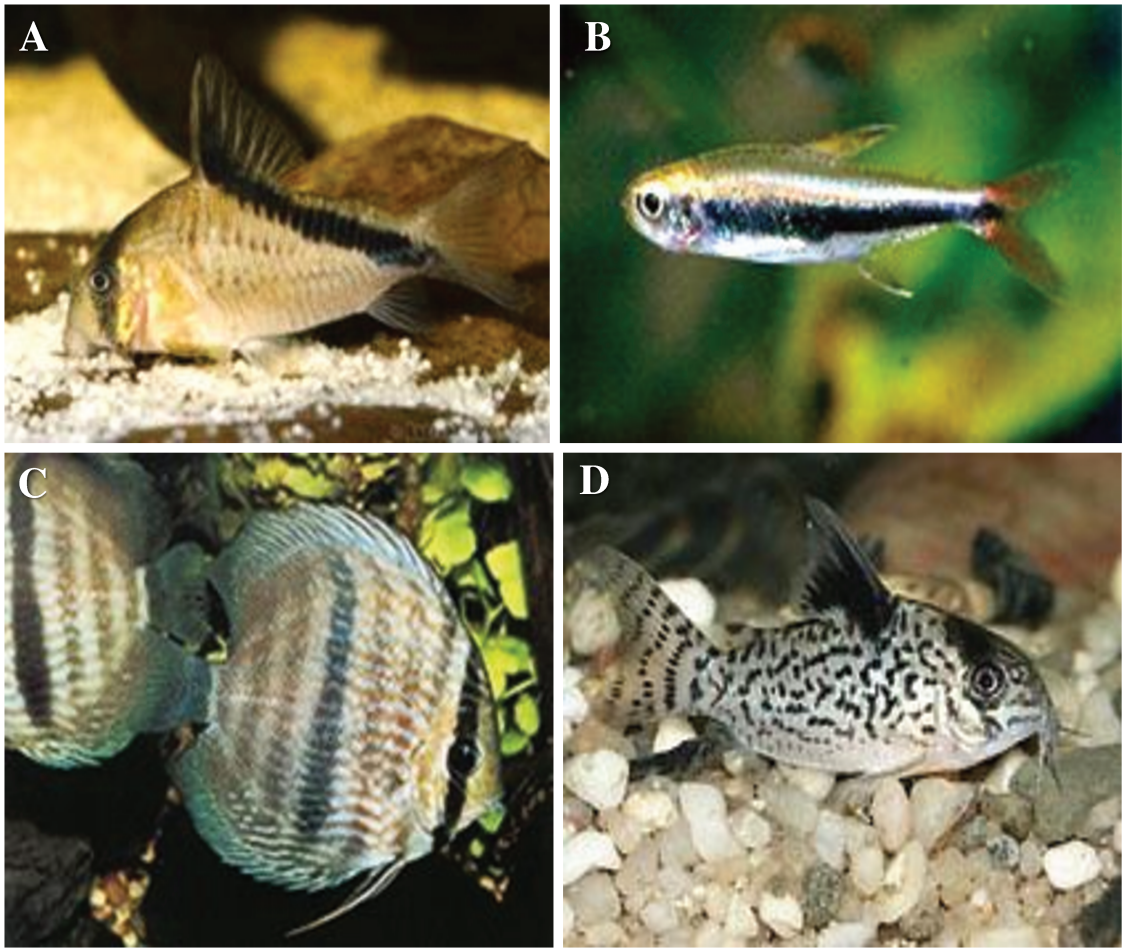


**Figura 2**- Espécies de peixes ornamentais analisados. A- *Corydoras melini;* B- *Hyphessobrycon loretoensis;* C- *Symphysodon discus;* D- *Corydoras leucomelas*. Fonte: http://www.fishbase.org.

## 5. RESULTADOS

Os resultados desta tese estão divididos em 6 capítulos, os quais trazem importantes avanços no conhecimento da diversidade de mixosporídeos parasitos de peixes amazônicos, especialmente aqueles envolvidas no aquarismo nacional e internacional.

Nos capítulos de 1 a 4 são apresentados resultados referentes aos estudos morfológicos, ultraestruturais e moleculares de quatro novas espécies de mixosporídeos dos gêneros *Myxidium* Bütschli, 1882, *Myxobolus* Bütschli, 1882, *Henneguya* Thélohan, 1892 e *Ceratomyxa* Thélohan, 1892, os quais foram encontrados parasitando *C. melini* e *S. discus* oriundos da Amazônia brasileira. Os capítulos 5 e 6 versam sobre estudos

realizados com *C. leucomelas* e *H. loretoensis* capturados na Amazônia peruana, onde foram descritas duas novas espécies do Gênero *Henneguya.*

A formatação de cada captítulo segue o padrão dos periódicos onde os dados foram publicados ou para os quais serão submetidos.

**Capítulo 1.**

**Ultrastructure and ssrRNA sequencing of *Myxidium* n. sp. a myxosporean parasite of *Corydoras melini* from the Rio Negro river, Amazonas state, Brazil**

A formatação segue o padrão do periódico Parasitology Research

**Abstract** In a survey of myxozoan parasites of ornamental freshwater fish from the Rio Negro river, it was found that seven of 30 (23.3 %) *Corydoras melini* specimens examined had plasmodia of a new *Myxidium* species (*Myxidium* n. sp.) in the gallbladder. The fish were caught in the Rio Negro river, in the municipality of Santa Isabel do Rio Negro, in the state of Amazonas, Brazil. The plasmodia had a tubular shape, which was organized as a spiral spring with several turns in the gallbladder. The development of the myxospores was asynchronic, with disporic pansporoblasts. Mature myxospores were elongated, with 17.0±0.9 (16.1– 17.9) μm in length and 3.7±0.7 (3.0–4.4) μm in width, and lightly arcuate from the valval view, with their bodies tapering slowly until ending in rounded extremities. The valval surface had nine to ten grooves in each valve. The polar capsules, one at either end of the spore, had a length of 5.4±0.5 (4.9–5.9) μm and a width of 3.4±0.6 (2.8–4.0) μm. Ultrastructural analysis showed that the wall of the plasmodia had numerous microvilli-like structures, pinocytotic canals, and cytoplasmic bridges connecting the pansporoblasts to each other and to the ectoplasm zone. Phylogenetic analysis, based on a small subunit ribosomal DNA (ssrDNA), identified the new species as a sister species of *Myxidium ceccarelli*, the unique South American *Myxidium* species whose ssrDNA sequence is available in the NCBI database. This study is the first description of *Myxidium* species in ornamental freshwater fish from Amazon.

**Keywords** Myxozoa · *Myxidium* · Systematic · Gallbladder · Fish · Amazon

## Introduction

The global trade in aquarium fish involves the buying and selling of about 400 million specimens a year. In Brazil, about 100 million ornamental fish were exported from the state of Amazonas between 2002 and 2005 (Anjos et al. 2009), a number that included 169 marketed species. The sale of ornamental fish in the Amazon region is responsible for the subsistence of fishing communities in small towns in countries such as Brazil, Colombia, Peru, and Guyana (Ajiaco-Martinez et al. 2001; Moreau and Coomes 2006; Junk et al. 2007). In Brazil, the state of Amazonas is responsible for 93 % of the total number of ornamental fish exported, generating an annual income of about US $ 3.6 million and generating over ten thousand jobs (IBAMA 2007).

The genus *Corydoras* Lacepede, 1803, is the most species rich genus of Siluriformes, with approximately 196 nominal species (Eschmeyer 2013; Espíndola et al. 2014). *Corydoras melini* Lönnberg and Rendahl, 1930 (English name=bandit corydoras), is a tropical freshwater fish belonging to the Callichthyidae family, which is endemic to South America (Reis 1998). This species is found in the upper Rio Negro and Rio Meta river basins in Brazil and Colombia. It can reach up to 5.0 cm in length and is popular in the aquarium trade industry (Froese and Pauly 2011).

Myxozoans of the genus *Myxidium* Bütschli, 1882, are typically coelozoic, developing in the gallbladder, urinary bladder, or urinary tubules of their intermediate vertebrate hosts (Jirků et al. 2006; Bartholomew et al. 2008), and are known around 232 nominal species (Eiras et al. 2011). Despite of the wide variety of South American ichthyofauna (Albert and Reis 2011), only eight *Myxidium* species have so far been identified in this continent (Azevedo et al. 2011; Eiras et al. 2011; Adriano et al. 2014).

This study describes, based on morphologic, molecular, and ultrastructural data, a new *Myxidium* species parasitizing the gallbladder of *C. melini* caught in the Rio Negro river, Brazil.

## Materials and methods

Thirty wild *C. melini* specimens from the Rio Negro, in the municipality of Santa Isabel do Rio Negro, in the state of Amazonas, Brazil, were examined in June 2014. Fish were transported live to the field laboratory, where they were euthanized by benzocaine

overdose, in accordance with Brazilian law (Federal Law No. 11794, dated 8 October 2008). All organs and body fluids were examined for myxosporean infection. Mature myxospores were examined on fresh wet mounts by light microscopy. Morphological and morphometric analyses were performed on mature myxospores obtained from different plasmodia based on the criteria outlined by Lom and Arthur (1989). Measurements were taken of 30 myxospores using a computer equipped with Axivision 4.1 image capture software coupled to an Axioplan 2 Zeiss microscope. Smears containing free spores were air-dried and stained with Giensa solution and mounted in a low-viscosity mounting medium (CytosealTM) on permanent slides.

For molecular study, three plasmodia obtained from different gallbladders were preserved separately in absolute ethanol. DNA was extracted using a DNeasy® Blood & Tissue Kit (Qiagen, USA), in accordance with the manufacturer's instructions. The concentration of the DNAwas measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). Polymerase chain reactions (PCR) were conducted in accordance with Adriano et al. (2014). The final reaction volume was 25 µl, which comprised 10–50 ng of extracted DNA, 1× Taq DNA Polymerase buffer (Invitrogen by Life Technologies, Brazil), 0.2 mmol dNTPs, 1.5 mmol MgCl2, 0.2 pmol for each primer, 0.25 µl (1.25 U) Taq DNA polymerase (Invitrogen by Life Technologies, MD, USA), and ultrapure water. The amplification of fragments containing approximately 1.000 and 1.200 bp was performed with the primer pairs ERIB1-ACT1R and MYXGEN4f-ERIB10, respectively (Barta et al. 1997; Kent et al. 2000; Hallett and Diamant 2001). PCRs were performed in an AG22331 Hamburg Thermocycler (Eppendorf, Hamburg, Germany) with 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. PCR products were subjected to electrophoresis in 1.0 % agarose gel (BioAmerica, Miami, FL, USA) in a TBE buffer (0.045 MTris-borate, 0.001MEDTA, pH 8.0), stained with ethidium bromide, and then analyzed with a FLA-3000 scanner (Fuji Photo Film, Tokyo, Japan). PCR products were purified using USB® ExoSap-IT® (OH Cleveland, USA) in accordance with the manufacturer's instructions and sequenced using the same PCR primers. Sequencing was performed at the Human Genome Research Center (HGRC), at the University of São Paulo, with a BigDye®

Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., CA, USA) 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). Bioedit (Hall 1999) was used to visually assemble sequence fragments and to compare the coding sequence obtained with the 25 most closely related myxozoan taxa, as determined by the BLAST search. Phylogenetic analysis was conducted using maximum likelihood (ML) methods with a Kimura 2-parameter (K2P) evolution sequence model in the MEGA 6.0 program (Tamura et al. 2013). Bootstrap analysis (1000 replicates) was employed to assess the relative robustness of the tree branches. The *Tetracapsuloides bryosalmonae* species was used as an outgroup in phylogenetic analysis.

For transmission electron microscopy, plasmodia were fixed for at least 12 h in 2.5 % glutaraldehyde with 0.1 M buffered cacodylate (pH 7.4). After this, the plasmodia were washed in the same buffer and post-fixed with osmium tetroxide ($OsO_4$). 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). Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined in an LEO 906 electron microscope operating at 60 kV.

## Results

Of the 30 specimens of *C. melini* examined, seven (23.3 %) contained plasmodia from an unknown *Myxidium* species floating free in the bile of their gallbladders. These were not found in any other organs, and no pathological signs were observed in the parasitized gallbladder.

*Myxidium* n. sp. (Figs. 1, 2, 3, 4, 5, and 6).

Description—Plasmodia with a tubular shape organized as a spiral spring with several turns within the gallbladder (Fig. 1a–c). Mature myxospores were elongated, measuring 17.0±0.9 (16.1–17.9) μm in length and 3.7±0.7 (3.0–4.4) μm in width and lightly arcuate in valval view, with their bodies tapering slowly and ending in rounded extremities. The polar capsules, one at either end of the spore, were 5.4±0.5 (4.9–5.9) μm in length, 3.4±0.6 (2.8–4.0) μm in width, and four to five turns in polar filaments

(Fig. 1b–d). Light and electron microscopy showed numerous microvilli-like structures on the plasmodial surface, mainly in the extremity, which were in the young developmental stages (Figs. 1b, c and 2a, b). The wall was composed of a single membrane, and numerous pinocytotic canals connected it to the plasmodial ectoplasm zone, which was around 8 μm thick and contained a grouping of electron-dense granules (Fig. 2a–c). Below the ectoplasm, there were generative cells and disporic pansporoblasts in different developmental stages. These pansporoblasts were connected to each other through cytoplasmic filiform expansions and had numerous translucent spaces between them, which were empty or had amorphous structures, and in some cases, contained myelin figures (Figs. 3, 4, and 5). A single binuclear sporoplasm, which had large sporoplasmosomes lay between the polar capsules (Fig. 4). The valval surface of the myxopores had nine to ten grooves in each valve (Figs. 4, 5, and 6).

The result of *Myxidium* n. sp. ssrDNA sequencing was 1920 bp. The BLAST search revealed its closest affinity, 88 %, was with *Zschokkella soleae* (JX271832). Phylogenetic analysis, with 26 species in the ingroup and *Tetracapsuloides bryosalmonae* (U70623) as an outgroup showed the formation of two clades. The smaller clade was composed of four marine Myxidium species. The larger clade comprised marine and freshwater taxa from the Myxidiidae (*Myxidium*, *Zschokkella*, and *Cystodiscus* species) and Sphaeromyxidae (Sphaeromyxa spp.) families. *Myxidium* n. sp. appeared as a sister species of *Myxidium ceccarellii* Adriano, et al., 2014, a parasite of South America freshwater fish (Fig. 7).

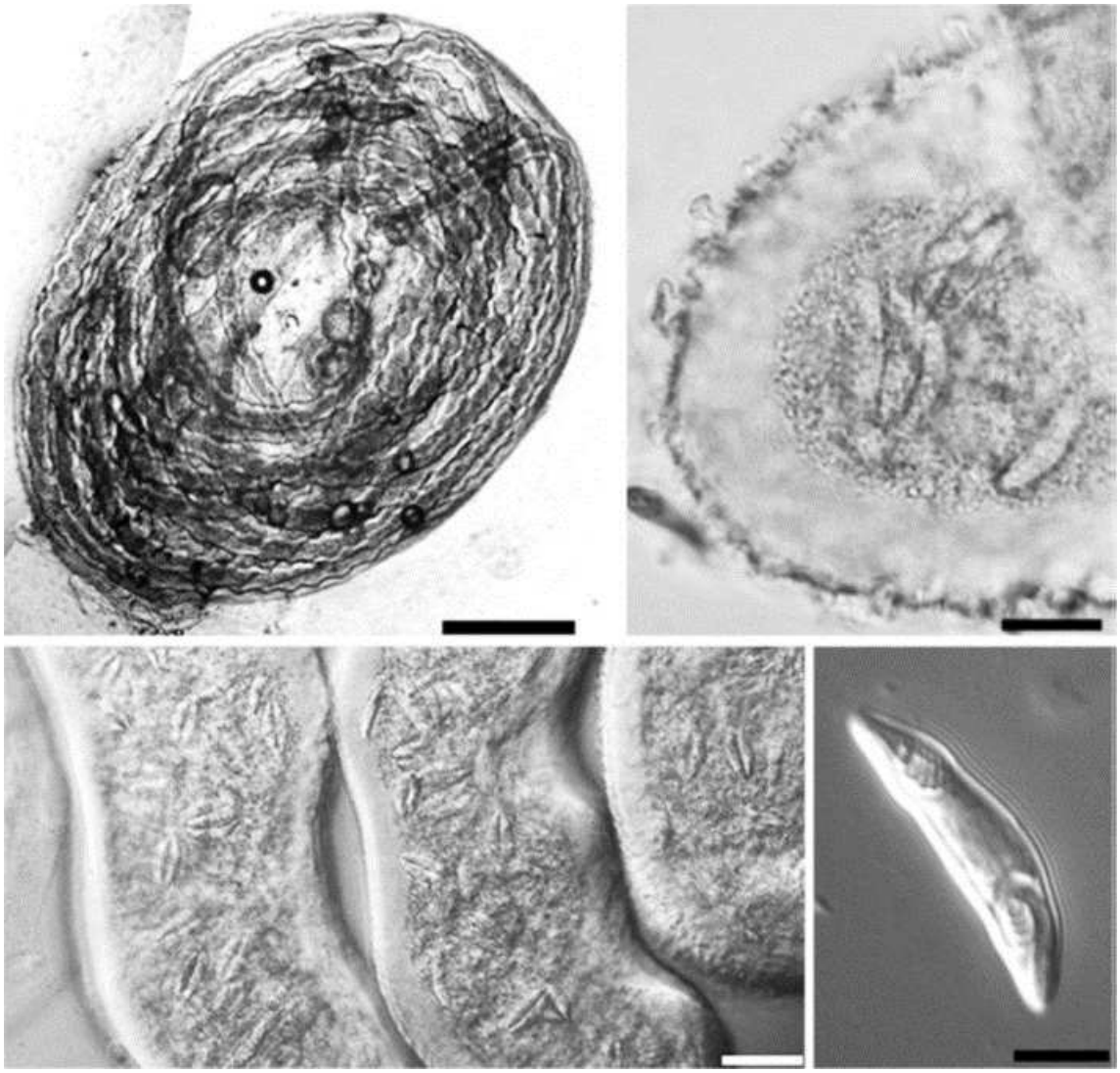

**Fig. 1** Photomicrograph of *Myxidium* n. sp. parasite of gallbladder of *Corydoras melini*. a filiform plasmodium (*arrow*) turning inside the gallbladder (G). Scale bar 400 μm. b tip of the plasmodium (tp) showing numerous microvilli-like structures (*arrows*) on the surface of the wall and mature myxospores (m) within. Scale bar 10 μm. c two segments of middle region of the plasmodium (mp) with few or without microvilli-like structures (*white arrows*) and a tip segment (tp) with numerous microvilli-like structures (*empty arrows*). Note the presence of numerous coupled myxospores. Scale bar 20 μm. d Mature fresh myxospore. Scale bar 5 μm.

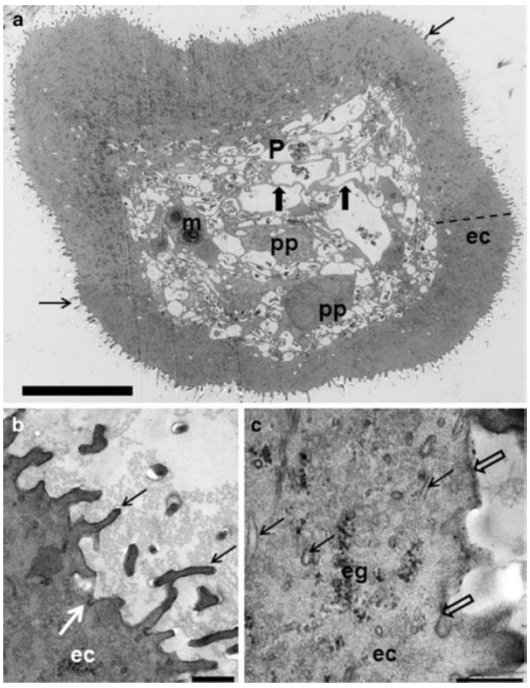


**Fig. 2** Electron micrograph of a plasmodium of *Myxidium* n. sp. parasite of gallbladder of *Corydoras melini*. a transversal section of the plasmodium (P) showing microvilli-like structures of the plasmodial wall (*thin arrows*), a wide ectoplasm area (ec), pansporoblasts (pp) connected by cytoplasmic bridges (*large arrows*), and a mature spore (m). Scale bar 10 µm. b, c: amplified area of the periphery of the plasmodium showing in b details of large and numerous microvilli-like structures (*black arrows*), a single membrane (*white arrow*), and ectoplasm (ec). Scale bar 500 nm and in c a single membrane (*large arrows*), ectoplasm containing electron dense granules (eg) and pinocytic channels (*thin arrows*). Scale bar 400 nm.

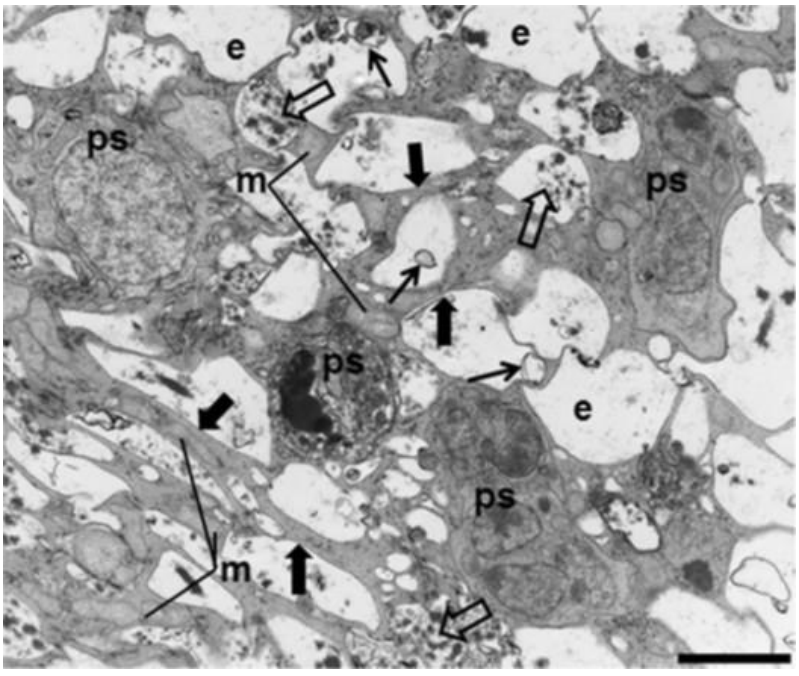


**Fig. 3** Electron micrograph of a plasmodium of *Myxidium* n. sp. parasite of gallbladder of *Corydoras melini* showing pansporoblasts (ps) connected by cytoplasmic bridges (*large black arrows*) with mitochondria (m). Among the cytoplasmic bridges are empty areas (e) containing amorphous electro-dense material (*large empty arrows*) and some myelin figures (*thin back arrows*). Scale bar 2 µm.

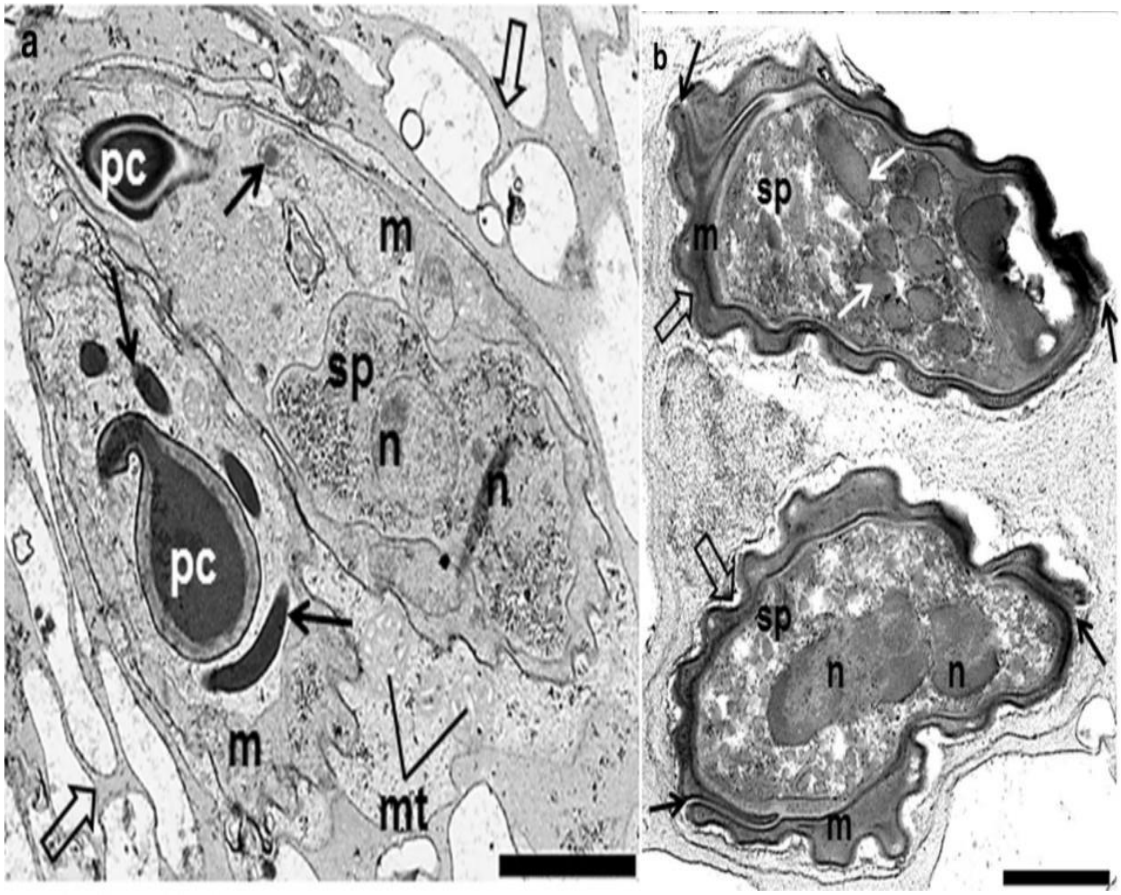


**Fig. 4** Electron micrographs of disporic pansporoblasts of *Myxidium* n. sp. parasite of gallbladder of *Corydoras melini*. a longitudinal section of immature myxospores (m) showing polar capsules with their still externalized polar filaments (*thin arrows*). Note sporoplasm (sp) with two nuclei (n) mitochondria (mt) and cytoplasmic bridges (*empty arrows*). Scale bar 2 μm. b transversal section of immature myxospores (m) in the sporoplasm (sp) level showing large sporoplasmosomes (*white arrows*) and two nuclei (n). In the valves, note the presence of grooves (*empty arrows*) and the points of valvar junctions (*black arrows*). Scale bar 1 μm.

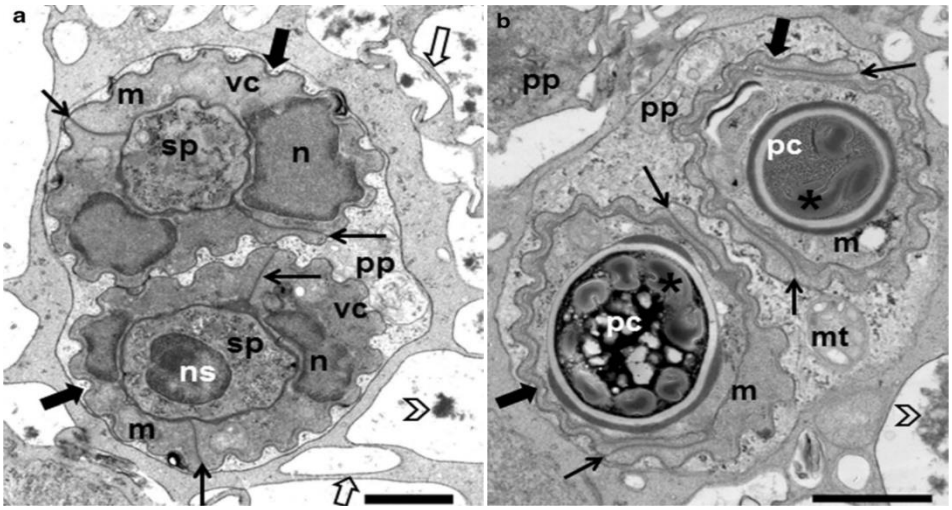


**Fig. 5** Electron micrographs of disporic pansporoblasts (pp) of *Myxidium* n. sp. parasite of gallbladder of *Corydoras melini*. a: young developmental stage myxospores (m) showing valval cells with their nuclei (n), sporoplasms (sp) and its nucleus (ns), point of valvar junctions (*thin arrows*), nine to ten grooves (*large arrows*) in the valvar surface and cytoplasmic bridges linking the pansporoblasts (*empty arrows*). b: transversal section of advanced developmental stage myxospores (m) in the polar capsule (pc) level. Note the polar filaments (*asterisks*) already internalized and thinner valves than in A. A and B show the visible points of valvar junctions (*thin arrows*), nine to ten grooves (*large black arrows*) in the valvar surface and empty areas outside the pansporoblasts containing amorphous electron-dense material (*head arrows*). Scale bars: 1μm.


**Fig. 6** Schematic representation of the mature myxospore *Myxidium* n. sp. parasite of gallbladder of *Corydoras melini*. an Internal view. b Eexternal view. Scale bars=5 μm.

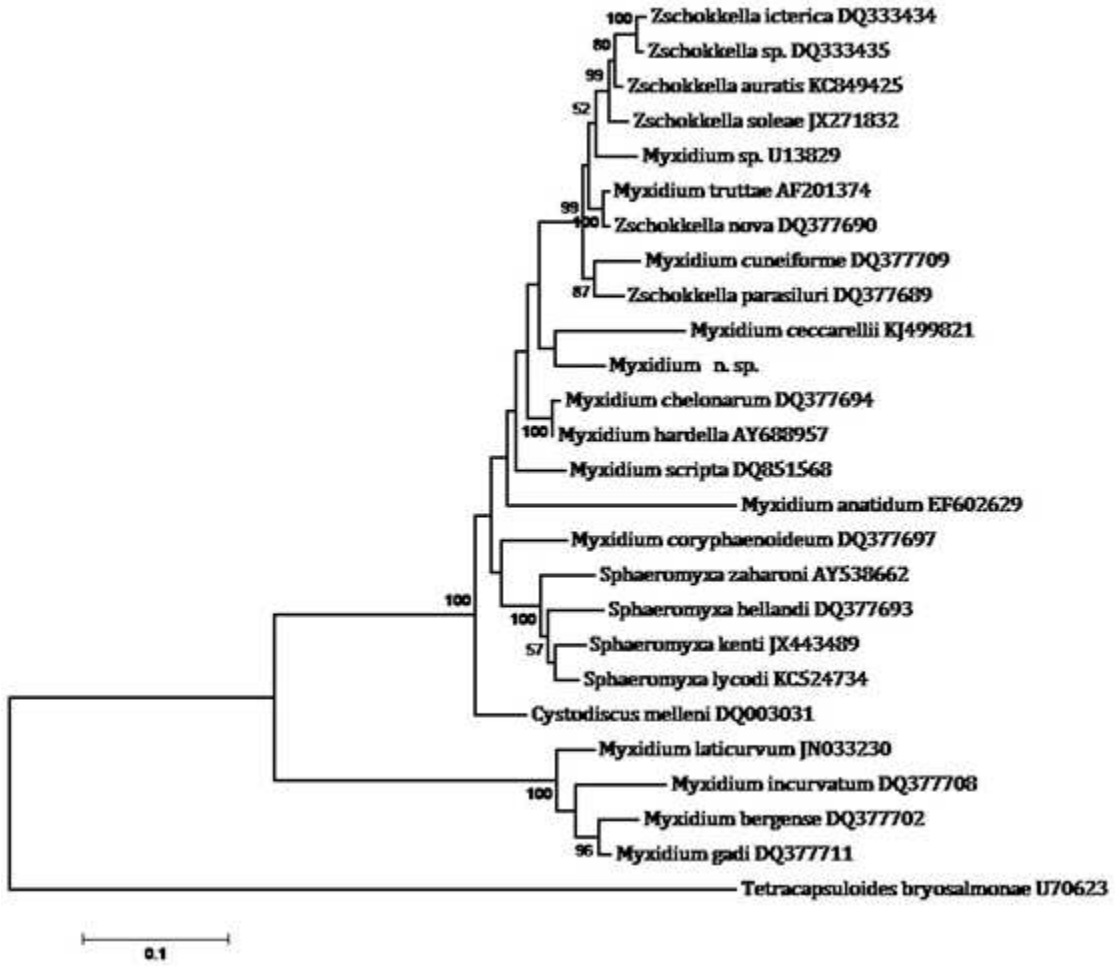


**Fig. 7** Maximum likelihood tree topology showing relationships between *Myxidium* n. sp. and other myxosporeans based on partial ssrRNA gene sequences. NCBI accession numbers are given after species names.

## Discussion

Several studies in different geographical areas around the world have shown the widespread distribution of *Myxidium* species infecting marine and freshwater fish, some reptiles, and birds (Lom and Dykova 2006; Bartholomew et al. 2008; Eiras et al. 2011). Despite the considerable information on *Myxidium* available in literature, with 232 known species (Eiras et al. 2011), little is known about these parasites in South American hosts (Adriano et al. 2014), particularly those from the Amazon, where there is a diverse assemblage of several hundred species of live fish (Albert and Reis 2011). Indeed, in South America only eight *Myxidium* species have been described parasitizing two marine and six freshwater fish (Azevedo et al. 2011; Eiras et al. 2011; Adriano et al. 2014).

The present study described *Myxidium n. sp.* infecting the gallbladder of *C. melini* from Rio Negro river, in the Brazilian Amazon. To our knowledge, this is the first report of *Myxidium* species in a South American ornamental freshwater fish. The morphologic comparison of mature myxospores of *Myxidium* n. sp. with those from the other six *Myxidium* species parasites of South American freshwater fishes, showed that the parasite was readily distinguishable by the shape of its spore, as well as by long tubular plasmodia organized as a spiral spring, free within the gallbladder. The most similar species to this new species was *Myxidium cruzi* Penido, 1927, which infects *Chalsinus nematurus* (Gióia and Cordeiro 1996; Eiras et al. 2011). However, this differs from *Myxidium* n. sp. in the shape of its spore (fusiform, with pointed ends), number of grooves in each valve (seven longitudinal grooves) and because it is substantially wider (5–7 μm). Furthermore, the six species of *Myxidium* reported as infecting the gallbladders of South American freshwater fish were found parasitizing Gymnotiform, Characiform, and Osmeriform fish (Viozzi and Flores 2003; Eiras et al. 2011; Adriano et al. 2014). This study however, describes for the first time a *Myxidium* species infecting the gallbladder of a fish of the Callichthyidae family, which is endemic to the South American continent (Reis 1998). Compared with freshwater *Myxidium* species from other regions of the world, *Myxidium* n. sp. differed in at least one characteristic (shape of plasmodia, size of spore or polar capsule, shape of spore, presence or absence or number of valve striations, and number of polar filament turns). However, besides morphological and biological characteristics, molecular characterization is highly recommended when characterizing a new myxospore species (Gunter et al. 2009; Heiniger and Adlard 2014). As such, a BLAST search of the ssrRNA gene sequence of *Myxidium* n. sp. found that it did not match any other myxidiidae sequences available in the NCBI database, with the most similar sequence that of *Z. soleae*, with an affinity of only 88 %. This lack of closely related sequences was also observed by Adriano et al. (2014) for *M. ceccarellii*.

Phylogenetic analysis of the myxozoan taxa most closely related to *Myxidium n. sp.*, as determined by BLAST search, produced a tree with two main clades. The smaller was composed exclusively of *Myxidium* species of marine fishes, while the larger clade divided further into a branch containing only *Cystodiscus melleni* Jirků et al., 2006, which infects amphibians (Jirků et al. 2006), and another large subclade clustering mainly parasites of freshwater hosts (fish, reptiles and birds) of the *Myxidium*,

*Zschokkella* and *Sphaeromyxa* genera. In this subclade, despite low bootstrap support, *Myxidium* n. sp. appears as a sister species of *M. ceccarellii*, a parasite of South American freshwater Characiformes of the Anastomidae family (Adriano et al. 2014). This is the first phylogenetic study of a myxidid parasite of Pimelodidae fish, and only the seventh *Myxidium* spp. reported in the South American continent (Azevedo et al. 2011; Adriano et al. 2014). Therefore, understanding of the phylogenetic relationship of these myxosporeans will only be improved with the availability of molecular data of new species parasiting distinct host taxa, infection sites, and watersheds.

Ultrastructural analysis of *Myxidium* n. sp. showed the presence of numerous microvilli-like and pinocytic channels in the plasmodial wall. Large multinucleate plasmodia from coelozoic and histozoic myxosporeans usually develop microvilli-like, pinocytic channels in walls (Feist 1995; Adriano et al. 2005; Casal et al. 2006; Azevedo et al. 2011; Naldoni et al. 2009, 2015), which clearly increases the absorptive area (Canning et al. 1999; Naldoni et al. 2009). These strategies, by which the parasite obtains nutrients, support the developmental forms inside the plasmodia (Canning et al. 1999; Hallett and Diamant 2001; Moreira et al. 2014). According to Feist (1995), in *Myxidium gadi* Georqevltch, 1916, the microvilli gradually vanish when the parasite reaches advanced stages of development. *Myxidium* n. sp. showed a similar profile. Once the plasmodia were extremely elongated, they showed intense microvilli-like structures in the extremity of the plasmodia, places with a greater prevalence of parasites in young developmental stages, while little or absence of microvilli-like structures were observed in the middle of the plasmodia, a region with more advanced or mature myxospores (Figs. 1b, c and 2b). Also, according to Feist (1995), those dynamic of these microvilli may be due to the approximation of sporogenesis completion, when the nutritional demands of the plasmodia are reduced and hence the microvilli are resorbed.

Other interesting features observed in the sporogenesis of *Myxidium* n. sp. was the presence of a cytoplasmic filiform connection among pansporoblasts. These cytoplasmic bridges permit the passage of nutrients from the ectoplasm zone to the different developmental stages in the deep part of the plasmodium. Similar cytoplasmic bridges have been observed by Upenskaya (1969) in *Myxidium gasterostei* Noble, 1943, and by Azevedo et al. (2011) in *Myxidium volitans* Azevedo et al., 2011.

**Capítulo 2.**

**Morphological and ultrastructural aspects of *Myxobolus* n. sp. (Myxozoa) gill parasite of *Corydoras melini* (Siluriformes: Callichthyidae) from Brazilian Amazon**

A formatação segue o padrão do periódico Acta Tropica


## ABSTRACT

*Myxobolus* n. sp. (Myxozoa) is described in the connective tissue of the serosa layer of the gill arch of *Corydoras melini* (Callichthyidae) captured from the Negro River, Amazonas State, Brazil. The prevalence of the parasite was 20% and the range intensity was 1–2 cysts per fish. The plasmodia were white and spherical to ellipsoidal, measuring 175 m in diameter and were surrounded by a well-defined capsule of host connective tissue, with distinct delicate and interlaced collagen fibers. The myxospores body was ellipsoidal in frontal view and biconvex in sutural view. Spore dimensions were 11.3 ± 0.4 m in length, 6.8 ± 0.2 m in width and 4.1 ± 0.2 m in thickness. The valves were symmetrical and smooth. The two polar capsules were elongated as pyriform and equal in size, measure 5.0 ± 0.3 m in length and2.0 ± 0.1 m in width. The polar capsule had six to seven polar filament turns. Some aberrant spores were round in shape and had three polar capsules. The sporoplasm was binucleated and contained moderated number of sporoplasmosomes. The development of the plasmodia was asynchronic, with mature and immature spores. The plasmodium had moderated pynocitic channels. There were no projections, no invaginations and no microvilli in the plasmodial wall. This study is the first description of *Myxobolus* species in the fish of the Callichthyidae family.


***Keywords:*** Myxozoa; Fish parasite; Ultrastructure; Ornamental fish; Amazon

## 1. Introduction

Myxozoa is a clade of cnidarians that diverged from their free-living cnidarian ancestors becoming miniaturized, morphologically simplified and with complex parasitic life cycle and has been enabled to exploit freshwater, marine and terrestrial hosts (Bartholomew et al., 2008; Székely et al., 2015; Okamura et al., 2015). Among the myxozoans, the genus *Myxobolus* Bütschli, 1882 is the most abundant, with more than 850 known species described within a wide geographical range (Eiras et al., 2005; Eiras et al., 2014). Around 41 species of *Myxobolus* have been reported parasitizing fishes in South America (Eiras et al., 2014), the continent with the most diversified freshwater ichthyofauna (Reis et al., 2003).

The ornamental fish sector is a valuable wildlife industry (FAO, 2015). In Brazil, the commerce of ornamental fish is responsible for subsistence of fishing communities of small towns of the Amazonas State (e.g., Barcellos and Manacapuru). In fact, this State is responsible for 93% of the exported ornamental fishes generating an annual income of about US$3.6 million (IBAMA, 2007). The genus *Corydoras* Lacepede, 1803, is a callichthyid freshwater catfish endemic to South America, which are very popular in the market of ornamental fishes (Reis, 1998, Froese and Pauly, 2011). Despite, the commercial importance of *Corydoras* spp., *Myxidium amazonense*, is the unique myxozoan so far reported to infect fishes of this genus (Mathews et al., 2015). Various studies have reported several species of myxosporeans causing considerable pathological changes in the gill of their hosts (Molnár, 1998; Pote et al., 2000; Adriano et al., 2005; Naldoni et al., 2009). Indeed, when present in sufficient number in the gill, myxosporeans can compromise respiratory capacity, because the gill is the major respiratory organ and plays an important role in nitrogenous waste excretion and in the ionic balance (Noga, 2000).

In the present study, morphological and ultrastructural analyses were used to describe a new *Myxobolus* species found in the membrane of the gill arch of the bandit corydoras *Corydoras melini* Lönnberg and Rendahl, 1930 from the Negro River, Amazonas State, Brazil.

## 2. Materials and methods

In June 2014, thirty specimens of *C. melini* were collected from the Negro River, near the town of Santa Isabel do Rio Negro, Amazonas State, Brazil. Fish were transported alive to the field laboratory, where they were euthanized following the methodology approved by the ethics research committee of the Federal University of São Paulo – UNIFESP, CEUA N 9209080214, and all organs and body fluids were examined for myxosporean infections. Mature myxospores were examined on fresh wet mounts by light microscopy. Morphological and morphometric analysis were performed on mature myxospores obtained from different plasmodia based on the criteria outlined by Lom and Arthur (1989). A total of 30 myxospores were measured using a computer equipped with Axiovision 4.1 image software coupled to an Axioplan 2 Zeiss microscope (Göttingen, Germany). Smears containing free spores were air-dried and stained with Giemsa solution and mounted in a low-viscosity mounting medium (CytosealTM) on permanent slides.

For ultrastructural analysis, small fragments of tissues containing plasmodia were fixed in 2.5% glutaraldehyde with 0.1 M buffered cacodylate (pH 7.4), washed in the same buffer and post-fixed with osmium tetroxide ($OsO_4$). 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). Ultrathin sections were double stained with uranyl acetate and lead citrate and examined in an LEO 906 electron microscope operated at 60 kV.

## 3. Results

Plasmodia of an unknown species of *Myxobolus* were found in the serosa layer of the gill arch of wild specimens of *C. melini*. The prevalence of the parasite was 20% (6/30) and the intensity ranged from 1 to 2 cysts per fish. They were not found in any other organs.

***Myxobolus* n. sp. (Figs. 1 – 5).**

Plasmodia 180±0.5 µm in diameter in the serosa layer of the gill arch were spherical to ellipsoidal, which contained numerous myxospores (Fig. 1A - B). The spores were elongated to ellipsoidal in frontal view and biconvex in sutural view (Fig. 2 A - C),

measuring 11.3±0.4 µm in length, 6.8±0.2 µm in width and 4.1±0.2 µm in thickness. Two equal and elongated polar capsules (Figs. 2A and 5), measuring 5.0±0.3 µm in length and 2.0±0.1µm in width. Polar filaments had six to seven turns aligned perpendicularly to the longitudinal axis of the capsule (Figs. 2A and 5). Some aberrant spores had three polar capsules and presents the same measurements of normal spore (Fig. 2C).

The analysis of semi-thin and ultra-thin sections of infected tissue showed plasmodia surrounded by a conspicuous capsule of host connective tissue, which was composed by layers of delicate collagen fibers disposed in distinct orientation and fibroblasts intermingled (Figs. 1A - B and 3A - B). The plasmodial wall had pinocytic channels (Fig. 3B), but projections, invaginations and microvilli were not observed. The development of the plasmodia was asynchronic, with sporoblasts in different developmental stages and immature and mature myxospores (Figs. 3A and 4A - C). The spores had a binucleated sporoplasm and contained moderated number of sporoplasmosomes (Figs. 3A and 4A). In immature spores, conspicuous desmosomal junctions were observed promoting the junction of the valvogenic cells (Fig. 4C).

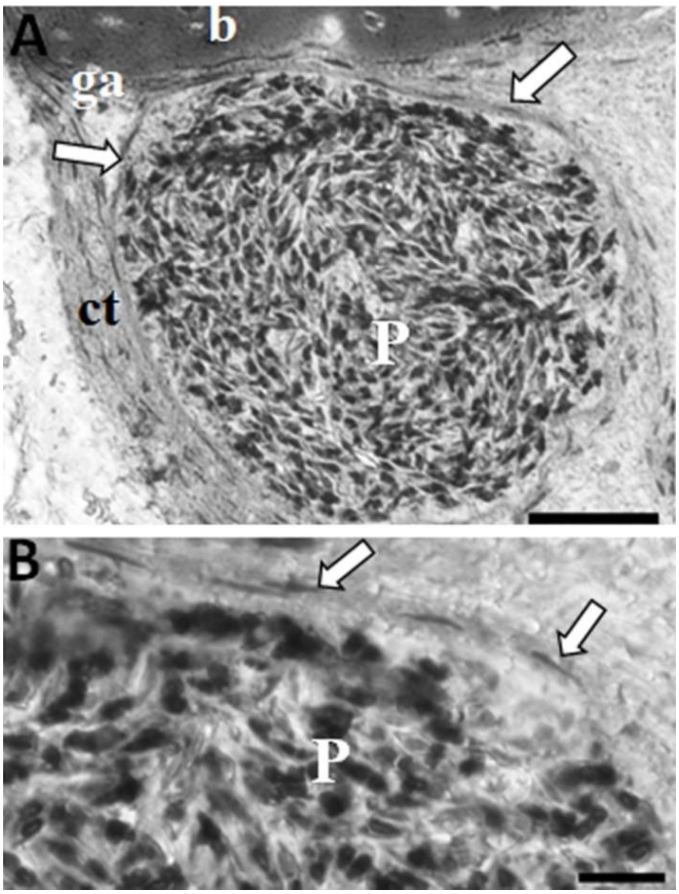


**Fig. 1.** Photomicrograph of semi fine section of gill arch of *Corydoras melini* showing plasmodium of *Myxobolus* n. sp. A: Plasmodium (P) in the connective tissue (ct) of the gill arch (ga). Note the thin connective tissue surrounding the plasmodium (*arrows*) and the bone (b) of the gill arch. Scale bar: 40 µm. B: amplified area of the plasmodium (P) showing the layer of connective tissue (*arrows*). Scale bar: 10 µm.

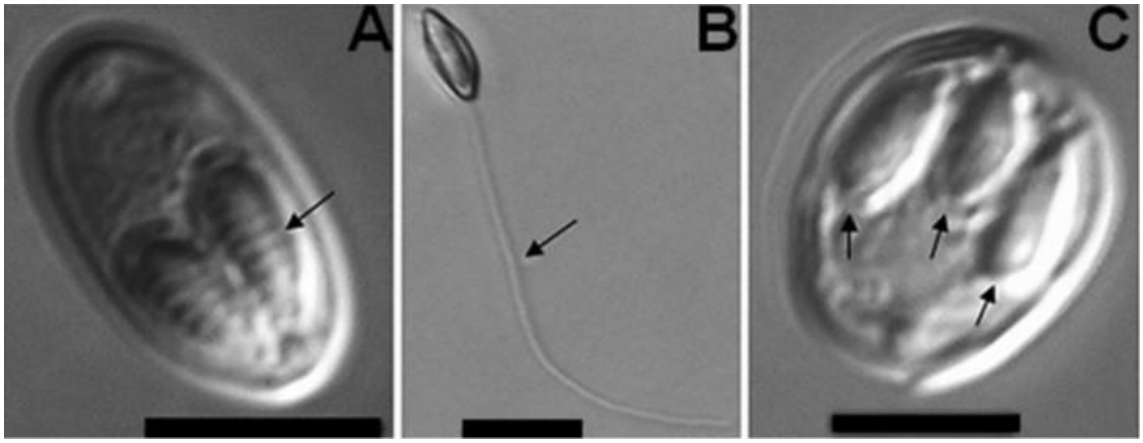


**Fig. 2**. Photomicrograph of mature myxospores of *Myxobolus* n. sp. parasite of gill arch of *Corydoras melini*. A: myxospore with conspicuous polar filaments turns (arrow). Scale bar: 5 µm. B: myxospore in lateral view and with the polar filament extruded (arrow). Scale bar: 10 µm. C: aberrant myxospore with three polar capsules (arrows). Scale bar: 5 µm.

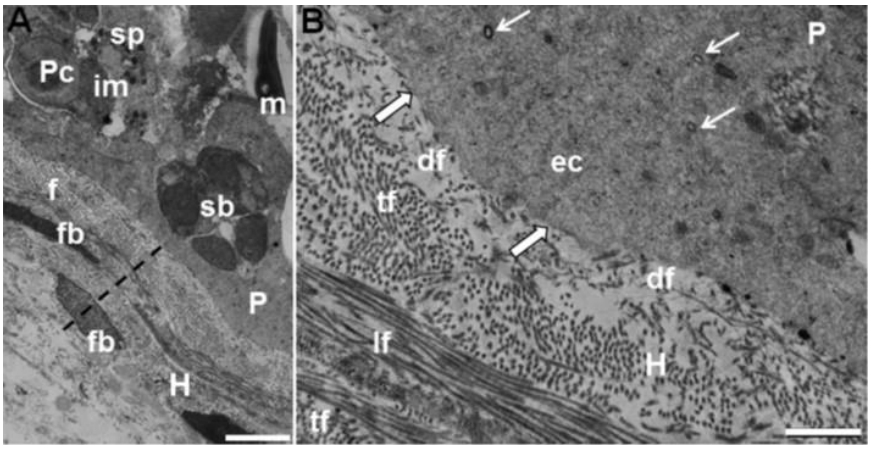


**Fig. 3**. Electron micrograph of gill arch of *Corydoras melini* infected with a plasmodium of *Myxobolus* n. sp. A: section showing host cells (H)-parasite interface with a thin layer of connective tissue (dotted line) composed by fibers of collagen (f) and fibroblasts (fb) surrounding the plasmodium (P). Inside of the plasmodium (P) are seen young sporoblasts (sb), fragments of mature (m) and immature (im) myxospores. Pc—Polar capsule, sp-sporoplasmosomes. Scale bar: 2µm. B: Amplified area of host cells (H)-parasite interface showing collagen fibers disposed in longitudinal (lf) and transversal (tf) layers. Note that nearest to the plasmodial wall, the collagen fibers are more loose and disorganized (df). Inside the plasmodium (P) may be seen transversal sections of pinocytic channels (*thin arrows*) in the ectoplasm (ec) and greater magnification showing the plasmodial wall composed by a single membrane (*thick arrows*). Scale bar: 500 nm.

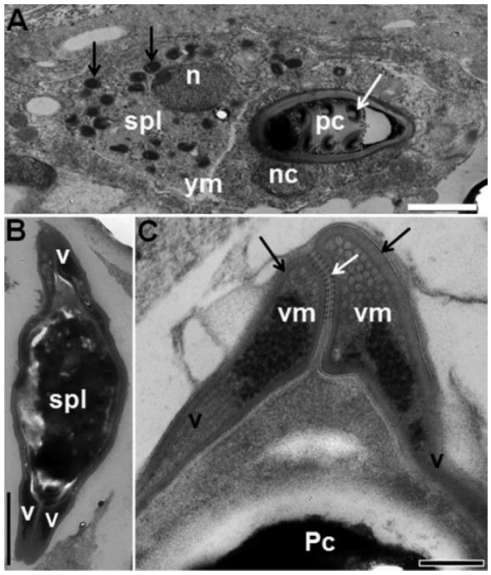


**Fig. 4.** Electron micrographs of myxospores of *Myxobolus* n. sp. parasite of gill arch of *Corydoras melini*. A: young developmental stage myxospores (ym) showing sporoplasm (spl) with its nucleus (n) and sporoplasmosomes (black arrows) and polar capsule (pc) with polar filaments (*white arrow*) and nucleus of capsulogenic cell (nc). Scale bar: 1µm. B: transversal section of the myxospore in sporoplasm (spl) level showing each one of the valves (v). Scale bar: 1µm. C: Detail region of junction of the valves (v) showing valve-forming material (vm), walls of the valves (*black arrows*) and desmosome-like junctions (*white arrow*). Pc−polar capsule. Scale bar: 200 nm.

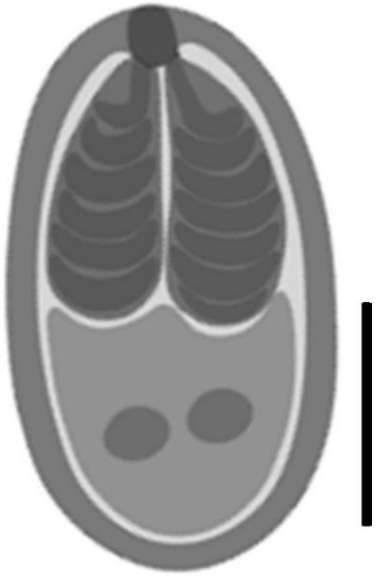

**Fig. 5.** Schematic representation of the mature myxospore of *Myxobolus* n. sp. parasite of gill arch of *Corydoras melini*. Scale bar: 5 µm.

## 4. Discussion

Previous studies in South America have shown widespread distribution of myxosporeans in many cultivated and wildlife fishes (Adriano et al., 2002; Azevedo et al., 2012; Carriero et al., 2013; Eiras et al., 2014; Zatti et al., 2015; Mathews et al., 2015). However, to our knowledge, this is only the second report of a myxosporean species infecting fishes of the genus *Corydoras*, as well as in Amazonian ornamental fish in Brazil (Mathews et al., 2015). Furthermore, of the approximately 850 nominal species of *Myxobolus* reported infecting fish around the world (Eiras et al., 2014), our study is the first report of presence of *Myxobolus* parasiting a fish of the Callichthyidae family.

The morphological data and morphometric parameters of the *Myxobolus* n. sp. were compared with *Myxobolus* spp. described previously, infecting Siluriformes fish: *Myxobolus absonus* Cellere, Cordeiro and Adriano, 2002, *Myxobolus gariepinus* Reed, Basson and Van As, 2003, *Myxobolus pangasii* Molnár, Székely, Mohamed, Shaharom-Harrison, 2006, *Myxobolus baskai* Molnár, Székely, Mohamed and Shaharom-Harrison, 2006, *Myxobolus cordeiroi* Adriano, Arana, Alves, Silva, Ceccarelli, Henrique-Silva and Maia, 2009, *Myxobolus hakyi* Baska, Voronin, Eszterbauer, Müller, Marton and Molnar, 2009 and *Myxobolus flavus* Carriero, Adriano, Silva, Ceccarelli and Maia, 2013 (Cellere et al., 2002; Reed et al., 2003; Molnár et al., 2006; Adriano et al., 2009; Baska

et al., 2009; Carriero et al., 2013). *Myxobolus hakyi* is similar in shape (ellipsoidal) and contains the same amount of polar filaments (six to seven filament turns). However, the spores of this species present larger size (15.9±0.59 µm), are substantially thicker (5.6±0.53 µm) and have two unequal polar capsules. Nevertheless, when comparing to the other six *Myxobolus* species mentioned above, it was noticed that *Myxobolus* n. sp. was readily distinguishable by the shape of its myxospore (elongated/ellipsoidal) and by higher number of polar filament turns (six to seven). When comparing with *Myxobolus* spp. described previously, the myxospores of the *Myxobolus* n. sp. resembled those of *Myxobolus heckelii* Azevedo, Casal, Matos, Ferreira and Matos, 2009, which is a parasite of *Centromochlus heckelii*, *Myxobolus colossomatis* Molnár and Békési, 1993, parasite of *Colossoma macropomum* and the spores of *Myxobolus metynnis* Casal, Matos and Azevedo, 2006, a parasite of *Metynnis argenteus*. However, *M. heckelii* has fewer filament turns (four to five), is thinner (3.1 µm) and is greater in length (12.7 µm). The polar capsules are smaller in length (2.9 µm) and width (1.7 µm) (Azevedo et al., 2009). *M. colossomatis* has thinner myxospores (3.7 µm) and two larger polar capsules (6.0 µm) (Molnár and Békési, 1993). *M. metynnis* has longer (13.1 µm) and wider (7.8 µm) myxospores and also presents two unequal valves and more polar filaments (eight to nine filaments) (Casal et al., 2006). Furthermore, these three species of *Myxobolus* were found parasitizing Characidae and Auchenipteridae fishes. In the same way, considering the shape and size of myxospores and polar capsules, number of polar filament turns, host and tissue affinity, *Myxobolus* n. sp. differs from all others *Myxobolus* spp. parasites of freshwater fish from other continents at least in one of those characteristics.

The ultrastructural analysis of the plasmodia of myxozoan species is very important for understanding the interactions between these parasites and their hosts (El-Matbouli et al., 1990; Adriano et al., 2009; Abdel-Azeem and Abdel-Baki, 2011; Azevedo et al., 2013). In the present study, *Myxobolus* n. sp. plasmodia were surrounded by a capsule of connective tissue composed of layers of collagen fibers with distinct orientation and fibroblasts intermingled, as also observed by Casal et al. (2006) and Azevedo et al. (2009), a host reaction commonly reported in several myxosporean species found parasitizing fishes (Sitjá-Bobadilla, 2008).

As observed in other *Myxobolus* species, the sporogenesis of *Myxobolus* n. sp. showed different developmental stages, including young sporoblasts with

undifferentiated cells to immature spores and mature myxospores (El-Matbouli et al., 1990; Casal et al., 2006; Adriano et al., 2009; Milanin et al., 2010; Abdel-Azeem and Abdel-Baki, 2011). Concerning the mechanisms through which myxosporeans obtain nutrients, in *Myxobolus* n. sp. the presence of few pinocytic channels was observed in comparison to *Myxobolus* spp as also reported by Casal et al. (2002), Milanin et al. (2010) and Zatti et al. (2015).

In the present study, *Myxobolus* n. sp. was observed only in the serosa layer of the gill arch and plasmodial development occurred in the connective tissue, suggesting the affinity of the parasite to this organ and tissue. However, several studies showed that myxosporean infections could have a tissue affinity rather than specificity by organ of infection (Molnár and Békési, 1993; Eszterbauer, 2004; Cone and Easy, 2005). This fact was widely observed in *Myxobolus* species parasitizing simultaneously different organs but a same tissue type, e.g. connective tissue in the same host (Molnár and Békési, 1993; Adriano et al., 2006; Adriano et al., 2009).

Host-specificity has been observed in the majority of the myxozoans (Lom and Dyková, 2006) and data have suggested that mainly histozoic freshwater platysporines myxosporeans have usually showed high host specificity (Molnár and Eszterbauer, 2015). So, based on morphological characteristics of the myxospores, in the high host specificity demonstrated by histozoic freshwater platysporines, and by the fact that this is the first report of a *Myxobolus* species infecting fish of the family Callichthyidae, one may consider *Myxobolus* n. sp. as a new myxozoan species. Despite the importance of molecular data in the description of new myxosporeans taxa, due to the few samples obtained (only seven plasmodia to perform morphological, ultrastructural and molecular analysis), difficulties in standardizing PCR reactions for this species, and limitations in accessing new samples of the same regions, we were not able to provide sequencing data of the new species. However, future molecular and phylogenetic studies are highly recommended, since this would permit stronger taxonomic comparison, as well as show the phylogenetic position of this species in the evolutionary context of myxosporeans.

**Capítulo 3.**

**Morphology and small subunit rDNA-based phylogeny of *Ceratomyxa* n. sp. parasite of *Symphysodon discus*, an ornamental freshwater fish from Amazon**

A formatação segue o padrão do periódico Parasitology Research

**Abstract** The specious genus *Ceratomyxa* Thélodan, 1892, infect mainly gallbladder of marine fishes, with only five species reported infecting species from freshwater environment. This study performed morphological and phylogenetic analyses involving a new *Ceratomyxa* species (*Ceratomyxa* n. sp.) found in gallbladder of *Symphysodon discus* Heckel, 1840 (Perciformes: Cichlidae), an important ornamental fish endemic to Amazon basin. Mature spores were strongly arcuate shaped and measured 7.0 ± 0.3 (6.2–7.6) μm in length, 15.8 ± 0.4 (15.0–16.7) μm in thickness, and polar capsules 3.22 ± 0.34 (2.4–3.6) μm in length and 2.63 ± 0.17 (2.4–2.9) μm in width. This was the first small subunit ribosomal DNA (SS rDNA) sequencing performed to *Ceratomyxa* species parasite of freshwater fish, and the phylogenetic analysis showed *Ceratomyxa* n. sp. clustering in the early diverging subclade of the ceratomyxids, together with species of parasites of amphidromous/estuaries fishes, suggesting some role of the transition of the fishes between marine/freshwater environments in the evolutionary history of these parasites.

**Keywords** Systematic - Myxozoa - Myxosporea - Fish parasite - Ornamental fish - Ceratomyxidae evolution

## Introduction

Among the myxosporeans, the genus *Ceratomyxa* Thélodan, 1892, is one of the most specious, with around 230 species which infect mainly gallbladder of marine fishes, despite only five species reported infecting species from freshwater environment (Eiras 2006; Gunter et al. 2009; Azevedo et al. 2013; Mansour et al. 2015). South America is the continent with the greatest diversity of freshwater fishes (Schaefer 1998) and has almost 120 myxosporeans species reported, the large majority in freshwater fish (Adriano and Oliveira 2016). In this myxosporean fauna, the number of species belonging to the genus *Ceratomyxa* is restrict to four, and only *Ceratomyxa microlepis* Azevedo, Rocha, Casal, São-Clemente, Matos, Al-Quraishy & Matos, 2013, parasite of Amazonian *Hemiodus microlepis*, has been reported in freshwater fish (Azevedo et al. 2013). In this context, while numerous small subunit (SS) rDNA sequences are available to marine *Ceratomyxa* species worldwide, there exist no available data about species from freshwater fishes (Fiala et al. 2015), making it impossible to understand their evolutionary history in the context of the marine lineage.

The present study provides morphological and SS rDNA data of a *Ceratomyxa* species found infecting gallbladder of the freshwater *Symphysodon discus* Heckel, 1840, an important perciform fish in the global aquarium trade (Crampton 2008), endemic to the lowland Amazon basin (Ready et al. 2006; Bleher et al. 2007). The parasite was considered a new species and its phylogenetic relationship was discussed in the context of marine *Ceratomyxa* lineage.

## Materials and methods

In December 2014, 30 wild *S. discus* specimens were collected by seine netting from the Rio Negro, near the city of Manaus, in the state of Amazonas, Brazil. The catches were authorized by Brazilian Ministry of the Environment (SISBIO n ° 44268–4), and fish were transported live to a field laboratory where they were euthanized following the methodology approved by the ethics research committee of the Federal University of São Paulo – UNIFESP, CEUA N 9209080214. All organs and body fluids were examined for myxosporeans infection using a light microscope. Morphological and morphometric analyses were performed on mature spores based on the criteria outlined by Lom and Arthur (1989) and Gunter et al. (2009). Measurements were taken from 30

spores using a computer equipped with Axivision 4.1 image capture software coupled to an Axioplan 2 Zeiss microscope (Göttingen, Germany). The dimensions of the spores (in micrometers) were expressed as the mean ± standard deviation followed by the range in parentheses. Smears containing free spores were air-dried and stained with Giensa solution and mounted in a low-viscosity mounting medium (CytosealTM) on permanent slides to be deposited in the collection of the Museum of Zoology "Adão Jose Cardoso" State University of Campinas, São Paulo, Brazil.

Extraction of DNA was performed from infected bile preserved in ethanol. The sample was pelleted at 8000 rpm for 5 min and the ethanol removed. DNAwas extracted from the pellet using a DNeasy® Blood & Tissue Kit (animal tissue protocol) (QIAGEN Inc., California, USA), in accordance with the manufacturer's instructions. The product was eluted in 50 µl Buffer AE and then quantified in a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA) at 260 nm.

Polymerase chain reactions (PCRs) were conducted with a final reaction volume of 25 µl, which comprised 10–50 ng of extracted DNA, 1× Taq DNA Polymerase buffer, 0.2 mmol dNTPs, 1.5 mmol MgCl2, 0.2 pmol for each primer, 0.25 µl (1.25 U) Taq DNA polymerase (all reagents used were from Invitrogen By Life Technologies, Maryland, USA), and ultrapure water. The SS rRNA gene of *Ceratomyxa* n. sp. was amplified as described by Fiala et al. (2015), with universal eukaryotic primers ERIB1 (ACCTGGTTGATCCTGCCAG; Barta et al. 1997) with ERIB10 (CTTCCGCAGGTTCACCTACGG; Barta et al. 1997). The amplification was performed in the Mastercycler® nexus (Eppendorf, Hamburg, Germany), with an initial denaturation step at 95 °C for 5 min, followed by 35 denaturation cycles at 95 °C for 60 s, annealing at 60 °C for 60 s, and extension at 72 °C for 120 s, following by a terminal extension at 72 °C for 5 min. A control reaction was processed in order to check for possible contamination. The amplicons were analyzed via 1.5 % agarose gel electrophoresis Tris–borate–EDTA (0.045 M Tris–borate, 0.001M EDTA, pH 8.0) stained with GelRed® (Biotium™) and analyzed on a Compact Digimage System transilluminator (Major Science™). The sizes of the fragments were estimated by comparison with 1 kb Plus DNA Ladder (Invitrogen By Life Technologies, Maryland, USA). The PCR products were purified with QIAquick® PCR Purification Kit (QIAGEN Inc., California, USA) according to manufacturer's instructions. Sequencing was performed at the Human Genome Research Center (HGRC), at the University of

São Paulo, with a BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., California, USA) in an ABI 3730 DNA sequencing analyzer (Applied Biosystems).

A standard nucleotide BLAST (blastn) search was conducted to verify the similarity of the sequence obtained in this study with other sequences available in the GenBank database (Altschul et al. 1997). All *Ceratomyxa* species sequences plus *Ceratonova shasta* AF001579 and *Ceratonova gasterostea* KF751186, *Myxodavisia bulani* KM273030 and *Palliatus indecorus* DQ377712 were aligned by ClustalW (Thompson et al. 1997) using the BioEdit program (Hall 1999) and used in the phylogenetic analysis. *Tetracapsuloides bryosalmonae* KF731712 and *Buddenbrockia plumatellae* AY074915 sequences were used as out group.

The phylogenetic analysis was performed using the maximum likelihood (ML) method by PhyML software (Guindon et al. 2010) of online execution, with NNI search, automatic model selection by SMS (Smart Model Selection), under a GTR+G6+I substitution model, optimized equilibrium frequencies, estimated transition/transversion ratio, fixed proportion of invariable sites (0.095), and fixed Gamma shape parameter (0.397).

## Results

Of 30 *S. discus* examined in December 2014, size ranging from 9.3 to 12.1 cm, 22 (73.3 %) had spores of a not described *Ceratomyxa* species in the gallbladder. The vegetative stages were not observed, being numerous spores observed in the bile. These were not found in any other organs and no pathological signs were observed in the parasitized gallbladder.

Mature spores are strongly arcuate shaped in valval view. Valve cells approximately equal-size, slightly tapering, and ending in rounded extremities. Surface without discernable ornamentation measured 7.0 ± 0.3 (6.2–7.6) μm in length, 15.8 ± 0.4 (15.0–16.7) μm in thickness, and posterior angle 105–115°. Polar capsules sub spherical in shape and equal size situated on either side of the sutural line, with 3.22 ± 0.34 (2.4–3.6) μm in length and 2.63 ± 0.17 (2.4–2.9) μm in width. Polar filament appears with 3 to 4 turns. The sporoplasm occupies nearly half of each valve (Fig. 1).

**Phylogenetic analysis**

The SS rDNA sequencing of *Ceratomyxa* n. sp. resulted in a sequence with 1591 nt that did not match none myxozoans sequence available in GenBank in the BLAST search. The phylogenetic analysis showed *Ceratomyxa* n. sp. clustering in the early diverging subclade, which also have *Ceratomyxa leatherjacketi* Fiala, Hlavnickova, Kodadkova, Freeman, Bartošova-Sojkova and Atkinson, 2015, *Ceratomyxa tunisiensis* Thabet, Mansour, Al Omar & Tlig-Zouari, 2016, and the basal species *M. bulani* Fiala, Hlavnickova, Kodadkova, Freeman, Bartošova-Sojkova and Atkinson, 2015 (Fig. 2).

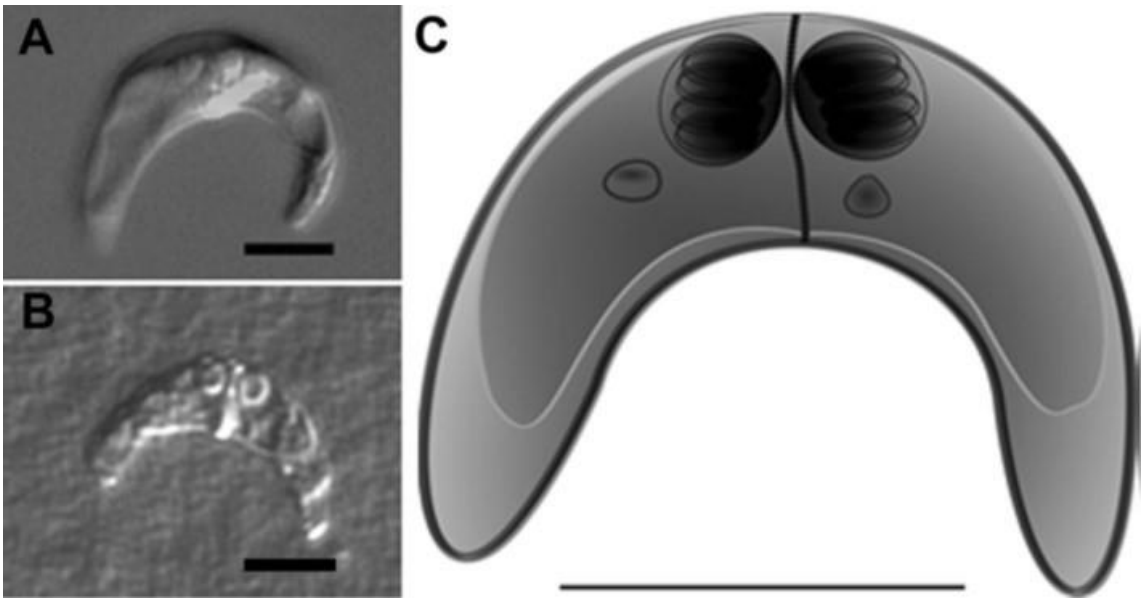


**Fig. 1** *Ceratomyxa* n. sp. parasite of gallbladder of *Symphysodon discus*. a and b Micrographs of mature spores, scale bars = 5 μm. c Line drawing of spore. Scale bar = 10 μm.


**Fig. 2** Phylogram based on maximum likelihood analysis using the SS rDNA dataset showing the position of *Ceratomyxa* n. sp. Bootstrap values above 70 are indicated at the nodes. GenBank accession numbers are after the species name

## Discussion

Although there are several studies for *Ceratomyxa* spp. infecting a wide range of fishes worldwide (Eiras 2006), information about these parasites in South America ichthyofauna is scarce and so far, only four species has been reported: *Ceratomyxa curvata* Cunha & Fonseca, 1918, from *Odontaspis americanus*, *Ceratomyxa hippocampi* Cunha & Fonseca, 1918, from *Hippocampus punctulatus*, both from Brazilian Atlantic coast, *Ceratomyxa dissostichi* Brickle, Kalavati & MacKenzie, 2001,

from *Dissostichus eleginoides* from the Falklands islands and *C. microlepis* from *H. microlepis* from Amazon basin, being this latter the unique species from fresh water (Eiras 2006; Azevedo et al. 2013). Worldwide, besides *C. microlepis* only four other species are known to infect hosts in freshwater environment worldwide: *Ceratomyxa anguillae* Tuzet & Ormières, 1957, from *Anguilla anguilla* from France, *Ceratomyxa hilsae* Chakravarty, 1939, from *Hilsa ilisha* from India, *Ceratomyxa hongtzensis* Hsieh & Chen, 1984, from *Pelteobagrus eupogon* from China and *Ceratomyxa hungarica* Molnár, 1992, from *Proterorhinus marmoratus* from Hungary.

*Ceratomyxa* n. sp. was found infecting a cichlid freshwater fish endemic from the Amazon Basin (Ready et al. 2006; Bleher et al. 2007) and the morphology sharply arched of its spore diverges from all others *Ceratomyxa* species from freshwater fishes, including *C. microlepis*. Regarding *Ceratomyxa* species from marine environment worldwide, the morphology of spores of *Ceratomyxa* n. sp. resembles those of *Ceratomyxa protopsettae* Fujita, 1923, *Ceratomyxa flexa* Meglitsch 1960, *Ceratomyxa hokarari* Meglitsch 1960, *Ceratomyxa uncinata* Meglitsch 1960, *Ceratomyxa trichinocephali* Kpatcha, Diebakate, Faye & Toguebaye, 1996, *Ceratomyxa trachinocephali* Kpatcha, Diebakate, Faye & Toguebaye, 1996, and *Ceratomyxa costata* Aseeva, 2001. However, besides occurring in hosts of marine environment, these species differ from *Ceratomyxa* n. sp. in at least one characteristic, as size of spore and/or polar capsule and/or number of polar filament turns (Meglitsch 1960; Kpatcha et al. 1996; Eiras 2006).

In a detailed phylogenetic analysis, Fiala et al. (2015) showed that the majority of *Ceratomyxa* species clustered in a more recent taxon-rich subclade, with *M. bulani* and *C. leatherjacketi* forming the older ceratomyxid lineage. In our analysis, this older evolutionary subclade was enriched with the insertions of the sequences of the marine species *C. tunisiensis*, found infecting *Caranx rhonchus* and *Trachurus trachurus* from the Mediterranean Sea (Thabet et al. 2016) and that of *Ceratomyxa* n. sp., the first of freshwater environment. The subclade also shows clustering of species from different geographical areas, being *C. leatherjacketi* and *M. bulani* from Indian Ocean and *C. tunisiensis* from Mediterranean Sea (Fiala et al. 2015; Thabet et al. 2016), a picture previously observed by Gunter et al. (2009) and Fiala et al. (2015). However, in a more detailed exam of this subclade, it can be observed that of the four species that compose it, the basal *M. bulani* was described in *Megalps cyprinoides*, an amphidromous fish, *C.*

*tunisiensis* infects *C. rhonchus*, that inhabits brackish-water lagoons and estuaries (Froese and Pauly 2009) and *Ceratomyxa* n. sp. parasites S. discus, endemic to floodplain habitats of the lowland Amazon basin. These data suggest some ancient, but still obscure evolutionary interaction between marine *Ceratomyxa* lineage and freshwater host, and as suggested by Fiala et al. (2015), stress the important role of the transition of the host fishes between marine/freshwater environments in the evolutionary history of these parasites.

Nevertheless, being this the unique available sequence of a *Ceratomyxa* species from freshwater environment, future studies resulting in sequences of other freshwater species, from Amazon and/or other regions, will be very important in the enlightenment of the actual evolutionary relation of the freshwater *Ceratomyxa* species to the specious marine group.

**Capítulo 4.**

***Henneguya* n. sp. 1 (Myxosporea: Myxobolidae), a parasite of *Corydoras melini* (Teleostei: Siluriformes) in the Amazon region: Morphological and ultrastructural aspects**

A formatação segue o padrão do periódico Parasitology Research

**Abstract** A new species of myxozoan, *Henneguya* n. sp. 1 (Myxosporea: Myxobolidae), was described based on morphologic and ultrastructural features. This is a parasite of the ornamental freshwater fish *Corydoras melini* from the Rio Negro, and it was found in five of 30 (16.7 %) *C. melini* examined. The parasite was found in the gill filaments, and the plasmodia had form of round to ellipsoid, with mature and immature spores inside them. The average spore body was 15.5±0.2 µm in length, 4.7±0.1 µm in width, and the tail measured 25.3± 0.1 µm in length. The spores showed typical features of the genus *Henneguya,* with two valves of equal size and two symmetrical polar capsules of 4.8±0.7 µm in length and 1.7± 0.3 µm in width. Each polar capsule had a polar filament with five to 6 turns. Based on morphology (morphologic and ultrastructural data) of the plasmodia and spores and the fact that this

is the first report of a *Henneguya* species in a fish species of the genus *corydoras*, it was considered a new myxozoan species.

**Keywords** Myxozoa, *Henneguya*, Taxonomy, Fish, Amazon

## Introduction

Interest in the Myxozoa as significant fish parasites is still increasing with the growing importance of fish farm and wild fisheries around of the world. *Henneguya* Thélodan, 1982 (Myxozoa: Myxosporea) is one of the genera from family Myxobolidae with the highest diversity (Eiras 2002). Currently more than 200 species are known (Eiras 2002; Eiras and Adriano 2012), and in recent years, South America has been the continent where more than 50% of the new species reported (Eiras and Adriano 2012). Species of the *genus Henneguya* are predominantly histozoic, invading different organs and causing considerable pathological changes (Dyková and Lom 1978; Molnár 1998; Pote et al. 2000; Adriano et al. 2005; Naldoni et al. 2009; Morsy et al. 2009; Barassa et al. 2012).

*Corydoras melini* Lönnberg and Rendahl, 1930 (English name=bandit corydoras), is a Siluriformes freshwater fish belonging to the Callichthyidae family, that harbor freshwater fishes with occurrence restricted to the Neotropical Region (Panama and South America) (Reis 1998). *C. melini* is endemic to the upper Rio Negro and Rio Meta basins in Brazil and Colombia (Reis 1998; Froese and Pauly 2011), and it is popular in the aquarium trade industry (Froese and Pauly 2011). Despite the commercial importance of species of the genus *Corydoras,* only two myxozoans have been reported infecting them (Mathews et al. 2015, 2016).

Herein, as part of a continuous study of freshwater myxozoans biodiversity, was described a new species of Myxozoa, *Henneguya* n. sp. 1, which was found in the gill filaments of *C. melini* from the Rio Negro, Amazonas State, Brazil. The parasite was characterized based on critical morphological features using light and transmission electron microscopy.

## Materials and methods

Thirty wild *C. melini* specimens (ranging from 4.5 to 5 cm in length) caught in the Rio Negro, municipality of Santa Isabel do Rio Negro (0°24′50″S, 65°01′08″O), Amazonas state, Brazil, were examined in June 2014. Fish were transported live to the field laboratory, where they were euthanized following the methodology approved by the

ethics research committee of the Federal University of São Paulo – UNIFESP, CEUA N 9209080214.

All organs and body fluids were examined for myxosporeans infection. Mature myxospores were examined on fresh wet mounts by light microscopy. Morphological and morphometric analyses were performed on mature myxospores obtained from two different plasmodia based on the criteria outlined by Lom and Arthur (1989). Measurements were taken of 30 myxospores using a computer equipped with Axivision 4.1 image capture software coupled to an Axioplan 2 Zeiss microscope. Smears containing free spores were air-dried and stained with Giensa solution and mounted in a low-viscosity mounting medium (CytosealTM) on permanent slides.

For ultrastructural examination, plasmodia were fixed for at least 12 h in 2.5 % glutaraldehyde with 0.1 M buffered cacodylate (pH 7.4). After this, the plasmodia were washed in the same buffer and post-fixed with osmium tetroxide ($OsO_4$). 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). Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined in an LEO 906 electron microscope operating at 60 kV.

## Results

Five out of 30 (16.7 %) specimens of *C. melini* caught from the Rio Negro had plasmodia of an unknown *Henneguya* species in the gill filaments. The intensity ranged of one to three cysts per fish and they were not found in any other organs.

*Henneguya* n. sp. 1 (Figs. 1, 2, 3and 4).

Round to ellipsoidal cysts measured up to 260 µm in diameter and with numerous myxospores were found in the gill filaments of *C. melini* (Fig. 1a). Mature myxospores were ellipsoidal in shape from the frontal view, and had a total length of 40.8±0.3 (40.3−41.1) µm, a body length of 15.5±0.2 (15.3− 15.7) µm, width of 4.7±0.1 (4.6 − 4.8) µm and the tail measured 25.3±0.1 (25.2− 25.4) µm in length. The two polar capsules were elongated and equal in size, and have 4.8±0.5 (4.3−5.3) µm in length and 1.7±0.3 (1.4−2.0) µm in width. They occupied only the anterior third of the myxospore body and helical filament with five to six coils was arranged obliquely to the longitudinal axis (Figs. 1b-c and 4).

Ultrastructural analysis showed a connective capsule surrounding the plasmodia, which was composed by collagen fibrils and some fibroblasts (Figs. 2 and 3a and b). The wall of the plasmodia consisted of a single membrane and the periphery of the ectoplasm had several pinocytic channels (Figs. 2 and 3b). The development of the plasmodia was asynchronous, with numerous mature spores in the central area and young developmental in the periphery (Figs. 1a, 2 and 3). Sections of immature myxospores indicated a polar capsule with five to 6 coils positioned obliquely to the longitudinal axis of the capsule and binucleated sporoplasms that contained several sporoplasmosomes (Figs. 2 and 3a). Capsulogenic and valvogenic cells were readily recognized in the developing sporoblast by the presence of bulbous capsular primordia and associated external tube in the first and by valve-forming materials in the second (Fig. 2).

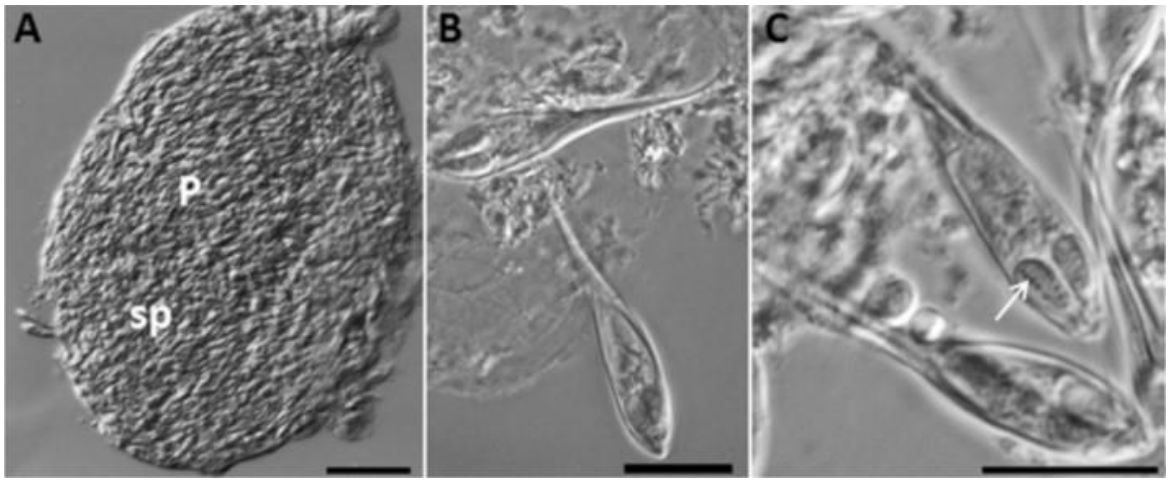


**Fig. 1** Light photomicrographs of *Henneguya* n. sp. 1 parasite of the gill filaments of *Corydoras melini*. a Plasmodium (P) with numerous spores inside (sp). Scale bar=40 μm. b, c Mature spores. Note in c the polar filaments inside of the polar capsules (*white arrow*). Scale bars=10 μm

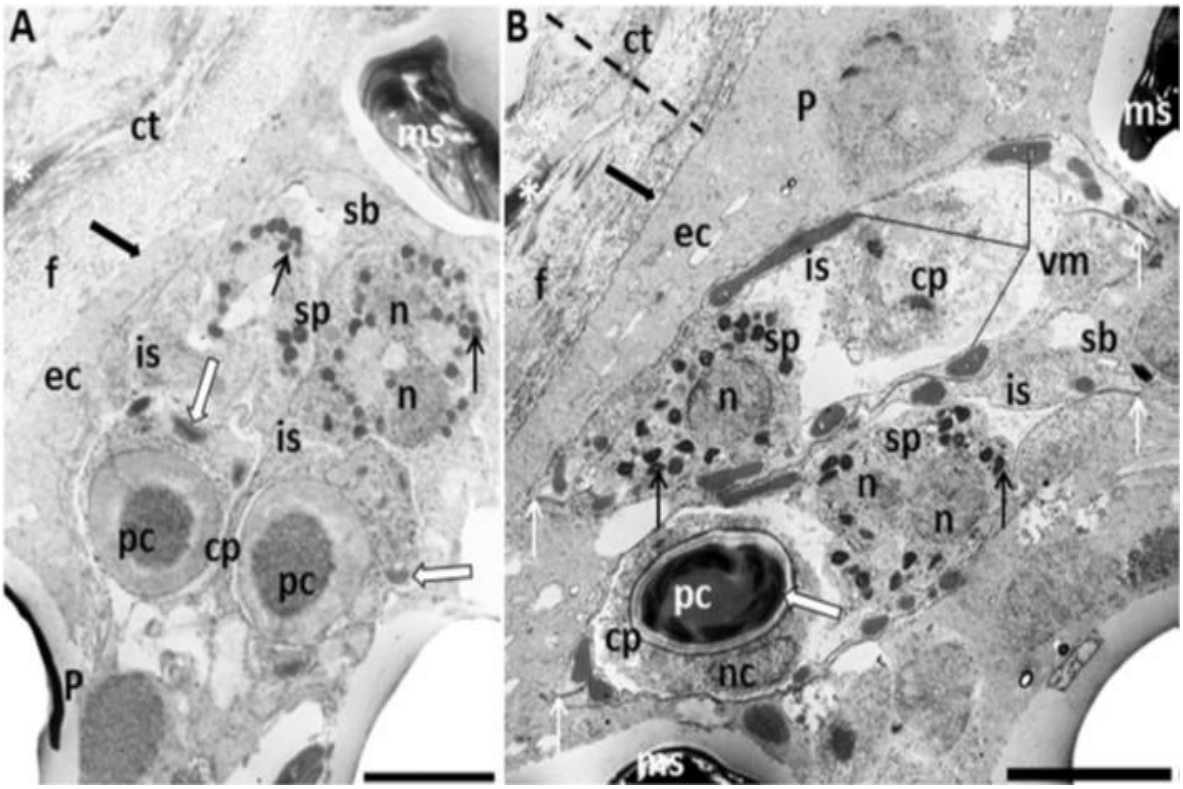


**Fig. 2** Electron micrography of plasmodia (P) of *Henneguya* n. sp. 1 parasite of the gill filaments of *Corydoras melini* showing the host–parasite interface. Externally can be seen connective tissue layer (ct) with collagenic fibers (f) and fibroblasts cells (*asterisk*), surrounding the plasmodial wall (*large black arrows*). Internally are seen a thin ectoplasm (ec), fragments of mature spores (ms), and disporic sporoblasts (sb) with immature spores (is). In a, note young sporoblast developmental stage with polar filaments (large white arrows) still out of the polar capsules (pc). In b, the sporoblast is in more advanced developmental stage, with the polar filament inside of the polar capsule, abundant valve-forming material (vm), and conspicuous sutural line (*white thin arrows*). The binucleate sporoplasms (sp) have numerous sporoplasmosomes (*thin black arrows*). cp capsulogenic cells, nc nucleus of capsulogenic cell, n nucleus Scale bars=2 μm.

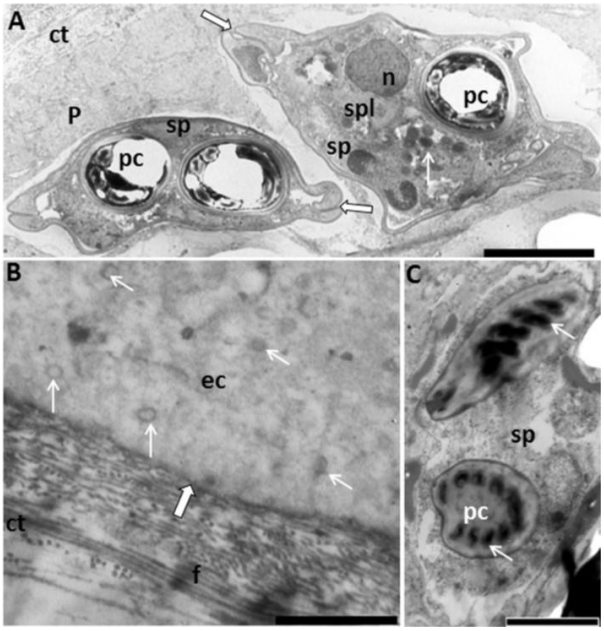


**Fig. 3** Electron micrography of plasmodia (P) of *Henneguya* n. sp. 1 parasite of the gill filaments of *Corydoras melini*. a Almost mature spores (sp). Note the sutural lines (*large arrows*), polar capsules (pc), and sporoplasm (spl) with its nucleus (n) and sporoplasmosomes (*thin arrow*). Scale bar = 2 μm. b Amplified area of a showing longitudinal and transversal distributions of the collagenic fibers ( f ) in the connective tissue layer (ct), single membrane of the plasmodial wall (*large arrow*), and pinocytotic channels (*thin arrows*) in the ectoplasm (ec). Scale bar=1 μm. c Spores (sp) with details of the polar filaments (*arrows*) in the polar capsules (pc). Scale bar=2 μm.

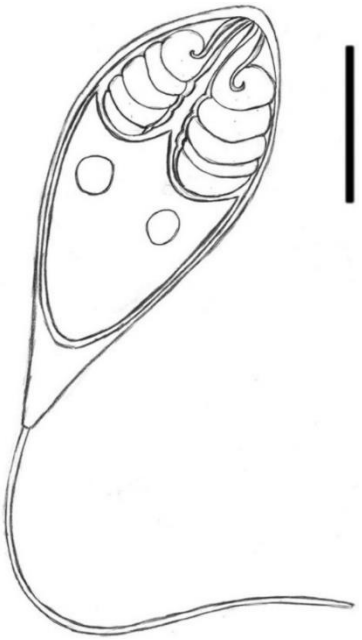

**Fig. 4** Schematic representation of mature spores of *Henneguya* n. sp. 1 from the gill filaments of *Corydoras melini*. Scale bar=5 μm.

**Discussion**

Several studies have reported infections of species of the genus *Henneguya* Thélodan, 1982 in Amazonian fishes (Rocha et al. 1992; Azevedo and Matos 1995; Casal et al. 1997; Vita et al. 2003; Feijó et al. 2008; Videira et al. 2015). However, to our knowledge, this is the first report of a *Henneguya* species parasitizing fish of the family Callichthyidae in the Amazon basin and the first report of this parasite infection in *Corydoras* fish in South America. In the present study, based on morphological features obtained by light and electron microscopy, *Henneguya* n. sp. 1 is described parasitizing gill filaments of *C. melini*, a callichthyid fish endemic to the upper Rio Negro and Rio Meta basins in Brazil and Colombia (Reis 1998; Froese and Pauly 2011).

The morphological and morphometric data of *Henneguya* n. sp. 1 were compared firstly with all *Henneguya* spp. described previously infecting South America siluriforms freshwater fish (Matos et al. 2005; Abdallah et al. 2007; Eiras et al. 2009; Naldoni et al. 2009; Naldoni et al. 2011; Adriano et al. 2012; Carriero et al. 2013; Naldoni et al. 2014). The most similar species to the new species were *Henneguya maculosus*, Carriero, Adriano, Silva, Ceccarelli and Maia, 2013, which infects *Pseudoplatystoma corrucans* and *Henneguya eirasi*, Naldoni, Maia, Silva, Carriero, Ceccarelli, Tavares and Adriano, 2011, which infect *Pseudoplatystoma fasciatum* and *P. corrucans*. However, these differ from *Henneguya* n. sp. 1 in the size of the spore (13.3±0.7 μm in length to *H. maculosus*, 37.1±1.8 μm to *H. eirasi* and 40.8±0.3 μm to the new species), number of coils in the polar filament (six to seven to *H. maculosus*, 12 to 13 *H. eirasi* and five to six to the new species) and because the tail is substantially greater in length (25.3±0.1 μm in the new species and 17.5±1.0 μm in *H. maculosus* and 23.6±2.2 μm in *H. eirasi*). Considering *Henneguya guanduensis* Addallah, Azevedo, Luque and Bomfin, 2007, which was described from *Hoplosternum littorale,* the closest host species to *C. melini*, the comparison showed shorter and wider spores for *H. guanduensis*, with 33.6 x 6.5 μm, while 40.8 x 4.7 μm for *Henneguya* n. sp. 1 The tail was smaller for *H. guanduensis* with 19.0 μm, versus 25.3 μm for *Henneguya* n. sp. 1 and still the two polar capsules of *H. guanduensis* are unequal in size, while they are equal in *Henneguya* n. sp. 1.

Compared with *Henneguya* species which infect siluriformes fish from other regions of the world (Sarkar 1985; Landsberg 1987; Konstoïngue et al. 2001; Reed et al. 2003;

Molnar et al. 2006; Iwanowicz et al. 2008; Griffin et al. 2008; Rabie et al. 2009; Morsy et al. 2012), *Henneguya* n. sp. 1 resembles those of *Henneguya suprabranchiae* and *Henneguya gurlei*, which infect the freshwater catfish *Clarias lazera* and *Ameiurus nebulosus*, respectively (Landsberg 1987; Iwanowicz et al. 2008). However, a small number of noticeable morphologic differences were observed, such as spore tail length (25.3 μm for *Henneguya* n. sp. 1; 41.1 μm for *H. gurlei* and 29.0 μm for *H. suprabranchiae*) and the number of turns of the polar filaments (five to six turns in *Henneguya* n. sp. 1, 10 to 11 turns in *H. suprabranchiae* and nine turns in *H. gurlei*). Other differences may also be observed in the width of the spores (4.7 μm for *Henneguya* n. sp. 1; 3.0 μm for *H. suprabranchiae* and 5.4 μm for *H. gurlei*).

The morphology of *Henneguya* n. sp. 1 also was compared with *Henneguya* species that have been reported infecting gills of not siluriformes fishes from Amazon basin. The species described in this paper has a total size similar to that of *Henneguya aequidens*, Videira, Velasco, Azevedo, Silva, Gonçalves, Matos and Matos, 2015 and *Henneguya striolata*, Casal, Matos and Azevedo, 1997. However, *H. aequidens* has longer tail (27±0.6 μm), is greater in width (6±0.8 μm) and the polar capsules are smaller in length (3±0.3 μm) (Videira et al., 2015). In the same way, *H. striolata* has more number of filament turns (13 to14) and the polar capsules are smaller in width (1.1-1.3 μm) (Casal et al., 1997). Regarding the body width of spore, polar capsule width or length and shape, *Henneguya* n. sp. 1 resembles *Henneguya astyanax*, Vita, Matos, Azevedo, 2003, *Henneguya friderici*, Casal, Matos and Azevedo, 2003, *Henneguya amazonica*, Rocha, Matos and Azevedo, 1992, *Henneguya adherens*, Azevedo and Matos, 1995, *Henneguya malabarica*, Azevedo and Matos, 1996 and *Henneguya schizodon*, Eiras, Malta, Varella and Pavanelli, 2004. Nevertheless, it has been noticed that the parasite was readily distinguishable by lower number of polar filament turns (five to 6 for *Henneguya* n. sp. 1; 8-9 for *H. astyanax*, 7-8 for *H. friderici*, 6-7 for *H. malabarica* and 8-10 for *H. schizodon*). Differences may also be observed in the length of the tail of the spores (25.3 μm for *Henneguya* n. sp. 1; 18.5 μm for *H. adherens*, 41.7 μm for *H. amazonica*, 32.6 μm for *H. astyanax*, 19.1 μm for *H. friderici* and 16.3 μm for *H. schizodon*). In the same way, considering the shape and size of myxospores and polar capsules, number of polar filament turns, host and tissue affinity, *Henneguya* n. sp. 1 differs from all others *Henneguya* spp. parasites of freshwater fish

found out of the Amazon basin, at least, in one of those characteristics (Eiras 2002; Eiras and Adriano 2012).

Ultrastructural studies on the plasmodial wall of species of the *Henneguya* genus are of major important for understanding host–parasite interactions and to show the characteristic features of this important group of parasites (Current and Janovy 1976; Hallett and Diamant 2001; El-Mansy and Bashtar 2002; Abdel-Ghaffar et al. 2008). The plasmodial wall of *Henneguya* n. sp. 1 is bordered by a single membrane and presents pinocytotic channels in the ectoplasma zone of the plasmodium, which are clearly involved in nutrient aquisition, to supporting the developmental forms inside the plasmodia (Hallett and Diamant 2001; El mansy and Bashtar 2002; Naldoni et al. 2014). Sporogenesis of *Henneguya* n. sp. 1 has many similarities with other previously described *Henenguya* species (Ali 1999; Casal et al. 2003; Abdel-Ghaffar et al. 2008; Naldoni et al. 2011), however, there was only a thin layer of the plasmodia with young developmental stages and mature spores viewed near the peripheric area, characterizing asynchronic development, but with slow development. The plasmodia were surrounded by connective tissue capsule, with fibroblast and collagen fibers slow layers disposed transversal and longitudinally, as observed in other studies (Adriano et al. 2005; Matos et al. 2005; Sitjà-Bobadilla 2008; Iwanowicz et al. 2008).

The new species has been characterized only on morphological/morphometric, host, and geographic area data, we recognize the importance of molecular data in the descriptions of new myxosporeans taxa. However, some difficulties were encountered to standardizing PCR reactions to this species, which, added to the few samples obtained and limitations to accessing new samples of the same regions, making it impossible to provide sequencing data of *Henneguya* n. sp.1 So, future molecular and phylogenetic studies are highly recommended, since they will permit stronger taxonomic comparison, as well as show the phylogenetic position of this species in the evolutionary context of myxosporeans.

In the present study, the prevalence and intensity of *Henneguya* n. sp. 1 was relatively low, with only 16.7% of the specimens of *C. melini* being infected and with an intensity of infection ranging from one up to three plasmodia. This finding is in agreement with other *Henneguya* spp. from South American siluriforms from natural environment. Martins et al. (2004), reported a prevalence of 13.4% for *Henneguya* sp. parasite of *Pimelodus maculatus*, Naldoni et al. (2011), who reported for *Henneguya*

*eirasi* a prevalence of 17.1% in specimens of *Pseudoplatystoma corrucans* and *P. fasciatum*, and Carriero et al. (2013), who reported a prevalence of 16% and 19% for *Henneguya maculosus* in *P. reticulatum* and *P. corrucans*. However, the results in the present study are in contrast with those described by Naldoni et al. (2009), who reported a high prevalence, which ranged of 75 up to 100% for *Henneguya Pseudoplatystoma* infecting the farmed pintado hybrid (*P. corrucans* x *P. fasciatum*).

In our study, no disease symptoms were observed in the infected fish specimens. However, several species of the family Myxobolidae have showed to induce pathogeny in their hosts (Molnár 1998; El Mansy and Bashtar 2002; Mohammed et al. 2002; Adriano et al. 2005; Feist and Longshaw 2006; Ali et al. 2007; Naldoni et al. 2009). Thus, as *C. melini* is commonly used in aquariums, there is a need of constant monitoring of the fish for diagnosis and timely control of infections by myxosporean to better prevent future disease outbreaks.

**Capítulo 5.**

**Morphology and small subunit rDNA-based phylogeny of a new *Henneguya* species, infecting the ornamental fish *Corydoras leucomelas* from the Peruvian Amazon**

A formatação segue o padrão do periódico Acta Tropica


## ABSTRACT

A new species of Myxosporea, *Henneguya* n. sp. 2 is described parasitizing the gill filaments from 17 of 35 specimens (48.5%) of *Corydoras leucomelas* (Siluriformes: Callichthyidae) caught in the Nanay River, near village Ninarumi, in the Loreto state, Peru. Mature spores were ellipsoidal in shape from the frontal view, measuring 36.2 ± 0.1 μm (36.1 – 36.3) in total length, 14.3 ± 0.1 μm (14.2 – 14.4) in body length, 5.1 ± 0.1 μm (4.9 – 5.3) in width and 21.9 ± 0.1 μm (21.8 – 22.0) in the caudal process. The two polar capsules were symmetrical and elongated, measuring 5.1 ± 0.1 μm (4.9 – 5.3) in length and 2.4 ± 0.2 μm (2.1 – 2.7) in width, containing a polar filament with five coils arranged obliquely to the longitudinal axis. The sporoplasm was binucleate. Partial sequencing of the ssu-rDNA of *Henneguya* n. sp. 2 resulted in a total of 1676

nucleotides, and this sequence did not match any of the myxozoan available in the GenBank. The phylogenetic analysis shows *Henneguya* n. sp. 2 as a sister species of *Henneguya paraensis*, another amazonian myxozoan parasite of *Cichla temensis* (Perciformes: Cichlidae).

***Keywords:*** *Henneguya*; Myxozoa; Cnidaria; Callichthyidae; Ornamental fish; fish parasite

## 1. Introduction

In the last years, several studies have shown widespread distribution of myxosporeans in many Amazonian fish that have high importance for human nutrition. Nevertheless, almost all reported data so far originate from the Brazilian part of the Amazon basin (Eiras and Adriano, 2012; Videria et al., 2015; Mathews et al., 2015; Mathews et al., 2016a; Adriano and Okamura, 2017). In the Peruvian Amazon, despite the importance of being the second largest portion of the Amazon rainforest, information about myxosporean infection is still very scarce (Espinoza et al., 2017).

Among the myxosporeans that infect fishes, the genus *Henneguya* Thelodan, 1892, has more than 200 known species (Eiras, 2002; Eiras and Adriano, 2012), and in recent years, South America has been the geographical region for where more than 40 new species were reported (Eiras and Adriano, 2012; Carriero et al., 2013; Naldoni et al., 2014; Velasco et al., 2016). Notably, *Henneguya* species are predominantly histozoic and these parasites can cause considerable pathological changes in their hosts (Dyková and Lom, 1978; Molnár, 1998; Pote et al., 2000; Adriano et al., 2005; Naldoni et al., 2009; Morsy et al., 2012).

Several members of the Family Callichthyidae Bonaparte, 1838, are commercially important in wild fisheries and pisciculture in many South American countries, e.g., the genus *Corydoras* Lacepede, 1803, the most diversified catfish genus (Reis, 1998), which is endemic to South America, is highly valued and very popular in the market of ornamental fish. Among the *Corydoras* spp., *Corydoras leucomelas* Eigenmann & Allen, 1942, is a tropical freshwater fish with occurrence restricted in the Upper Amazon River basin in Colombia, Ecuador, and Peru, and it is also common in the aquarium trade industry (Nijssen and Isbrücker, 1986; Prang, 2007). Despite the commercial importance and diversity of species of the genus *Corydoras*, to our knowledge only three myxozoans have been reported infecting them (Mathews et al., 2015; Mathews et al., 2016a, 2016b). In the present article, based on morphologic and small subunit rDNA (ssu-rDNA) sequencing data, we further identified and described a new *Henneguya* species parasitizing the gill filaments of *C. leucomelas* caught in the Nanay river, Loreto State, Peru.

## 2. Materials and methods

Thirty-five wild *C. leucomelas* specimens (ranging from 4.1 to 4.5 cm in length) caught in the Nanay River, near village Ninarumi (3°50'33" S, 73°23'15" W), Loreto State, Peru, were examined in November 2014. Immediately after capture, the fish were transported alive to the field laboratory, where they were euthanized following the methodology approved by the ethics research committee of the Federal University of São Paulo – UNIFESP, CEUA N 9209080214. All organs and body fluids (pericardial, and perivisceral fluids) were examined for myxosporean infections. Morphological and morphometric analysis were performed on formalin-fixed mature myxospores obtained from different plasmodia (Lom and Arthur, 1989). A total of 30 myxospores were measured using a computer equipped with Axiovision 4.1 image software coupled to an Axioplan 2 Zeiss microscope (Zeiss, Göttingen, Germany). Smears containing free spores were air-dried and stained with Giemsa solution and mounted in a low viscosity mounting medium (Cytoseal$^{TM}$) (Thermo Fisher Scientific, Massachusetts, USA) on permanent slides.

For the ssu-rDNA sequencing, genomic DNA extracting was performed on myxospores preserved in absolute ethanol, using a DNeasy® Blood & Tissue Kit (Qiagen, California, USA), in accordance with the manufacturer's instructions. Polymerase chain reactions (PCRs) were conducted in accordance with Adriano et al. (2014). The reaction final volume was 25 μL, which comprised 10–50 ng of extracted DNA, 1× Taq DNA Polymerase buffer (Invitrogen by Life Technologies, Maryland, USA), 0.2 mmol dNTPs, 1.5 mmol $MgCl_2$, 0.2 pmol for each primer, 0.25 μL (1.25 U) Taq DNA polymerase (Invitrogen by Life Technologies, Maryland, USA), and ultrapure water. The amplification of fragments containing approximately 1.000 pb and 1.200 bp was performed with the primer pairs ERIB1-ACT1R and MYXGEN4f-ERIB10, respectively (Barta et al., 1997; Kent et al., 2000; Hallett and Diamant, 2001). PCRs were performed in an AG22331 Hamburg Thermocycler (Eppendorf, Hamburg, Germany) with 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. PCR products were subjected to electrophoresis in 1.0 % agarose gel (BioAmerica, Florida, USA) in a TBE buffer (0.045 M Tris-borate, 0.001 M EDTA, pH 8.0), stained with ethidium bromide, and then analyzed with a FLA-3000 scanner (Fuji Photo Film, Tokyo, Japan). PCR products

were purified using USB® ExoSap-IT® (Thermo Fisher Scientific, Ohio, USA) in accordance with the manufacturer's instructions and sequenced using the same PCR primers. Sequencing was performed at the Human Genome Research Center (HGRC), at the University of São Paulo, with a BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., California, USA) in an ABI 3730 DNA sequencing analyzer (Applied Biosystems).

A standard nucleotide-nucleotide BLAST (blastn) search was conducted to verify the similarity of the sequence obtained in this study with other sequences available in the GenBank database (Altschul et al., 1997). Bioedit (Hall, 1999) was used to visually assemble sequence fragments and to compare the coding sequence obtained with the 38 most closely related *Henneguya* species, as determined by the BLAST search.

Phylogenetic analysis was performed using maximum likelihood (ML) methods with a Kimura 2-parameter (K2P) evolution sequence model in the MEGA 6.0 program (Tamura et al., 2013). Bootstrap analysis (1000 replicates) was employed to assess the relative robustness of the tree branches. *Ceratonova shasta* (AF001579) sequence was used as outgroup. Other alignment, including the species that cluster together with the parasite here described, was used to produce a pairwise similarity using MEGA 6.0.

## 3. Results

Of the 35 specimens of *C. leucomelas* examined, 17 (48.5%) contained plasmodia of an unknown *Henneguya* species in the gill filaments. The intensity ranged of one to two plasmodia per fish and they were not found in any other organs.

*Description of Henneguya* n. sp. 2 (Figs. 1A-D and 2).

Rounded plasmodia with myxospores were found in the gill filaments of *C. leucomelas* (Fig. 1A). Mature myxospores were ellipsoidal in shape from the frontal view and had a total length of 36. 2 ± 0.1 μm (36.1 – 36.3), a body length of 14.3 ± 0.1 μm (14.2–14.4) and 5.1 ± 0.1 μm (4.9–5.3) in width (Figs. 1B-C and 2). The tail measured 21.9 ± 0.1 μm (21.8–22.0) in length. The two polar capsules in the anterior pole of spore were symmetrical, elongated and presented 5.1 ± 0.1 μm (4.9–5.3) in length and 2.4 ± 0.2 μm (2.1–2.7) in width (Fig. 1C and 2) (Table 1). They occupied only the anterior third of the myxospore body and contained an helical filament with five coils arranged

obliquely to the longitudinal axis (Fig. 1C and 2). The sporoplasm was noticeably binucleate (Fig. 1D).

The sequencing of the ssu-rDNA of *Henneguya* n. sp. 2 obtained from gill filaments of specimens of *C. leucomelas* resulted in a total of 1676 nucleotides that did not match any myxozoan species sequences available in GenBank. The most similar sequences identified by a blastn search was *Henneguya ictaluri* Pote, Hanson and Shivaji, 2000 (query coverage 91%, maximum identities 92%), reported in the gills of *Ictalurus punctatus*, from the commercial catfish pond in Brooksville, Mississippi State, USA (Pote et al., 2000). Phylogenetic analysis, with 38 species in the ingroup and *C. shasta* as an outgroup showed the formation of two *Henneguya* clades. The smaller clade was formed by *Henneguya* species reported infecting freswater salmoniforms, cypriniforms, and siluriformes hosts, showing a strong tendency among *Henneguya* species to form clades according to the taxonomic affinity of the fishes (order, and more specifically, family of the host). The larger clade was composed by parasites of several families of marine and freshwater fishes. In this large clade, *Henneguya* n. sp. 2 appeared in a small sub-calde cluster together with *H. paraensis* Velasco, Videira, Nascimento, Matos, Gonçalves and Matos, 2016, and had *Henneguya pellucida* Adriano, Arana and Cordeiro, 2005, as the basal species (Fig. 3). The pairwise comparisons among the ssu-rDNA sequences of *Henneguya* species of this sub-clade showed that the genetic similarity of the new species was of 90.7% to *H. paraensis* and 87.3% to *H. pellucida*.

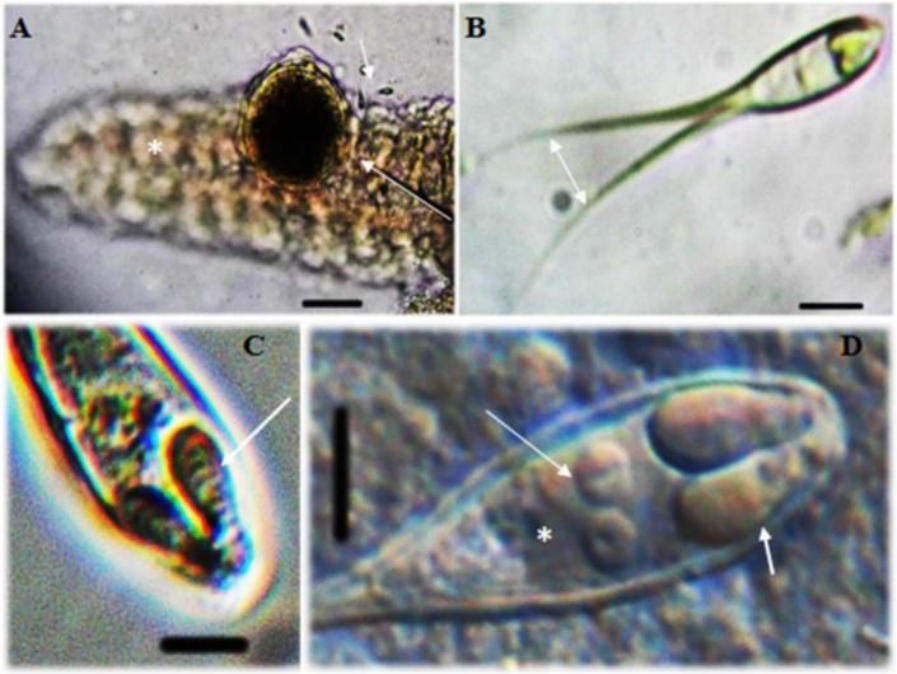


**Fig. 1.** Photomicrograph of *Henneguya* n. sp. 2 parasite of *Corydoras leucomelas*. A, Plasmodium (*long white arrow*) infecting gill filaments (*) with some out spores (small white arrow). Scale bar: 40 µm. B, Fresh spores with a view of the tails (*thin wthite arrows*). Scale bar: 10 µm. C–D, Differential interference-contrast (DIC) microscopy image of spore body showing the polar capsules with five coils arranged obliquely to the longitudinal axis (*large white arrow*) and sporoplasm (*) noticeably binucleate (*thin white arrow*). Scale bar: 10 µm.

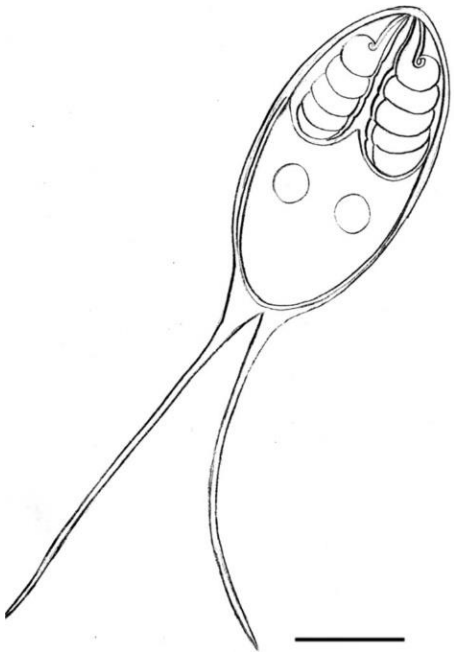

**Fig. 2.** Schematic representation of mature spore of *Henneguya* n. sp. 2 parasite of gill filaments of *Corydoras leucomelas*. Scale bar: 5 µm.

| Species | Total length | Spore length | Spore width | Thickness | LPC | WPC | Tail length | NCF | ite of infection and host | Locality | Source |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Henneguya* n. sp. 2 | 36. 2 ± 0.2 (36.1 – 36.3) | 14.3 ± 0.1 (14.2–14.4) | 5.1 ± 0.2 (4.9–5.3) | – | 5.1 ± 0.2 (4.9–5.3) | 2.4 ± 0.3 (2.1–2.7) | 21.9 ± 0.1 (21.8–22.0) | 5 | Gill filaments of *Corydoras leucomelas* | Nanay river, Peru | Thsi study |
| *Henneguya* n. sp. 1 | 40.8 ± 0.3 (40.3–41.1) | 15.5 ± 0.2 (15.3–15.7) | 4.7 ± 0.1 (4.6–4.8) | – | 4.8 ± 0.5 (4.3–5.3) | 1.7 ± 0.3 (1.4–2.0) | 25.3 ± 0.1 (25.2–25.4) | 5-6 | Gill filaments of *Corydoras melini* | Negro river, Brazil | Mathews et al. 2016 |
| *Henneguya guanduensis* | 33.6 (27.3 - 38.1) | 14.6 (11.4 - 16.7) | 6.5 (4.9 - 7.9) | – | 4.4 (3.3-5.6) 4.1 (3.3 - 5.3) | 2.0 (1.6-2.3) 2.2 (1.5-2.8) | 19.0 (15.6-22.5) | 3-6 | Gill of *Hoplosternum littorale* | Guandu river, Brazil | Abdallah et al. 2007 |
| *Henneguya cuniculator* | 29.4 ± 1.9 (23.3−32.4) | 12.13 ± 0.69 (10.0−14.7) | 4,8 ± 0.29 (4.0−5.9) | 4.23 ± 0.15 (3.9−4.9) | 6.18 ± 0.28 (5.2−6.2) | 1.8 ± 0.12 (1.4−1.9) | 16.7 ± 1.9 (12.3−19.4) | 10-11 | Gill filaments of *P. corruscans* | São Francisco river, Brazil | Naldoni et al. 2014 |
| *H. multiplasmodialis* | 30.8 ± 1.3 | 14.7 ± 0.5 | 5.2 ± 0.3 | 4.4 ± 0.1 | 6.1 ± 0.1 | 1.4 ± 0.1 | 15.4 ± 1.3 | 6-7 | Large cysts in the gill of *P. corruscans* | Brazilian Pantanal wetland, Brazil | Adriano et al. 2012 |
| *H. multiplasmodialis* | 30.6 ± 1.2 | 14.5 ± 0.4 | 5.2 ± 0.2 | 4.2 ± 0.3 | 6.2 ± 0.2 | 1.5 ± 0.2 | 14.8 ± 1.4 | 6-7 | Large cysts in the gill of *P. fasciatum* | Brazilian Pantanal wetland, Brazil | Adriano et al. 2012 |
| *H. pseudoplatystoma* | 33.2 ± 1.9 | 10.4 ± 0.6 | 3.4 ± 0.4 | – | 3.3 ± 0.4 | 1.0 ± 0.4 | 22.7 ± 1.7 | 6-7 | Gill of hybrid pintado | Fish farms: São Paulo and Mato Grossso do Sul states, Brazil | Naldoni et al. 2009 |
| *H. corruscans* | 27.6 (25-29) | 14.3 (13-15) | 5.0 | – | 6.8 (6-7) | 2.0 | 13.7 (12-15) | 5-6 | Gill of *P. corruscans* | Paraná river, Brazil | Eiras et al. 2009 |
| *H. eirasi* | 37.1±1.8 | 12.9±0.8 | 3.4±0.3 | 3.1±0.1 | 5.4±0.5 | 0.7±0.1 | 24.6±2.2 | 12–13 | Gill filaments of *P. corruscans* and *P. fasciatum* | Brazilian Pantanal wetland, Brazil | Naldoni et al. 2011 |
| *H. maculosus* | 31.2 | 13.7±0.6 | 4.1±0.2 | 3.0±0.3 | 5.6±0.5 | 1.6±0.2 | 17.5±0.5 | 6-7 | Gill filaments of *P. corruscans* | Brazilian Pantanal wetland, Brazil | Carriero et al. 2013 |
| *H. maculosus* | 33.0 | 13.3±0.7 | 4.4±0.4 | 3.5±0.4 | 5.2±0.6 | 1.6±0.2 | 19.7±0.6 | 6-7 | Gill filaments of *P. reticulatum* | Brazilian Pantanal wetland, Brazil | Carriero et al. 2013 |

**Table 1.** Comparative data of *Henneguya* n. sp. 2 with other *Henneguya* spp. parasites of siluriform fishes from South America. Spore dimensions, infection sites, and collection sites are given. LPC: length of polar capsules; WPC: width of polar capsules; NCF: number of coils of polar filaments; −: no data. All measurements are means ± SD and/or range, in μm.

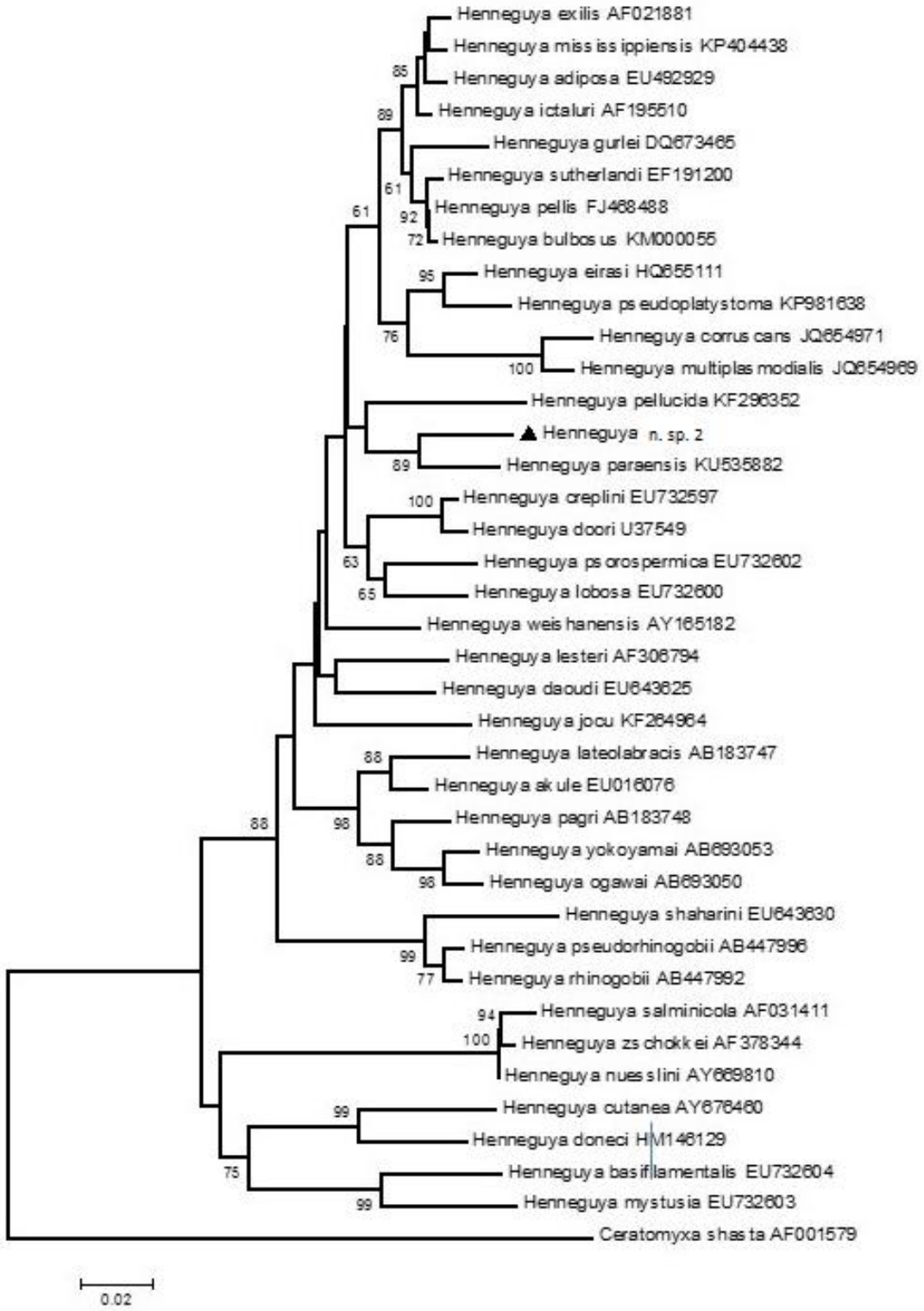


**Fig. 3.** Maximum likelihood tree, based on partial ssu-rDNA sequencing, showing the relationship between *Henneguya* n. sp. 2, parasite of *Corydoras leucomelas* and others *Henneguya* spp. The numbers above the nodes indicate bootstrap confidence levels.

## 4. Discussion

Although previous studies in South America have shown a considerable number of *Henneguya* spp. infecting farmed and wild fishes (Eiras and Adriano, 2012; Carriero et al., 2013; Moreira et al., 2014; Velasco et al., 2016), there is only one species described in an ornamental fish from Amazon region (Mathews et al., 2016b). The present study reports, for the first time, the infection of a myxosporean in *C. leucomelas*, being notably as well the first report of these parasites infecting fish from Peruvian Amazon.

The morphological features of *Henneguya* n. sp. 2 were compared with those of all *Henneguya* species reported to infect South American freshwater fishes (Naldoni et al., 2009; Eiras and Adriano, 2012; Carriero et al., 2013; Moreira et al., 2014; Videira et al., 2015; Velasco et al., 2016). Among the ten *Henneguya* species found in South American siluriforms fish, two species have been described infecting fish of the Family Callichthyidae: *H. melini* Mathews, Maia and Adriano, 2016, infects the gill filaments of *Corydoras melini*, and *H. guanduensis* Abdallah, Azevedo, Luque and Bonfim, 2007, a reported parasite of the gills of *Hoplosternum littorale* (Abdallah et al., 2007; Mathews et al., 2016b). The morphological and morphometric comparison of the new species with parasite species of callichthyids fishes indicated that the spores of this parasite were more similar to those of *H. guanduensis,* with regard to dimensions of body length (14.6 μm to *H. guanduensis* and 14.3 μm to the new species). However, they differ in total spore length (33.6 μm to *H. guanduensis* and 36.2 μm to *Henneguya* n. sp. 2), body width (6.5 μm to *H. guanduensis* and 5.1 to the new species) and in the tail length (19.0 μm to *H. guanduensis* and 21.9±0.1 μm to the new species). Furthermore, the two polar capsules in *H. guanduensis* are unequal in size, while they are equal in *Henneguya* n. sp. 2, and the tail in *H. guanduensis* is not bifurcated at the end of spore body, but in the new species the tail bifurcates near the spore body end. Thus, based on differences in morphometric and morphology data, likewise differences in the host genus (*Hoplosternum* to *H. guanduensis* and *Corydoras* to the new species), emphasizing as well the different geographic area of occurrence (Paraíba do Sul basin to *H. guanduensis* and Amazon basin to *Henneguya* n. sp. 2), these arguments are sufficient to strongly support the separation of these species. Considering *H. melini*, a closest host species to *C. leucomelas*, the comparison showed larger dimentions of the total spore length and tail lenght for *H. melini* (respectively 40.8 and 25.3 μm), compared to 36.2 and 21.8 μm of the new species, and a wider spores of *Henneguya* n.

sp. 2 (5.1 μm) versus 4.7 μm to *H. melini*. Furthermore, the tail is not bifurcated for *H. melini* in contrast to the new species. Unfortunatly there are not ssu-rDNA data available for *H. guanduensis* and *H. melini* in GenBank, rendering imposible the genetic comparation of them with *Henneguya* n. sp. 2 and the evaluation of the phylogenetic relationships of these three *Henneguya* species parasites of the siluriforms of the Family Callichthyidae within of the evolutionary context of myxobolids. Therefore, future molecular and phylogenetic analysis are highly recommended to *H. guanduensis* and *H. melini* for a definitive comparison with these species. In the same way, for a better interpretation of myxosporean phylogeny from Amazon basin will need the the combination of studies on morphological features, host specificity, tissue affinity, and molecular analyses (Abdel-Gaber et al., 2017).

Compared with the other *Henneguya* species that infect other families of siluriforms fish in South America, as related in Table 1, the new species differed in at least one character (size of spore or polar capsules, shape of spore, bifurcation of the tail and number of polar filament turns). The morphology of the new species was besides compared with species of *Henneguya* that parasitize gills of not siluriforms freshwater fish from South America. Morphologically, it resembles the spores of *Henneguya visibilis* Moreira, Adriano, Silva, Ceccarelli and Maia, 2014, which infects the freshwater fish *Leporinus obtusidens* (Moreira et al., 2014). However, morphometrically, the spores of this species are shorter and narrower (26.8 μm long and 3.9 μm wide) and the polar capsules are shorter (4.9 μm) and narrower (1.4 μm) than those of the new species. Furthemore, they also differ in the number of coils of the polar filament (eight to nine to *H. visibilis* and five to *Henneguya* n. sp. 2). Moreover, these species differ also in the ssu-rDNA data. Thus, based on differences in morphometrical and morphological and ssu-rDNA sequences data, the parasite here reported was considered a new taxon.

In phylogenetic analysis, *Henneguya* n. sp. 2 appeared as sister species of *H. paraensis*, a parasite of the gills of *Cichla temensis* (Perciformes: Cichlidae), and the unique other amazonian species in this analysis. This data points out the geographic affinity, however, due to the few ssu-rDNA data available about Amazonian myxosporeans, only the addition of molecular data from other taxa will enable a better understanding about the evolutionary context of *Henneguya* n. sp. 2, as well of the freshwater myxosporeans of that region as a whole.

**Capítulo 6.**

**High infection, morphology and SSU rDNA sequencing of *Henneguya* n. sp. 3 (Myxosporea), a parasite of the Amazonian ornamental fish *Hyphessobrycon loretoensis* from Peru: A myxosporean dispersal approach**

A formatação segue o padrão do periódico Aquaculture.


## ABSTRACT

Myxosporean are endoparasitic cnidarians of wide distribution and responsible for important economic losses on fisheries and aquaculture. A new myxosporean species, *Henneguya* n. sp. 3, is described parasitizing the gill filaments of the ornamental fish *Hyphessobrycon loretoensis* caught in the Nanay River, Department of Loreto, Peru. The parasite was found in 37 of 45 (82.2%) *H. loretoensis* examined and the new species was characterized based on morphological features and SSU rDNA sequence data. The sequencing of the SSU rDNA from the spores of *Henneguya* n. sp. 3 resulted in 1632 nucleotides and this sequence did not match any of the myxozoan available in the GenBank. Phylogenetic analysis showed *Henneguya* n. sp. 3 closed together with *H. leporinicola.* Nonetheless, the SSU rDNA sequences of *Henneguya* n. sp. 3 and *H. leporinicola* have only 82% similarity. This is the first description and molecular study of a Myxozoa parasitizing fish of the genus *Hyphessobrycon* in the Amazon basin. Given the importance of the ornamental fish industry in moving aquatic organisms worldwide, the international movement of myxosporeans in infected fish is discussed in terms of disease outbreaks and the need for preventative action.


**Keywords:** Myxosporean; Fish parasites; *Henneguya*; Gill filaments; Ornamental fish

## 1. Introduction

As a function of growing demand of the aquatic pet trade, the risk of transboundary spread of several pathogens has increased in the last years (Whittington and Chong, 2007; Camus et al., 2013; Becker et al., 2014; Hallet et al., 2015). Nowadays, the aquarium trade industry moves millions of fishes each year around the world and besides, the global connectivity has raised with shorter transportation times, thus favoring that myxosporeans arrive viable to new regions (Evans and Lester, 2001; Biosecurity, 2005; Garner et al., 2008; Camus et al., 2017), in some cases causing pathological changes and mortalities (Garner et al., 2008; Camus and Griffin, 2010). In this scenario, myxosporeans survey on ornamental fish becomes important for diagnosis and timely control to prevent that these parasites will be introduced and disseminated to foreign locations.

Myxosporean are endoparasitic cnidarians of wide distribution around the world and they are responsible for important economic losses on fisheries and aquaculture (Pote et al., 2000; Foott et al., 2004; Hallet and Bartholomew, 2011). Annelids are the definitive hosts, excreting actinospores in faeces into water. Mostly fish and some other vertebrates such as amphibians, reptiles, waterfowl and small mammals may become infected by infective actinospores and can be intermediate hosts of myxosporeans (Eiras et al., 2005; Bartholomew et al., 2008; Székely et al., 2015; Abdel-Ghaffar et al., 2016; Espinoza et al., 2017).

Among myxosporean, species belonging to the genus *Henneguya* Thélodan, 1982, are typically histozoic parasites that cause considerable pathological changes and severe diseases in wild and cultured fish (Naldoni et al., 2009; Dykova et al., 2011; Morsy et al., 2012). In South America, despite several studies have reported *Henneguya* species in many species of wild and farmed freshwater fish (Eiras and Adriano, 2012; Carriero et al., 2013; Naldoni et al., 2014; Velasco et al., 2016), information in ornamental fish is still scarce and only two *Henneguya* species have been reported infecting ornamental fish from Amazon basin (Mathews et al., 2016a; Mathews et al., 2017).

The genus *Hyphessobrycon* Durbin, 1908, is the most species-rich genus in Characiformes species, with approximately 150 valid species and one of the largest characid genera with a wide distribution in all major watersheds of the Neotropical Region (García-Alzate et al., 2013). The tetra loreto *Hyphessobrycon loretoensis*

Ladiges, 1938, is a native fish from upper Amazon River basin in Peru, Ecuador and Colombia and inhabits slow to moderate flow rivers of black waters and is internationally exported in the aquarium trade industry (Jimenez-Prado and Arguello, 2016). Despite the commercial importance of *H. loretoensis,* information about the parasite fauna is still scarce and nothing is known about myxosporean parasites. Therefore, in the present study we describe a novel freshwater *Henneguya* species from the gills of *H. loretoensis*, caught in the Nanay River, Department of Loreto, Peru. We characterized the new species based on morphological features and SSU rDNA sequencing, emphasizing the importance of identification and diagnosis of myxosporean infections and warning the introduction in foreign locations, prevention of dissemination and future disease outbreaks.

## 2. Materials and methods

Forty-five wild *H. loretoensis* specimens (ranging from 2.1 to 2.4 cm in length) were caught in the Nanay River, near village Santa Maria de Nanay (03° 53′ 13″ S, 73° 42′ 01″ W), Department of Loreto, Peru. These samples were caught in July 2016 during the dry season in the Amazon region. After captured, alive fish were placed in plastic bags containing one-fourth water and three-fourths oxygen, and immediately transported to the Limnology Research Laboratory at Research Institute of Peruvian Amazon. The fish were stocked in two aquariums with water free of chemical pollutants, and with temperature at 26 °C and pH of 5.9.

For myxosporeans survey, fish were euthanized following the methodology approved by Ethics Committee on Animal Use of Federal University of São Paulo - UNIFESP (CEUA no 9209080214). Gill filaments infected with myxosporeans were removed and fixed in formalin (10%) for morphological analysis (Lom and Arthur, 1989) and in 100% ethanol for DNA sequencing. Morphological characterization of myxospores was performed using differential interference contrast (DIC) microscopy 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, New York, USA). Permanent slides were performed with smears containing free spores, which were air-dried and stained with Giensa solution and mounted in a low

viscosity mounting medium (Cytoseal™) (Thermo Fisher Scientific, Massachusetts, USA).

For the molecular characterization genomic DNA was extracted using the DNeasy® Blood & Tissue Kit (Qiagen, California, USA), in accordance with the manufacturer's instructions. DNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Delaware, USA). The SSU rDNA was amplified by polymerase chain reaction (PCR) in accordance to Mathews et al. (2015). Fragments of 1000 bp were amplified using the primers ERIB1+ACT1R (Barta et al., 1997), and fragments of 1200 bp were amplified using the primers MYXGEN+ERIB10 (Kent et al., 2000; Hallett and Diamant, 2001). PCR was carried out using a final volume of 25 μL, which comprised 10–50 ng of extracted DNA, 1× Taq DNA Polymerase buffer (Invitrogen by Life Technologies, Maryland, USA), 0.2 mmol dNTPs, 1.5 mmol $MgCl_2$, 0.2 pmol for each primer, 0.25 μL (1.25 U) Taq DNA polymerase (Invitrogen by Life Technologies, Maryland, USA), and ultrapure water. PCRs were performed in an iCycler (Bio-Rad, Hercules, California, USA) with 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. PCR products were electrophoresed in 0.8% agarose gel (BioAmerica, Florida, USA) in a TAE buffer (Tris–Acetate EDTA, Tris 40 mM, acetic acid 20 mM, EDTA 1 mM), stained with 0.5 mg/mL of Sybr Safe DNA gel stain (Invitrogen by Life Technologies, California, USA), and then analyzed with an FLA-3000 scanner (Fuji Photo Film, Tokyo, Japan). Sizes of the amplified fragments were compared using a standard 1 kb DNA Ladder marker (Invitrogen by Life Technologies, California, USA) to verify the presence of appropriate sized fragments. PCR products were purified using USB® ExoSap-IT® (Thermo Fisher Scientific, Ohio, USA) in accordance with the manufacturer's instructions and sequenced using the same PCR primers and additionally MC5 and MC3 primers (Molnár et al., 2002). Sequencing was performed at the Human Genome Research Center (HGRC), at University of São Paulo, with a BigDye® Terminator ver. 3.1 cycle sequencing kit (Applied Biosystems Inc., California, USA) in an ABI 3730 DNA sequencing analyzer (Applied Biosystems). A standard nucleotide BLAST (blastn) search was conducted to verify the similarity of the sequence obtained in this study with other sequences available in the GenBank database (Altschul et al., 1997). Sequences were visualized, assembled and edited using BioEdit

version 7.1.3.0 (Hall, 1999) in order to visually assemble sequence fragments and to compare the coding sequence obtained with the 45 most closely related myxozoan taxa, as determined by the BLAST search.

Phylogenetic analysis was conducted using maximum likelihood (ML) methods with a Kimura 2-parameter (K2P) evolution sequence model in the MEGA 6.0 program (Tamura et al., 2013). Bootstrap analysis (1000 replicates) was employed to assess the relative robustness of the tree branches. Sequences of *Ceratonova shasta* AF001579 and *Ceratomyxa seriolae* AB530265, were used as outgroups. Additionally, a second alignment including only the *Henneguya* species clustering together with the new parasite here described was performed to evaluate the genetic distance between them, using the pairwise method with the p-distance model in MEGA 6.0 software (Tamura et al., 2013).

## 3. Results

Among the 45 specimens of *H. loretoensis* examined in the present study, 37 (82.2%) had plasmodia of an undescribed parasite from the genus *Henneguya* in the gill filaments. These were not found in any other organs and no clinical signs were observed in the parasitized organ.

*Henneguya* n. sp. 3 (Figs. 1A-C and 2).

Plasmodia rounded in shape and measuring up to 300 µm were found in the gill filaments of specimens of *H. loretoensis* caught from the Nanay River (Fig. 1A). The mature spores were ellipsoidal in shape from the frontal view and laterally biconvex and had a total length of 24.2±1.3 µm (22.9-25.5), a body length of 13.4±0.9 µm (12.5-14.3), 3.9±0.1 µm (3.8-4.0) width and a caudal process of 10.7±1.2 µm (9.5-11.9) (Figs. 1B-C and 2). The two polar capsules in the anterior pole of spore were symmetrical, elongated aubergine in shape, with 3.3±0.2 µm (3.1-3.5) in length and 1.6±0.2 µm (1.4-1.8) in width and helical filament with four to five coils arranged obliquely to the longitudinal axis (Fig. 1B-C and 2).

The sequencing of the SSU rDNA from the spores of *Henneguya* n. sp. 3 resulted in a total of 1632 pb with a GC content of 49%. The BLAST search revealed that the sequence did not match any myxozoan species sequences available in GenBank. The highest sequence similarity with *Henneguya* n. sp. 3 was *Henneguya maculosus* (query

coverage: 85%; maximum identities: 89%), reported in the gill filaments of two South American freshwater siluriforms belonging to *Pseudoplatystoma* genus: *P. corrucans* and *P. reticulatum* (Carriero et al., 2013). The sequences similarity of *Henneguya* n. sp. 3 with other *Henneguya* species was low (≤ 86%). In the phylogenetic analysis using ML, *Henneguya* n. sp. 3 appeared in a subclade as a closed taxon of *Henneguya leporinicola* reported parasitizing the gill lamellae of *Leporinus macrocephalus* from São Paulo State, Brazil (Martins et al., 1999). In this same clade, *Henneguya* n. sp. 3 clustered with *Henneguya visibilis* Moreira, Adriano, Silva, Ceccarelli and Maia, 2014, and *Henneguya rotunda* Moreira, Adriano, Silva, Ceccarelli and Maia, 2014 (Fig. 3). Analysis of the genetic distance of the *Henneguya* species that cluster together in the same subclade with *Henneguya* n. sp. 3 showed 83% similarity to *H. leporinicola*, 82% to *H. visibilis* and 83.3% to *H. rotunda.*

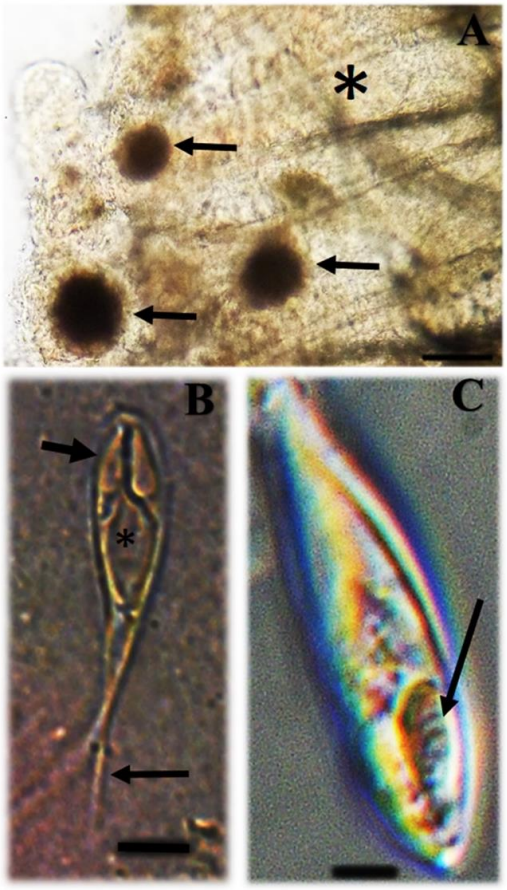


**Fig 1.** Photomicrograph of *Henneguya* n. sp. 3 parasite of *Hyphessobrycon loretoensis*. A, Plasmodium (long black arrows) infecting gill filaments (*). Scale bar: 40 µm. B, Differential interference-contrast (DIC) microscopy image of spore body showing two equal polar capsules (small black arrow), view of the tails (thin black arrow) and sporoplasm (*). Scale bar: 10 µm. C, Fresh spores showing the polar capsules with five coils arranged obliquely to the longitudinal axis (large black arrow). Scale bar: 10 µm.

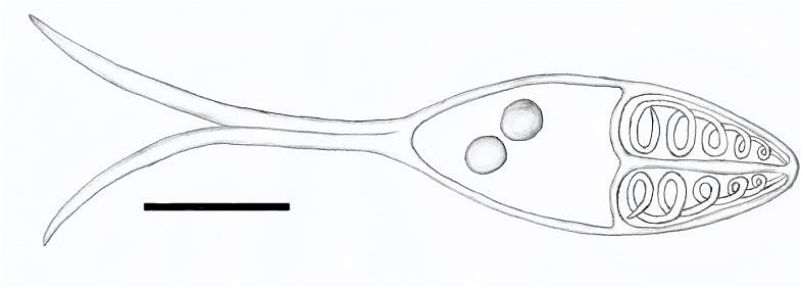

**Fig 2**. Schematic representation of mature spore of *Henneguya* n. sp. 3 parasite of gill filaments of *Hyphessobrycon loretoensis*. Scale bar: 5 μm.

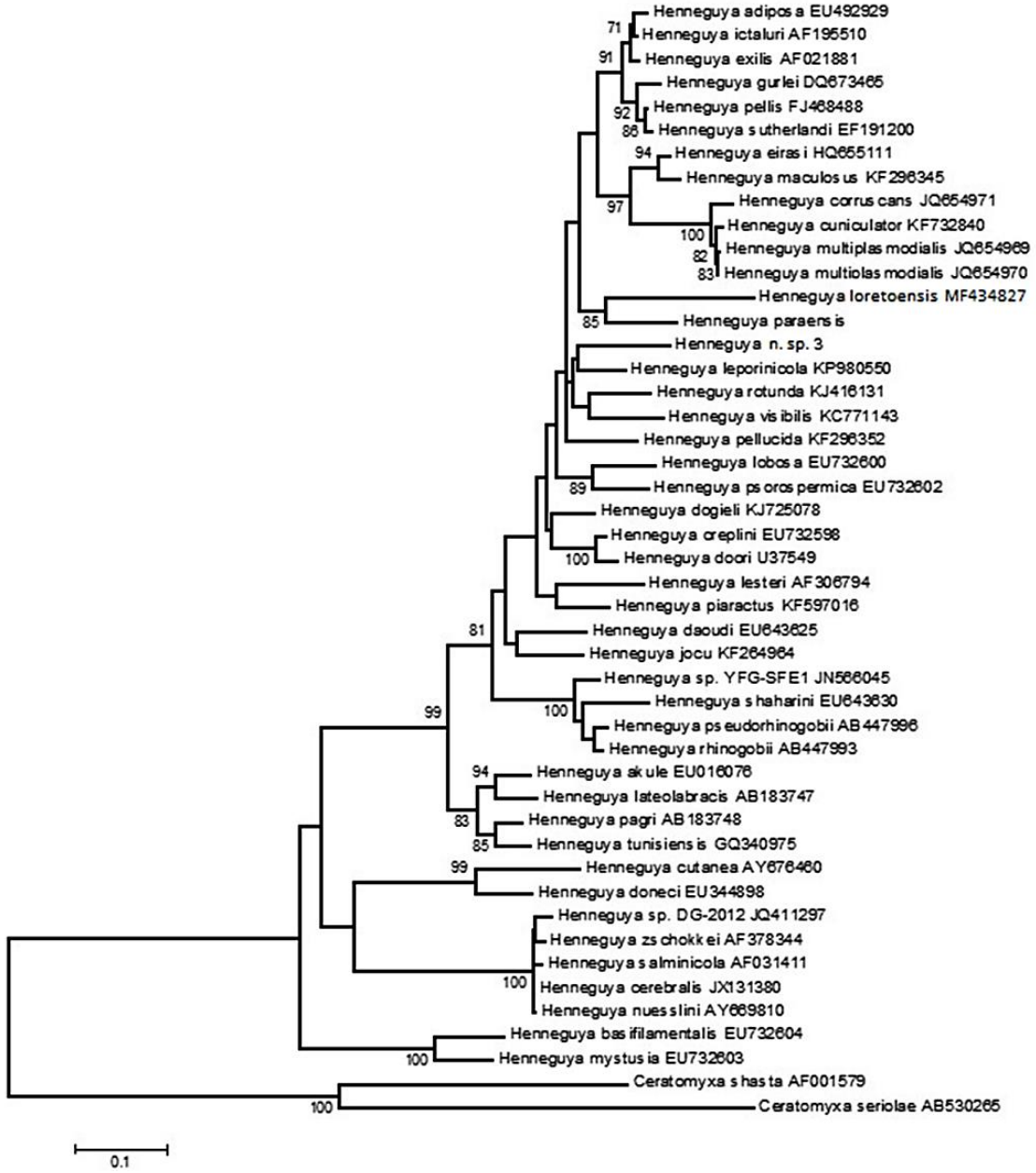


**Fig 3.** Maximum likelihood tree, based on partial SSU rDNA sequencing, showing the relationship between *Henneguya* n. sp. 3, parasite of *Hyphessobrycon loretoensis* and others *Henneguya* spp. The numbers above the nodes indicate bootstrap confidence levels.

## 4. Discussion

Previous studies in South America have shown great diversity and wide distribution of myxosporeans in many species of wild and farmed fish (Eiras and Adriano, 2012; Carriero et al., 2013; Azevedo et al., 2013; Adriano et al., 2014; Mathews et al., 2016b, 2016c, Adriano and Okamura, 2017). However, there is few information about myxosporean infections in ornamental fish, particularly from the Amazon basin (Mathews et al., 2017), considering that in this region there is a great diversity, being many fish species native and endemic (Hubert and Renno, 2006). To our knowledge, this is the first report of a *Henneguya* species parasitizing fish of the genus *Hyphessobrycon* in the Amazon basin and the second report of this parasite infection in an ornamental fish from Peru. The results showed a high prevalence of *Henneguya* n. sp. 3 in *H. loretoensis* specimens caught from Nanay River during the dry season, indicating that the infection by this parasite is frequent. Surely, seasonal occurrence survey is necessary to establish the pattern of infection for this parasite, taking in account that seasonal changes represent a combination of many factors influencing the success of a parasite to penetrate a host (Abdel-Gaber et al., 2017).

In the Amazon region, there have been reported more than 29 species of aquatic oligochaetes (Bevilacqua, 2014), with the majority of species showing preference for lentic environments, and with accumulation of abundant aloctone organic matter in the sediment (Bevilacqua, 2014). Aquatic oligochaetes involved as definitive hosts in the life cycles of myxosporean parasites may spread infective actinospores in the water (Eszterbauer et al., 2015). Furthermore, oligochaetes may release thousands actinospores which can remain viable at 10-30 °C from three days to about four weeks (Eszterbauer et al., 2015; Kallert et al., 2015). The Nanay River, where specimens of *H. loretoensis* had been living, is a black water river with slow current and with abundant organic matter and it divides in many canals, providing adequate conditions for oligochaetes establishment. Furthermore, the climate in the region is tropical humid with annual average temperature of 28 °C, favoring that worms release actinospores for shorter periods and at higher intensity (Rácz, 2004), 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, and this picture cans has a key role in the large prevalence of *Henneguya* n. sp. 3 here observed.

The morphological characteristics of *Henneguya* n. sp. 3 were compared with those of all *Henneguya* spp. that parasitize South America freshwater fish (Eiras and Adriano, 2012; Velasco et al., 2016; Mathews et al., 2016a; Mathews et al., 2017). Mature spores of *H. visibilis* Moreira, Adriano, Silva Ceccarelli and Maia, 2014, which infects *Leporinus obtusidens,* had a resemblance to those of *Henneguya* n. sp. 3 However, these differ from the new species in the body size of the spore (10.8±0.6 μm in length to *H. visibilis* and 13.4±0.9 μm to the new species). Differences may also be observed in the length of the polar capsules (4.9±0.3 μm to *H. visibilis* and 3.3±0.2 μm to the new species), number of coils in the polar filament (eight to nine to *H. visibilis* and four to five to the new species), and in the length of caudal process (18±1.2 μm to *H. visibilis* and 10.7±1.2 μm to the new species). Furthermore, differences related to the host family and site of infection, with *H. visibilis* parasitizing fins of an anostomid fish (Moreira et al., 2014a), while *Henneguya* n. sp. 3 infect gill filaments on a characid fish, are important to support the taxonomic difference, considering that histozoic freshwater platysporines has shown high organ/tissue and host specificity (Molnár and Eszterbauer, 2015). Morphological features of *Henneguya* n. sp. 3 were also compared with *Henneguya pisciforme* described parasitized the gill filaments of the congener *Hyphessobrycon anisitsi* (Cordeiro et al., 1983). However, the comparison showed small number of noticeable morphologic differences, such as spore body length and wide (20.40±1.5 × 6.12±0.4 μm for *H. pisciforme* and 13.4±0.9 × 3.9±0.1μm for *Henneguya* n. sp. 3). The two polar capsules of *Henneguya* n. sp. 3 are equal in size, while in *H. pisciforme* they are unequal. Another important difference is related to geographic distribution of the hosts, with *H. loretoensis* inhabiting rivers from Amazon basin, while *H. anisitsi* is distributed in La Plata basin. Considering that long periods of geographical isolation had promoted allopatric speciation in South American freshwater fish (Hubbert and Renno, 2006; Carvalho-Costa et al., 2011), it may also have contributed to the divergence of these two parasites found in isolated watersheds (Naldoni et al., 2014; Moreira et al., 2014a), with *H. pisciforme* found in La Plata basin and *Henneguya* n. sp. 3 in the Amazon basin, both basins separated by 10 Ma (Hubbert and Renno, 2006). Although the absence of SSU rDNA sequence of *H. pisciforme* in the National Center for Biotechnology (NCBI) prevented the genetic comparison with *Henneguya* n. sp. 3, the morphological/morphometric data were able to differentiate these species parasites. However, future molecular analysis is highly recommended to *H. pisciforme* to elucidate the phylogenetic relationship between these two *Henneguya*

spp. parasites of congener's hosts habiting distinct watersheds. In this way, in our study the new species was described based on morphological, SSU rDNA sequencing, host and geographic area data, and the arguments are sufficient to justify the establishment of *Henneguya* n. sp. 3 as a new species.

Analysis of the SSU rDNA sequence of *Henneguya* n. sp. 3 using BLAST search revealed no identical sequences in the NCBI database. Phylogenetic analysis showed *Henneguya* n. sp. 3 closed together with *H. leporinicola* reported infecting the gills of *Leporinus microcephalus* (Anostomidae) from fish farm in the state of São Paulo, Brazil (Martins et al., 1999). In this same subclade, *Henneguya* n. sp. 3 appeared together with *H. visibilis* and *H. rotunda*, reported parasitizing two characiforms fish belonging to the Anostomidae and Bryconidae family, respectively (Moreira et al., 2014a, 2014b). However, analysis of the genetic similarity among the SSU rDNA sequence of *Henneguya* n. sp. 3 and *Henneguya* species that cluster together in the same clade revealed only 83% similarity to *H. leporinicola* and *H. rotunda* and 82% to *H. visibilis.* Another important feature observed in the subclade where *Henneguya* n. sp. 3 appears is the absence of geographic and host family affinity, although this picture may only to reflects the low number of taxa of parasites of characids host, as well as of the studied region. Therefore, future molecular studies of new species parasitizing distinct taxa of ornamental fish will enable a better understanding about the evolutionary context of *Henneguya* n. sp. 3 and further improve knowledge of the phylogenetic relationship of these myxosporeans of that region as a whole.

Although in our study, no clinical signs were observed in the infected specimens of *H. loretoensis*, several studies have shown myxosporeans species inducing pathological changes and diseases in ornamental fish (Crawshaw and Sweeting, 1986; Camus and Griffin, 2010; Mathews et al., 2016a). Moreover, imported ornamental fish have been reported to introduce and disperse myxosporean species to new locations. For instance, Hallett et al. (2006) described a wide dispersion of four myxosporean species (*Sphaerospora* sp*., Myxobolus diversus*, *Myxobolus cultus*, and *Hoferellus carassii*) by means of infected specimens of *Carasiaus auratus* from a national USA pet store chain. Likewise, *Chloromyxum* sp. has been introduced to Australia by transportation of specimens of *Gyrinocheilus aymonieri* imported from Singapore (Evans and Lester, 2001). In the pathological outbreak point of view, renal myxozoosis caused by *Sinuolinea phyllopteryxa* in *Phyllopteryx taeniolatus* exported from Australia to the

United States was reported (Garner et al., 2008). Mortalities were also reported for ornamental Koi from aquaculture caused by *Myxobolus Koi* introduced with *Cyprinus carpio* from Asia to the UK and the USA (Crawshaw and Sweeting, 1986; Camus and Griffin, 2010). Hence, considering that international trade of ornamental fish is a major factor for transboundary spreading of myxosporeans (Biosecurity, 2005; Hallet et al., 2015), is indispensable increase the knowledge about the diversity of these parasites found infecting wild ornamental fish from regions as the Amazon basin, that provide annually millions of specimens for the ornamental market. This knowledge is an important tool constant monitoring for diagnosis and timely control of infections by myxosporean in order to better prevent introduction in new environments, dissemination and future disease outbreaks.

## 6. CONSIDERAÇÕES FINAIS

Os resultados obtidos durante as pesquisas realizados para o desenvolvimento desta tese permitiram concluir que:

Espécies de peixes amazônicos utilizados no aquarismo são infectados por diferentes *taxa* de mixosporídeos até aqui desconhecidas pela ciência.

Seis novas espécies foram registradas a partir dos peixes ornamentais analizados, as quais foram distribuídas em quatro gêneros. *Corydoras melini* apresentou infecção por *Myxidium* n. sp. na vesícula biliar, *Henneguya* n. sp. 1 nos filamentos branquiais e *Myxobolus* n. sp. no arco da brânquia. *Ceratomyxa* n. sp. foi encontrado na vesícula biliar de *Symphyssodon discus* e *Henneguya* n. sp. 2 e *Henneguya* n. sp. 3 foram encontrados infectando as brânquias de *Corydoras leucomelas* e *Hyphessobrycon loretoensis*, respectivamente.

Através da análise ultraestrutural, foi possível concluir que as espécies de mixosporídeos descritas a partir dos peixes ornamentais examinados, apresentam diferentes estratégias pelas quais os parasitos obtêm nutrientes necessários para o desenvolvimento dos plasmódios. Em *Henneguya* n. sp. e *Myxobolus* n. sp. numerosos canais de pinocitosis auxiliam na incorporação de nutrientes para o interior do plasmódio. Em *Myxidium* n. sp., além da presença de canais de pinocitosis, numerosos microvilosidades foram observadas na parede do plasmódio, aumentando claramente a área de absorção de nutrientes. Adicionalmente, a presença de pontes filiformes

citoplasmáticas entre pansporoblastos permitem a passagem de nutrientes da zona do ectoplasma para os diferentes estágios de desenvolvimento na parte profunda do plasmódio. Análises ultraestruturais também permitiram concluir que a presença de *Henneguya* n. sp. 1 e *Myxobolus* n. sp. induziram à formação de uma cápsula de tecido conjuntivo, composta por fibroblastos e fibras de colágeno, que envolveram os plasmódios.

Com base na análise filogenética, podemos concluir que as espécies de mixosporídeos analisadas apresentaram tendência por agrupar de acordo com os sítios de infecção, pela afinidade de hospedeiro e área geográfica.

Considerando a importância das espécies de peixes amazônicos no comércio internacional de peixes ornamentais, e a diversidade de mixosporídeos encontrados nas espécies estudadas, pode-se concluir que somente o monitoramento constante poderá prevenir a introdução e disseminação de mixosporídeos em outras regiões do mundo.

## 7. REFERENCES

Abdel-Ghaffar, F., Abdel-Gaber, R., Maher, S., El Deeb, N., Kamel, R., Al Quraishy, S., Mehlhorn, H., 2017. Morphological and ultrastructural characteristics of *Myxobolus ridibundae* n. sp. (Myxosporea: Bivalvulida) infecting the testicular tissue of the marsh frog *Rana ridibunda* (Amphibia: Ranidae) in Egypt. Parasitol. Res. 116, 133-141.

Abdel-Gaber, R., Abdel-Ghaffar, F., Maher, S., El-Mallah, A.M., Al Quraishy, S., Mehlhorn, H., 2017. Morphological re-description and phylogenetic relationship of five myxosporean species of family Myxobolidae infecting the Nile tilapia *Oreochromis niloticus* (Perciformes: cichlidae). Dis. Aquat. Org. 124, 201–214.

Adriano, E.A., Okamura, B., 2017. Motility, morphology and phylogeny of the plasmodial worm, *Ceratomyxa vermiformis* n. sp. (Cnidaria: myxozoa: Myxosporea). Parasitol. 144, 158–168.

Adriano, E.A., Silva, M.R., Atkinson, S.D., Bartholomew, J.L., Maia, A.A., 2014. *Myxidium ceccarellii* n. sp. (Myxosporea) from the gallbladder of *Leporinus elongates* (Anastomidae) from the São Francisco River, Brazil. Parasitol. Res. 113, 2665–2670.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Azevedo, C., Rocha, S., Casal, G., São Clemente, S.C., Matos, P., Al-Quraishy, S., Matos, E., 2013. Ultrastructural description of *Ceratomyxa microlepis* sp. nov (Phylum Myxozoa): a parasite infecting the gall bladder of *Hemiodus microlepis*, a freshwater teleost from the Amazon river. Mem. Inst. Oswaldo Cruz. 108, 150–154.

Barta, J.R., Martin, D.S., Liberator, P.A., Dashkevicz, M., Anderson, J.W., Feighner, S.D., Elbrecht, A., Perkins-Barrow, A., Jenkins, M.C., Danforth, H.D., Ruff, M.D., Profous-Juchelka, H., 1997. Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J. Parasitol. 83, 262–271.

Bartholomew, J.L., Atkinson, S.D., Hallett, S.L., Lowenstine, L.J., Garner, M.M., Gardiner, C.H., Rideout, B.A., Keel, M.K., Brown, J.D., 2008. Myxozoan parasitism in waterfowl. Int. J. Parasitol. 38, 1199–1207.

Becker, J.A., Tweedie, A., Rimmer, A., Landos, M., Lintermans, M., Whittington, R.J., 2014. Incursions of *Cyprinid herpesvirus* 2 in goldfish populations in Australia despite quarantine practices. Aquaculture 432, 53-59.

Bevilacqua, M.S., 2014. Oligochaeta em riachos (Igarapés) da Floresta Nacional Saracá-Taquera (PA): Abundância, Riqueza, Diversidade e potencial como indicador de impacto antrópico. Ph.D. dissertation, Universidade Federal do Rio de Janeiro.

Biosecurity, N.Z., 2005. Import risk analysis: ornamental fish. Ministry of Agriculture and Forestry, New Zealand.

Camus, A.C., Griffin, M.J., .2010. Molecular characterization and histopathology of *Myxobolus koi* infecting the gills of a koi, *Cyprinus carpio*, with an amended morphological description of the agent. J. Parasitol. 96, 116–124.

Camus, A.C., Dill, J.A., McDermott, A.J., Clauss, T.M., Berliner, A.L., Boylan, S.M., Soto, E., 2013. *Francisella noatunensis* subsp. *orientalis* infection in Indo-Pacific reef fish entering the United States through the ornamental fish trade. J. Fish Dis. 36, 681–684.

Camus A.C., Dill, J.A., Rosser, T.G., Pote, L.M., Griffin, M.J., 2017. *Myxobolus axelrodi* n. sp. (Myxosporea: Myxobolidae) a parasite infecting the brain and retinas of the cardinal tetra *Paracheirodon axelrodi* (Teleostei: Characidae). Parasitol. Res. 116, 387-397.

Carriero, M.M., Adriano, E.A., Silva, M.R.M., Ceccarelli, P.S., Maia, A.A.M., 2013. Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American species. PLoS One 8, e73713.

Carvalho-Costa, L.F., Piorski, N.M., Willis, S.C., Galetti, P.M.Jr., Ortí, G., 2011. Molecular systematics of the neotropical shovelnose catfish genus *Pseudoplatystoma* Bleeker 1862 based on nuclear and mtDNA markers. Mol. Phylogenet. Evol. 59, 177−1.

Crawshaw, M.T., Sweeting, R.A., 1986. *Myxobolus koi* Kudo, 1919: a new record for Britain. J. Fish Dis. 9, 465–467.

Cordeiro, N.S., Artigas, P.T., Gióia, I., Lima, R.S., 1983. *Henneguya pisciforme* n. sp., mixosporídeo parasito de brânquias do lambari *Hyphessobrycon anisitsi* (Pisces, Characidae). Mem. Inst. Butantan. 47, 61-69.

Dyková, I., de Buron, I., Roumillat, W., Fiala, I., 2011. *Henneguya cynoscioni* sp. n. (Myxosporea: Bivalvulida), an agent of severe cardiac lesions in the spotted seatrout, *Cynoscion nebulosus* (Teleostei: Sciaenidae) Folia Parasitol. 58, 169–177.

Eiras, J.C., 2005. An overview on the myxosporean parasites in amphibians and reptiles. Acta Parasitol. 50, 267–275.

Eiras, J.C., Adriano, E.A., 2012. Checklist of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa, myxosporea, myxobolidae) described between 2002 and 2012. Syst. Parasitol. 83, 95–104.

Espinoza, L.L., Mertins, O., Gama, G.S., Fernandes-Patta, A.C.M., Mathews, P.D., 2017. A new *Myxidium* species (Myxozoa: myxosporea) infecting the gallbladder of the turtle *Podocnemis unifilis* (Testudines: podocnemididae) from Peruvian Amazon. Acta Trop. 172, 75–79.

Eszterbauer, E., Atkinson, S., Diamant, A., Morris, D., El-Matbouli M., Hartikainen, H., 2015. Myxozoan Life Cycles: Practical Approaches and Insights. In: Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), Myxozoan Evolution, Ecology and Development. Springer, Switzerland, pp. 175-198.

Evans, B.B., Lester, R.J.G., 2001. Parasites of ornamental fish imported into Australia. Bull. Eur. Assoc. Fish Pathol. 2, 51–55.

Fiala, I., 2006. The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. Int. J. Parasitol. 36, 1521–1534.

Foott, J.S., Harmon, R., Stone, R., 2004. Effect of water temperature on non-specific immune function and ceratomyxosis in juvenile Chinook salmon and steelhead from the Klamath River. Cal. Fish Game. 90, 71–84.

García-Alzate, C.A., Urbano-Bonilla, A., Taphorn, D.C. 2017, A new species of *Hyphessobrycon* (Characiformes, Characidae) from the upper Guaviare River, Orinoco River Basin, Colombia. ZooKeys. 668, 123–138.

Garner, M.M., Atkinson, S.D., Hallett, S.L., Bartholomew, J.L., Nordhausen, R.W., Reed, H., Adams, L., Whitaker, B., 2008. Renal myxozoanosis in weedy sea dragons, *Phyllopteryx taeniolatus* (Lacepède), caused by *Sinuolinea phyllopteryxa* n. sp. J. Fish Dis. 31, 27–35.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.

Hallett, S.L., Diamant, A., 2001. 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. Organ. 46, 197–212.

Hallett, S.L., Atkinson, S.D., Holt, R.A., Banner, C.R., Bartholomew, J.L., 2006. A new myxozoan from feral goldfish (*Carassius auratus*). J. Parasitol. 92:357–363.

Hallett, S.L., Hartigan, A., Atkinson, S.D., 2015. Myxozoans on the move: Dispersal modes, exotic species and emerging diseases. In: Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), Myxozoan Evolution, Ecology and Development. Springer, Switzerland, pp. 343-362.

Hallett, S.L., Bartholomew, J.L., 2011. *Myxobolus cerebralis* and *Ceratomyxa shasta*. In: Buchmann, K., Woo, P.T.K. (Eds.), Fish Parasites, Pathobiology and Protection. CABI International, pp. 141-172.

Hubert, N., Renno J.F., 2006. Historical biogeography of South American freshwater fishes. J. Biogeogr. 33, 1414–1436.

Kallert DM, Eszterbauer E, Erséus C et al (2005b) Life cycle studies of *Myxobolus parviformis* sp. n. (Myxozoa, Myxobolidae) from bream. Dis. Aquat. Org. 66, 233–243.

Kent, M.L., Khattra, J., Hedrick, R.P., Devlin, R.H., 2000. *Tetracapsula renicola* n. sp. (Myxozoa: saccosporidae); the PKX myxozoan the cause of proliferative kidney disease in salmonid fishes. J. Parasitol. 86, 103–111.

Jimenez-Prado, P., Arguello, P., 2016. *Hyphessobrycon loretoensis*. The IUCN Red List of Threatened Species. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T49829519A53817098.en

Lom, J., Arthur, J.R., 1989. A guideline for the preparation of species descriptions in Myxosporea. J. Fish Dis. 12, 151–156.

Martins, M.L., Souza, V.N., Moraes, J.R.E., Moraes, F.R., 1999. Gill infection of *Leporinus macrocephalus* Garavello et Britski, 1988 (Osteichthyes: Anostomidae) by

*Henneguya leporinicola* n. sp. (Myxozoa: Myxobolidae). Description, Histopathology and Treatment. Ver. Bras. Biol. 59, 527–534.

Mathews, P.D., Silva, M.R.., Maia, A.A., Adriano, E.A., 2015. Ultrastructure and ssrRNA sequencing of *Myxidium amazonense* n. sp. a myxosporean parasite of *Corydoras melini* from the Rio Negro river, Amazonas state, Brazil. Parasitol. Res. 114, 4675–4683.

Mathews, P.D., Maia, A.A., Adriano, E.A., 2016a. *Henneguya melini* n. sp. (Myxosporea: myxobolidae), a parasite of *Corydoras melini* (Teleostei: siluriformes) in the Amazon region: morphological and ultrastructural aspects. Parasitol. Res. 115, 3599–3604.

Mathews, P.D., Maia, A.A., Adriano, E.A., 2016b. Morphological and ultrastructural aspects of *Myxobolus niger* n. sp. (Myxozoa) gill parasite of *Corydoras melini* (Siluriformes: callichthyidae) from Brazilian Amazon. Acta Trop. 158, 214–219.

Mathews, P.D., Naldoni, J., Maia, A.A., Adriano, E.A., 2016c. Morphology and small subunit rDNA-based phylogeny of *Ceratomyxa amazonensis* n. sp. parasite of *Symphysodon discus*, an ornamental freshwater fish from Amazon. Parasitol. Res. 115, 4021–4025.

Mathews, P.D., Naldoni, J., Adriano, E.A., 2017. Morphology and small subunit rDNA-based phylogeny of a new *Henneguya* species, infecting the ornamental fish *Corydoras leucomelas* from the Peruvian Amazon. Acta Trop. 176, 51–57.

Molnár K., Eszterbauer, E., 2015. Specificity of Infection Sites in Vertebrate Hosts. In: Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), Myxozoan Evolution, Ecology and Development. Springer, Switzerland, pp. 295-313.

Molnar, K., Eszterbauer, E., Szczekely, C., Benko, M., Harrach, B., 2002. Morphological and molecular biological studies on intramuscular *Myxobolus* spp. of cyprinid fish. J. Fish Dis. 25, 643–652.

Moreira, G.S.A., Adriano, E.A., Silva, M.R.M., Ceccarelli, P.S., Maia, A.A., 2014a. Morphology and 18S rDNA sequencing identifies *Henneguya visibilis* n. sp., a parasite of *Leporinus obtusidens* from Mogi Guaçu River. Brazil. Parasitol. Res. 113, 81–90.

Moreira, G.S.A., Adriano, E.A., Silva, M.R.M., Ceccarelli, P.S., Maia, A.A.M., 2014b. The morphological and molecular characterization of *Henneguya rotunda* n. sp., a parasite of the gill arch and fins of *Salminus brasiliensis* from the Mogi Guacu River, Brazil. Parasitol. Res. 113, 1703–1711.

Morsy, K., Abdel-Ghaffar, F., Bashtar, A.R., Mehlhorn, H., Al Quraishy, S., Abdel-Gaber, R., 2012. Morphology and small subunit ribosomal DNA sequence of *Henneguya suprabranchiae* (Myxozoa), a parasite of the catfish *Clarias gariepinus* (Clariidae) from the River Nile, Egypt. Parasitol. Res. 111, 1423–1435.

Naldoni, J., Arana, S., Maia, A.A.M., Ceccarelli, P.S., Tavares, L.E.R., Borges, F.A., Pozo, C.F., Adriano, E.A., 2009. *Henneguya pseudoplatystoma* n. sp. causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: histopathology and ultrastructure. Vet. Parasitol. 166, 52–59.

Naldoni, J., Maia, A.A.M., Da Silva, M.R.M., Adriano, E.A., 2014. *Henneguya cuniculator* sp. nov., a parasite of spotted sorubim *Pseudoplatystoma corruscans* in the São Francisco Basin, Brazil. Dis. Aquat. Org. 107, 211–221.

Pote, L.M., Hanson, L.A., Shivaji, R., 2000. Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to *Henneguya* n. sp. (Myxozoa: Myxosporea). J. Aquat. Anim. Health 12, 230–240.

Rácz, O.Z., 2004. Experimental study of intrapiscin and intraoligochaete developmental stages of fish-parasitic myxozoans. Ph.D. dissertation, Szent István University.

Székely, C., Cech, G., Atkinson, S.D., Molnár, K., Egyed, L., Gubányi, A., 2015. A novel myxozoan parasite of terrestrial mammals: description of *Soricimyxum minuti* sp. n. (Myxosporea) in pygmy shrew *Sorex minutus* from Hungary. Folia Parasitol. 62, http://dx.doi.org/10.14411/fp.2015.045.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA 6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Velasco, M., Videira, M., Nascimento, L.C., Matos, P., Gonçalves, E.C., Matos, E., 2016. *Henneguya paraensis* n. sp. (Myxozoa; Myxosporea), a new gill parasite of the Amazonian fish *Cichla temensis* (Teleostei: cichlidae): morphological and molecular aspects. Parasitol. Res. 115, 1779–1787.

Whittington, R., Chong, R., 2007. Global trade in ornamental fish from an Australian perspective: the case for revised import risk analysis and management strategies. Prev. Vet. Med. 81, 92–116.

Adriano A, Arana S, Cordeiro NS (2005) Histophatology and ultrastructure of *Henneguya caudalongula* sp. n. infecting *Prochilodus lineatus* (Pisces: Prochilodontidae) cultivated in the state of São Paulo, Brazil. Mem Inst Oswaldo Cruz 100(2):177–181

Adriano EA, Silva MR, Atkinson SD, Bartholomew JL, Maia AA (2014) *Myxidium ceccarellii* n. sp. (Myxosporea) from the gallbladder of *Leporinus elongatus* (Anastomidae) from the São Francisco River, Brazil. Parasitol Res 113(7):2665–2670

Ajiaco-Martinez RE, Blanco-Castaneda MC, Barreto-Reyes CG, Ramírez-GIL H (2001) Las exportaciones de peces ornamentales. In: Ramirez-Gil H, Ajiaco-Martinez RE (eds) La pesca en la baja Orinoquia colombiana: una vision integral. Instituto Nacional de Pesca y Acuicultura-INPA, Bogotá, pp 79–121

Albert JS, Reis RE (2011) Historical biogeography of Neotropical freshwater fishes. University of California Press, Berkeley.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402

Anjos HDBD, Amorim RMDS, Siqueira JA, Anjos CR (2009) Ornamental fish export of the State of Amazonas, Amazon Basin, Brazil. B Inst Pesca 35(2):259–274

Azevedo C, Casal G, Clemente SC, Lopes LMS, Matos P, Abdel-Baki AA, Oliveira E, Matos E (2011) *Myxidium volitans* sp. nov., a parasite of the gallbladder of the fish, *Dactylopterus volitans* (Teleostei: Triglidae) from the Brazilian Atlantic coast morphology and pathology. Mem Inst Oswaldo Cruz 106:557–561

Barta JR, Martin DS, Liberator PA, Dashkevicz M, Anderson JW, Feighner SD, Elbrecht A, Perkins-Barrow A, Jenkins MC, Danforth D, Ruff MD, Profous-Juchelka H (1997) Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J Parasitol 83:262–271

Bartholomew JL, Atkinson SD, Hallett SL, Lowenstine LJ, Garner MM, Gardiner CH, Rideout BA, Keel MK, Brown JD (2008) Myxozoan parasitism in waterfowl. Int J Parasitol 38:1199–1207

Canning EU, Curry A, Anderson CL, Okamura B (1999) Ultrastructure of *Myxidium trachinorum* sp. nov. from the gallbladder of the lesser weever fish *Echiichthys vipera*. Parasitol Res 85:910–919

Casal G, Matos E, Azevedo C (2006) A new myxozoan parasite from the amazonian fish *Metynnis argenteus* (Teleostei, Characidae): light and electron microscope observations. J Parasitol 92(4):817–821

Eiras JC, Saraiva A, Cruz CF, Santos MJ, Fiala I (2011) Synopsis of the species of *Myxidium* Bütschli, 1882 (Myxozoa: Myxosporea: Bivalvulida). Syst Parasitol 80:81–116

Eschmeyer WN (2013) Catalog of Fishes. California Academy of Sciences. Disponível: (http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Accessed 25 May 2015

Espíndola VC, Spencer MRS, Rocha LR, Britto MR (2014) A new species of *Corydoras* Lacépède (Siluriformes: Callichthyidae) from the Rio Tapajós basin and its phylogenetic implications. Pap Avulsos de Zool 54(3):25–32

Feist SW (1995) Ultrastructural aspects of *Myxidium gadi* (Georgévitch, 1916) (myxozoa: Myxosporea): infections in pollack (*Pollachius pollachius* L.) and saithe (*P. virens* L). Europ J Protistol 31:309–317

Froese R, Pauly D (2011) FishBase. World Wide Web electronic publication. Version (02/2011) www.fishbase.org. Accessed 10 May 2015

Gióia I, Cordeiro NS (1996) Brazilian Myxosporidians' check-list (Myxozoa). Acta Protozool 35:137–149

Gunter NL, Whipps CM, Adlard RD (2009) *Ceratomyxa* (Myxozoa: Bivalvulida): robust taxon or genus of convenience? Int J Parasitol 39:1395–1405

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp 41:95–98

Hallett SL, Diamant A (2001) 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 46:197–212

Heiniger H, Adlard RD (2014) Relatedness of novel species of *Myxidium* Bütschli, 1882, *Zschokkella* Auerbach, 1910 and *Ellipsomyxa* Køie, 2003 (Myxosporea: Bivalvulida) from the gall bladders of marine fishes (Teleostei) from Australian waters. Syst Parasitol 87:47–72

IBAMA (2007) Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Brasília: Relatório Técnico sobre o Diagnóstico geral das práticas de controle ligadas a exploração, captura, comercialização, exportação e uso de peixes para fins ornamentais e de aquariofilia - Diretoria de uso sustentável da biodiversidade e florestas, Brasilia, p 214

Jirků M, Bolek MG, Whipps CM, Janovy J Jr, Kent ML, Modry D (2006) A new species of *Myxidium* (Myxosporea: Myxidiidae), from the western chorus frog, *Pseudacris triseriata triseriata*, and Blanchard's cricket frog, *Acris crepitans blanchardi* (Hylidae), from eastern Nebraska: morphology, phylogeny, and critical comments on amphibian *Myxidium* taxonomy. J Parasitol 92:611–619

Junk WJ, Soares MGM, Bayley PB (2007) Freshwater fishes of the Amazon River basin: their biodiversity, fisheries, and habitats. Aquat Ecosys Health Manag 10(2):153–173

Kent ML, Khattra J, Hedrick RP, Devlin RH (2000) *Tetracapsula renicola* n. sp. (Myxozoa: Saccosporidae); the PKX myxozoan the cause of proliferative kidney disease in salmonid fishes. J Parasitol 86:103–111

Lom J, Arthur JR (1989) A guideline for the preparation of species descriptions in Myxosporea. J Fish Dis 12:151–156

Lom J, Dykova I (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol 53(1):1–36

Moreau MA, Coomes OT (2006) Potential threat of the international aquarium fish trade to silver arawana *Osteoglossum bicirrhosum* in the Peruvian Amazon. Oryx 40:152–160

Moreira GSA, Adriano EA, SilvaMRM, Ceccarelli PS, Maia AA (2014) The morphological and molecular characterization of *Henneguya rotunda* n. sp., a parasite of the gill arch and fins of *Salminus brasiliensis* from the Mogi Guaçu River, Brazil. Parasitol Res 113: 1703–1711

Naldoni J, Arana S, MaiaAAM, Ceccarelli OS, Tavares LER, Borges FA, Pozo CF, Adriano EA (2009) *Henneguya pseudoplatystoma* n. sp. causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: histopathology and ultrastructure. Vet Parasitol 166:52–59

Naldoni J, Zatti SA, Capodifoglio KRH, Milanin T, Maia AAM, Silva MRM, Adriano EA (2015) Hostparasite and phylogenetic relationships of *Myxobolus filamentum* sp. n. (Myxozoa: Myxosporea), a parasite of *Brycon orthotaenia* (Characiformes: Bryconidae) in Brazil. Folia Parasitol 62:014

Reis RE (1998) Systematic, Biogeography, and the fossil record of the Callichthyidae: a review of the available data. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (eds) Phylogeny and classification of neotropical fishes. Edipucrs, Porto Alegre, pp 351–362

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30: 2725–2729

Upenskaya AV (1969) Ultrastructure of some stages of *Myxidium gasterostei*. Acta Protozool 7:71–79

Viozzi GP, Flores VR (2003) *Myxidium biliare* sp. n. (Myxozoa) from gall bladder of *Galaxias maculatus* (Osmeriformes: Galaxiidae) in Patagonia (Argentina). Folia Parasitol 50:190–194

Abdallah, V.D., Azevedo, R.K., Luque, J.L., Bomfim, T.C.B., 2007. Two new species of *Henneguya* Thélohan, 1892 (Myxozoa, Myxobolidae), parasitic on the gills of *Hoplosternum littorale* (Callichthyidae) and *Cyphocharax gilbert* (Curimatidae) from the Guandu River, State of Rio de Janeiro, Brazil. Parasitol. Latinoam. 6, 35–41.

Abdel-Gaber, R., Abdel-Ghaffar, F., Maher, S., El-Mallah, A., Al Quraishy, S., Mehlhorn, H., 2017. Morphological re-description and phylogenetic relationship of five myxosporean species of family Myxobolidae infecting the Nile tilapia *Oreochromis*

*niloticus* (Perciformes: Cichlidae). Dis. Aquat. Org. 124,201-214. doi:10.3354/dao03118.

Adriano, E.A., Arana, S., Cordeiro, N.S., 2005. An ultrastructural and histopathological study of *Henneguya pellucida* n. sp. (Myxosporea: Myxobolidae) infecting *Piaractus mesopotamicus* (Characidae) cultivated in Brazil. Parasite 12, 221–227. doi: 10.1051/parasite/2005123221.

Adriano, E.A., Silva, M.R., Atkinson, S.D., Bartholomew, J.L., Maia, A.A., 2014. *Myxidium ceccarellii* n. sp. (Myxosporea) from the gallbladder of *Leporinus elongatus* (Anastomidae) from the São Francisco River, Brazil. Parasitol. Res. 113, 2665–2670. doi:10.1007/s00436-014-3921-x.

Adriano E.A., Okamura, B., 2017. Motility, morphology and phylogeny of the plasmodial worm, *Ceratomyxa vermiformis* n. sp. (Cnidaria: Myxozoa: Myxosporea), Parasitol. 144, 158-168. doi:10.1017/S0031182016001852.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Barta, J.R., Martin, D.S., Liberator, P.A., Dashkevicz, M., Anderson, J.W., Feighner, S.D., Elbrecht, A., Perkins-Barrow, A., Jenkins, M.C., Danforth, D., Ruff, M.D., Profous-Juchelka, H., 1997. Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J. Parasitol. 83, 262–271.

Carriero, M.M., Adriano, E.A., Silva, M.R.M., Ceccarelli, P.S., Maia, A.A.M., 2013. Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American species. *PLoS ONE*, 8: e73713. doi: 10.1371/j0073713.

Dyková, I, Lom, J., 1978. Histopathological changes in fish gills infected with myxosporidian parasites of the genus *Henneguya*. J. Fish. Biol. 12, 197–202.

Eiras, J.C., 2002. Synopsis of the species of the genus *Henneguya* Thelohan, 1892 (Myxozoa: Myxosporea: Myxobolidae. Syst. Parasitol. 52, 43–54. doi: 10.1023/A:1015016312195.

Eiras, J.C., Adriano, E.A., 2012. Checklist of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa, Myxosporea, Myxobolidae) described between 2002 and 2012. Syst. Parasitol. 83, 95–104. doi:10.1007/s11230-012-9374-7.

Espinoza, L.L., Mertins, O., Gama, G.S., Fernandes-Patta. A.C.M., Mathews, P.D., 2017. A new *Myxidium* species (Myxozoa: Myxosporea) infecting the gallbladder of the turtle *Podocnemis unifilis* (Testudines: Podocnemididae) from Peruvian Amazon. Acta Trop. 172, 75-79. doi: 10.1016/j.actatropica.2017.04.021.

Hall, T.A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95– 98.

Hallett, S.L., Diamant, A., 2001. 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. Organ. 46, 197–212. doi: 10.3354/dao046197.

Kent, M.L., Khattra, J., Hedrick, R.P., Devlin, R.H., 2000. *Tetracapsula renicola* n. sp. (Myxozoa: Saccosporidae); the PKX myxozoan the cause of proliferative kidney disease in salmonid fishes. J. Parasitol. 86, 103–111. doi: 10.1645/0022-3395(2000)0860103:TRNSMS]2.0.CO;2.

Lom, J., Arthur, J.R., 1989. A guideline for the preparation of species descriptions in Myxosporea. J. Fish. Dis. 12,151–156.

Mathews, P.D., Silva, M.R.M., Maia, A.M.A., Adriano, E.A, 2015. Ultrastructure and ssrRNA sequencing of *Myxidium amazonense* n. sp. a myxosporean parasite of *Corydoras melini* from the Rio Negro river, Amazonas state, Brazil. Parasitol. Res. 114, 4675–4683. doi:10.1007/s00436-015-4715-5.

Mathews, P.D., Maia, A.A.M., Adriano, E.A., 2016a. Morphological and ultrastructural aspects of *Myxobolus niger* n. sp. (Myxozoa) gill parasite of *Corydoras melini* (Siluriformes: Callichthyidae) from Brazilian Amazon. Acta Trop. 158, 214–219. doi: 10.1016/j.actatropica.2016.03.016.

Mathews, P.D., Maia, A.A.M., Adriano, E.A., 2016b. *Henneguya melini* n. sp. (Myxosporea: Myxobolidae), a parasite of *Corydoras melini* (Teleostei: Siluriformes) in the Amazon region: morphological and ultrastructural aspects. Parasitol. Res. 115, 3599-3604. doi:10.1007/s00436-016-5125-z.

Molnár, K., 1998. Taxonomic problems, seasonality and histopathology of *Henneguya creplini* (Myxosporea) infection of the pikeperch *Stizostedion lucioperca* in Lake Balaton. Folia Parasitol. 45, 261–269.

Moreira, G.S.A., Adriano, E.A., Silva, M.R.M., Ceccarelli, P.S., Maia, A.A., 2014. Morphology and 18S rDNA sequencing identifies *Henneguya visibilis* n. sp., a parasite of *Leporinus obtusidens* from Mogi Guaçu River, Brazil. Parasitol. Res. 113,81–90. doi: 10.1007/s00436-013-3629-3.

Morsy, K., Abdel-Ghaffar, F., Bashtar, A.R., Mehlhorn, H., Al Quraishy, S., Abdel-Gaber R., 2012. Morphology and small subunit ribosomal DNA sequence of *Henneguya suprabranchiae* (Myxozoa), a parasite of the catfish *Clarias gariepinus* (Clariidae) from the River Nile, Egypt. Parasitol Res 111,1423–1435. doi: 10.1007/s00436-012-2976-9.

Naldoni, J., Arana, S., Maia, A.A.M., Ceccarelli, P.S., Tavares, L.E.R., Borges, F.A., Pozo, C.F., Adriano, E.A., 2009. *Henneguya pseudoplatystoma* n. sp. causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: histopathology and ultrastructure. Vet. Parasitol. 166, 52–59. doi:10.1016/j.vetpar.2009.07.034.

Naldoni, J., Maia, A.A.M., Da Silva, M.R.M., Adriano, E.A., 2014. *Henneguya cuniculator* sp. nov., a parasite of spotted sorubim *Pseudoplatystoma corruscans* in the São Francisco Basin, Brazil. Dis. Aquat. Org. 107,211–221. doi: 10.3354/dao02685.

Nijssen, H., Isbrücker, I.J.H., 1986. Review of the genus *Corydoras* from Peru and Ecuador (Pisces, Siluriformes, Callichthyidae). Stud. Neotrop. Fauna Environ. 21,1-68.

Pote, L.M., Hanson, L.A., Shivaji, R., 2000. Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to *Henneguya* n. sp. (Myxozoa: Myxosporea). J. Aquati. Anim. Health 12,230–240. doi: 10.1577/1548-8667(2000)012<0230:SSRRSL>2.0.CO;2.

Prang, G., 2007. An industry analysis of the freshwater ornamental fishery with particular reference to the supply of Brazilian freshwater ornamentals to the UK market. Uakari 3,7-51.

Reis, R.E., 1998. Systematic, Biogeography, and the fossil record of the Callichthyidae: a review of the available data. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS,

Lucena CAS (eds) Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, Brasil, pp. 351‑362.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA 6: Molecular evolutionary genetics analysis Version 6.0. Mol. Biol. Evol. 30, 2725–2729. doi: 10.1093/molbev/mst197.

Velasco, M., Videira, M., Nascimento, L.C., Matos, P., Gonçalves, E.C., Matos, E., 2016. *Henneguya paraensis* n. sp. (Myxozoa; Myxosporea), a new gill parasite of the Amazonian fish *Cichla temensis* (Teleostei: Cichlidae): morphological and molecular aspects. Parasitol. Res. 115,1779-1787. doi:10.1007/s00436-016-4916-6.

Videira, M., Velasco, M., Azevedo, R., Silva, R., Gonçalves, E., Matos, P., Matos, E., 2015. Morphological aspects of *Henneguya aequidens* n. sp. (Myxozoa: Myxobolidae) in *Aequidens plagiozonatus* Kullander, 1984 (Teleostei: Cichlidae) in the Amazon region, Brazil. Parasitol. Res. 114, 1159–1162. doi:10.1007/s00436-014-4295-9.

Abdallah VD, Azevedo RK, Luque JL, Bomfim TCB (2007) Two new species of *Henneguya* Thélohan, 1892 (Myxozoa, Myxobolidae), parasitic on the gills of *Hoplosternum littorale* (Callichthyidae) and *Cyphocharax gilbert* (Curimatidae) from the Guandu River, State of Rio de Janeiro, Brazil. Parasitol Latinoam 62: 35 - 41

Abdel-Ghaffar F, Abdel-Baki AS, Bayoumy EM, Bashtar AR, Qurieshy SA,MorseyKS, Alghamdy A, Mehlhorn H (2008) Light and electron microscopic study on *Henneguya suprabranchiae* Landsberg, 1987 (Myxozoa: Myxosporea) infecting *Oreochromis niloticus*, a new host record. Parasitol Res 103:609–617

Adriano EA, Arana S, Cordeiro NS (2005) An ultrastructural and histopathological study of *Henneguya pellucida* n. sp. (Myxosporea: Myxobolidae) infecting *Piaractus mesopotamicus* (Characidae) cultivated in Brazil. Parasite 12(3):221–227

Adriano EA, Carriero MM, Maia AAM, Silva MRM, Naldoni J, Ceccarelli OS, Arana S (2012) Phylogenetic and host−parasite relationship analysis of *Henneguya multiplasmodialis* n. sp. infecting *Pseudoplatystoma* spp. in Brazilian Pantanal wetland. Vet Parasitol 185: 110−120

Ali MA (1999) *Henneguya ghaffari* sp. n. (Myxozoa: Myxosporea), infecting the Nile perch *Lates niloticus* (Teleostei: Centropomidae). Dis Aquat Org 38: 225–230.

Ali MA, Abdel-Baki AS, Sakran TH, Entzeroth R, Abdel-Ghaffar F (2007) *Myxobolus lubati* n. sp. (Myxosporea: Myxobolidae), a new parasite of haffara seabream *Rhabdosargus haffara* (Forsskal, 1775), Red Sea, Egypt: a light and transmission electron microscopy. Parasitol Res 100:819-827.

Azevedo C, Matos E (1995) *Henneguya adherens* n. sp. (Myxozoa, Myxosporea), parasite of the Amazon fish, *Acestrorhynchus falcatus*. J Eukaryot Microbiol 42:515–518

Barassa B, Adriano EA, Cordeiro NS, Ceccarelli PS (2012) Morphology and host-parasite interaction of *Henneguya azevedoi* n. sp., parasite of gills of *Leporinus obtusidens* from Mogi-Guaçu River, Brazil. Parasitol Res 110: 887−894

Casal G, Matos E, Azevedo C (1997) Some ultrastructural aspects of *Henneguya striolata* sp. nov. (Myxozoa, Myxosporea), a parasite of the Amazonian fish *Serrasalmus striolatus*. Parasitol Res 83(1):93–95

Casal G, Matos E, Azevedo C (2003) Light and electron microscopic study of the myxosporean, *Henneguya friderici* n. sp. from the Amazonian teleostean fish, *Leporinus friderici*. Parasitology 126: 313–319

Carriero MM, Adriano EA, Silva MRM, Ceccarelli PS, Maia AAM (2013) Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American species. PLoS ONE 8: e73713

Current WL, Janovy J Jr (1976) Ultrastructure of interlamellar *Henneguya exilis* in the channel catfish. J Parasitol 62:975–981

Dyková I, Lom J (1978) Histopathological changes in fish gills infected with myxosporidian parasites of the genus *Henneguya*. J Fish Biol 12: 197-202

Eiras JC (2002) Synopsis of the species of the genus *Henneguya* Thelohan, 1892 (Myxozoa: Myxosporea: Myxobolidae. Syst Parasitol 52(1):43–54

Eiras JC, Takemoto RM, Pavanelli GC (2009) *Henneguya corruscans* n. sp. (Myxozoa, Myxosporea, Myxobolidae), a parasite of *Pseudoplatystoma corruscans* (Osteichthyes, Pimelodidae) from the Parana River, Brazil: a morphological and morphometric study. Vet Parasitol 159: 154−158

Eiras JC, Adriano EA (2012) Checklist of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa, Myxosporea, Myxobolidae) described between 2002 and 2012. Syst Parasitol 83(2):95–104

El-Mansy AIE, Bashtar AR (2002) Histopathological and ultrastructural studies of *Henneguya suprabranchiae* Landsberg, 1987 (Myxosporea: Myxobolidae) parasitizing the suprabranchial organ of the freshwater catfish *Clarias gariepinus* Burchell, 1822 in Egypt. Parasitol Res 88:617–626

Feist SW, Longshaw M (2006) Phylum Myxozoa. In: Woo PTK (ed) Fish diseases and disorders: Protozoan and Metazoan infections, 2nd ed. CAB International, UK, pp 230–296

Feijó MM, Arana S, Ceccarelli PS, Adriano EA (2008) Light and scanning electron microscopy of *Henneguya arapaima* n. sp. (Myxozoa: Myxobolidae) and histology of infected sites in pirarucu (*Arapaima gigas*: Pisces: Arapaimidae) from the Araguaia River, Brazil. Vet Parasitol 157:59–64

Froese R, Pauly D (2011) FishBase. World Wide Web electronic publication. Version (02/2011) www.fishbase.org (accessed 10 May 2015)

Griffin MJ, Pote LM, Wise DJ, Greenway TE, Mauel MJ, Camus AC (2008) A novel *Henneguya* species from channel catfish described by morphological, histological, and molecular characterization. *J Aquat Anim Health* 20: 127−135

Hallett SL, Diamant A (2001) 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 46:197–212

Iwanowicz LR, Iwanowicz DD, Pote LM, Blazer VS, Schill WB (2008) Morphology and 18S rDNA of *Henneguya gurlei* (Myxosporea) from *Ameiurus nebulosus* (Siluriformes) in North Carolina. J Parasitol 94: 46−57

Kostoïngue B, Diebakate C, Faye N, Toguebaye S (2001) Presence of Myxosporidea (Myxozoa: Myxosporea) of the genus *Henneguya* Thélohan, 1892 in freshwater fishes from Chad (Central Africa). Acta Protozool 40: 117–123

Landsberg JH (1987) Myxosporean parasites of the catfish, Clarias lazera (Valenciennes). Syst Parasitol 9: 73−81

Lom J, Arthur JR (1989) A guideline for the preparation of species descriptions in Myxosporea. J Fish Dis 12: 151–156

Martins ML, Onaka EM, Bozzo FR, Fenerick JrJ (2004) *Henneguya* sp. (Myxozoa: Myxobolidae) in *Pimelodus maculatus* (Osteichthyes: Siluridae) from Volta Grande reservoir, Minas Gerais, Brazil. B Inst Pesca 30: 1-7

Matos E, Tajdari J, Azevedo C (2005) Ultrastructural studies of *Henneguya rhamdia* n. sp. (Myxozoa) a parasite from the Amazon teleost fish, *Rhamdia quelen* (Pimelodidae). J Eukaryot Microbiol 52: 532−537

Mathews PD, Silva MRM, Maia AMA, Adriano EA (2015) Ultrastructure and ssrRNA sequencing of *Myxidium amazonense* n. sp. a myxosporean parasite of *Corydoras melini* from the Rio Negro river, Amazonas state, Brazil. Parasitol Res 114: 4675-4683

Mathews PD, Maia AMA, Adriano EA (2016) Morphological and ultrastructural aspects of *Myxobolus niger* n. sp. (Myxozoa) gill parasite of *Corydoras melini* (Siluriformes: Callichthyidae) from Brazilian Amazon. Acta Trop 158: 214–219

Mohammed AA, AL-Rasheid KA, Sakran T, Abdel-Baki AA, Abdel-Ghaffar FA (2002) Some species of the genus *Myxobolus* (Myxozoa: Myxosporea) infecting freshwater fish of the River Nile, Egypt, and the impact on their hosts. Parasitol Res 88: 9-15

Molnár K (1998) Taxonomic problems, seasonality and histopathology of *Henneguya creplini* (Myxosporea) infection of the pikeperch *Stizostedion lucioperca* in Lake Balaton. Folia Parasitol 45: 261–269

Molnár K, Székely C, Mohamed K, Shaharom-Harrison F (2006) Myxozoan pathogens in cultured Malaysian fishes. I. Myxozoan infections of the sutchi catfish Pangasius hypophthalmus in freshwater cage cultures. Dis Aquat Org 68: 209−218

Morsy K, Abdel-Ghaffar F, Bashtar AR, Mehlhorn H, Al Quraishy S, Abdel-Gaber R. (2012). Morphology and small subunit ribosomal DNA sequence of *Henneguya suprabranchiae* (Myxozoa), a parasite of the catfish *Clarias gariepinus* (Clariidae) from the River Nile, Egypt. Parasitol Res 111:1423-35

Naldoni J, Arana S, Maia AAM, Ceccarelli PS, Tavares LER, Borges FA, Pozo CF, AdrianoEA (2009) *Henneguya pseudoplatystoma* n. sp. causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: histopathology and ultrastructure. Vet Parasitol 166: 52–59

Naldoni J, Arana S, Maia AAM, Silva MRM and others (2011) Host–parasite–environment relationship, morphology and molecular analysis of *Henneguya eirasi* n. sp. parasite of two wild *Pseudoplatystoma* ssp. in Pantanal Wetland, Brazil. Vet Parasitol 177: 247−255

Naldoni J, Maia AAM, Da Silva MRM, Adriano EA (2014) *Henneguya cuniculator* sp. nov., a parasite of spotted sorubim *Pseudoplatystoma corruscans* in the São Francisco Basin, Brazil. Dis Aquat Org 107: 211–221

Pote LM, Hanson LA, Shivaji R (2000) Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to Henneguya n. sp. (Myxozoa: Myxosporea). J Aquat Anim Health 12:230–240

Rabie SA, Mohammed NI, Hussein AA, Hussein NM (2009) The infection of freshwater fishes with three species of Henneguya in Qena, Upper Egypt. Egypt Acad J Biol Sci 1: 11−19

Reed CC, Basson L, Van As LL (2003) Myxozoans infecting the sharptooth catfish, *Clarias gariepinus* in the Okavango River and Delta, Botswana, including descriptions of two new species, *Henneguya samochimensis* sp. n. and *Myxobolus gariepinus* sp. n. Folia Parasitol 50:183–189

Reis RE (1998) Systematic, Biogeography, and the fossil record of the Callichthyidae: A review of the available data. In Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (eds) Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, Brasil, pp 351-362

Rocha E, Matos E, Azevedo C (1992) *Henneguya amazonica* n. sp. (Myxozoa, Myxobolidae), parasitizing the gills of *Crenicichla lepidota* Heckel, 1840 (Teleostei, Cichlidae) from Amazon River. Eur J Protistol 28(3):273–278

Sarkar NK, (1985) Myxosporidan *Henneguya mystusia* sp. n. (Myxozoa: Myxosporea) from the gill of a freshwater teleost fish *Mystus* sp. Acta Protozool, 241: 55-58

Sitjà-Bobadilla A (2008) Fish immune response to myxozoan parasites. Parasite 15:420–425

Videira M, Velasco M, Azevedo R, Silva R, Gonçalves E, Matos P, Matos E (2015) Morphological aspects of *Henneguya aequidens* n. sp. (Myxozoa: Myxobolidae) in *Aequidens plagiozonatus* Kullander, 1984 (Teleostei: Cichlidae) in the Amazon region, Brazil. Parasitol Res 114:1159–1162

Vita P, Corral L, Matos E, Azevedo C (2003) Ultrastructural aspects of the myxosporean *Henneguya astyanax* n. sp. (Myxozoa: Myxobolidae), a parasite of the Amazonian teleost *Astyanax keithi* (Characidae). Dis Aquat Org 53:55–60

Abdel-Azeem, Sh., Abdel-Baki, A.S., 2011. Light and electron microscopic studies on *Myxobolus egyptica* sp. nov. (Myxozoa, Myxosporea), infecting the hornlip mullet *Oedalechilus labiosus* from the Red Sea. Acta Parasitol. 56, 255–262.

Adriano, E.A., Arana, S., Cordeiro, N.S., 2005. Histology, ultrastructure and prevalence of *Henneguya piaractus* (Myxosporea) infecting the gills of *Piaractus mesopotamicus* (Characidae) cultivated in Brazil. Dis. Aquat. Org. 64, 229–235.

Adriano, E.A., Arana, S., Ceccarelli, P.S., Cordeiro, N.S., 2002. Light and scanning electron microscopy of *Myxobolus porofilus* sp. n. (Myxosporea: Myxobolidae) infecting the visceral cavity of *Prochilodus lineatus* (Pisces: Characiformes: Prochilodontidae) cultivated in Brazil. Folia Parasitol. 49, 259–262.

Adriano, E.A., Arana, S., Cordeiro, N.S., 2006. *Myxobolus cuneus* n. sp. (Myxosporea) infecting the connective tissue of *Piaractus mesopotamicus* (Pisces: Characidae) in Brazil: histopathology and ultrastructure. Parasite 13, 137–142.

Adriano, E.A., Arana, S, Alves, A.L., Silva, M.R.M., Ceccarelli, P.S., Henrique-Silva, F., Maia, A.A.M., 2009. *Myxobolus cordeiroi* n. sp., a parasite of *Zungaro jahu* (Siluriformes: Pimelodidae) from Brazilian Pantanal: morphology, phylogeny and histopathology. Vet. Parasitol. 162, 221–229.

Azevedo, C., Casal, G., Matos, P., Ferreira, I., Matos, E., 2009. Light and electron microscopy of the spore of *Myxobolus heckelii* n. sp., (Myxozoa), parasite from the Brazilian fish *Centromochlus heckelii* (Teleostei, Auchenipteridae). J. Eukaryot. Microbiol. 56, 589–593.

Azevedo, C., São Clemente, S.C., Casal, G., Matos, P., Oliveira, E., Al-Guraishy, S., Matos, E., 2012. Light and ultrastructural analysis of *Myxobolus insignis* (Myxozoa), infecting the Amazonian fish *Semaprochilodus insignis* (Prochilodontidae). Zootaxa 3182, 51–56.

Azevedo, C., São Clemente, S.C., Casal, G., Matos, P., Oliveira, E., Al-Quraishy, S., Matos, E., 2013. Ultrastructure of the plasmodial development of *Myxobolus insignis* (Myxozoa), Infecting the Amazonian Fish *Semaprochilodus insignis* (Prochilodontidae). Acta Protozool. 52: 91–97.

Bartholomew, J.L., Atkinson, S.D., Hallett, S.L., Lowenstine, L.J., Garner, M.M., Gardiner, C.H., Rideout, B.A., Keel, M.K., Brown, J.D., 2008. Myxozoan parasitism in waterfowl. Int. J. Parasitol. 38, 1199–1207.

Baska, F., Voronin, V.N., Eszterbauer, E., Müller, L., Marton, S., Molnár, K., 2009. Occurrence of two myxosporean species, *Myxobolus hakyi* sp. n. and *Hoferellus pulvinatus* sp. n., in *Pangasianodon hypophthalmus* fry imported from Thailand to Europe as ornamental fish. Parasitol. Res. 105,1391–1398.

Carriero, M.M., Adriano, E.A., Silva, M.R.M., Ceccarelli, P.S., Maia, A.A.M., 2013. Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American species. PloS ONE 8, e73713.

Casal, G., Matos, E., Azevedo, C., 2002. Ultrastructural data on the spore of *Myxobolus maculatus* n. sp. (phylum Myxozoa), parasite from the Amazonian fish *Metynnis maculatus* (Teleostei). Dis. Aquat. Org. 51, 137–149.

Casal, G., Matos, E., Azevedo, C., 2006. A new myxozoan parasite from the Amazonian fish *Metynnis argenteus* (Teleostei, Characidae). Light and electron microscope observations. J. Parasitol. 92, 817–821.

Cellere, E.F., Cordeiro, N.S., Adriano, E.A., 2002. *Myxobolus absonus* sp. n. (Myxozoa: Myxosporea) parasitizing *Pimelodus maculatus* (Siluriformes: Pimelodidae), a South American freshwater fish. Mem. Inst. Oswaldo Cruz 97, 79–80.

Cone, D.K., Easy, R.H., 2005. Supplemental diagnosis and molecular taxonomy of *Myxobolus diaphanous* (Fanthan, Porter et Richardson, 1940) (Myxozoa) parasitizing *Fundulus diaphanous* (Cyprinodontiformes) in Nova Scotia, Canada. Folia Parasitol. 52, 217–222.

Eiras, J.C., Molnár, K., Lu, Y.S., 2005. Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). Syst. Parasitol. 61, 1–46.

Eiras, J.C., Zhang, J., Molnár, K., 2014. Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea, Myxobolidae) described between 2005 and 2013. Syst. Parasitol. 88, 11–36.

El-Matbouli, M., Fischer-Scherl, T., Hoffmann, R.W., 1990. Light and electron microscopic studies on *Myxobolus cotti* El- Matbouli and Hoffmann, 1987 infecting the central nervous system of the bullhead (*Cottus gobio*). Parasitol. Res. 76, 219–227.

Eszterbauer, E., 2004. Genetic relationship among gill-infecting *Myxobolus* species (Myxosporea) of cyprinids: molecular evidence of importance of tissue specificity. Dis. Aquat. Org. 58, 35–40.

FAO, 2015. Food and Agriculture Organization of the United Nations. Fisheries and Aquaculture topics. Ornamental fish. Topics Fact Sheets. Text by Devin Bartley. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 27 May 2005. [Cited 15 August 2015].

Froese, R., Pauly, D., 2011. FishBase. World Wide Web electronic publication. Version (02/2011) www.fishbase.org (accessed 10 May 2015).

IBAMA, 2007. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Brasília: Relatório Técnico sobre o Diagnóstico geral das práticas de controle ligadas a exploração, captura, comercialização, exportação e uso de peixes para fins ornamentais e de aquariofilia - Diretoria de uso sustentável da biodiversidade e florestas, Brasilia, Brasil, p 214

Lom, J., Arthur, J.R., 1989. A guideline for the preparation of species descriptions in Myxosporea. J. Fish. Dis. 12, 151–156.

Lom, J., Dykova, I., 2006. Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol. 53, 1–36.

Mathews, P.D., Silva, M.R.M., Maia, A.M.A., Adriano, E.A., 2015. Ultrastructure and ssrRNA sequencing of *Myxidium amazonense* n. sp. a myxosporean parasite of *Corydoras melini* from the Rio Negro river, Amazonas state, Brazil. Parasitol. Res. 114, 4675-4683.

Milanin, T., Eiras J.C., Arana, S., Maia, A.M.M., Alves, A.L., Silva, M.R.M., Carriero, M.M., Ceccarelli, P.S., Adriano, E.A., 2010. 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 105,762-769.

Molnár, K., Békési, L., 1993. Description of a new *Myxobolus* species, *M. colossomatis* n. sp. from the teleost *Colossoma macropomum* of the Amazon river basin. J. Appl. Ichthyol. 9, 57–63.

Molnár, K., 1998. Taxonomic problems, seasonality and histopathology ok *Henneguya creplini* (Myxosporea) infection of the pikeperch *Stizoztedion lucioperca* in Lake Balaton. Folia Parasitol. 45,261–269.

Molnár, K., Eszterbauer, E., 2015. Specificity of Infection Sites in Vertebrate Hosts: in Okamura, B., Gruhl, A., Bartholomew, J.L. (eds) Myxozoan Evolution, Ecology and Development, Springer. Switzerland, pp 295-313.

Molnár, K., Székely, C., Mohamed, K., Shaharom-Harrison, F., 2006. Myxozoan pathogens in cultured Malaysian fishes. I. Myxozoan infections of the sutchi catfish *Pangasius hypophthalmus* in freshwater cage cultures. Dis. Aquat. Org. 68, 209–218.

Naldoni, J., Arana, S., Maia, A.A.M., Ceccarelli, P.S., Tavares, L.E.R., Borges, F.A., Pozo, C.F., Adriano, E.A., 2009. *Henneguya pseudoplatystoma* n. sp. causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: histopathology and ultrastructure. Vet. Parasitol. 166, 52–59.

Noga, E.J., 2000. Fish disease: diagnosis and treatment. Blackwell, Ames, pp 173–178.

Okamura, B., Gruhl, A., Bartholomew, J.L., 2015. An Introduction to Myxozoan Evolution, Ecology and Development: in Okamura, B., Gruhl A., Bartholomew, J.L. (eds) Myxozoan Evolution, Ecology and Development. Springer, Switzerland, pp 1-20.

Pote, L.M., Hanson, L.A., Shivaji, R., 2000. Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to *Henneguya* n. sp. (Myxozoa: Myxosporea). J. Aquat. Anim. Health 12, 230–240.

Reed, C.C., Basson, L., Van As L.L., 2003. Myxozoans infecting the sharptooth catfish, *Clarias gariepinus* in the Okavango River and Delta, Botswana, including descriptions

of two new species, *Henneguya samochimensis* sp. n. and *Myxobolus gariepinus* sp. n. Folia Parasitol. 50,183–189.

Reis, R.E., 1998. Systematic, Biogeography, and the fossil record of the Callichthyidae: A review of the available data. In Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (eds) Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, Brasil, pp 351-362.

Reis, R.E., Kullander, S.O., Ferraris, Jr.C.J., (eds). 2003. Check list of the freshwater fishes of South and Central America. Edipucrs, Porto Alegre, Brasil, 729p.

Székely, C., Cech, G., Atkinson, S.D., Molnár, K., Egyed, L., Gubányi, A., 2015. A novel myxozoan parasite of terrestrial mammals: description of *Soricimyxum minuti* sp. n. (Myxosporea) in pygmy shrew *Sorex minutus* from Hungary. Folia Parasitol. 62, doi:10.14411/fp.2015.045

Sitjà-Bobadilla, A., 2008. Fish immune response to myxozoan parasites. Parasite 15, 420-425.

Zatti, S.A., Naldoni, J., Silva, M.R.M., Maia, A.A.M., Adriano, E.A., 2015. Morphology, ultrastructure and phylogeny of *Myxobolus curimatae* n. sp. (Myxozoa: Myxosporea) a parasite of *Prochilodus costatus* (Teleostei: Prochilodontidae) from the São Francisco River, Brazil. Parasitol. Int. 64, 362–368.

Adriano EA, Oliveira OMP (2016) Myxosporea. In Catálogo Taxonômico da Fauna do Brasil. PNUD. http://fauna.jbrj.gov. br/fauna/faunadobrasil/152799.Access in: April 14th 2016

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402

Azevedo C, Rocha S, Casal G, São Clemente SC, Matos P, Al-Quraishy S, Matos E (2013) Ultrastructural description of *Ceratomyxa microlepis* sp. nov. (Phylum Myxozoa): a parasite infecting the gall bladder of *Hemiodus microlepis*, a freshwater teleost from the Amazon River. Mem Inst Oswaldo Cruz 108:150–154

Barta JR, Martin DS, Liberator PA, Dashkevicz M, Anderson JW, Feighner SD, Elbrecht A, Perkins-Barrow A, Jenkins MC, Danforth D, Ruff MD, Profous-Juchelka H

(1997) Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J Parasitol 83:262–271

Bleher H, Stölting KN, Salzburger W, Meyer A (2007) Revision of the genus *Symphysodon* Heckel, 1840 (Teleostei: Perciformes: Cichlidae) based on molecular and morphological characters. Aquaculture 12:133–174

Crampton WGR (2008) Ecology and life history of an Amazon floodplain cichlid: the discus fish *Symphysodon* (Perciformes: Cichlidae). Neotrop Ichthyol 6:599–612

Eiras JC (2006) Synopsis of the species of the genus *Ceratomyxa* Thélohan, 1892 (Myxozoa: Myxosporea: Ceratomyxidae). Syst Parasitol 65:49–71

Fiala I, Hlavničková M, Kodádková A, FreemanMA, Bartošová-Sojková P, Atkinson SD (2015) Evolutionary origin of *Ceratonova Shasta* and phylogeny of the marine myxosporean lineage. Mol Phylogenet Evol 86:75–89

Froese R, Pauly D (2009) FishBase. http://www.fishbase.org (accessed 25.03.2016)

Guindon S, Dufayard JF, Lefort V, Anisimova M, HordijkW, Gascuel O (2010) New algorithms and methods to estimate maximum likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321

Gunter NL, Whipps CM, Adlard RD (2009) *Ceratomyxa* (Myxozoa: Bivalvulida): robust taxon or genus of convenience? Int J Parasitol 39:1395–1405

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Kpatcha TK, Diebakate C, Faye X, Toguebaye BS (1996) Qualques nouvelles espèces de Myxosporidies, du genre *Ceratomyxa* Thélohan, 1895 parasites des poissons marins du Sénégal, Afrique de L'Ouest. Parasite 3:223–228

Lom J, Arthur JR (1989) A guideline for the preparation of species descriptions in Myxosporea. J Fish Dis 12:151–156

Mansour L, Abdel-Baki AA, Al-Qahtani HA, Al-Quraishy S (2015) Morphological and molecular aspects of *Ceratomyxa mehlhorni* n. sp., a parasite of the golden trevally

Gnathanodon speciosus in the Arabian Gulf off the Saudi Arabian coast, with data on its seasonal prevalence. Parasitol Res 114(10):3783–3789

Meglitsch PA (1960) Some coelozoic Myxosporidia from New Zealand fishes. I. General and family Ceratomyxidae. Trans R Soc N Z 88: 265–365

Ready JS, Ferreira EJG, Kullander SO (2006) Discus fishes: mitochondrial DNA evidence for a phylogeographic barrier in the Amazon genus *Symphysodon* (Teleostei: Cichlidae). J Fish Biol 69:200–211

Schaefer SA (1998) Conflict and resolution: impact of nov taxa on phylogenetic studies of the neotropical cascudinhos (Siluroidea: Loricariidae). In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (eds) Phylogeny and Classification of Neotropical Fishes. EDIPUCRS, Porto Alegre, pp 375–400

Thabet A, Mansour L, Al Omar SY, Tlig-Zouari S (2016) *Ceratomyxa tunisiensis* n. sp. (Myxosporea: Bivalvulida) from the gallbladders of two Carangid fish caught of the coast of Tunisia. J Eukaryot Microbiol 63:86–92

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

# 8. ANEXOS

## 8.1. Certificado da Comissão de Ética no uso de Animais (CEUA/UNIFESP)

UNIFESP
UNIVERSIDADE FEDERAL DE SÃO PAULO

*Comissão de Ética no Uso de Animais*

**CERTIFICADO**

Certificamos que a proposta intitulada "Sistemática e interação parasito-hospedeiro de parasitos do filo Myxozoa em peixes de importância econômica da Bacia Amazônica", protocolada sob o CEUA nº 9209080214, sob a responsabilidade de **Edson Aparecido Adriano** *e equipe; Antônio Augusto Mendes Maia* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de São Paulo (CEUA/UNIFESP) na reunião de 11/05/2015.

We certify that the proposal "", utilizing 15 Fishes (15 males), protocol number CEUA 9209080214, under the responsibility of **Edson Aparecido Adriano** *and team; Antônio Augusto Mendes Maia* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Sao Paulo (CEUA/UNIFESP) in the meeting of 05/11/2015.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 03/2014 a 12/2015 Área: Ciências Biológicas

| | | | | | |
|---|---|---|---|---|---|
| Origem: | Não aplicável | | | | |
| Espécie: | Peixes | sexo: | Machos | idade: | 0 a 100 anos |
| Linhagem: | indefinida | | | Peso: | 0 a 20000 g |

Resumo: Peixes, em ambiente natural ou sistemas de criação, são hospedeiros de várias espécies de parasitos, que podem causar importantes patogenias. Entre estes parasitos, os mixosporídeos, devido à grande diversidade e ao grande potencial patogênico de algumas espécies, vem recebendo cada vez mais atenção por parte dos pesquisadores de várias regiões do mundo. Atualmente são conhecidas mais de 2.300 espécies de mixosporídeos, das quais a grande maioria infecta peixes, sendo algumas espécies responsáveis por altas taxas de mortalidade em várias partes do mundo. Este projeto tem como foco a diversidade e a interação parasito-hospedeiro de parasitos do filo Myxozoa que infectam peixes amazônicos de importância na área de peixes ornamentais (Paracheirodon axelroldi [cardinal] Paracheirodon innesi [néon], Carnegiella strigata [borboleta], Corydoras spp. [coridora] e Symphysodon discus [acara disco]) e espécies de peixes importantes para o mercado de alimentos (Colossoma macropomum [tambaqui], Piaractus brachypomum [pirapitinga], Prochilodus nigricans [curimatã], Brachyplatystoma rousseauxii [dourada], Brachyplatystoma filamentousum [piraíba], Pseudoplatystoma spp. [cachara e cacharari], Phractocephalus hemioliopterus [pirarara] e (Leiarius marmoratus) [jundiá da Amazônia]). Os peixes serão de oriundos de ambiente natural, mas no caso das espécies que já são utilizadas em sistemas de criação, também serão examinadas a partir de pisciculturas. O estudo da interação parasito-hospedeiro dar-se-á a partir de análises ultra estruturais e histopatológicas. Para o estudo da identificação e descrição das espécies serão realizadas análises morfológicas utilizando microscopia de luz, microscopia eletrônica de transmissão e análises moleculares, através do sequenciamento do gene 18S rDNA. Os peixes ornamentais serão capturados na região de Manaus, AM e os peixes importantes para o mercado de alimento serão obtidos na região de Santarém, PA. ANIMAIS 15 Peixes de cada espécie, Machos ou Fêmeas (depende da coleta) Procedência: Os peixes coletado serão examinados em laboratório de campo montado próximo ao local de coleta. Conforme destacado no projeto, serão examinados várias espécies de peixes, sendo 15 exemplares de cada espécie. Manutenção: Os peixes coletado serão examinados em laboratório de campo montado próximo ao local de coleta.

Local do experimento:

São Paulo, 06 de setembro de 2017

UNIFESP
UNIVERSIDADE FEDERAL DE SÃO PAULO

*Comissão de Ética no Uso de Animais*

Profa. Dra. Monica Levy Andersen
Coordenadora da Comissão de Ética no Uso de Animais
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Profa. Dra. Tatiana Helfenstein
Vice-Coordenadora da Comissão de Ética no Uso de Animais
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## 8.2. Declaração de Direitos Autoriais

**Declaração**

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **SISTEMÁTICA E INTERAÇÃO PARASITO-HOSPEDEIRO DE MIXOSPORÍDEOS PARASITOS DE PEIXES ORNAMENTAIS DA BACIA AMAZÔNICA** , não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 25 de Janeiro de 2018

Assinatura : _____

Nome do(a) autor(a): **Patrick Mathews Delgado**

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