UNIVERSIDADE ESTADUAL DE CAMPINAS

Instituto de Biologia

PROGRAMA DE PÓS-GRADUAÇÃO EM PARASITOLOGIA

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TAXONOMIA E INTERAÇÃO PARASITO-HOSPEDEIRO NA INFECÇÃO DE MIXOSPORÍDEOS EM PINTADO (*Pseudoplatystoma corruscans*) E CACHARA (*Pseudoplatystoma fasciatum*) ORIUNDOS DE AMBIENTE NATURAL E DE SISTEMAS DE CULTIVO

Dissertação apresentada ao Instituto de Biologia, da Universidade Estadual de Campinas (UNICAMP), como requisito para obtenção do título de Mestre em Parasitologia.

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Campinas

2010

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECA DO INSTITUTO DE BIOLOGIA – UNICAMP

Naldoni, Juliana

N146t Taxonomia e interação parasito-hospedeiro na infecção de mixosporídeos em pintado (*Pseudoplatystoma corruscans*) e cachara (*Pseudoplatystoma fasciatum*) oriundos de ambiente natural e de sistemas de cultivo / Juliana Naldoni. – Campinas, SP: [s.n.], 2010.

Orientador: Edson Aparecido Adriano.

Dissertação (mestrado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. Myxozoa. 2. Ultraestrutura (Biologia). 3. Peixe - Parasito. I. Adriano, Edson Aparecido. II. Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

(rodt/ib)

Título em inglês: Taxonomy and host-parasite interaction in the infection of myxosporeans in pintado (*Pseudoplatystoma corruscans*) and cachara (*Pseudoplatystoma fasciatum*) from the natural environment and fish farms.

Palavras-chave em inglês: Myxozoa; Ultrastructure (Biology); Fish - Parasite.

Área de concentração: Parasitologia.

Titulação: Mestre em Parasitologia.

Banca examinadora: Edson Aparecido Adriano, Maria José Tavares Ranzani de Paiva, Marlene Tiduko Ueta.

Data da defesa: 08/07/2010.

Programa de Pós-Graduação: Parasitologia.

Campinas, 08 de Julho de 2010.

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À minha família, em especial a minha mãe Clotilde Ap. Honor Naldoni, por todo carinho, apoio e incentivo.

Agradecimentos

À Deus, por mais esta grande conquista em minha vida.

Ao Professor Doutor Edson Aparecido Adriano pela orientação, apoio e incentivo, sem o qual este trabalho não seria possível.

Á Professora Doutora Sarah Arana, do Departamento de Histologia e Embriologia do Instituto de Biologia da UNICAMP pelo auxílio na análise histológica.

Aos Professores que fizeram parte da defesa prévia deste trabalho, Dr. Rúbens Riscala Mandi, Profa. Dra. Marlene Tiduko Ueta e a Profa. Dra. Maria Jose Tavares Ranzani de Paiva, pela análise e alterações sugeridas.

Aos demais professores do curso de parasitologia da UNICAMP por todo conhecimento que compartilharam durante as disciplinas ministradas.

Aos colegas do curso de parasitologia, em especial à Carina Mara de Souza e ao Tiago Milanin, pela amizade e apoio durante esta etapa.

Ao Prof. Dr. Antônio Augusto Mendes Maia por disponibilizar o laboratório de parasitologia do Departamento de Ciências Básicas da Faculdade de Zootecnia e Engenharia de Alimentos-FZEA/USP em Pirassununga, permitindo o processamento do material coletado.

Aos funcionários do Centro Nacional de Pesquisa e Conservação de Peixes Continentais – CEPTA, vinculado ao Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio , em especial ao Dr. Paulo Sérgio Ceccarelli e ao Ricardo Afonso Torres de Oliveira, pelo auxílio na captura dos peixes.

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RESUMO

Parasitos do filo Myxozoa são cosmopolitas, infectam peixes em diversas regiões e estão entre os mais importantes patógenos de peixes, tanto em ambiente natural, como em sistemas de criação, onde podem causar prejuízos importantes. Este trabalho teve por objetivo o estudo da interação parasito-hospedeiro em infecções de peixes do gênero *Pseudoplatystoma*, pintado (*Pseudoplatystoma corruscans*), cachara (*Pseudoplatystoma fasciatum*) e o híbrido (pintado x cachara), causadas por parasitos do filo Myxozoa. Os peixes estudados foram oriundos de ambiente natural, Pantanal Mato-Grossense e de sistemas de criação, pisciculturas dos Estados de São Paulo e do Mato Grosso do Sul. Durante este trabalho foi possível estudar e descrever duas novas espécies de *Henneguya* infectando *Pseudoplatystoma* spp. *Henneguya* sp. 1 foi descrito infectando pintado híbrido de sistemas de criação nos Estados de São Paulo e Mato Grosso e as respectivas prevalências foram 100 e 36, 7%. Devido à intensidade de infecção, com vários plasmódios em um mesmo filamento, o desenvolvimento do parasito produziu importante redução de área do epitélio branquial. A análise ultra-estrutural revelou uma única parede plasmodial ligada à zona do ectoplasma por vários canais de pinocitose. A outra espécie (*Henneguya* sp. 2) foi encontrado infectando simultaneamente brânquia de exemplares de pintado e cachara obtidos em ambiente natural, no Pantanal Mato-Grossense. A prevalência foi de 17,1% para ambas espécies de peixes examinados. A análise histopatológica revelou o desenvolvimento do parasito no tecido conectivo sub-epitelial do filamento branquial, estando os plasmódios envolvidos por uma cápsula de tecido conectivo. O plasmódio produziu uma leve compressão dos tecidos adjacentes, mas nenhum infiltrado inflamatório foi observado no sítio da infecção. A análise ultra-estrutural mostrou uma única parede plasmodial conectada com o ectoplasma através de numerosos canais de pinocitose.

Palavras-chaves: Mixozoa, Pimelodídeos, peixes cultivados, peixes selvagens, Brasil.

ABSTRACT

Myxozoa are cosmopolitan parasites and are among the most important pathogens of wild and cultivated fish. The aim of the present study was to investigate the host-parasite interaction in fish of the genus *Pseudoplatystoma* infected by Myxozoan parasites: the spotted sorubim (*Pseudoplatystoma corruscans*), barred sorubim (*Pseudoplatystoma fasciatum*) and a hybrid of the two species. Specimens were obtained from the natural environment in the Mato Grosso wetlands and fish farms in the states of São Paulo and Mato Grosso do Sul, Brazil. Two news species of *Henneguya* were described infecting *Pseudoplatystoma* ssp. *Henneguya* sp.1 was described infecting the hybrid on fish farms in the states of São Paulo and Mato Grosso do Sul (Brazil), with a prevalence of 100 and 36.7%, respectively. Due to the intensity of the infection, with several plasmodia in a single gill filament, the development of the parasite caused an important reduction in the area of functional epithelium. The ultrastructural analysis revealed a single plasmodial wall connected to the ectoplasma zone through several pinocytotic canals. *Henneguya* sp.2 was found infecting the gills of both the spotted and barred sorubim caught in the natural wetland environment. The prevalence was 17.1% in both species of fish. The histopathological analysis revealed that the parasite develops in the sub-epithelial connective tissue of the gill filaments and the plasmodium is surrounded by a capsule of connective tissue. The plasmodia caused slight compression of the adjacent tissues, but no inflammatory infiltrate was observed at the infection site. The ultrastructural analysis revealed a single plasmodial wall connected to the ectoplasma zone through numerous pinocytotic canals.

Keywords: Myxozoa, Pimelodidae, fish farm, wild fish, Brazil

INTRODUÇÃO

A aqüicultura é um dos sistemas de produção de alimentos que mais tem crescido nos últimos anos no mundo. Para a Organização das Nações Unidas para a Agricultura e Alimentação - FAO, aquicultura é a atividade que envolve o cultivo de organismos aquáticos, incluindo peixes, moluscos, crustáceos e plantas (FAO, 1997). Conforme o tipo de organismo envolvido, a aquicultura recebe diferentes denominações: piscicultura (criação de peixe), carcinicultura (criação de camarões), ranicultura (criação de rãs) e malacocultura (criação de moluscos) (Scorvo Filho, 2009).

A piscicultura, o setor da aqüicultura de maior importância econômica, desenvolveu-se gradativamente, mediante o aumento da demanda de alimento. Antigamente, comer peixe era privilégio das pessoas que moravam no litoral ou próximas de rios, devido à dificuldade de se manter vivos os peixes fora de seu ambiente natural, portanto raramente mantinham-se peixes em cativeiro. Provavelmente os primeiros peixes mantidos em cativeiros pertenciam à família das carpas (Mills, 1998).

De acordo com Sousa e Ceci (1985), a piscicultura teve início na China há cerca de 4 mil anos, quando a partir da observação dos peixes no seu ambiente natural construíram-se viveiros para criá-los. No Ocidente, os primeiros registros sobre piscicultura foram realizados no início da era cristã, com os romanos. Descreviam-se "piscinas", onde eram armazenados os peixes capturados no mar para serem consumidos na época em que determinadas espécies não fossem encontradas. A criação de peixes na Europa só foi registrada a partir do século XIV, quando monges passaram a criar carpas no interior do continente para serem consumidas nos períodos de abstinência, evitando com isto o transporte de peixes marinhos por longas distâncias. Na América do Sul, o primeiro país a introduzir a piscicultura foi a Argentina, importando os primeiros reprodutores de carpa (*Cyprinus carpio*) em 1870, sendo logo após introduzida a truta arco-íris (*Salmo irideus*) na região de Bariloche (Costa, 1978). No Brasil, as primeiras iniciativas voltadas para a criação de peixes foram por volta de 1904, sendo que já a partir de 1927 foram realizados os primeiros estudos liderados por Rodolfo Von Ihering, visando à reprodução de peixes autóctones (*Salminus maxillosus*),

utilizando hipófise para provocar a desova de dourados oriundos dos rios Mogi-Guaçu e Piracicaba, Estado de São Paulo. Mas a piscicultura só foi oficializada no Brasil em 1932, e, em 1939 surgiu a primeira Estação de Piscicultura do país, na cidade de Pirassununga, estado de São Paulo (Barbosa, 1992), onde hoje está localizado o Centro Nacional de Pesquisa e Conservação de Peixes Continentais (CEPTA/ICMBio).

Atualmente, o Brasil é considerado o país de maior potencial para a aqüicultura no mundo. Contribuem para isso os 8.400 Km de costa marítima, os 5.500.000 hectares de reservatórios de águas doces, aproximadamente 12% da água doce disponível no planeta, o clima favorável (quente o ano todo) na maior parte do país, a vasta extensão territorial, mão-de-obra abundante, crescente demanda por pescado no mercado interno (www.ana.gov.br) e a grande diversidade de espécies com potencial para cultivo (Castagnolli, 1992).

Dentre as espécies da ictiofauna brasileira, várias espécies se destacam com potencial para a piscicultura, como o pintado (*Pseudoplatystoma corruscans* Spix & Agassiz, 1829) (Kubitza *et al*., 1998) e a cachara (*Pseudoplatystoma fasciatum* Linnaeus, 1766) (Campos, 2005).

Estas são espécies que pertencem à ordem Siluriformes, a qual é representada pelos peixes de couro, cuja principal característica é a ausência de escamas sobre o corpo, sendo este revestido por uma pele espessa (Tavares, 1997). Geralmente possui três pares de barbilhões e o primeiro raio da nadadeira dorsal e das nadadeiras peitorais transformado em acúleo forte e pungente (Britski *et al*., 1999). A subordem Siluroidei constitui treze famílias na região neotropical, uma delas, a Pimelodidae encerra o gênero *Pseudoplatystoma*, que compreende os maiores peixes desta família, podendo ser encontrado nas principais bacias hidrográficas sul americanas (Tavares, 1997). As espécies deste gênero apresentam o corpo alongado e roliço, cabeça deprimida e largura ao nível da boca apenas ligeiramente menor do que a largura total do corpo, mandíbula mais curta que a maxila superior e dentes viliformes no palato (Tavares, 1997).

O *P. corruscans* ocorre nas bacias do Prata e do São Francisco e *P. fasciatum* ocorre nas bacias Amazônica, Corantijn, Essequibo, Orinoco e do Prata (Froese & Pauly,

2009). Na região do Pantanal Mato-Grossense, a maior área alagada do mundo, estas espécies de peixes apresentam grande importância econômica (Resende, 2003). Entretanto, apesar da grande importância ecológica e econômica nas regiões onde ocorrem, e do crescente interesse que estas espécies despertam nos piscicultores, o conhecimento da biologia e ecologia destes pimelodídeos é ainda relativamente precário (Prioli *et al.,* 2009).

Sabe-se que os peixes silvestres apresentam uma variedade de espécies de parasitos, porém raramente apresentam sinais clínicos de patogenia, por apresentarem um equilíbrio nutricional e fisiológico com o ambiente e também pelo fato da predação eliminar os peixes doentes. No entanto, se há alterações no ambiente, estas refletem na diminuição da imunidade do peixe, o qual torna-se sujeito a ação dos patógenos e pode apresentar sinais clínicos (Pavanelli *et al*., 2002). Em pisciculturas a pressão por parte dos patógenos é ainda maior devido à alta densidade populacional, o que facilita a transmissão, o desenvolvimento dos parasitos e o surgimento de doenças (Bakke & Harris, 1998).

O filo Myxozoa (mixosporídeos) compreende mais de 2.180 espécies, das quais, a grande maioria é parasito de peixes (Sitjá-Bobadilla, 2008), tanto de ambiente natural como de sistemas de criação, sendo algumas espécies responsáveis por altas taxas de mortalidade em várias partes do mundo (Lom & Dykova, 2006). Os gêneros *Myxobolu*s e *Henneguya* são os mais comumente encontrados (Adriano *et al*., 2002a), sendo registradas aproximadamente 37 espécies de *Henneguya* (Eiras *et al.* 2008; 2009; Azevedo *et al*., 2008; 2009a; Naldoni *et al*., 2009) e 27 espécies do gênero *Myxobolus* em peixes de água doce na América do Sul (Eiras *et al*, 2005, Martins & Onaka, 2006; Flores & Viozz, 2007; Adriano *et al.,* 2006; 2009a; 2009b; Azevedo *et al*., 2009b), as quais infectam peixes de ambiente natural e mantidos em sistemas de criação, causando prejuízos ao atingir espécies de importância econômica.

Durante seu desenvolvimento no hospedeiro vertebrado, os mixosporídeos formam plasmódios repletos de esporos, em vários tecidos de seus hospedeiros (Untergasser, 1989), sendo que os tamanhos dos plasmódios podem variar desde poucos micrometros até vários milímetros. O desenvolvimento pode ser histozóico

(plasmódios localizados intracelularmente ou intercelularmente) ou celozóico (localizados nas cavidades dos órgãos, soltos ou aderidos ao epitélio interno) (Lom, 1987; Eiras, 1994). Sendo assim, são encontrados comumente nas brânquias, na pele e em órgãos internos e estruturais, como cartilagens, músculos, fígado, baço, parede intestinal, vesícula biliar e bexiga natatória. Alguns plasmódios, como os localizados no tecido subcutâneo, causam deformações visíveis a olho nu (Thatcher & Neto, 1994).

Dentre os mixosporídeos, a espécie mais conhecida é o *M. cerebralis* Hofer, 1903, agente etiológico da doença do rodopio, a qual se manifesta em exemplares de salmonídeos jovens, onde o parasito ataca e deforma a cartilagem da cabeça e a coluna vertebral, provocando altas taxas de mortalidade desses peixes em várias regiões do mundo (Eiras, 1994). Nos Estados Unidos, na região das Montanhas Rochosas, este parasito é visto como o responsável pelo declínio de populações de truta arco-íris em ambiente natural (Allen & Bergersen, 2002).

Existem registros de várias outras espécies de mixosporídeos causando danos a diversas espécies de peixes em todo o mundo, sendo numerosos os estudos sobre mixosporídeos parasitos de peixes na América do Norte, Europa e Ásia (Casal *et al.,* 1996), mas ainda são poucos aqueles realizados visando o conhecimento da fauna e da importância desses parasitos na América do Sul (Azevedo & Matos, 2002).

Os primeiros estudos realizados com mixosporídeos da América do Sul ocorreram nas primeiras décadas do século XX e foram desenvolvidos por Splendore (1910) e Migone (1916). A partir de então, a presença de tais parasitos em peixes desta região foi assinalada por vários pesquisadores, sendo descritas várias espécies. Gióia e Cordeiro (1996) publicaram uma “check-list” das espécies de mixosporídeos descritos parasitando peixes do Brasil, onde relataram a existência de 52 espécies distribuídas em 11 gêneros. Mais recentemente, mixosporídeos do gênero *Henneguya* foram observados infectando diversas espécies de peixes de importância comercial: *H. piaractus* e *H. pellucida* infectando *Piaractus mesopotamicus* em sistemas de criação e em ambiente natural (Martins & Souza, 1997; Adriano *et al*., 2002a; Adriano *et al*., 2005a; 2005b); *H. caudalonga* em *Prochilodus lineatus* (Adriano *et al*., 2005c), *H. arapaima* infectando pirarucu (*Arapaima gigas*) das bacias dos Rios Araguaia-Tocantins (Feijó *et al*., 2008) e

H. corruscans infectando *P. corruscans* provenientes do rio Paraná (Eiras *et al*., 2009). No que se refere ao gênero *Myxobolus* foram encontrados *M. cuneus* infectando *P. mesopotâmicus* de sistemas de cultivo (Adriano *et al*., 2006), *M. colossomatis em P. mesopotamicus* oriundos de ambiente natural no Pantanal (Adriano *et al*. 2002a), *M. porofilus* infectando *P. lineatus* de sistemas de criação e de ambiente natural nos rios do Pantanal Mato-Grossense (Adriano *et al*., 2002b; Campos *et al*., 2005), *M. cordeiroi* parasitando *Zungaru jahu* (Adriano *et al.* 2009a) e *M. salminus* infectando *Salminus brasiliensis* (Adriano *et al*., 2009b), ambos no Pantanal Mato-Grossense.

A literatura mostra que as brânquias estão entre os órgãos mais acometidos pelas infecções por mixosporídeos, e como estes são órgãos de importância vital no metabolismo e homeostase dos peixes, a infecção parasitária neste local pode acarretar um déficit das trocas gasosas e iônicas, comprometendo a saúde do animal (Roberts, 2001).

Em estudos histológicos realizados por vários autores, as alterações branquiais foram caracterizadas por hemorragia, desorganização e hiperplasia epitelial e de células mucosas, aumento da produção de muco, hipertrofia das células, necrose e focos inflamatórios (Takashima & Hibiya, 1995, Martins & Souza, 1997, Adriano *et al.,* 2005c)

De acordo com Lom e Dyková (1992) o estudo da fauna parasitária dos peixes de água doce é de alta relevância econômica, pois é inviável pensar no desenvolvimento da indústria de peixes de água doce sem dispor do conhecimento sobre as enfermidades que os afetam.

JUSTIFICATIVA

Devido à grande demanda de pescados, a piscicultura tem despertado grande interesse e apresenta importante expansão globalmente. A obtenção do pescado a partir de sistemas de criação produz alimento de qualidade e ajuda a diminuir o impacto da pesca extrativista sobre os estoques de peixes na natureza. Neste contexto, porém, para que a cadeia produtiva se estabeleça de forma sustentável e segura, é necessário

alargar os conhecimentos sobre o manejo sanitário e sobre os patógenos que podem interferir de forma negativa na produtividade do setor.

Entre os agentes causadores de enfermidades em peixes, merecem destaque os mixosporídeos, que podem afetar peixes em ambientes naturais e também aqueles mantidos em sistemas de várias partes do mundo. Peixes do gênero *Pseudoplatystoma* como *P. corruscans* e *P. fasciatum* sempre tiveram grande destaque na pesca extrativista das regiões onde ocorrem, e mais recentemente, tem se firmado como espécies nativas de grande interesse para a piscicultura brasileira, sendo hoje criados em diversas regiões do país. Dessa forma, o estudo das espécies de mixosporídeos que parasitam pintado e cachara tanto em ambiente natural como em sistemas de criação, terá grande valia para o manejo dessas espécies de peixes e para o desenvolvimento de técnicas de controle e tratamento destes parasitos.

OBJETIVOS

Objetivo Geral

Estudar parasitos do filo Myxozoa infectando *Pseudoplatystoma corruscans* (pintado) e *Pseudoplatystoma fasciatum* (cachara) oriundos de ambiente natural no Pantanal Mato Grossense e o híbrido (*P. corruscans* X *P. fasciatum*) de sistemas de criação (pisciculturas do Centro Nacional de Pesquisa e Gestão de Recursos Pesqueiros Continentais – CEPTA/ICMBio de Pirassununga, SP e de duas pisciculturas comerciais do estado de São Paulo e uma do estado de Mato Grosso do Sul).

Objetivos Específicos

- Realizar estudos taxonômicos de mixosporídeos parasitos de pintado e cachara oriundos de ambiente natural e do híbrido de sistemas de cultivo utilizando microscopia de luz e microscopia eletrônica
- Avaliar a ocorrência e a prevalência de mixosporídeos no Pantanal Mato-Grossense e em pisciculturas do estado de São Paulo e do Mato Grosso do Sul.
- Analisar a interação parasito-hospedeiro em peixes do gênero *Pseudoplatystoma* infectados por parasitos do filo Myxozoa.

MATERIAL E MÉTODOS

Este projeto foi desenvolvido em parceria entre os Departamentos de Biologia Animal do Instituto de Biologia da Unicamp, o Laboratório de Parasitologia do Departamento de Ciências Básicas-ZAB da Faculdade de Zootecnia e Engenharia de Alimentos-FZEA/USP, campus de Pirassununga e o Centro Nacional de Pesquisa e Gestão de Recursos Pesqueiros Continentais CEPTA/ICMBio de Pirassununga, SP. Assim, para o desenvolvimento desta pesquisa foram utilizados instalações e equipamentos da USP (laboratório, micrótomo e microscópio de luz), do CEPTA (material de pesca) e da UNICAMP (microscópio eletrônico de transmissão).

Em ambiente natural, as coletas foram em três regiões do Pantanal Mato-Grossense: rio Aquidauana, rio Miranda e rio Paraguai na região Sul do Pantanal Mato-Grossense; Parque Nacional do Pantanal (PARNA-Pantanal) na região Central; rios Cuiabá e Manso, no município de Nobres, parte mais ao Norte do Pantanal Mato-Grossense. As áreas de coletas, na região Sul estão aproximadamente 150 km distantes dos locais de captura do PARNA-Pantanal, na região Central, que por sua vez, está aproximadamente 200 km distante das áreas de captura da região Norte. Nos locais de captura da região Sul existem intensas atividades antrópicas, baseada na pecuária e na agricultura. Na região Norte, as atividades econômicas relacionadas com os locais de coleta são agricultura, pecuária e também a extração de calcário. Na região central, o PARNA-Pantanal é uma área de proteção ambiental.

Em sistemas de criação as coletas foram realizadas em 3 pisciculturas do Estado de São Paulo (município de Mogi Mirim, Mococa e Águas Claras) e 1 no Estado do Mato Grosso do Sul (município de Bandeirantes).

O desenvolvimento deste trabalho foi realizado com aprovação do Comitê de Ética (Anexo 1).

Como a dissertação está dividida em capítulos, a metodologia específica para cada um deles encontra discriminada nos mesmos.

RESULTADOS

Neste trabalho foram descritas duas novas espécies de mixosporídeos: uma infectando exemplares de pintado híbrido (pintado x cachara) oriundos de pisciculturas do Estado de São Paulo e do Mato Grosso do Sul e a outra infectando simultaneamente exemplares de pintado e cachara coletados em ambiente natural no Pantanal Mato-Grossense. Cada capítulo a seguir aborda a descrição de uma espécie de mixosporídeo, tratando dos aspectos taxonômicos e da interação parasito-hospedeiro.

(Publicado na Revista Veterinary Parasitology, 2009)

Capítulo 1

Henneguya sp. 1 causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: histopathology and ultrastructure

ABSTRACT

The present study is part of an ongoing investigation into the characteristics of Myxozoan parasites of Brazilian freshwater fish and was carried out using morphology, histophathology and electron microscopy analysis. A new Myxosporea species (*Henneguya* sp. 1) is described causing an important reduction in gill function in the farmed pintado (a hybrid fish from a cross between *Pseudoplatystoma corruscans* and *Pseudoplatystoma fasciatum*), which is a commercially important South American catfish. From a total of 98 pintado juveniles from fish farms in the states of São Paulo and Mato Grosso do Sul (Brazil), 36 samples (36.7%) exhibited infection of the gill filaments. Infection was intense, with several plasmodia occurring on a same gill filament. The plasmodia were white and measured up to 0.5 mm in length; mature spores were ellipsoidal in the frontal view, measuring 33.2 ± 1.9 μm in total length, 10.4 ± 0.6 μm in body length, 3.4 ± 0.4 μm in width and 22.7 ± 1.7 μm in the caudal process. The polar capsules were elongated, measuring 3.3 ± 0.4 μm in length and 1.0 ± 0.1 μm in width and the polar filaments had six to seven turns. Histopathological analysis revealed the parasite in the connective tissue of the gill filaments and lamella. No inflammatory infiltrate was observed, but the development of the plasmodia reduced the area of functional epithelium. Ultrastructural analyses revealed a single plasmodial wall, which was in direct contact with the host cells and had numerous projections in direction of the host cells as well as extensive pinocytotic canals. A thick layer (2 to 6 μm) of fibrous material and numerous mitochondria were found in the ectoplasm. Generative cells and the earliest stage of sporogenesis were seen more internally. Advanced spore

developmental stages and mature spores were found in the central portion of the plasmodia.

Keywords: Myxozoa, Myxosporea, Pimelodidae, catfish, fish farm, Brazil

INTRODUCTION

Pimelodidae from the genus *Pseudoplatystoma* are found in the main hydrographic basins of South America (Lundberg & Littmann, 2003): *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) in the São Francisco and Prata River basins; *Pseudoplatystoma fasciatum* (Linnaeus, 1766) in the Amazon, Corantijn, Essequibo, Orinoco and Prata River basins; *Pseudoplatystoma magdaleniatum* Buitrago-Suárez and Burr, 2007 in the drainage area of the Magdalena River; *Pseudoplatystoma metaense* Buitrago-Suárez and Burr, 2007 and *Pseudoplatystoma orinocoense* Buitrago-Suárez and Burr, 2007 in the Orinoco River basin; and *Pseudoplatystoma tigrinum* (Valenciennes, 1840) in the Amazon and Orinoco River basins (Froese & Pauly, 2009). These are carnivorous, migratory fish species that attain large sizes and play an important role in the fishery economy of the regions in which they occur. *Pseudoplatystoma corruscans*, popularly known in Brazil as the pintado or surubim (English name = spotted sorubim) and *P. fasciatum*, known as the cachara (English name = barred sorubim) reach up to 100 kg and 20 kg, respectively (Campos, 2005). Due to the quality of their meat, these species have a high market value (Campos, 2005). Fisheries targeting these species landed approximately 3.570.000 kg in 2006 (about 2.597.000 kg of *P. corruscans* and 973.000 kg of *P. fasciatum*) (Ibama, 2008). This high market value, together with the rapid growth of the species, has led to an increased interest in farming the pintado (Campos, 2005).

In brazilian fish farms, *P. corruscans* and *P. fasciatum* are often crossbred to produce hybrid fingerlings. This strategy has led to a greater larva survival rate than that obtained in pure *P. corruscans* and greater growth than that obtained in pure *P. fasciatum.* This hybrid fish is sold as the pintado and its production in brazilian fish farms reached 1,094,000 kg in 2006 (Ibama, 2008), with exports to several countries (Mar & Terra, 2009).

With the increase in fish farm activities, there is growing concern over fish diseases. Parasites are the most common and important cause of disease in farmed fish, among which species from the Myxozoa phylum figure prominently (Schmahl *et al.,* 1989; Feist & Longshaw, 2006). The genus *Henneguya* is the second greatest in species diversity (Eiras, 2002) and a number of species have been reported infecting wild and farmed fish in many regions around the world (Azevedo & Matos, 2003; Yokoyama *et al.,* 2003; Martins & Onaka, 2006; Eiras *et al.* 2008). A large number of these species are important pathogenic parasites that can have considerable economic impact (Martins *et al*., 1997**;** Yokoyama *et al*., 2003; Adriano *et al*., 2005c; Feist & Longshaw, 2006). The genus *Henneguya* includes ~150 species (Eiras, 2002), 35 of which have been reported infecting South American fish (Azevedo & Matos, 2003; Azevedo *et al*., 2008; Eiras *et al.,* 2008; Feijó *et al.,* 2008) and one species has been described infecting wild *P. corruscans* (Eiras *et al.,* 2009).

This paper describes the morphological, histopathological and ultrastructural characteristics of a new parasitic species of *Henneguya* in the gills of the *P. corruscans* x *P. fasciatum* hybrid in fish farms in the states of São Paulo and Mato Grosso do Sul, Brazil.

MATERIAL AND METHODS

Ninety-eight juveniles of the *P. corruscans* x *P. fasciatum* hybrid were collected in 2008 from three fish farms in the state of São Paulo [Centro Nacional de Pesquisa e Gestão de Recursos Pesqueiros Continentais-CEPTA in the municipality of Pirassununga (n = 30), and two commercial fish farms, one in the municipality of Mogi Mirim (n = 30) and another in Mococa (n = 30)] and one fish farm in the state of Mato Grosso do Sul [in the municipality of Bandeirantes (n = 08)]. Immediately after being caught, the fish were transported alive to the field laboratory, measured and necropsied. Plasmodia with mature spores were examined on fresh mounts with a light microscope. The measurements of the spores (n = 53) obtained from plasmodia in different specimens were performed on a computer equipped with the Axivision 4.1 image capturing program

and coupled to an Axioplan 2 Zeiss Microscope. Spore dimensions (in µm) were expressed as mean ± standard deviation (SD). Smears containing free spores were stained with Giemsa solution and mounted in low-viscosity mounting medium (Cytoseal^{TM}) as permanent slides.

For the histological analysis, fragments of infected organs were fixed in 10% buffered formalin, embedded in paraffin, cut into serial sections (4 µm in thickness) and stained with haematoxylin/eosin and Sirius red (Adriano *et al*., 2002).

For transmission electron microscopy, plasmodia were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 12 h, washed in a glucose-saline solution for 2 h and post-fixed in 1% OsO4 – all done at 4 ^{0}C. After dehydration in an acetone series, the material was embedded in EMbed 812 resin. Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined in an LEO 906 electron microscope operated at 60 kV.

RESULTS

Among a total of 98 hybrid pintado juveniles from fish farms in the states of São Paulo and Mato Grosso do Sul (Brazil), 36 samples (36.7%) had gill filaments infected by an unknown species from the genus *Henneguya* Thélohan, 1892. Among the three fish farms in the State of São Paulo, only the one in the municipality Mogi Mirim exhibited the infection, for which the prevalence was 100% (30/30). In the fish farm in the state of Mato Grosso do Sul, the prevalence was 75% (6/8). In both fish farms in which the parasite occurred, the infection was intense, with several plasmodia occurring in a single filament, occupying the medial portion of the filament core and occasionally the lamella (Figs. 1a-b and 2a).

Description of *Henneguya* sp. 1 (Figs. 1 – 5).

The plasmodia are white and measure up to 0.5 mm in length. Mature spores are ellipsoidal in the frontal view, measuring 33.2 ± 1.9 µm in total length, 10.4 ± 0.6 µm in body length, 3.4 ± 0.4 µm in width and 22.7 ± 1.7 µm in the caudal process. In the lateral

view, the spores are biconvex, measuring 4.5 ± 0.1 µm in thickness; the valves are symmetrical, with a smooth surface. The polar capsules are elongated and equal in size, measuring 3.3 ± 0.4 µm in length and 1.0 ± 0.1 µm in width. The anterior ends of polar capsules are close one another and occupy only the anterior third of the spore body. The polar filaments have six to seven turns and are arranged perpendicularly to the longitudinal axis of the capsules (Figs. 4c and 5).

Histological analysis revealed that the parasite develops in the connective tissue of the gill filaments and lamella, frequently surrounding the blood vessels. The development of the plasmodia leads to the stretching and deformation of these structures, thereby reducing the area of functional epithelium (Figs. 1b and 2). No inflammatory infiltrate was found in the infection site and the plasmodia were not enveloped by a host capsule. The ectoplasm of the plasmodia exhibited an intense eosinophilic material associated with a less eosinophilic area (Fig. 2b).

Ultrastructural analyses revealed a single plasmodial wall, connected to a plasmodial ectoplasm zone through numerous and extensive pinocytotic canals (Fig. 3d). The plasmodial wall was in direct contact with the host cells and the plasmodial surface had numerous projections (Fig. 3a). A thick layer (2 to 6 µm) of fibrous material was found in the periphery of the plasmodia. This fibrous material occurred throughout the extension of the plasmodial wall and within the projections in direction of the host cells (Figs. 3a - c). Just below this fibrous material, there was an area with numerous mitochondria (Fig. 3a). Generative cells and the earliest stages of sporogenesis were seen more internally (Fig. 3a). Advanced spore developmental stages and mature spores were found in the central portion of the plasmodia (Fig. 4).

Type host: Hybrid of *Pseudoplatystoma corruscans* Agassiz, 1829 and *Pseudoplatystoma fasciatum* Linnaeus, 1766 (Siluriformes: Pimelodidae).

Site of infection: gill filaments and occasionally gill lamellae.

Prevalence: 36/98 (36.7%) of the total of fish examined were infected. Considering the fish farms separately, the prevalence was 100% (30/30) for that of the municipality of Mogi Mirim, 75% (6/8) for that of the municipality of Bandeirantes and 0% (0/30) for those ones of the municipalities of Pirassununga and Mococa.

Locality: Fish farms in the municipality Mogi Mirim in the state of São Paulo and in the municipality Bandeirantes in the state of Mato Grosso do Sul, Brazil.

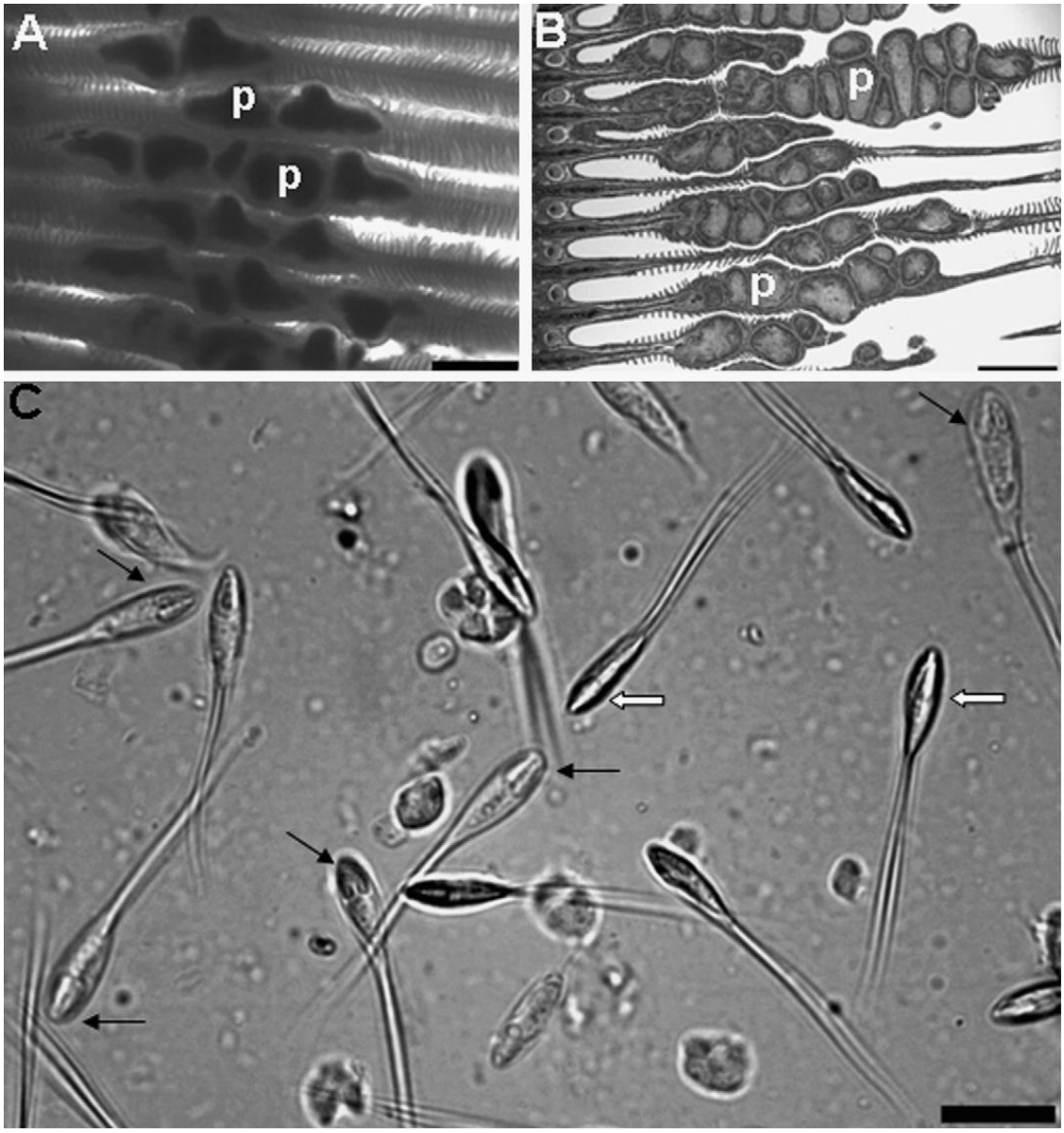

Figure 1. Light photomicrographs of *Henneguya* sp. 1, parasite of the pintado. (A) Formalin-fixed gill arch showing plasmodia (p) in the gill filaments. Scale bar = 400µm. (B) Histological section of gill arch showing high infection intensity. Note the development of the plasmodia (p) stretching and deforming of the filaments and lamellae structures. Scale bar = 500 µm. Sirius red staining. (C) Mature fresh spores in frontal view (black arrows) and lateral view (white arrows). Scale bar = 10 µm.

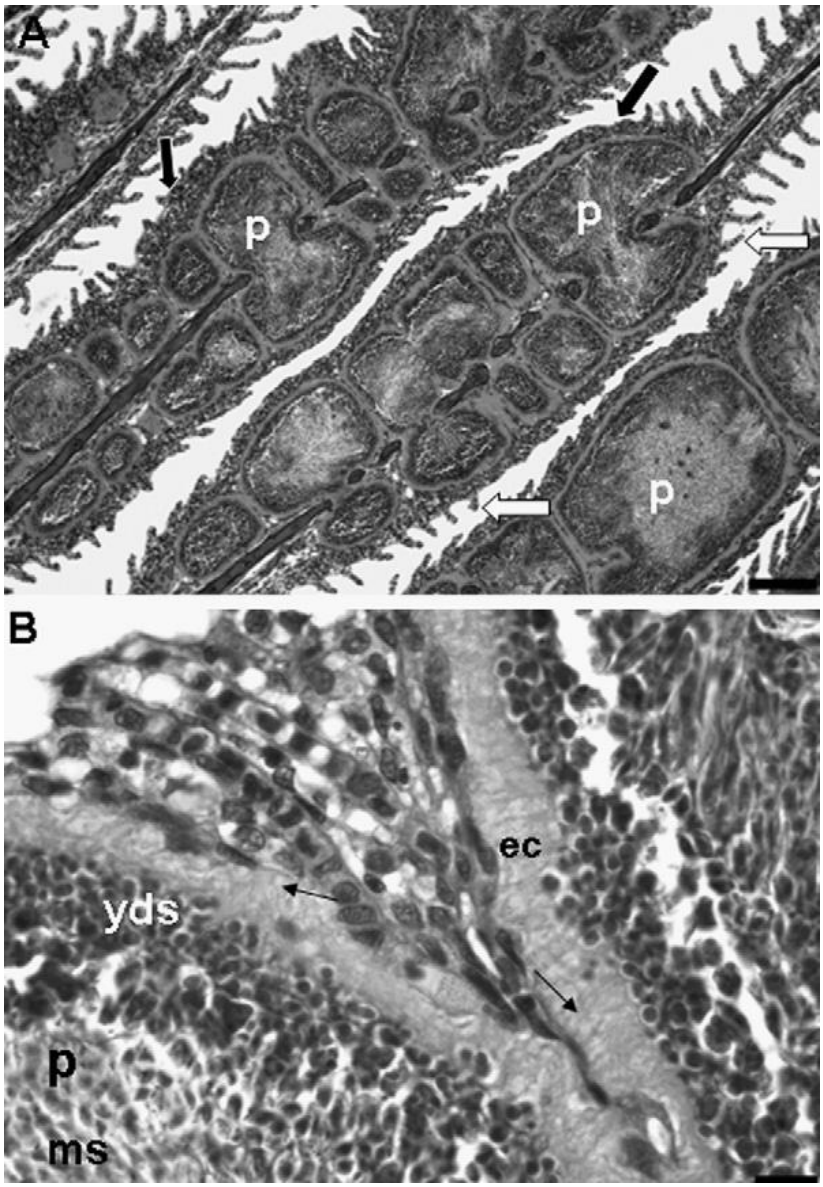

Figure 2. Histological sections of gill filaments of pintado infected by plasmodia of *Henneguya* sp. 1, (A) showing numerous large plasmodia (p) causing deformation of the filaments structures (black arrows) and disorganization and displacement of the gill lamellae (white arrows). Note the reduction in area of functional epithelium. Scale bar = 100µm. Sirius red staining. (B) Large plasmodia (p) causing displacement and grouping of the lamellae (large arrow). Note intensely eosinophilic areas associated to other, less eosinophilic areas (thin arrows) in the plasmodial ectoplasm (ec). (yds, young developmental stages; ms, mature spores). Scale bar = 10µm. Sirus red staining.

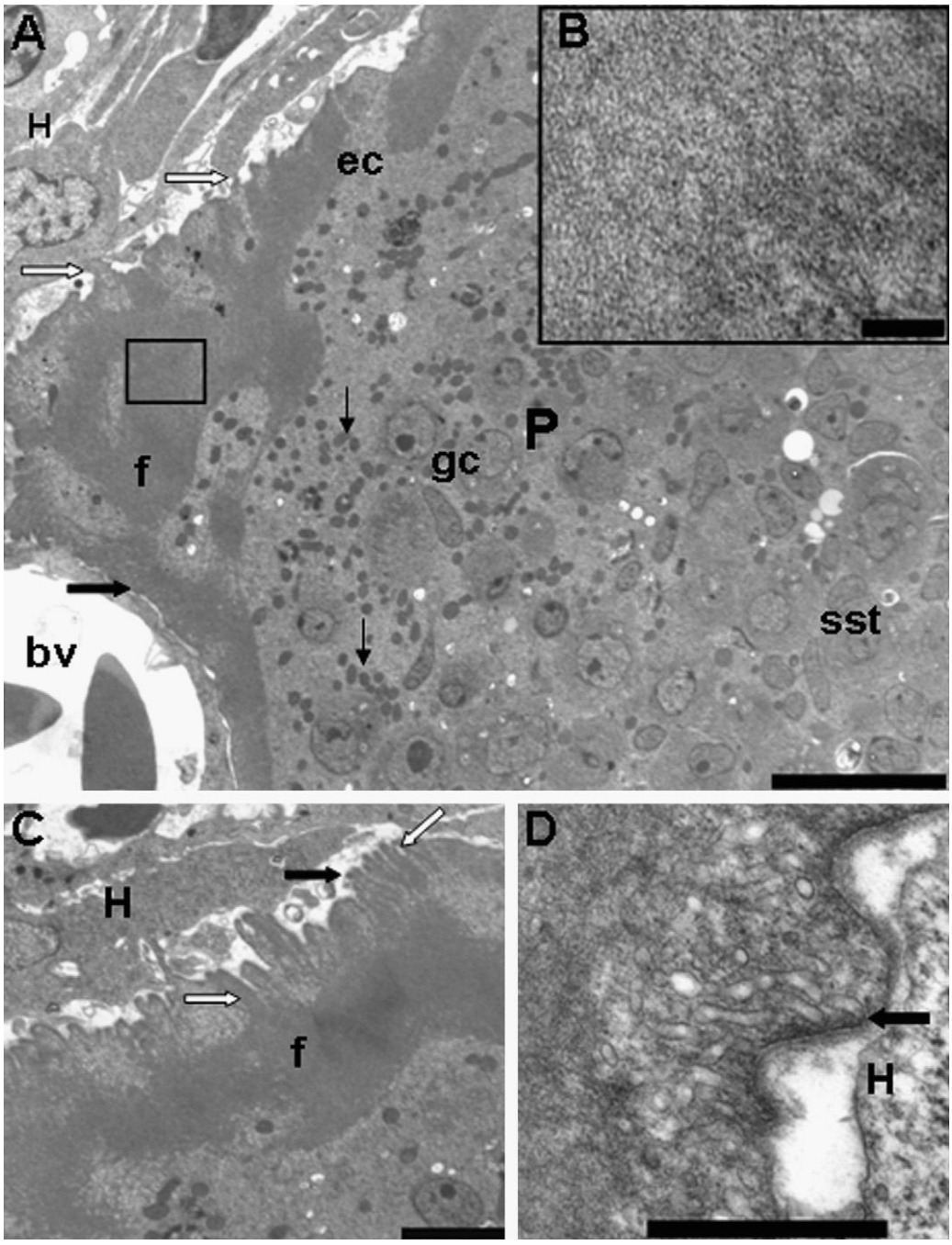

Figure 3. Electron micrography of gills of pintado infected by *Henneguya* sp.1. (A–D) Host–parasite interface showing: (A) large portion of the periphery of a plasmodium (P) with the plasmodial wall in direct contact (black arrow) with the endothelium of a blood vessel (bv) and projections of the plasmodial wall (white arrows) in direction of the host cells (H). In the ectoplasm (ec), a layer of fibrous material (f) and more internally numerous mitochondria (thin arrow), generative cells (gc) and stages of sporogenesis (sst). Scale bar = 5µm; (B) insert of (A) showing amplified layer of the fibrous material. Scale bar = 0.2µm; (C) the plasmodial wall with digitiform projections wall in direction of the host cells (black arrows). Note the presence of the fibrous material (f) in the projections (white arrows). Scale bar = 2 µm; (D) periphery of the plasmodia showing it limited by a single membrane and numerous and extensive pinocytotic canals (arrow). Scale bar = 5 µm.

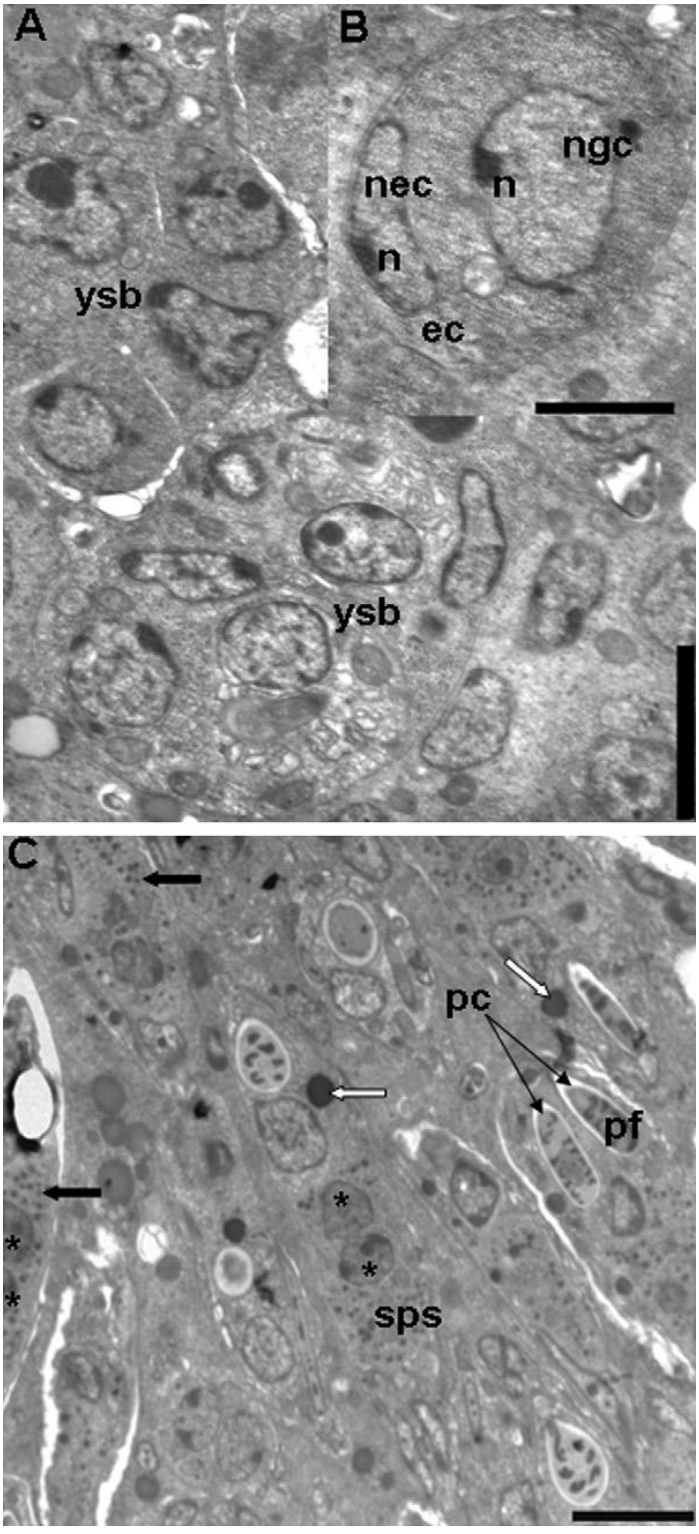

Figure 4. Electron micrography of internal zone of plasmodia of *Henneguya* sp.1 showing (A) two young sporoblasts (ysb). Scale bar = 2 µm; (B) generative cell (gc) in the enveloping cell (ec). Nucleus of the generative cell (ngc). Nucleus of the enveloping cell (nec). Nucleolus (n). Scale bar = 1 µm; (C) longitudinal sections of immature spores showing the binuclear (*) sporoplasm (sps) with small sporoplasms (black arrows). Note the polar capsule (pc) with its polar filaments (pf) and spherical electron-dense inclusions in the capsulogenic cell (white arrows). Scale bar = 2.5 µm.

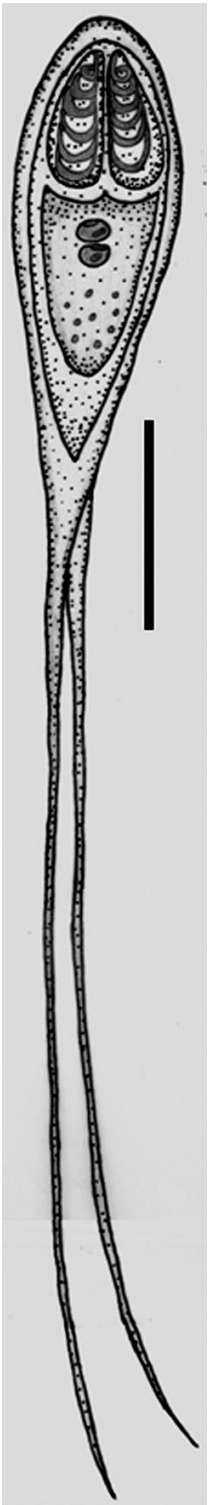

Figure 5. Schematic representation of mature spores of *Henneguya* sp.1. Scale bar = 5µm.

Table 1. Features of the *Henneguya* species similar to *Henneguya* sp. 1.

Species	Total length	Spore length	Spore width	LPC	WPC	Tail length	NCF	Site of infection and host	Locality
Henneguya sp. 1	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	Gills of hybrid pintado	São Paulo and Mato Grossso do Sul states, Brazil
H. adherens	30.7–35.0	10.5–13.8	5.1–6.5	2.8–3.5	1.0–1.6	18.0–21.7	3-4	Gills of *Acestrorhynchus falcatus*	Pará state, Brazil
H. corruscans	27.6 (25-29)	14.3 (13-15)	5.0	6.8 (6-7)	2.0	13.7 (12-15)	5-6	Gills of *Pseudoplatystoma corruscans*	Paraná state, Brazil
H. curimata	35.4 (34.2–36.1)	16.6 (16.0–17.4)	6.2 (5.8–6.6)	6.5 ± 03	1.2 ± 02	19.1 (18.3–19.9)	10–11	Kidney of *Curimata inormata*	Pará state, Brazil
H. intracornea	42.4 (36.5-45.9)	-	6.6 (5.6-9.9)	8.5 (6.9-9.9)	2.3 (18-39)	24.2 (17.8-28.9)	-	Eye of *Astyanax scabripinnis*	São Paulo state, Brazil
H. leporine	28.0–33.0	13.0–15.0	5.0	5.0–8.0	-	15.0–18.0	-	Urinary duct of *Leporinus mormyrops*	São Paulo state, Brazil
H. pellucida	33.3 ± 1.5	11.4 ± 0.3	4.1 ± 0.4	4.0 ± 0.4	1.6 ± 0.2	24.1 ± 1.5	6–7	Serosa visceral cavity and swimblader *Piaractus mesopotamicus*	São Paulo state, Brazil
H. mbourensis	29.6 (28.0-33.0)	10.2 (10.0-11.0)	7.9 (6.5-9.0)	4.7 (3.5-5.0)	2.4 (2-3.2.0)	20.6 (20-22.5)	-	Kidney of *Dentex canariensis*	Senegal, Africa

PCL = length of the polar capsules; WPC = width of the polar capsules; NCF = number of coils of the polar filaments.

DISCUSSION

The morphology and dimensions of *Henneguya* sp. 1 were compared with all *Henneguya* spp. described infecting South American freshwater fish (Martins & Onaka, 2006, Eiras *et al*., 2008; 2009; Feijó *et al.,* 2008; Azevedo *et al.,* 2008) as well as those of other geographic regions listed by Eiras (2002). Among the 36 species described so far in South American fish (Eiras *et al*., 2008), only *Henneguya corruscans* Eiras, Takemoto and Pavanelli, 2009, infects fish from the genus *Pseudoplatystoma*. However, *H. corruscans* differs from *Henneguya* sp. 1 in the smaller total length of its spores (27.6 μm), larger body length (14.3 μm), smaller caudal process (13.7 μm), smaller number of polar filament turns (5 to 6) and spore morphology.

The total dimensions of the spores from the parasite species described here are very similar to other South American *Henneguya* species from other hosts, such as: *Henneguya adherens* Azevedo and Matos, 1995, parasitic in gill filaments of *Acestrorhynchus falcatus, Henneguya leporini* Nemeczeck, 1926, parasitic in the urinary ducts of *Leporinus mormyrops, Henneguya curimata* Azevedo and Matos, 2002, parasitic in the kidney of *Curimata inornata, Henneguya intracornea* Gióia, Cordeiro and Artigas, 1986, parasitic in the eyes of *Axtyanax scabripinnis* and *Henneguya pellucida* Adriano, Arana and Cordeiro 2005, parasitic in the serosa of visceral cavity and surface of the swimblader of *Piaractus mesopotamicus* (Table 1). However, *H. adherens* has a wider spore body and smaller number of polar filaments turns, *H. leporini* has a wider and longer spore and longer polar capsules. *H. curimata* has a wider and longer spore body, longer polar capsules and smaller caudal process. Total length and width of the spores and polar capsule length are larger in *H. intracornea* than the dimensions found in *Henneguya* sp.1 spores. The spore dimensions in *H. pellucida* are very similar to those found in *Henneguya* sp.1, but the morphology of the spores is very different. Among the species cited above, only *H. adherens* infects gills and all infect Characiformes fish, while *Henneguya* sp.1 infects a Siluriformes fish.

Comparing *Henneguya* sp.1 with species from other continents (Eiras, 2002), only *Henneguya mbourensis* Kpatcha, Faye, Diebakate, Fall and Toguebaye, 1997 – a

parasite of the kidney of *Dentex canariensis* in Senegal – has similar dimensions regarding body length and total length of the spores. However, this species has other features that differentiate it from *Henneguya* sp.1, such as the size of polar capsules, width of the spores, spore morphology and infection site. Thus, based on these data, *Henneguya* sp.1 is proposed as a new species of Myxosporea.

The development site of *Henneguya* sp.1 is the gill filaments, core and lamella. The ultrastructural analyses revealed that the plasmodia have single wall, which is in direct contact with the host cells and has extensive, multiple pinocytic canals connecting the wall to the plasmodial ectoplasm, which are commonly reported characteristics in *Henneguya* spp. (Current & Janovy, 1978; Rocha *et al*., 1992, Azevedo & Matos, 2002; El-Mansy & Bashtar, 2002; Adriano *et al*., 2005b). However, in addition to the pinocytic canals, the plasmodia of *Henneguya* sp.1 has numerous projections in its wall, which clearly increases the area of contact with host cells and, consequently, its capacity to obtain nutrients. The plasmodium wall is seen as the organelle responsible for feeding the entire plasmodium and knowledge on the structure of the plasmodium wall is necessary to understanding the stress incurred on the host by the parasite (El-Mansy & Bashtar, 2002). The plasmodia frequently seen surrounding the endothelium and the projections in the direction of the capillaries suggest an important interaction between the parasite and blood vessels, possibly characterizing an important source of plasmodium nutrition.

The gill is the major respiratory organ, the primary site of nitrogenous waste excretion and plays an important role in ionic balance (Noga, 2000). Species from the genus *Henneguya* have different manners of interaction with fish gill structures, resulting in different levels of disease (Current & Janovy, 1978; Dykova & Lom, 1978; Bowser & Conroy, 1985; Kalavati & Narasimhamurti, 1985; Duhamel *et al*., 1986; Martins *et al*., 1997; 1999; Molnár, 1998; Adriano *et al*., 2005a; 2205b; Molnár *et al*., 2006a; 2006b). No inflammatory infiltrate was observed in the gills infected with *Henneguya* sp.1, which is a common finding in infections by *Henneguya* spp. (Barassa *et al*., 2003; Adriano *et al*., 2005a; 2005b; Eiras *et al*., 2008; Eiras *et al*., 2009; Feijó *et al.,* 2008), despite the

responses induced by some species (Dykova & Lom, 1978, Martins *et al*., 1997; 1999; Molnár, 1998; Work *et al.,* 2008).

Regardless of the absence of inflammatory infiltrate, the development of the plasmodia of *Henneguya* sp.1 cause stretching and deformation of the filament and lamella structures, thereby substantially reducing the area of functional epithelium, which certainly has a negative effect on the development of infected pintado specimens and may lead to significant losses, especially if a high prevalence and high infection intensity of the parasite are associated with poor environmental and biological conditions, which are common in fish farms.

The histopathological analysis revealed a layer in the plasmodial ectoplasm near the plasmodial wall, with intensely eosinophilic areas associated to other, less eosinophilic areas. In the ultrastructural study, this layer corresponded to fibrous material, which, based on the thickness of the microfibrils (~ 7 nm), resembles aggregated actin. Actin is one of the most abundant proteins in eukaryotic cells and has an important role as a major component of the cytoskeleton in the cell (Kabsch & Vandekerckhove, 1992). In *Henneguya* sp.1, this fibrous material appears to act as support to the plasmodium and the projections observed on its surface. Fibrous material resembling aggregated actin has also been reported anchoring the mural cells of the presporogonic cells of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) and presumably helping stabilize the spore sac (Morris & Adams, 2007). Casal *et al*. (1997) also report the presence of microfilaments occupying the entire space of pericyte cells in *Henneguya striolata*. The authors suggest, however, that these are myosin filaments.

Just below of the fibrous material found in the plasmodial ectoplasm of *Henneguya* sp.1, there were numerous mitochondria, generative cells and the earliest stages of sporogenesis, whereas far from periphery there were different spore developmental stages, which is a similar characteristic to other parasites from this group (Casal *et al*., 1997; Abdel-Ghaffar, 2008; Azevedo & Matos, 2002; Azevedo & Matos, 2003; Casal *et al*., 2003; Adriano *et al* 2005a; 2005b).

The present study describes an interesting host-parasite relationship in *Henneguya* sp.1 infections. The reduction in the area of functional epithelium in the gills

demonstrates that this species has considerable pathogenic potential. Thus, its presence and dispersion in fish farms need to be monitored closely.

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(Submetido à Revista Veterinary Parasitology, 2010)

Capítulo 2

Host-parasite-environment relationship and morphology of *Henneguya* sp. 2 parasite of two wild *Pseudoplatystoma* spp. in pantanal wetland, Brazil

ABSTRACT

A new myxosporean species, *Henneguya* sp. 2, is described parasitizing the gill filaments of *Pseudoplatystoma coruscans* and *Pseudoplatystoma fasciatum* (Siluriformes: Pimelodidae) caught in the Pantanal Wetland, Brazil. The parasite formed white, elongated plasmodia measuring up to 3 mm. Mature spores were ellipsoidal in the frontal view, measuring 37.1 ± 1.8 µm in total length, 12.9 ± 0.8 µm in body length, 3.4 ± 0.3 µm in width, 3.1 ± 0.1 µm in thickness and 24.6 ± 2.2 µm in the caudal process. Polar capsules were elongated and equal in size, measuring 5.4 ± 0.5 µm in length and 0.7 ± 0.1 µm in width. Polar filaments had 12 to 13 coils. Histopathological analysis revealed that the parasite developed in the sub-epithelial connective tissue of the gill filaments and the plasmodia were surrounded by a capsule of host connective tissue. The plasmodia caused slight compression of the adjacent tissues, but no inflammatory infiltrate was observed in the infection site. Ultrastructure analysis revealed a single plasmodial wall connected to the ectoplasmic zone through numerous pinocytotic canals. The plasmodial wall exhibited numerous projections and slightly electron-dense material was found in the ectoplasm next to the plasmodial wall, forming a line just below the wall. The prevalence of the parasite was 17.1% in both fish species examined. Parasite prevalence was not influenced by season or host sex.

Keywords: Myxozoa, Myxosporea, Pimelodidae, Catfish Fish, Pantanal, Brazil

INTRODUCTION

The Pimelodidae *Pseudoplatystoma corruscans* Spix & Agassiz, 1829, popularly known in Brazil as “pintado” or “surubim”, and *Pseudoplatystoma fasciatum* Linnaeus, 1766, known as “cachara”, are carnivorous, migratory fish that play an important role in the fishing economy where they occur. These fish attain large sizes (up to 100 kg for *P. corruscans* and up to 20 kg for *P. fasciatum*) and are among the most important freshwater fish in Brazil (Campos, 2005). *Pseudoplatystoma corruscans* occurs in the São Francisco and Prata River basins, while *P. fasciatum* occurs in the Amazon, Corantijn, Essequibo, Orinoco and Prata River basins (Froese & Pauly, 2009). Due to the quality of the meat and high market value (Campos 2005), fisheries targeting these species attained approximately 3,570,000 kg (2,597,000 kg for *P. corruscans* and 973,000 kg for *P. fasciatum*) in 2006 (Ibama, 2008). The rapid growth of these species has also caught the interest of fish farmers (Campos, 2005). The production of these species in fish farms reached approximately 1,094,000 kg in 2006 (Ibama, 2008), representing an important option for Brazilian fish farmers.

Regarding infection by Myxosporeans, *Henneguya corruscans* and *Henneguya* sp. 1 have been reported infecting Pimelodidae of the genus *Pseudoplatystoma* (Eiras *et al*., 2009, Chapter 1). The genus *Henneguya* includes 204 known species (Lom & Dyková, 2006), 36 of which have been reported to infect South American fish (Eiras *et al*. 2008; 2009; Azevedo *et al*., 2008, 2009).

As part of ongoing research on the characteristics of myxosporean parasites of freshwater fish in Brazil, a new species of *Henneguya* found infecting wild specimens of “pintado” and “cachara” in the Pantanal wetland (Brazil) is described in the present study, using morphological, ultrastructural and histological analyses.

MATERIALS AND METHODS

Eighty-two wild young and adults specimens of *P. corruscans* and seventy of *P. fasciatum* were collected in the Pantanal wetland: Aquidauna River (20º 29’ 19’’ S/ 55º 46’ 49’’ W), Miranda River (20º 11’ 27’’ S/ 56º 30’ 19’’ W), Paraguay River (17º 54’ 58’’

S/ 57º28' 01'' W) and Cuiabá River (17º 50' 32'' S/ 57º 23' 46 W). Exams were performed in the rainy season (spring 2001, 2002, 2003, 2004 and 2009) and dry season (autumn 2003, 2004, 2005 and 2008).

Immediately after capture, the specimens were transported alive to the field laboratory mounted nearby, where they were measured, weighed and submitted to necropsy. Plasmodia with mature spores were examined on fresh mounts with a light microscope. The morphological and morphometric studies of the spores were based on mature spores obtained from different specimens (38 from *P. corruscans* and 41 from *P. fasciatum*). Measurements were performed on a computer equipped with an Axivision 4.1 image capture software coupled to an Axioplan 2 Zeiss Microscope. The dimensions of the spores were expressed in µm as the mean ± standard deviation (SD). Smears containing free spores were stained with Giemsa solution and mounted in a low-viscosity mounting medium (Cytoseal^{TM}) as permanent slides. For the histological analysis, fragments of infected organs were fixed in 10% buffered formalin and embedded in paraffin. Serial sections 4 µm in thickness were stained with hematoxylin/eosin and Sirius Red. For the transmission electron microscopy, plasmodia were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 12 h, washed in a glucose-saline solution for 2 h and post-fixed in OsO4, all done at 4^{o} C. After dehydration in an acetone series, the material was embedded in EMbed 812 resin. Ultrathin sections, double stained with uranyl acetate and lead citrate were examined in an LEO 906 electron microscope operated at 60 kV.

The possible effects of season and host sex on the prevalence of the parasite and possible differences in prevalence between the two fish species were assessed using the Fisher' exact test, with the level of significance set at $P< 0.05$ (Zar, 1999).

RESULTS

Plasmodia of an unknown species of *Henneguya* were found in the gill filaments of wild specimens of *P. corruscans* and *P. fasciatum* from the Pantanal wetland of Brazil.

The general prevalence of the parasite was 17.1% in both *P. corruscans* (14/82) and *P. fasciatum* (12/70).

In *P. corruscans,* the prevalence among male and female specimens was 18.6% (8/43) and 15.6% (5/32), respectively, with no statistically significant difference between sexes (P = 0.7693). In *P. fasciatum,* the prevalence was 15.1% among males (5/33) and 17.6% among females (6/34), also with no statistically significant difference between sexes (P = 1.0). The sex of seven specimens of *P. corruscans* and one specimen of *P. fasciatum* was undetermined.

In the rainy season of 2001, nine specimens of *P. corruscans* and seven of *P. fasciatum* were examined; the *P. corruscans* specimens were not infected and only one *P. fasciatum* specimen (14.3%) was infected. In the rainy season of 2002, 13 specimens of each fish species were caught; two (15.4%) *P. corruscans* specimens and three (23.1%) *P. fasciatum* specimens were infected. In the dry season of 2003, five specimens of *P. corruscans* and 8 of *P. fasciatum* were examined; only one *P. fasciatum* specimen was infected (12.5%). In the rainy season of 2003, seven specimens of *P. corruscans* and nine of *P. fasciatum* were caught and none was infected. In the dry season of 2004, seven specimens of *P. fasciatum* and one of *P. corruscans* specimens were examined; only one *P. fasciatum* specimen was infected (14.3%). In the rainy season of 2004, 14 specimens of *P. corruscans* were examined, four (28.6%) of which were infected, and 12 specimens of *P. fasciatum* were examined, only one (8.3%) of which was parasitized. In the dry season of 2005, 11 specimens of *P. corruscans* were examined, two (18.2%) of which were parasitized, and three specimens of *P. fasciatum* were examined, two (66.6%) of which were infected. In the dry season of 2008, only *P. corruscans* was caught and four of the 11 specimens (36.4%) were infected. In the rainy season of 2009, 11 specimens of each species were examined; three (27.3%) of the *P. corruscans* specimens were infected and two (18.2%) of the *P. fasciatum* specimens were infected.

For the evaluation of the possible influence of the season on the prevalence of the parasite, these data were grouped into rainy season (n = 54 *P. corruscans* specimens and 52 *P. fasciatum* specimens) and dry season (n = 28 *P. corruscans* specimens and

18 *P. fasciatum* specimens). The prevalence of infected *P. corruscans* was 14.8% (8/54) in the rainy season and 21.4% in the dry season (6/28); this difference did not achieve statistical significance (P = 0.5394). In *P. fasciatum*, the prevalence was 15.4% (8/52) in the rainy season and 22.2% (4/18) in the dry season; this difference also did not achieve statistical significance (P = 0.7177).

Description of *Henneguya* sp. 2 (Figs. 1-5)

Plasmodia whitish, elongate in shape and measuring up to 3 mm were located in the gill filaments of *P. corruscans* and *P. fasciatum* (Fig.1 A). The histopathological analysis showed that the parasite develops in the sub-epithelial connective tissue of the gill filaments (intrafilamental-epithelial type) (Figs. 2 A-B) and the plasmodia were surrounded by a capsule of host connective tissue (Figs. 2 C and 3 A). The plasmodia had asynchronous development, with young sporogonic stages occurring in a thin layer (~ 50 µm) in the periphery. Due to its localization, the development of the parasite produced only slight compression of the adjacent tissues and no inflammatory infiltrate was observed in the infection site (Figs. 2 A-B-C).

Mature spores were ellipsoidal in the frontal view, measuring 37.1 ± 1.8 µm in total length, 12.9 ± 0.8 µm in body length, 3.4 ± 0.3 µm in width, 3.1 ± 0.1 µm in thickness and 24.6 ± 2.2 µm in the caudal process. The polar capsules were elongated and equal in size, measuring 5.4 ± 0.5 µm in length and 0.7 ± 0.1 µm in width. The anterior ends of polar capsules were close to one another, with the anterior end reaching the second half of the body of the spore (Fig. 1 B).

Ultrastructure analysis revealed a plasmodial wall formed by a single membrane, which had numerous projections into the host tissue and also was connected to the ectoplasmic zone through numerous pinocytotic canals (Figs. 3 B and C). Slightly electron-dense material was found in the ectoplasm next to the plasmodial wall, forming a line just below the wall: several mitochondria, numerous vacuoles and some rounded electron-dense structures (Fig. 4 A). Just below, there were generative cells, the earliest stages of sporogenesis and advanced spore developmental stages (Fig.4 B).

Sporogenesis exhibited sporoblasts with two spores, which had binucleate sporoplasm, with few and small sporoplasmossomes (Fig. 4 B). Immature and mature spores were found in central part of the plasmodia (Fig. 4 B). The polar capsule was elongated and filled with granular material. The polar filaments had a half-moon shape, 12 to 13 coils and were immersed in this granular material (Figs. 4 C and 5).

Type host: Pseudoplatystoma coruscans Agassiz, 1829 and *Pseudoplatystoma fasciatus* Linnaeus, 1766 (Osteichthyes: Pimelodidae)

Site of infection: gill filaments

Prevalence: 14 of the 82 specimens (17.1%) of *P. coruscans* and 12 of the 70 specimens (17.1%) of *P. fasciatus* were infected.

Locality: Pantanal Wetland, Central Brazil

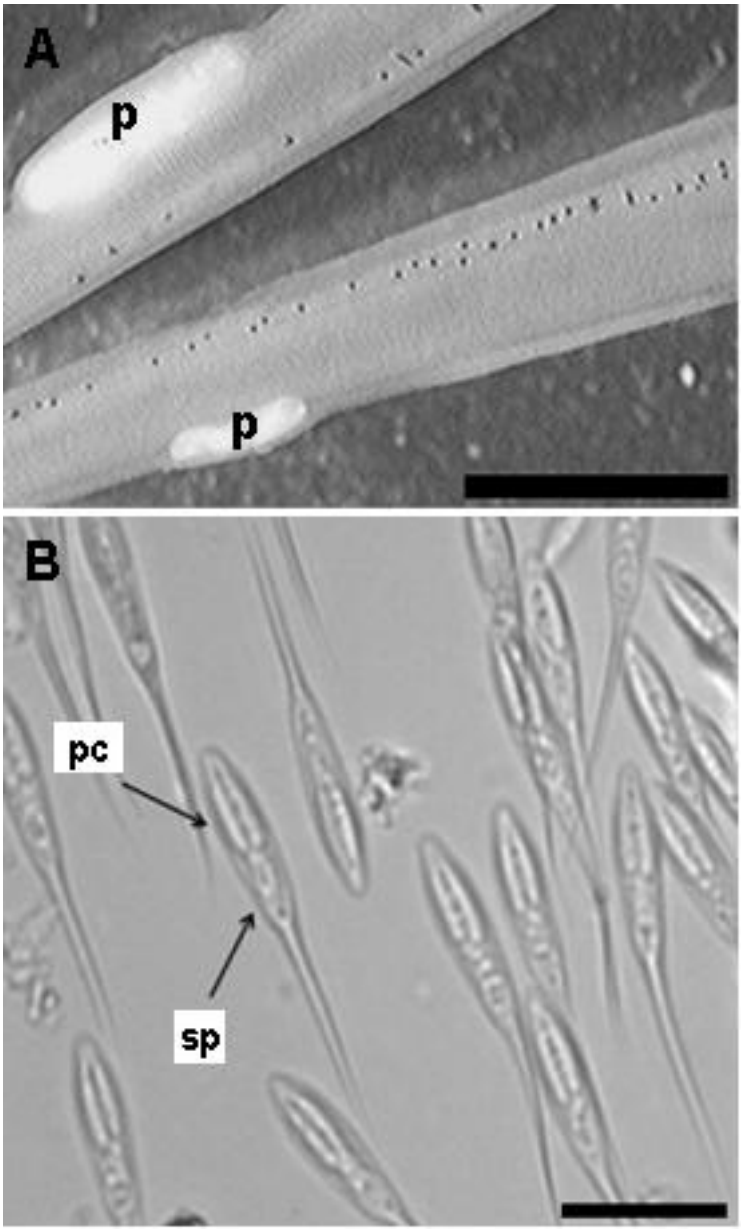

Figure 1. Light photomicrographs of *Henneguya* sp. 2, parasite of *Pseudoplatystoma corruscans* and *Pseudoplatystoma fasciatum*; (A) Formalin-fixed gill filaments from *P. corruscans* showing plasmodia (p), scale bar = 1 mm; (B) Mature fresh spores in frontal view, showing polar capsule (pc) and sporoplasm (sp), scale bar = 10 µm

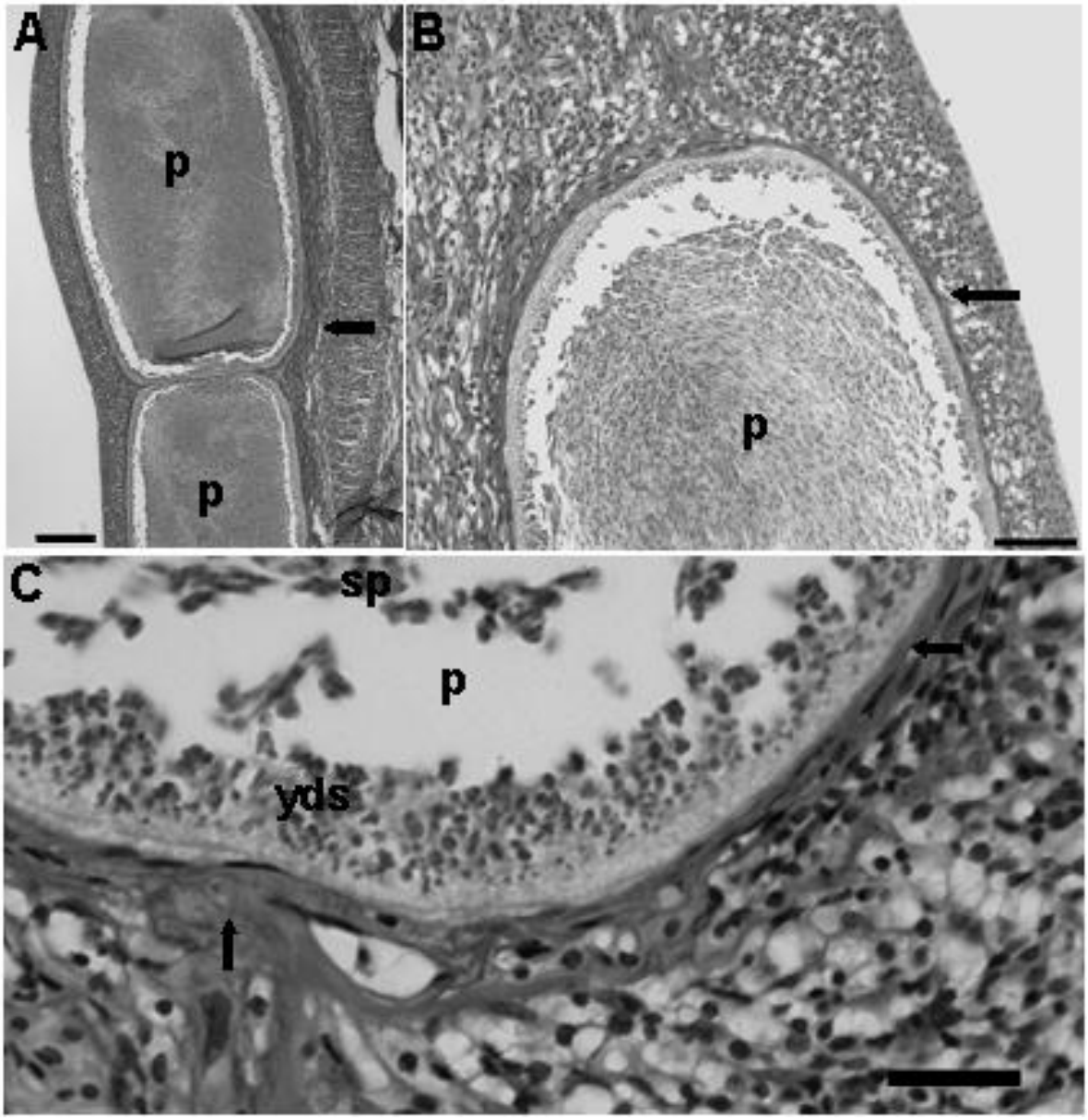

Figure 2. Histological sections of gill filaments from *Pseudoplatystoma corruscans* infected by plasmodia of *Henneguya* sp. 2; (A) Plasmodia (p) producing slight compression of adjacent tissues (black arrow), scale bar =100 µm; (B) Development of plasmodia (p) in sub-epithelial connective tissue of gill filaments (black arrow), scale bar = 50 µm; (C) Plasmodia (p) surrounded by capsule of host connective tissue (white arrows); Note development of young sporogonic stages in a thin layer in the peripheary and mature spores (sp) in central region, scale bar = 20 µm; staining with Sirius red

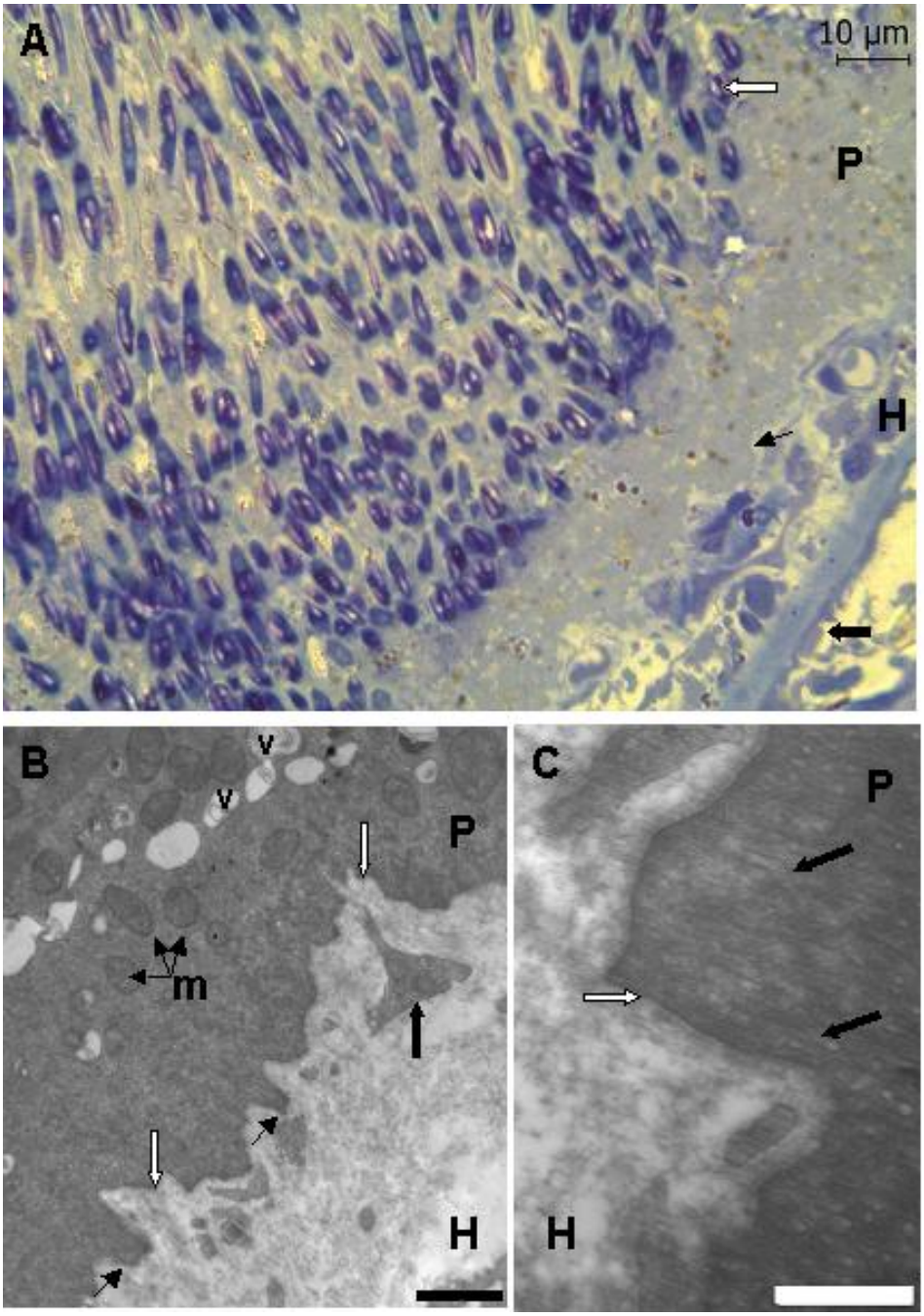

Figure 3. Semi-thin cut electron micrography of gill filaments from *Pseudoplatystoma corruscans* and *Pseudoplatystoma fasciatum* infected by plasmodia (p) of *Henneguya* sp. 2; (A) Semi-thin cut of gill filament from *P. fasciatum* infected by plasmodium of *Henneguya* sp. 2 showing mature spores in central region (white arrow), plasmodial ectoplasm (ec) and plasmodial wall (thin arrow), scale bar = 10 µm; Staining with toluidine blue; (B and C) Electron micrography of gill filaments from *P. corruscans* infected by *Henneguya* sp. 2 showing host-parasite interface; (B) Invaginations (white arrows) and expansions (thin arrows) of the plasmodial wall; Within the plasmodium: mitochondria (m) and numerous vacuoles (v), scale bar = 1 µm; (C) Detail of periphery of plasmodia showing single membrane (white arrow) and numerous pinocytotic canals (black arrows), scale bar = 0.5 µm; Host (H)

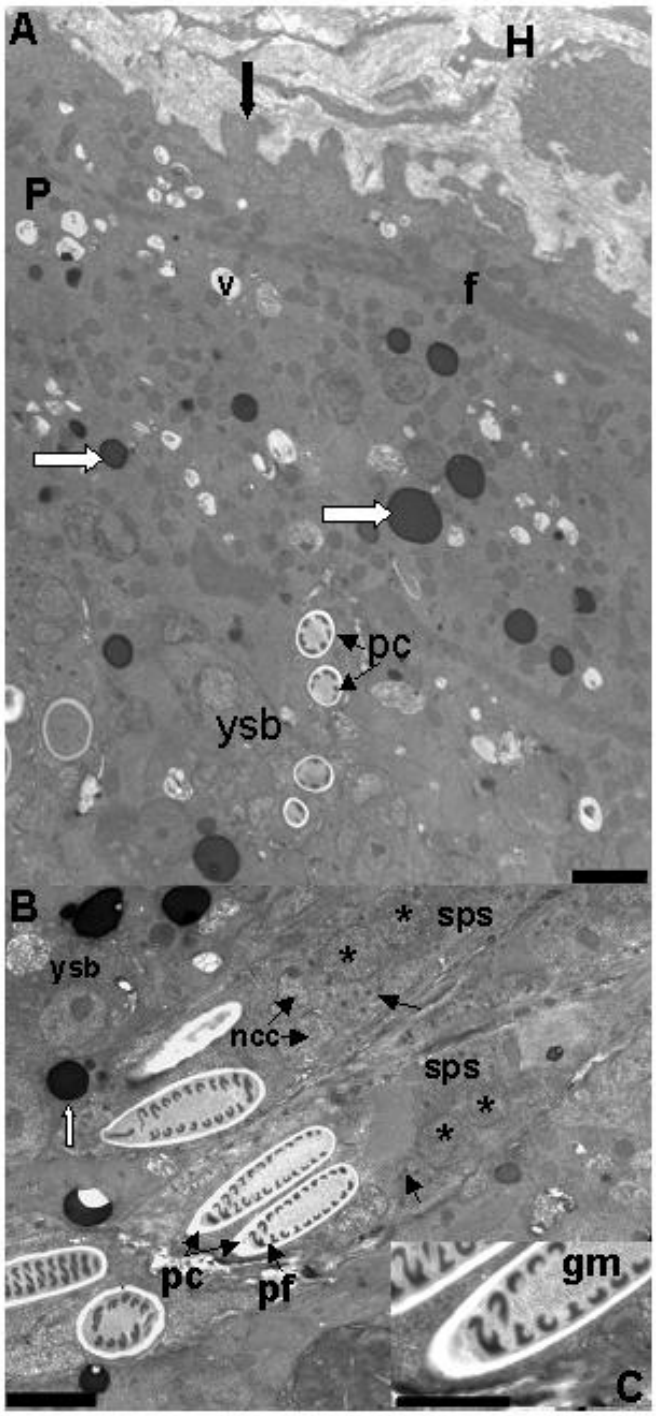

Figure 4. Electron micrography of plasmodia (p) of *Henneguya* sp. 2; (A) Projections of plasmodial wall (black arrow), fibrous material (f), rounded electron-dense structures (white arrows), vacuoles (v), young sporoblast (ysb) and polar capsule (pc), scale bar = 2 µm; (B) Longitudinal sections of immature spores showing binuclear (*) sporoplasm (sps) with small sporoplasmosomes (thin arrows), polar capsule (pc) with polar filament (pf), nuclei of caspulogenic cells (ncc) and young sporoblasts (ysb), scale bar = 2 µm; (C) Detail of polar capsule with granular material, scale bar = 0.5 µm

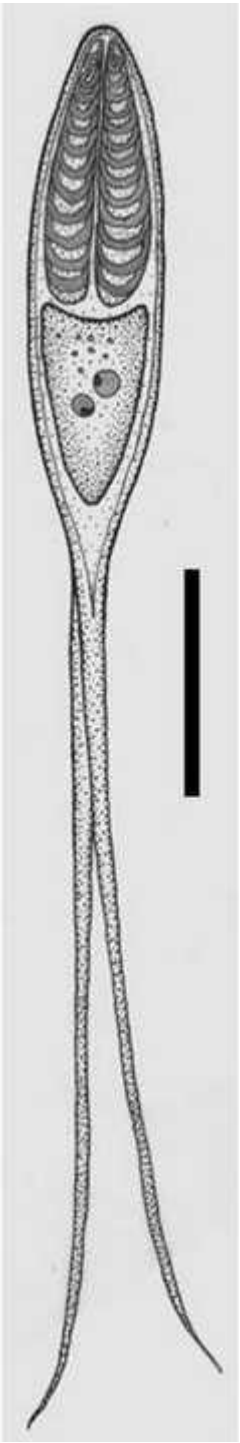

Figure 5. Schematic representation of mature spore of *Henneguya* sp.2, scale bar = 5 µm

DISCUSSION

The characteristics of *Henneguya* sp. 2 were compared with all *Henneguya* spp. described parasitizing freshwater fish in South America (Azevedo *et al.,* 2008, Eiras 2008; 2009; Feijó *et al*., 2008; Azevedo *et al*., 2009) as well as other continents (Eiras, 2002). Among the South American *Henneguya* ssp. parasites of freshwater fish, only *H. corruscans* and *Henneguya* sp. 1 have been found in fish belonging to the genus *Pseudoplatystoma. Henneguya* sp. 2, however, exhibits important morphological differences in comparison to *H. corruscans* and *Henneguya* sp. 1. *H. corruscans* has a smaller total spore length (27.6 μm), larger body length (14.3 μm), larger spore width (5.0 μm), smaller caudal process (13.7 μm) and smaller number of polar filament turns (5 to 6). *Henneguya*sp.1 has a smaller total spore length (33.2 μm), smaller body length (10.4 μm), smaller polar capsule length (3.3 μm) and smaller number of polar filament turns (6 to 7).

Among the species parasitic to other species of South American freshwater fish, spore length and width in *Henneguya* sp. 2 resemble those of the following species: *H. adherens* Azevedo & Matos, 1995, parasite of gill filaments in *Acestrorhynchus falcatus*; *H. curimata* Azevedo & Matos, 2002, parasite of the kidneys in *Curimata inormata*; and *H. pellucida* Adriano, Arana & Cordeiro 2005, parasite of the serosa of the visceral cavity and surface of the swimbladder in *Piaractus mesopotamicus*. However, these parasites are very different from *Henneguya* sp. 2 with respect to other characters and all have been reported infecting Characiformes, whereas the species described here infects two species of Siluriformes.

Henneguya sp. 2 differs from *Henneguya* spp. parasites of freshwater fish species on other continents (Eiras, 2002) in at least one of the following characteristics: total spore length, spore body length, length of caudal process, spore width, length or width of polar capsules, number of polar filament turns, infection site, shape and size of the plasmodia, and the infection of phylogenetically distant hosts.

Henneguya sp. 2 is the third myxosporean species to be described parasitizing species of *Pseudoplatystoma*, following the descriptions of *H. corruscans* (Eiras *et al*.,

2009) and *Henneguya* sp. 1 (Chapter 1). Other South America fish have also been host to numerous myxosporean species: *Prochilodus lineatus* (likely *P. corruscans*) has been infected by three species (Adriano et al., 2002a; 2005a); *P. mesopotamicus* has been host to four species (Martins & Sousa, 1997; Adriano *et al*., 2002b; 2005b; 2005c); and *Brycon hilarii* has been host to five species (Adriano *et al*., 2005d). This indicates that each South American fish may be host to a number of different myxosporean species. According to Vari and Malabarba (1998), the Neotropical region is estimated to have 8000 species of freshwater fish, with the overwhelming majority found in South America. Based on this huge number of potential host species for myxosporeans, it is possible to estimate the myxosporean fauna in this biogeographic region. About 90 myxosporean species have been described thus far in South America. However, considering the host-specificity of these parasites (Molnar *et al*., 1998), a hypothetical average number of two myxosporean species per host species (perfectly possible considering the Brazilian fish studied thus far) and Vari and Malabarba's estimate (1998) of species of freshwater fish in the Neotropics, there be as many as 16,000 myxosporean species. This incredible but possible number of species represents roughly eight times the number of species described thus far for all continents [around 2180 species, according to Sitjá-Bobadilla (2008)] and would mean that the 90 myxosporean species described for species of South American freshwater fish thus far represent only 0.56% of the estimated number of Neotropical myxosporeans, thereby indicating a promising field of study with regard to taxonomy, phylogeny, host parasite interaction and parasite ecology.

The statistical analysis revealed that the prevalence of *Henneguya* sp. 2 in the Pantanal wetland of Brazil was not significantly influenced by season (rainy or dry), host species (*P. corruscans* or *P. fasciatum*) or host gender. Regarding the host species, the two pimelodids species infected by *Henneguya* sp. 2 are phylogenetically close (both are from the genus *Pseudoplatystoma*), thereby corroborating the theory put forth by Molnár (1998) and Molnár *et al.* (1998) that myxosporeans are host specific or can infect phylogenetically close fish species, such as the congener species analyzed in the present study.

Following the localization pattern of the plasmodia of myxosporean parasites in gills proposed by Molnár *et al*. (2002), the plasmodia of *Henneguya* sp. 2 proved to be the intrafilamental-epithelial type, developing in the sub-epithelial connective tissue of the gill filaments, and were surrounded by a capsule of host connective tissue, but with no inflammatory infiltrate in the infection site. However, the development of the parasite caused slight compression of the adjacent tissues. These alterations are similar to those observed in *H. mystusia* (Molnár *et al.,* 2006) and *Henneguya* sp. 1 (Chapter 1).

The ultrastructural characterization of the plasmodial wall of myxosporeans is of fundamental importance to the study of the host-parasite relationship (Current & Janoyy, 1978), as the structure of this wall differs between different myxosporean species (El-Mansy & Bashtar, 2002; Adriano *et al.,* 2005a; Casal *et al.,* 2006). The plasmodial wall is a nutrient transport system, supplying the nutrients necessary to plasmodial development through pinocytic canals (Hallett & Diamant, 2001; El- Mansy & Bashtar, 2002) and/or by engulfing parts of the host cells through phagocytosis in some cases (Uspenskaya, 1982; Lom & Dyková, 1995, Adriano *et al.,* 2005a; Chapter 1).

The analysis of the host-parasite interface during infection by *Henneguya* sp. 2 revealed a capsule of collagen surrounding the parasite and the plasmodial wall composed of a single membrane connected to the ectoplasmic zone through numerous pinocytic canals, which is in agreement with the findings of previous studies on myxosporean species (Adriano *et al.,* 2005a, Adriano *et al*., 2009). Internally, slightly electron-dense material was found forming a line just below plasmodial wall, resembling the fibrous material described in the plasmodia of *Henneguya* sp. 1*,* which was suggested to be actin filaments (Chapter 1). These filaments are thought to have the function of supporting the plasmodium, as suggested by Morris and Adams (2007).

The present study describes a new myxosporean species infecting an economically important South American fish, focusing on histopathological, morphological and ultrastructural analyses and its interesting host-parasite-environment relationship, such as the lack of an influence from season, host gender and host species on the prevalence of the parasite.

ACKNOWLEDGEMENTS

The authors thank Ricardo Afonso Torres de Oliveira (CEPTA/ICMBio) for help in dissecting the fish; Dr. Laerte Batista de Oliveira Alves, manager of the National Center for Research and Conservation of Continental Fish (CEPTA/ICMBio) and Dr. José Augusto Ferraz de Lima, manager of Pantanal National Park, for support during the field work.

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CONCLUSÕES

Os resultados deste estudo possibilitaram as seguintes conclusões:

1) Em ambiente natural *Pseudoplatystoma corruscans* e *Pseudoplatystoma fasciatum* são hospedeiros de uma nova espécie do gênero Henneguya.

2) Em sistemas de criação, o pintado híbrido é hospedeiro de uma nova espécie do gênero *Henneguya* distinta daquela que infecta *P. corruscans* e *P. fasciatum* em ambiente natural.

3) Em espécimes de pintado oriundos de sistemas de criação, a infecção pela nova espécie de *Henneguya* causa importante redução da área funcional do epitélio da brânquia.

4) No Pantanal Mato-Grossense, a infecção de *P. corruscans* e *P. fasciatum* pela nova espécie de *Henneguya* não apresentou variação significativa com relação às estações secas e chuvosas, às espécies e sexo dos hospedeiros

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ANEXO 1: Declaração do comitê de ética.

UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
Departamento de Ciências Básicas

Pirassununga, 25 de fevereiro de 2009.

Assunto: Parecer da Comissão de Ética da FZEA.

Prezado Professor,

Tendo por base os princípios estabelecidos no Regimento Geral do Comitê de Ética em Experimentação Animal da FZEA/USP, informo que o projeto de pesquisa, "Mixosporídeos parasitos de *Pseudoplatystoma curruscans* (pintado), *Pseudoplatystoma fasciatum* (cachara), *Salminus brasiliensis* (dourado) e *Brycon hilarii* (piraputanga) oriundos de ambiente natural e de sistemas de criação: taxonomia e interação parasito-hospedeiro", coordenado pelo Prof. Dr Antônio A. M. Maia, recebeu parecer favorável de um pesquisador que atua na área. Deste modo, a Comissão de Ética da FZEA considerou-o aprovado.

Atenciosamente,

Prof. Dr. João Alberto Negrão
Presidente da Comissão de Ética

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