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INSTITUTO DE BIOLOGIA

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MYXOZOA EM PEIXES AUTÓCTONES MANTIDOS EM SISTEMAS DE CRIAÇÃO: TAXONOMIA E RELAÇÃO PARASITO-HOSPEDEIRO

Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas para a obtenção do título de Doutor em Parasitologia

Orientador: Prof. Dr. Nelson da Silva Cordeiro

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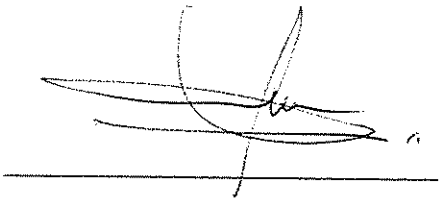
Prof. Dr. Nelson da Silva Cordeiro (Orientador) 

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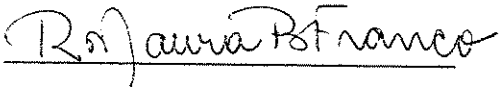
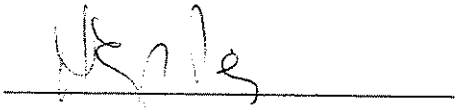
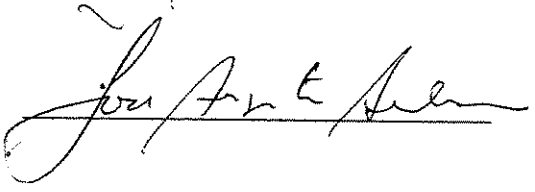
Profa. Dra. Regina Maura Bueno Franco 

Profa. Dra. Maria Josè Tavares Ranzani de Paiva 

Prof. Dr. José Augusto Senhorini 

Prof. Dr. Vanderlei Rodrigues _____

"Aos meus pais, Francisco Adriano e Maria Itamar Rodrigues Adriano, que são os meus exemplos de vida."

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RESUMO

A ocorrência de mixosporídeos parasitos de peixes foi avaliada em exemplares de pacu (*Piaractus mesopotamicus*), curimbatá (*Prochilodus lineatus*) piauçu (*Leporinus macrocephalus*) e matrinxã (*Brycon cephalus*) obtidos de desova artificial e mantidos em um tanque do Centro de Pesquisa e Gestão de Recursos Pesqueiros Continentais – CEPTA/IBAMA, localizado em Pirassununga, SP. Brasil. Mensalmente, de março de 2000 a fevereiro de 2002, cinco exemplares de cada espécie foram sacrificados para o exame parasitológico. A identificação do parasito foi mediante análise morfológica e comparação com as espécies já descritas na literatura. Órgãos ou parte de órgãos parasitados foram fixados para o estudo histopatológico e ultra-estrutural. Nos espécimes de curimbatá encontramos duas espécies de mixosporídeos: *Myxobolus porofilus* formando plasmódios esbranquiçados e redondos encontrados livres na cavidade visceral de 2,5% dos peixes examinados e *Henneguya* sp. 1. encontrado nas regiões intra e inter lamelar das brânquias, com prevalência de 48,3%. As análises histopatológica e ultra-estrutural revelaram a formação de uma fina cápsula de colágeno envolvendo os plasmódios de *Henneguya* sp. 1. O parasito produziu deformações nas estruturas lamelares, tais como deslocamento lateral das lamelas vizinhas, fusão lamelar e hiperplasia de células epiteliais. Em pacu observamos a ocorrência de três espécies de mixosporídeos: *Henneguya piaractus* infectando as brânquias com prevalência de 45%, sendo que a menor prevalência foi registrada em peixes mais jovens;. *Henneguya* sp 2. parasitando a bexiga natatória e membrana serosa da cavidade visceral com prevalência de 8,3% e a infecção foi registrada apenas em exemplares jovens, e *Myxobolus* sp. 1 que

apresentou prevalência de 60% e foi encontrado na vesícula biliar, bexiga urinária, brânquias, baço, nadadeiras, superfície da cabeça, fígado e coração. A ocorrência do parasito se deu durante todo o período de estudo e não houve variação de uma estação para outra nem com o tamanho do hospedeiro. Os plasmódios de *H. piaractus* foram do tipo intralamelar e o desenvolvimento dos mesmos resultou na dilatação das lamelas infectadas com discreta hiperplasia epitelial e as lamelas vizinhas foram deslocadas lateralmente. A análise ultra-estrutural mostrou canais de pinocitose e pontos de fagocitose na parede dos plasmódios de *H. piaractus* e houve contato direto das células hospedeiras com esta parede. Os plasmódios de *Henneguya* sp. 2 apresentaram coloração amarelada e forma arredondada, com tamanho variando entre 0,5 a 3,0 mm. As análises histopatológica e ultra-estrutural mostraram a formação de uma espessa cápsula de tecido conjuntivo envolvendo os plasmódios, e na bexiga natatória, o desenvolvimento do parasito produziu espessamento da túnica externa e uma reação granulomatosa. *Myxobolus* sp. 1 produziu importantes alterações apenas nas brânquias, onde causou deformação das arteríolas dos filamentos, com redução, e em alguns casos, obstrução destes vasos. Nos espécimes de piauçu e matrinxã não foram observadas infecções por mixosporídeos durante o decorrer deste trabalho.

ABSTRACT

The occurrence of myxosporean parasites in fish was studied in specimens of pacu (*Piaractus mesopotamicus*), curimbatá (*Prochilodus lineatus*), piauçu (*Leporinus macrocephalus*) and matrinxã (*Brycon cephalus*) obtained from artificial reproduction programs and released into a pond at the Center for the Research and Management of Continental Fishing Resources (CEPTA/IBAMA) located in the municipality of Pirassununga, in the state of São Paulo, Brazil. At monthly intervals from march 2000 to February 2002, five specimens of each species were sacrificed for parasitological examinations. The parasites were identified by morphological analysis and by comparison with the myxosporean species reported in the literature. Infected organs or fragments of them were fixed for histopathological and ultrastructural analysis. Two myxosporean species were found in curimbatá: *Myxobolus porofilus*, which produced white, round plasmodia found free in the visceral cavity of only young fish, with a prevalence of 2.5%, and *Henneguya* sp. 1 found in the interlamellar and intralamellar regions of the gills. Histopathological and ultrastructural analysis revealed the presence of a thin collagen capsule surrounding the plasmodia. The parasite caused deformation of the lamellar structures by pushing the neighbouring gill lamellae aside, to produce lamellar fusion and hyperplasia of the epithelial cells. The occurrence of the parasite showed no seasonal variation or preference for host size. Three myxosporean species occurred in pacu: *Henneguya piaractus* in the gills, with a total prevalence of 45%, but that decreased in younger fish, *Henneguya* sp. 2 in the swim bladder and in the serous membranes of the visceral cavity of only juvenile specimens with a prevalence of 8.3%, and *Myxobolus* sp. 1

which had a total prevalence of 60% and was found in the gall bladder, urinary bladder, gills, spleen, fins, head surface, liver and heart. The latter parasite was found throughout this study and showed no seasonal variation or preference for host size. The plasmodia of *H. piaractus* were of the intralamellar type, and their development resulted in dilatation of the infected gill lamellae, discreet epithelial hyperplasia, and lateral dislocation of the neighbouring lamellae. Ultrastructural analysis showed direct contact between the plasmodial wall of *H. piaractus* and the host cells. Pinocytic canals and points of phagocytosis were seen in the plasmodial wall. The plasmodia of *Henneguya* sp. 2 were yellow, round and 0.5 – 3.0 mm in diameter. Histopathological and ultrastructural analysis of these plasmodia showed the presence of a thick capsule of connective tissue surrounding the plasmodia and, in the swim bladder, the development of the parasite produced thickening of the tunica externa and a granulomatous reaction. Of the organs infected by *Myxobolus* sp. 1, the greatest damage was seen in the gills, since the development of the plasmodia reduced the vessel lumen and, in some cases, completely obstructed the lumen of the gill filament arterioles. No myxosporeans were found in specimens of piauçu and matrinxã.

1-INTRODUÇÃO

Peixe é fonte primária de proteína para humanos em muitas partes do mundo. A pesca industrial, entretanto, tem declinado significativamente nas últimas décadas e esse declínio é devido, principalmente, a fatores relacionados com a pesca predatória e a poluição ambiental. Nos últimos anos, governos de vários países encorajaram e continuam a encorajar indústrias do setor pesqueiro a envolverem-se com a aqüicultura, cultivando peixes e frutos-do-mar em condições artificiais e/ou semi-artificiais (Woo, 1995).

Diferente da pesca, que é uma atividade extrativista, a aqüicultura fundamenta-se na produção, geralmente em ambientes artificiais. No Brasil, segundo dados do CNPq (1998), a aqüicultura (piscicultura e maricultura) é uma das atividades produtivas que mais cresce. Em 1996 e 1997, a produção aqüicola brasileira saltou de 40,5 para 60,7 mil toneladas aproximadamente, representando, assim, um crescimento de quase 49%. Dentro do contexto do desenvolvimento da aqüicultura brasileira destaca-se a piscicultura, com ênfase para a intensificação dos sistemas de cultivo e a domesticação de espécies nativas com potencial para a criação (Martins & Romero, 1996).

A piscicultura é uma atividade produtiva e em franca expansão no Brasil. Devido ao fato de não carecer de grandes áreas para o seu desenvolvimento, apresenta grande importância econômica, principalmente para as regiões onde as propriedades rurais são de pequeno porte e utilizadas para o desenvolvimento da agricultura familiar, apresentando-se, portanto, como uma opção de renda para o pequeno agricultor, permitindo sua permanência no campo. Além da reconhecida importância sócio-econômica da atividade, a criação de pescado em sistemas de criação produz alimento de qualidade e ajuda a diminuir o impacto ambiental da pesca extrativista sobre as espécies de peixes selvagens de maior aceitação comercial.

Em várias regiões do Brasil, especialmente nos estados do Paraná, Santa Catarina e São Paulo, e mais recentemente na região Centro Oeste, é grande o número de proprietários rurais que têm se dedicado à piscicultura. Nas proximidades dos grandes centros urbanos tornou-se muito comum os pesque-pague, atividade que associa o comércio (venda de peixes diretamente ao consumidor) com a recreação (pesca esportiva), criando-se assim uma nova modalidade de comercialização de pescado.

Todavia, a criação intensiva de peixes, devido a fatores intrínsecos da atividade, favorece o aparecimento de algumas situações problemáticas, tais como enfermidades infecciosas e parasitárias. Segundo Thatcher (1981), a concentração de peixes de uma mesma espécie, como ocorre na piscicultura intensiva, geralmente com alta densidade populacional, facilita a transmissão de agentes patogênicos, favorecendo o surgimento de enfermidades. Entre os vários parasitos de peixes, aqueles do filo Myxozoa estão entre os que apresentam maior importância (Schmahl et al., 1989). Os Myxozoa parasitam principalmente peixes, mas algumas espécies infectam répteis, anfíbios e invertebrados (Lom & Dyková, 1995). Até os anos 90, este grupo de organismo estava inserido no Reino Protista, mas estudos filogenéticos baseados em biologia molecular mostraram que os Myxozoa são metazoários (Cox, 2002) e estão relacionados aos Cnidaria (Kent et al. 2001).

O filo Myxozoa é composto por duas classes. Aqueles encontrados parasitando peixes e menos freqüentemente répteis e anfíbios formam a classe Myxosporea, cujos esporos são considerados os estágios disseminadores do parasito e aqueles encontrados parasitando invertebrados (anelídeos) formam a classe Actinosporea (Eiras, 1994). Markiw & Wolf (1983) estudando a forma de transmissão de *Myxobolus cerebralis* Hofer, 1903 (agente causador da doença do rodopio), relataram o envolvimento de anelídeos oligoquetas no ciclo de vida deste parasito. No ano seguinte, Wolf & Markiw (1984), em publicação na

revista *Science* esclareceram a participação de anelídeos tubificídeos no ciclo de vida de *M. cerebralis*. Neste trabalho, os autores mostraram que tubificídeos em contato com esporos de *M. cerebralis* eram parasitados por actinosporídeos do gênero *Triactinomyxon,* indicando que esporos de *M. cerebralis* e de *Triactinomyxon* eram formas distintas do ciclo de vida do *M. cerebralis,* e que *Triactinomyxon* é, portanto, a forma que desencadeia a doença do rodopio em salmonídeos. Desde então, 25 espécies de Myxozoa são reconhecidas tendo anelídeos como hospedeiros intermediários, o que levou Kent et al. (1994, 2001) a proporem a supressão da classe Actinosporea. Segundo Kent et al. (2001), cerca de 1.350 espécies de Myxozoa, distribuídas por 52 gêneros foram descritas até o presente.

Myxosporea causam intensas infecções e doenças em peixes cultivados e de ambientes naturais. *M. cerebralis* é responsável por altas taxas de mortalidade em salmonídeos de várias partes do mundo. O parasito ataca principalmente a cartilagem da cabeça e coluna vertebral dos hospedeiros. *Myxidium truttae* Léger, 1930 produz infecções nos ductos biliares de salmonídeos em toda a Eurásia. *Myxobolus lintoni* Guerley, 1983 infecta *Cyprinodon variegatus* ao longo da costa leste dos Estados Unidos. O parasito caracteriza-se por formar agregados de cistos, originando grandes massas semelhantes a tumores no tecido subcutâneo e algumas vezes na musculatura (Lom & Dyková 1995). Kabata & Whitaker (1989) registraram *Kudoa thyrsites* Gilchrist, 1924 parasitando o músculo cardíaco de salmonídeos do Pacífico. Langdon (1991) associou liquefação muscular em *Coryphaena hippurus* com a mesma espécie de mixosporídeo na Austrália. Lom *et al.* (1989) relacionaram a presença de estruturas císticas nos corpúsculos renais de *Esox lucius* com a infecção de *Myxidium lieberkuehni* Bütschli, 1882. *Hoferellus carassii* Akhmerov, 1960 produz hipertrofia dos rins de goldfish, na Ásia, Europa e América do

Norte, sendo muito comum em animais cultivados. No Japão a espécie é responsável por mortalidade acima de 20% (Lom & Dyková 1995). Dyková & Lom (1982) descreveram *Sphaerospora renicola* no rim de carpa, na Tchecoslováquia (atual República Tcheca). Na ocasião, os autores observaram hipertrofia e necrose nos túbulos renais dos peixes infectados. *Ceratomyxa shasta* Noble, 1950 infecta o trato digestivo de salmonídeos da América do Norte, causando sérios danos em populações cultivadas e selvagens (Lom & Dyková, 1995).

Os primeiros registros de parasitos do filo Myxozoa em peixes do Brasil foram realizados por Splendore (1910) que encontrou mixosporídeos nas brânquias de peixes capturados nas proximidades da cidade de São Paulo, e Migone (1916) que relatou a presença desses parasitos em 5 espécies de peixes da Bacia do rio Paraguai. Desde então, diversas espécies de mixosporídeos foram catalogadas parasitando várias espécies de peixes no país.

Gioia & Cordeiro (1996) publicaram uma lista atualizada das espécies de mixosporídeos parasitos de peixes da fauna brasileira; foram reconhecidas até aquela data 52 espécies distribuídas em 11 gêneros. Os principais órgãos acometidos foram as brânquias, vesícula biliar, rins e fígado. No território brasileiro, espécies dos gêneros *Henneguya* e *Myxobolus* foram as mais comuns.

Azevedo & Matos (1995, 1996, 2002, 2003), Casal et al. (1996, 1997, 2002, 2003) e Vita et al. (2003) utilizaram características ultraestruturais na descrição de novas espécies de mixosporídeos de peixes da região do rio Amazonas. Martins & Romero (1996) estudando o efeito destes parasitos sobre o tecido branquial em peixes cultivados, registraram *Henneguya* sp. produzindo aderência entre as lamelas secundárias adjacentes, hiperplasia, congestão lamelar e edema causando desprendimento do epitélio respiratório.

Quando Martins et al. (1997) estudaram efeitos patológicos e comportamentais associados com *Henneguya* sp. em pacus confinados na mesmo centro de estudo, relataram comportamento anormal dos peixes parasitados. Segundo os autores, os peixes permaneciam próximos às margens ou agrupados, a atividade alimentar diminuiu com o passar do tempo e os animais tornaram-se apáticos, nadavam irregularmente, com aparente perda de equilíbrio, chegando por vezes à morte. Estudos histológicos revelaram hemorragias e severos focos inflamatórios no epitélio das brânquias dos peixes infectados. Ferraz de Lima *et al.* (1995) associaram modificações histológicas nos rins de exemplares de pacus criados em viveiros do Centro de Pesquisa e Gestão de Recursos Pesqueiros Continentais – CEPTA/IBAMA, localizado no município de Pirassununga, Estado de São Paulo, com a alta infecção por *Henneguya* sp. Ceccarelli *et al.* (1990) relataram a presença de *Henneguya* spp. parasitando pacu, tambaqui e carpa comum criados nos viveiros do CEPTA. Molnár *et al.* (1998) descreveram *Myxobolus macroplasmodialis* parasitando a cavidade visceral de dourados (*Salminus maxillosus*) capturados no rio Mogi-Guaçu, nas proximidades da Cachoeira das Emas, no município de Pirassununga. A susceptibilidade natural de pacu (*P. mesopotamicus*), tambaqui (*Colossoma macropomum*), carpa (*Cyprinus carpio*) e o híbrido tambacu (*C. macropomum* fêmea x *P. mesopotamicus* macho) à infecção por mixosporídeos foi relatada por Martins *et al.* (1999). Os exames parasitológicos revelaram *Myxobolus colossomatis* Molnár & Békési, 1992 parasitando os rins, figado, baço, músculos e vesícula biliar de pacu, bem como os rins de tambaqui. *Henneguya piaractus* Martins & Souza, 1997 foi encontrado parasitando as brânquias de pacu, tambaqui e tambacu. O estudo revelou alta susceptibilidade do pacu para infecções por mixosporídeos. A incidência de *H. piaractus* e *M. colossomatis* foi de 97,3% no pacu, 33,3% no tambacu, 5,7% no tambaqui e 0,0% na carpa. Mais recentemente, Cellere et al.

(2002) descreveram *Myxobolus absonus* parasitando *Pimelodus maculatus* oriundos do rio Piracicaba, Adriano et al. (2003) estudaram a prevalência de mixosporídeos em pacu do Pantanal e Barassa et al. (2003a e 2003b) descreveram *Henneguya curvata* e *Henneguya chydadea* infectando respectivamente brânquias de piranha (*Serrasalmus spilopleura*) e de lambari (*Astyanax altiparanae*), oriundos de ambiente natural na região de Campinas, SP.

Apesar do rápido desenvolvimento da piscicultura no Brasil, poucos estudos foram realizados no sentido de conhecer as espécies de mixosporídeos que acometem os peixes em sistemas de criação, bem como saber quais os danos provocados por estes parasitos em seus hospedeiros.

Para este trabalho, onde estudamos mixosporídeos em peixes mantidos em sistemas de criação examinamos exemplares de 4 espécies: pacu (*Piaractus mesopotamicus* Holmberg, 1887), matrinxã (*Brycon cephalus* Gunther, 1869), curimbatá (*Prochilodus lineatus* Valenciennes, 1836) e piauçu (*Leporinus macrocephalus* Garavello & Britski, 1988) mantidos em um viveiro do Centro de Pesquisa e Gestão de Recursos Pesqueiros Continentais – CEPTA/IBAMA. Estas espécies foram escolhidas porquê pertencem à fauna brasileira, apresentam grande potencial do ponto de vista da produção de pescado em condições artificiais no Brasil e têm boa aceitação no mercado.

2-OBJETIVOS:

1) Determinar as espécies de parasitos do filo Myxozoa encontrados nas 4 espécies de peixes estudadas: pacu (*Piaractus mesopotamicus*), matrinxã (*Brycon cephalus*), curimba (*Prochilodus lineatus*) e piauçu (*Leporinus macrocephalus*)
2) Determinar a prevalência destes parasitos e verificar a sua possível variação ao longo do tempo de estudo.
3) Estudar os efeitos do parasitismo sobre os tecidos dos órgãos afetados.

3-MATERIAL E MÉTODOS:

Este estudo foi realizado em parceria com o Centro de Pesquisa e Gestão de Recursos Pesqueiros Continentais – CEPTA/IBAMA de Pirassununga. Para o desenvolvimento do estudo foram utilizados instalações e equipamentos do Laboratório 1 de Protozoologia do Departamento de Parasitologia IB/UNICAMP, do laboratório de Histofisiologia e Histopatologia Experimental em Ectotérmicos do Departamento de Histologia e Embriologia IB/UNICAMP, do Laboratório de Microscopia Eletrônica IB/UNICAMP e do Laboratório de Sanidade, Bem Estar e Controle de Enfermidades de Peixes do CEPTA/IBAMA.

Os peixes utilizados foram provenientes de reprodução induzida realizada no Laboratório de Reprodução e Larvicultura de Peixes do CEPTA/IBAMA. Exemplares das quatro espécies, (500 pacus, 500 matrinxãs, 500 curimbatás e 500 piauçus) foram confinados em um viveiro do CEPTA/IBAMA com dimensão de 1000 m^2 (Figs. 1 e 2).

A água que abastece o viveiro é proveniente de uma represa alimentada por um riacho, cuja nascente está localizada no interior de uma mata, próxima à sede do CEPTA.

Fig. 1: Viveiro de criação de peixes do CEPTA.

Fig. 2: Captura dos peixes no viveiro de criação do CEPTA.

As características físicas e químicas da água do viveiro, como temperatura e oxigênio dissolvido foram determinados diariamente, às 9:00 horas, a 1 metro de profundidade com o emprego de oxigenômetro YSI 55. Outros dados como pH, amônia, alcalinidade e dureza foram medidas semanalmente no mesmo horário, sendo o pH com peagômetro digital, a amônia por espectrofotometria e a alcalinidade e dureza por titulação.

Durante dois anos ininterruptos foram realizadas coletas mensais de 5 exemplares de cada espécie de peixe. Os peixes foram sacrificados por transecção do cordão espinhal, e medidos usando-se o valor do comprimento total. Posteriormente, foram realizados exames macroscópicos das estruturas externas em busca de lesões e/ou cistos do parasito. Finalmente, foi realizou-se a necropsia visando o exame dos órgãos internos.

As metodologias referentes aos estudos taxonômicos, histológicos, ultra-estruturais e das análises estatística estão relatadas separadamente em cada capítulo dos resultados.

4-RESULTADOS

Os resultados desta tese foram divididos em cinco capítulos, nos quais são abordados aspectos taxonômicos, histopatológicos, ultra-estruturais e epidemiológicos dos mixosporídeos encontrados parasitando peixes mantidos em sistemas de criação.

Capítulo 1 (Publicado - Folia Parasitologica 49: 259-262, 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

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Key words: Myxosporea, *Myxobolus porofilus*, Prochilodontidae, *Prochilodus lineatus* Brazil

Abstract. *Myxobolus porofilus* sp. n. is described infecting the visceral cavity of *Prochilodus lineatus* (Valenciennes, 1836) cultivated in São Paulo State, Brazil. The plasmodial form of the parasite is 3-5 mm in length and appeared compressed between the wall of the visceral cavity and the pyloric caecum, reposing on this organ. The spores are small (length 5.7 ± 0.3 μm, width 4.8 ± 0.2 μm; mean ± SD) and round to elliptical in frontal view. The valve surfaces are smooth and have sutural folds. The polar capsules are

ovoid, small (length 1.6 ± 0.1 μm, width 1.1 ± 0.1 μm) and equal in size. The polar filaments have three turns aligned perpendicularly to the longitudinal axis of the capsule. A conspicuous polar filament pore is arranged at the anterior end of the spore. The only reaction observed upon histological analysis was the presence of a capsule of connective tissue surrounding the plasmodia. This is the first report of a myxosporean parasite in the Prochilodontidae.

INTRODUCTION

Wild and cultivated fishes in many regions of the world are infected by numerous myxosporean parasites of the genus *Myxobolus* Bütschli, 1882. Landsberg and Lom (1991) listed a total of 444 *Myxobolus* species, most of them found in North American, European and Asiatic fishes. Fomena and Bouix (1997) listed 48 *Myxobolus* species infecting freshwater fishes in Africa. In South America, 17 *Myxobolus* species have been reported to parasitise freshwater fishes. During a survey of myxosporean parasites of fish species cultivated in Brazil, we discovered a new species of *Myxobolus*. The parasite was found in *Prochilodus lineatus* (Valenciennes, 1836), a teleostean detritivorous species native to South America. This fish species, popularly known as curimba or curimbatá, is widely distributed in South American rivers and attains a relatively large size, with some specimens measuring 75 cm in length and weighing 8.2 kg (Godoy 1975). The curimba is one of the most consumed fish species in Brazil and is the principal source of income for fisheries in several regions of the country. This species' high reproductive capacity, its rapid growth, its detritivorous habit, i.e., the ingestion of mud, which reduces the

requirement for feeding, and its widespread acceptance on the market, have all contributed to the increased interest in its cultivation in fish farms throughout Brazil.

MATERIALS AND METHODS

Specimens of *P. lineatus* (curimba), *Piaractus mesopotamicus* (Holmberg, 1887) (pacu), *Brycon cephalus* (Günther, 1869) (matrinxã) and *Leporinus macrocephalus*, Garavello & Britski, 1988 (piauçu) four months old, were released in a pond at the Research and Management of continental fishing Resources CEPTA/IBAMA located in the municipality of Pirassununga, in the state of São Paulo, Brazil. Five specimens of each species were examined monthly for the presence of myxozoan parasites from March 2000 to February 2002. Immediately after collection, the fishes were transported alive to the laboratory where they were killed by transection of the spinal cord, before being measured, weighed and necropsied. When plasmodia were present, they were measured with a ruler. The measurements of 32 fresh mature spores (Lom and Arthur 1989) were obtained using a micrometer incorporated into the microscope eyepiece. The dimensions were expressed as the mean ± standard deviation (SD). India ink was used to detect the presence of a mucus envelope around the spores. The spores were checked for the presence of an iodophilous vacuole after adding a drop of Lugol solution. Smears containing free spores were stained with Giemsa's solution and mounted in mounting medium of low viscosity (Cytoseal^{TM}) as permanent preparations. For histological analysis, the plasmodia were fixed in 10% buffered formalin for 24 h, embedded in paraffin, cut into sections 4 μm thick and stained with haematoxylin and eosin or sirius red, a stain developed by Montes and Junqueira (1991) to study the distribution of collagen fibres. For scanning electron

microscopy, two methods were used: (1) Free spores were deposited on a coverslip coated with poly-L-lysine and fixed for 2 h at room temperature with glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). After washing in the same buffer, the preparations were dehydrated in ethanol, critical-point dried, coated with metallic gold and examined in a JEOL JSM-5900LV microscope operated at 30 kV. (2) Histological sections of a plasmodium fixed and embedded using routine histological procedures were deposited on a coverslip coated with albumin, dried in stove for 48 h, deparaffinized with xylol, dehydrated in ethanol, critical-point dried, coated with metallic gold and examined as above.

RESULTS

Of the four fish species studied, only three (2.5%) specimens of *P. lineatus* had plasmodia containing spores of an undescribed myxosporean species belonging to the genus *Myxobolus*. The infected fish were examined in August 2000 and had one or two plasmodia compressed between the wall of the visceral cavity and the pyloric caecum, reposing on this organ (Fig. 1A).

Myxobolus porofilus sp. n. Figs. 1-2

Plasmodial form 3-5 mm in size, white, spherical and polysporic, with spores in different developmental stages. Histologically, the only host reaction detected was the formation of a thin (2.0 μm) connective tissue capsule around the plasmodia (Fig. 1B). Young sporogonic stages present in ectoplasm, with a larger number of mature spores in the endoplasm (Fig. 1B).

Spores small (length 5.7 ± 0.3 μm, width 4.8 ± 0.2 μm) and sub-spherical in frontal view (Figs. 1C, E and 2). Valve walls smooth, symmetrical with sutural folds (Figs. 1D, E). In lateral view, spores discus-shaped (Figs. 1D and 2B); suture line emerges well over surface (Figs. 1D, E). A conspicuous polar filament pore situated on one side of sutural line, at anterior end (Figs. 1C - E). Polar capsules ovoid in shape, small and equal in size (length 1.6 ± 0.1 μm, width 1.1 ± 0.1 μm). Anterior ends of polar capsules closed; ratio of capsule length to spore length about 1:3. Polar filaments with three turns aligned perpendicularly to longitudinal axis of capsule (Fig. 2). A large, round vacuole present in sporoplasm and two nuclei discernible in unstained and Giemsa stained preparations. No mucus envelope was seen after treatment with India ink.

Type host: Prochilodus lineatus (Valenciennes, 1836) (Pisces, Characiformes, Prochilodontidae).

Site of infection: Compressed between the wall of the visceral cavity and the pyloric caecum, reposing on the pyloric caecum.

Prevalence: 3/120 (2.5%) of *P. lineatus* were infected.

Locality: National Center for Research in Tropical Fishes (CEPTA/IBAMA), Pirassununga, State of São Paulo, Brazil.

Type material: Slides with stained spores (syntyps) have been deposited in the collection of the Museum of Natural History, Institute of Biology, State University of Campinas (UNICAMP), State of São Paulo, Brazil (accession numbers ZUEC 04 and 05).

Etymology: The specific name is a combination of the words poro (derived from the Greek *poros* = hole) and filus (from the Latin *filus* = filament) in reference to the conspicuous polar filament pore.

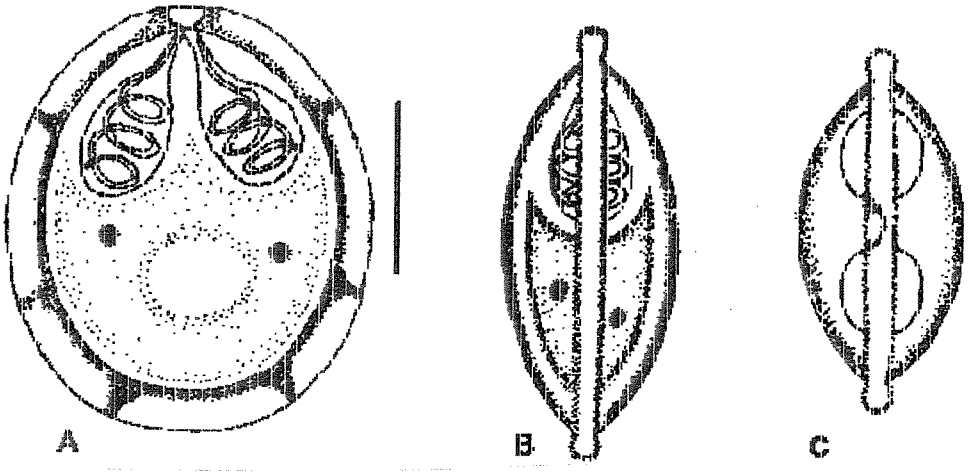

Fig. 2. Schematic representation of mature spores of *Myxobolus porofilus* sp. n.: **A** - frontal view, **B** - lateral view, **C** - apical view of anterior end. Scale bar = 3 μm.

DISCUSSION

Myxobolus porofilus was compared with all *Myxobolus* species previously reported in South American fishes. Only *Myxobolus macroplasmodialis* Molnár, Ranzani-Paiva, Eiras et Rodrigues, 1998, reported to have been found free in the visceral cavity of *Salminus maxillosus* (Characidae) (Molnár et al. 1998), and *Myxobolus absonus* Cellere, Cordeiro et Adriano, 2002, reported to have been found free in the opercular cavity of *Pimelodus maculatus* (Pimelodidae) (Cellere et al. 2002), resembled the species described here with regard to the site of infection (not incorporated in the host tissues) and the colour and structure of the plasmodia. The spores of *M. porofilus* are smaller than those of *M. macroplasmodialis* and *M. absonus*, and the polar capsules are small, of equal size and

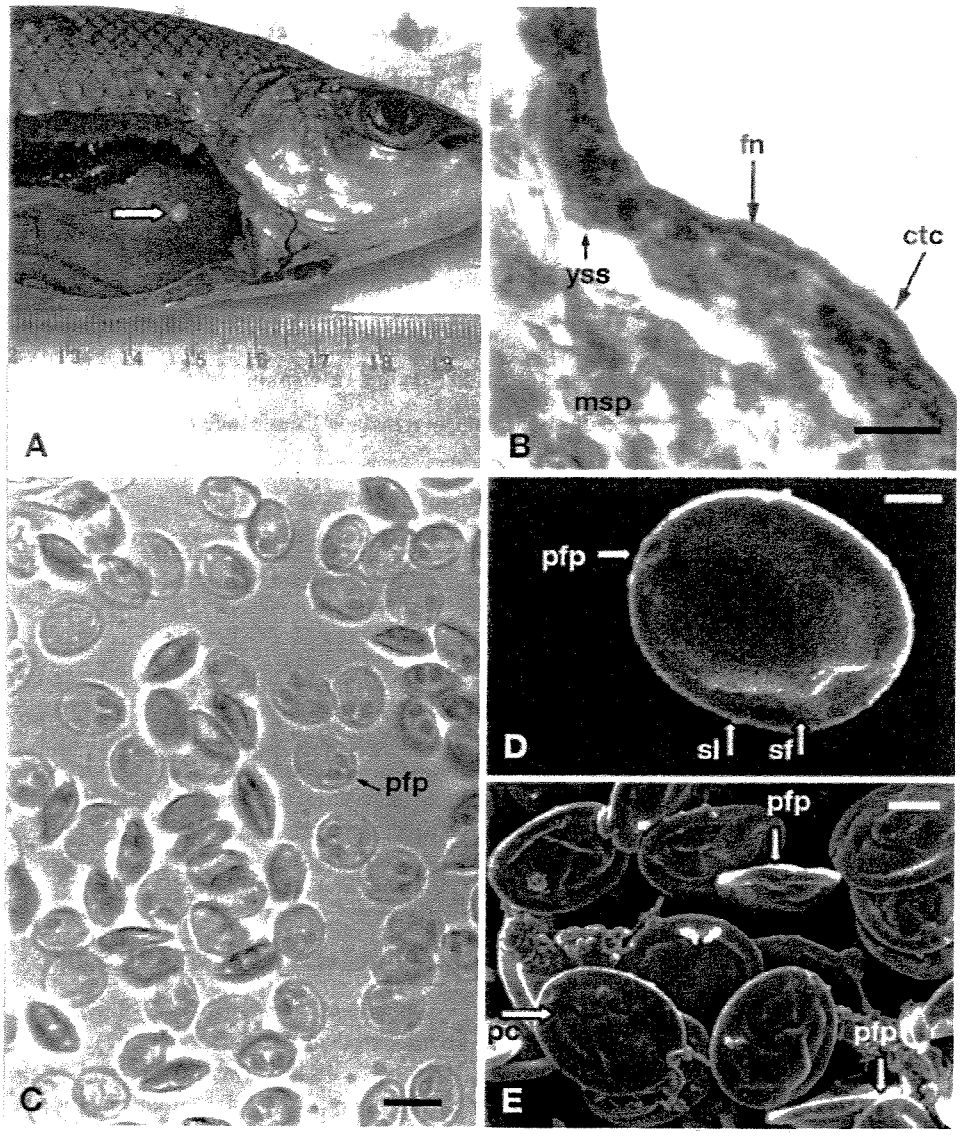

Fig. 1. *Myxobolus porofilus* sp. n. in the visceral cavity of *Prochilodus lineatus*. **A** - Plasmodium on the pyloric caecum of a young fish. **B** - Histological section of a plasmodium. Sirius red stain. **C** - Photomicrograph of spores in a fresh preparation. **D** - Scanning electron micrograph of a spore processed by method 1. **E** - Scanning electron micrograph of spores processed by method 2. Note that the spores appear shrunken, probably because of the processing artefact. Abbreviations: ctc - connective tissue capsule; fn - fibrocyte nucleus; msp – mature spores; pfp – polar filament pore; pc – polar capsules; sf – sutural folds; sl – robust suture line; yss - young sporogonic stages. Scales bars: B = 10 μm; C = 5 μm; D = 1 μm; E = 2 μm.

convergent anterior end (the polar capsules are of unequal size in *M. absonus* and the anterior end is divergent in *M. macroplasmodialis*). *M. porofilus* also has a conspicuous polar filament pore at the anterior end of one side of the sutural line. In addition to these features, *M. porofilus* was found in a host species of another family. According to Molnár et al. (1998), although little is known about the host specificity of *Myxobolus* species, the number of species with a large host range is low and most species appear to be strictly host-specific or capable of developing only in closely related fishes. The specimens of *P. lineatus* examined in this study were confined to a pond with two fish species of the family Characidae (*B. cephalus* and *P. mesopotamicus*) and one of the family Anostomidae (*L. macrocephalus*). However, *M. porofilus* was detected only in specimens of *P. lineatus*, indicating a possible host specificity, at least at the family level. Based on the morphological characteristics of the spores and plasmodia, the site of infection and the fact that this is the first report of a *Myxobolus* sp. in a species of the family Prochilodontidae, we consider *M. porofilus* to be a new myxosporean species.

The plasmodia of *M. porofilus* were found compressed between the wall of the visceral cavity and the pyloric caecum, but the site of initial development is unknown. Molnár et al. (1998) suggested that the initial development of *M. macroplasmodialis* occurred in the serous membrane of the visceral cavity or abdominal organs, and became detached from these sites only at an advanced stage of development. The same cycle may apply to the development of *M. porofilus*, with the serous membrane of the visceral cavity or more probably the pyloric caecum being the site of initial development since the plasmodia were always found repousing on this organ.

The prevalence and intensity of *M. porofilus* was low, with only 2.5% of *P. lineatus* being infected and the specimen with the highest parasitaemia had only two plasmodia,

making it difficult to draw conclusions about the epidemiology of this parasite. The fact that *M. porofilus* was found only in specimens examined in the winter initially suggested a seasonal incidence. However, since this parasite was not detected in fish examined in the winter of 2001, it is possible that only young fish are susceptible to infection.

ACKNOWLEDGMENTS. The authors thank the Laboratório de Microscopia Eletrônica at the Laboratório Nacional de Luz Síncrotron (LNLS), Campinas, SP, for use of the electron microscope.

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Capítulo 2

Histopathology and ultrastructure of *Henneguya* sp. 1, a new myxosporean infecting *Prochilodus lineatus* (Pisces; Prochilodontidae) cultivated in Brazil

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The histological and ultrastructural characteristics of a new species of Henneguya *and the host reactions to infection by this species are reported.* Henneguya *sp. 1 was found in the inter and intralamellar regions of the gills of* Prochilodus lineatus *(Valenciennes, 1836) obtained from a fish farm in Brazil. The plasmodia were white and round or ellipsoidal and measured 0.2 to 1 mm in length. The development of the parasite was asynchronous and the mature spores were fusiform, with a total length of 71 ± 1.4 μm, a width of 4.6 ± 0.2 μm, and a body length of 16.6 ± 0.54 μm. The caudal process was 52.6 ± 1.5 μm long, and the polar capsules were elongate (length - 6.1 ± 0.19 μm, width - 1.6 ± 0.15 μm) and of equal size. The polar filament was coiled in 10-11 turns. The prevalence of the parasite was 48.3% and did not vary significantly with the seasons or host size.*

Key words: *Henneguya* sp. 1, Myxosporea, Prochilodontidae, *Prochilodus lineatus*, gill, Brazil

INTRODUCTION

Prochilodus lineatus (Valenciennes, 1836) is a detritivorous teleost popularly known as curimba or curimbatá, is widely distributed in South American rivers and attains a relatively large size, with some specimens measuring 75 cm in length and weighing 8.2 kg (Godoy 1975). This species, which has a high reproductive capacity, rapid growth, and good acceptance on the market, is one of the most consumed fish species in Brazil. This consumption has led to increased interest in cultivation of *P. lineatus* on fish farms throughout the country.

Until recently, *Myxobolus porofilus* Adriano, et al., 2002, was the only myxosporean species reported to parasitise *P. lineatus.* However, during a survey of myxosporean parasites of fish species cultivated in Brazil, we found a new species of *Henneguya* parasitising the gills of *P. lineatus.* This species is described based on light microscopy, scanning electron microscopy, transmission electron microscopy and on the histopathological effects produced in the host.

MATERIALS AND METHODS

Four-month old specimens of *P. lineatus* (curimba), *Piaractus mesopotamicus* (Holmberg, 1887) (pacu), *Brycon cephalus* (Günther, 1869) (matrinxã) and *Leporinus macrocephalus* Garavello & Britski, 1988 (piauçu) obtained from artificial reproduction programs were released in a pond at Center for the Research and Management of Continental Fishing Resources (CEPTA/IBAMA) located in the municipality of Pirassununga, in the state of São Paulo, Brazil. Each month, five specimens of each species were examined for the presence of myxozoan parasites from March 2000 to February 2002.

Immediately after collection, the fish were transported alive to the laboratory where they were killed by transection of the spinal cord, before being measured and necropsied. The parasite was identified according to Lom and Arthur (1989), and measurements from 40 fresh mature spores were obtained using a micrometer incorporated into the microscope eyepiece and were expressed as the mean ± standard deviation (SD). Smears containing free spores were stained with Giemsa's solution and mounted in low viscosity mounting medium (Cytoseal^{TM}) as permanent preparations. For histological analysis, the gills were fixed in 10% buffered formalin for 24 h, embedded in paraffin, cut into sections 4 μm thick and stained with sirius red (Adriano et al. 2002), haematoxylin and eosin, and PAS. For scanning electron microscopy, free spores were deposited on a coverslip coated with poly-L-lysine and fixed for 2 h at room temperature with glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). After washing in the same buffer, the preparations were dehydrated in ethanol, dried by CO_2 critical point drying, coated with metallic gold and examined in a JEOL JSM 35 microscope operated at 15 kV. For transmission electron microscopy, fragments of gills containing plasmodia were fixed in 2.5% glutaraldeyde in cacodylate buffer (2 h), dehydrated in increasing concentrations of acetone and embedded in Epon-Araldite resin. Ultrathin sections double stained with uranyl acetate and lead citrate were examined in a LEO 906 electron microscope operated at 60 kV.

The chemical and physical properties of the pond water, including dissolved oxygen levels and temperature, were measured daily. Other properties, such as alkalinity, pH, NH_3 and hardness, were measured weekly. Pearson's correlation was used to determine whether there was any correlation between the chemical and physical characteristics of the water

and the prevalence of the parasite. The effect of season and host (fish) size on prevalence was tested using the χ^2 test.

RESULTS

Of the four fish species studied, only specimens of curimba had the parasite. Of 120 curimba examined, 20 were 7-15 cm long, 78 were 15,1-25 cm long and 22 were 25,1-30,5 cm long. Fifty-eight fish (48.3%) had plasmodia of an undescribed myxosporean species belonging to the genus *Henneguya* in their gills.

The parasite was found throughout the duration of the study and its occurrence did not vary significantly from one season to another ($\chi^2 = 10.82$, df = 7, ns: non significant) or with host size ($\chi^2 = 0.39$, df = 2, ns). There was no correlation between the prevalence of the parasite and the chemical and physical characteristics of the water, such as alkalinity (r = 0.0339: p = 0.8723), pH (r = 0.3745: p = 0.0651), hardness (r = -0.0326: p = 0.8770), NH_3 (r = -0.0406: p = 0.8472) and temperature (r = 0.1368: p = 0.5145), but there was a significant correlation with the dissolved oxygen levels (r = 0.5244: p = 0.0071).

Description

Henneguya sp. 1

(Figs 1-4)

Vegetative stage - Plasmodial form white, round or ellipsoidal, measured up to 1 mm. Ultrastructural analysis showed that the development of the parasite was asynchronous since generative cells, sporoblasts and mature spores were seen in the same plasmodium (Fig. 2B). The plasmodia were surrounded by a capsule of connective tissue (Fig. 1D, 2A,

B). The plasmodial wall consisted by two layers that communicated with the thin ectoplasmic zone of the plasmodium through numerous pinocytotic canals. These canals terminated in pinocytotic vesicles (Fig. 2A). The spores had a binucleate sporoplasm with a few dark randomly scattered sporoplamosomes (Fig. 2C, D). Electron-dense spherical inclusions were seen in the space between the polar capsules and valve on two sides of the spore (Fig. 3A).

Spores - Mature spores were fusiform in face view, with a total length of 71 ± 1.4 μm, a width of 4.6 ± 0.2 μm, and a body length of 16.6 μm ± 0.54 μm (Fig. 1A). The valve surfaces were smooth and prolonged by a caudal process 52.6 ± 1.5 μm long (Fig. 3D). The polar capsules were elongate (length - 6.1 ± 0.19 μm, width - 1.6 ± 0.15 μm) and of equal size. The polar filaments are thin and coiled in 10-11 turns aligned perpendicularly to the longitudinal axis of the capsule (Fig. 3C).

Type host - *Prochilodus lineatus* (Valenciennes, 1836) (Pisces, Characiformes, Prochilodontidae).

Site of infection - Gills (in the interlamellar and intralamellar space).

Prevalence - 58/120 (48.3%) of *P. lineatus* were infected.

Locality – Center for the Research and Management of Continental Fishing Resources CEPTA/IBAMA, Pirassununga, in the state of São Paulo, Brazil.

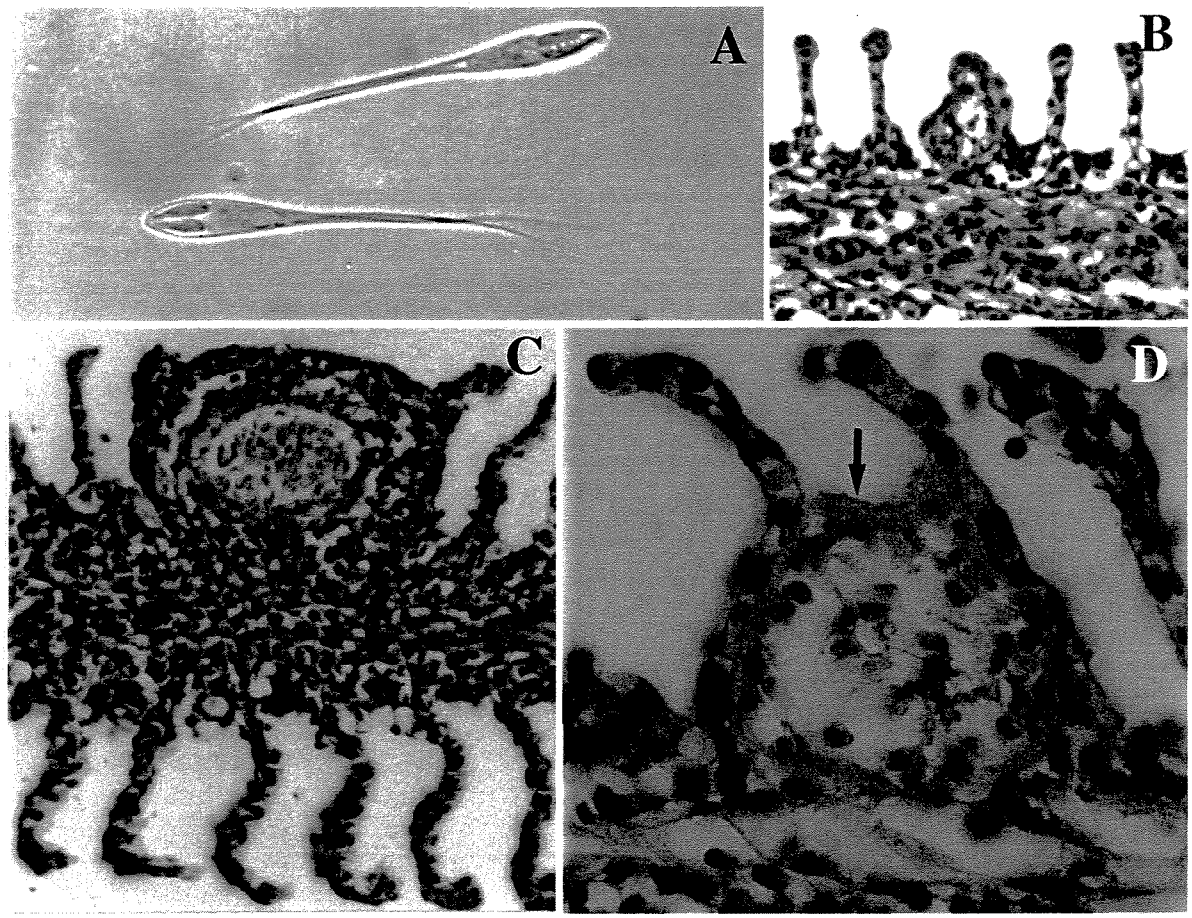

Fig. 1: *Heneguya* sp. 1 from *Prochilodus lineatus*. A: spores in a fresh preparation. 3700x. B-D: histological sections of gills. B: young plasmodium in the intralamellar epithelium. 1160x. C: interlamellar pasmodium. Note the severe hyperplasia of the epithelial cells. 1640x. D: young plasmodium in the interlamellar epithelium. Note the collagen capsule surrounding the plasmodium (arrow). 3400x.

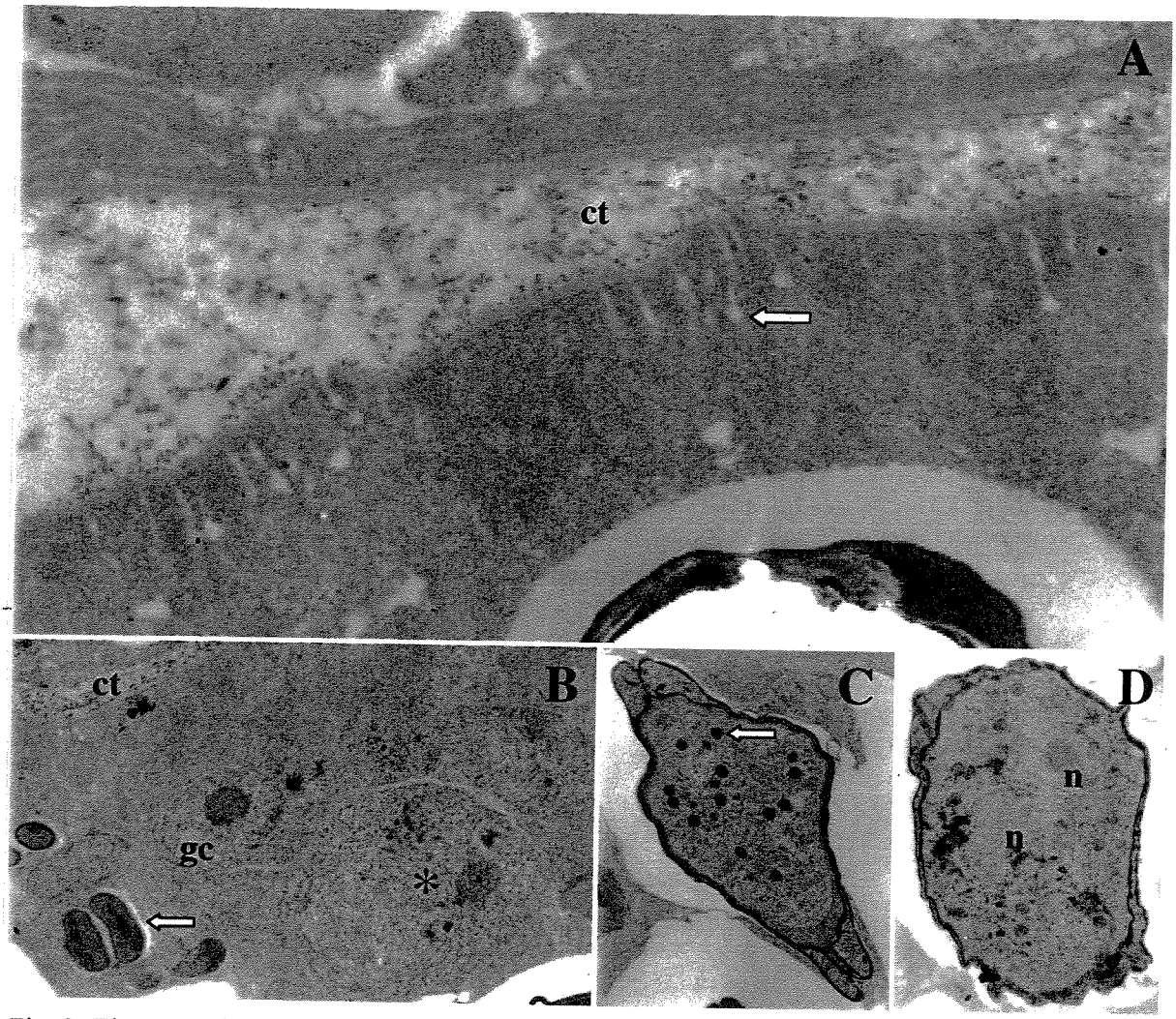

Fig. 2: Electron micrographs of *Henneguya* sp. 1 from *Prochilodus lineatus*. A: section of the host-parasite interface showing the pinocytotic canals terminating in pinocytotic vesicles (arrows) and the capsule of connective tissue surrounding the plasmodium (ct). 19200x. B: Periphery of a plasmodium showing generative cell (gc), spore in early developmental stage (*), mature spores in the caudal level (arrow) and the capsule of connective tissue (ct). 10680x. C-D: transversal sections of young spores in the sporoplasm level. C: showing the sporoplasmosomes (arrow). 10850x. D: two nuclei (n). 12930x

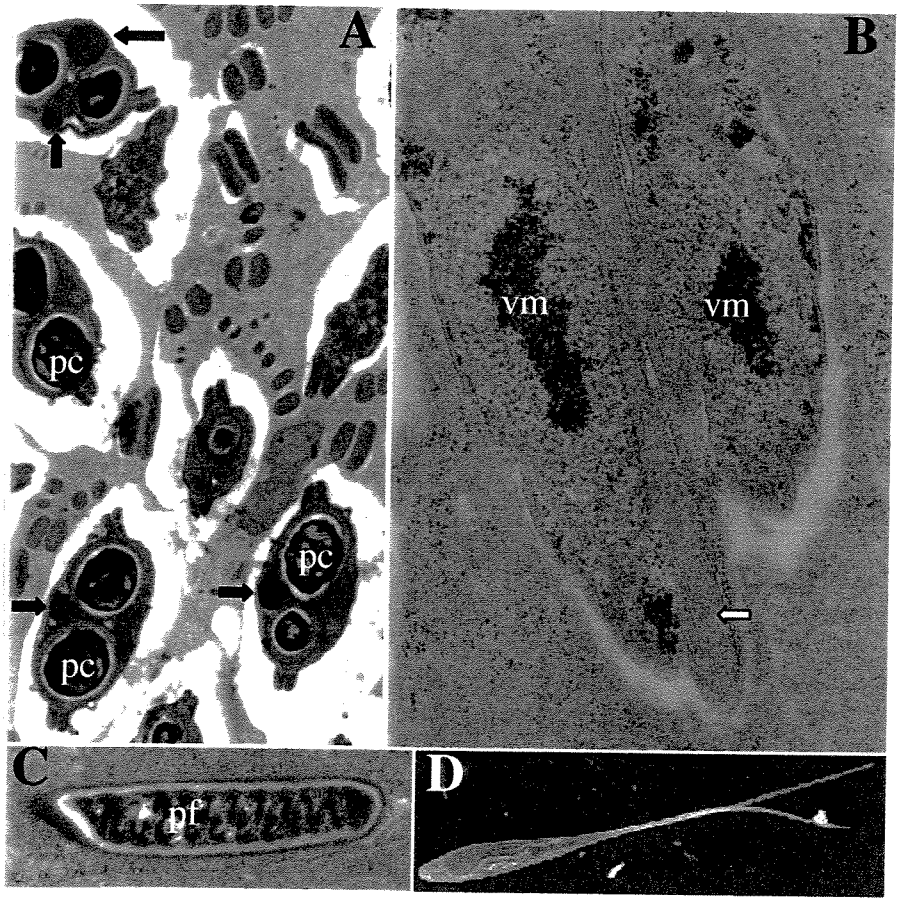

Fig. 3: Electron micrographs of spores of *Henneguya* sp. 1 A: transversal sections of mature spores in the polar capsule (pc) level showing the electron-dense spherical inclusions (arrows). 6120x. B: section of the tail showing longitudinal microtubules (arrows) and valve-forming material (vm). 28670x. C: longitudinal section of a polar capsule showing its polar filaments (pf). 14000x. D: scanning electron image of a mature spore. 3730x.

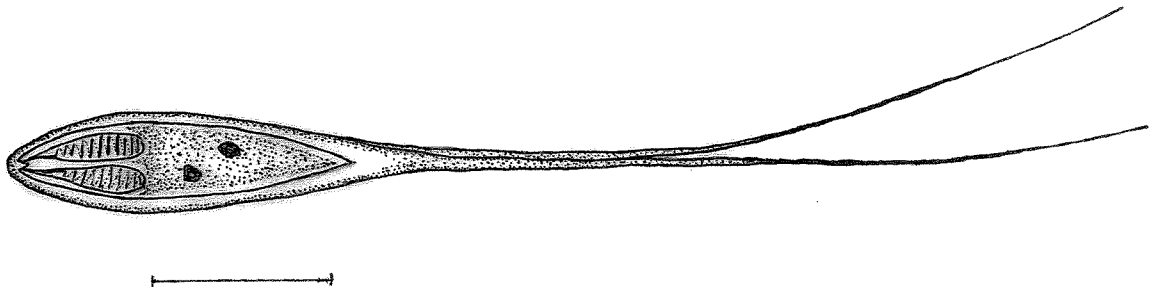

Fig. 4: Schematic representation of mature spores of *Henneguya* sp. 1 Bar = 10 μm.

Histopathology - Histological analysis showed that the parasite developed in the interlamellar and intralamellar gill regions (Figs. 1B, D) and that the plasmodia were surrounded by a thin collagen capsule (Fig. 1D). In an advanced developmental stage, the parasite caused deformation of the lamellar structures, pushed aside the neighbouring gill lamellae, produced fusion lamellar, and caused severe hyperplasia in epithelial cells, but not mucous cells (Fig. 1C). No inflammatory reaction was observed in infected fish.

DISCUSSION

The morphology and dimensions of *Henneguya* sp. 1 spores were compared with those of other *Henneguya* species identified in South American fishes. Only *Henneguya piaractus,* Martins & Souza, 1997, which parasites *P. mesopotamicus* and *Henneguya pilosa* Azevedo

& Matos, 2003, which infects *Serrasalmus altuvei*, showed similar morphology to *Henneguya* sp. 1. However, *Henneguya* sp. 1 had a greater spore body length (16.6 μm) than *H. piaractus* (12.7 μm) but smaller than *H. pilosa* (21.1 μm). The total length of *Henneguya* sp.1 (71 μm) was greater than *H. piaractus* and *H. pilosa* (52.5 μm and 54.2 μm, respectively). In addition, *Henneguya* sp. 1 had 10-11 turns in the polar filament, while *H. piaractus* had 8-9 and *H. pilosa* 11-13. In addition to these differences seen in light microscopy, ultrastructural analysis revealed a complex network of densely ramified granulo-fibrillar masses covering the spores of *H. pilosa* (Azevedo & Matos, 2003) and numerous lipid-like droplets in the sporoplasm of *H. piaractus* (see chapter 4). These structures were not seen in *Henneguya* sp. 1, but the spores of this species had electron-dense, spherical inclusions located in the space between the polar capsules and valve. Similar structures had also been reported in *H. pilosa* (Azevedo & Matos, 2003).

Henneguya sp. 1 is the largest South American species of this genus described so far. Compared with *Henneguya* species from other geographical regions, the dimensions of its spores are smaller only than those of *Henneguya clariae* Abolarin, 1971 (Nigeria), *Henneguya gigantea* Nemecek, 1911 (Hungary), *Henneguya pellis* Minchew, 1977 (USA), *Henneguya sebasta* Moser & Love, 1975 (USA) and *Henneguya tunghuensis* Chen, 1998 (China) (Eiras, 2002).

Ultrastructural analysis showed that sporogenesis in *Henneguya* sp. 1 differed from other *Henneguya* species (Current, 1979; Azevedo & Matos, 2002; El-Mansy & Bashtar, 2002; Vita et al., 2003) by the presence of electron-dense spherical inclusions located in the space between the polar capsules and valve, except perhaps for *H. pilosa* (Azevedo & Matos, 2003).

The plasmodia of *Henneguya* sp. 1 were surrounded by a thin layer of collagen fibres, that prevented direct contact of the plasmodium wall with the host cells. The plasmodia of *Henneguya adiposa* also were reported to be surrounded by collagen fibres that prevented direct contact with host cells (Current 1979). This lack of contact, suggested that this parasite incorporated only interstitial material (Current 1979). A similar condition was suggested for the intralamellar form of *Henneguya exilis* (Current & Janovy 1978). In contrast, the interlamellar form of this species, which is capable of direct contact with the host cells, can take up host cell cytoplasm and interstitial material through pinocytotic canals (Current & Janovy 1976). Thus, the presence of collagen fibres surrounding the plasmodia of *Henneguya* sp. 1 suggests that this species obtains nutrients by taking up interstitial material through pinocytotic canals.

Like *H. exilis*, *Henneguya* sp. 1 developed in the interlamellar and intralamellar space, but in contrast to *H. exilis* that present direct contact between the membrane and the host cells in some regions, in the interlamellar form, and has the membrane covered by a fine granular coat of uniform thickness, in the intralamellar form (Current & Janovy 1978), no ultrastructural difference was observed between the intralamellar and interlamellar forms of the plasmodia of *Henneguya* sp. 1.

Of the chemical and physical properties of the water evaluated, only the dissolved oxigen level presented a weak correlation with the prevalence of *Henneguya* sp. 1, but the prevalence did not vary significantly with season or host size. This finding indicate that the life cycle of this parasite is not influenced by environmental factors or host development, as also reported by Barassa et al. (2003) for *Henneguya chydadea* Barassa et al., 2003 infecting the gills of *Astyanax altiparanae.* In contrast, Molnár (1998) observed that the prevalence of *H. creplini* Gurley, 1894 varied with season and host size.

No important inflammatory response was observed in specimens of curimba infected by *Henneguya* sp. 1, but a massive infection by this parasite could compromise the gill functions by deforming of the lamellar structures and reducting the gill area.

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Capítulo 3

An ultrastructural and histopathological study of *Henneguya* sp. 2, a new myxosporean (Myxobolidae) infecting *Piaractus mesopotamicus* (Characidae) cultivated in Brazil

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Abstract During a study of myxosporean parasites of cultivated freshwater fish, a new myxosporean species, *Henneguya* sp. 2, was discovered. Of the 120 *Piaractus mesopotamicus* sampled, only 10 specimens (8.3%) were infected. Yellow, round plasmodia measuring 0.5 - 3 mm were found in the serous membrane of the visceral cavity and in the tunica externa of the swim bladder. The wall of the plasmodia consisted of only a single membrane. Adjacent to the inner surface of the plasmodium wall was a thick ectoplasm with an outer layer composed of thin granules and an internal layer rich in mitochondria. Pinocytotic canals were absent. Sporogenesis was asynchronous, with the earliest developmental stages aligned prevailingly along the endoplasmic periphery and mature spores in the central zone. The mature spores were pyriform, (total length – 33.3 ±

1.5 μm, men ± S.D; width – 4.1 ± 0.4 μm; body length – 11.4 ± 0.3μm; caudal process length – 24.1 ± 1.5 μm). The polar capsules were elongated (length – 4.0 ± 0.4 μm, width – 1.6 ± 0.2 μm). The development of the parasite in the swim bladder produced thickening of the tunica externa and a granulomatous reaction. There was no correlation between the prevalence of the parasite and the chemical and physical characteristics of the water. Infection was recorded only in juvenile specimens ranging in size from 9.5 to 20 cm.

Introduction

Cultivated and wild fishes are infected by numerous myxosporean species of the genus *Henneguya* Thelohan, 1892, some of which are important pathological agents (Kalavati and Narasimhamurti 1985, Lom and Dyková 1995, Martins et al. 1999). Eiras (2002) listed a total of 146 *Henneguya* species that parasitize fishes in different parts of the world. In South America, 32 *Henneguya* species have been reported in freshwater fishes (Barassa et al. 2003), and are the most common myxosporeans parasites of the fish, with the gills being the organ most frequently infected (Gioia and Cordeiro 1996).

A few histological (Martins et al. 1999, Adriano et al 2002, Barassa et al. 2003) and ultrastructural (Rocha et al. 1992, Azevedo et al. 1997, Azevedo and Matos, 1989, 1995, 1996, 2002, 2003, Vita et al. 2003) studies have been done on *Henneguya*, using Brazilian fish. The present study is part of an investigation into the ultrastructural and histopathological characteristics of myxosporean parasites of freshwater fish cultivated in Brazil. Using light, scanning electron and transmission electron microscopy, we describe a new species of *Henneguya* infecting *Piaractus mesopotamicus* (Holmberg, 1887), popularly known as pacu, a Brazilian fish species of considerable economic importance.

Materials and methods

During a survey of Myxozoa parasites done at the Research and Management of continental fishing Resources CEPTA/IBAMA, located in the municipality of Pirassununga, in the state of São Paulo, Brazil, specimens of four fish species (pacu, *P. mesopotamicus* (Characidae); matrinxã, *Brycon cephalus* (Gunther, 1869) (Characidae); curimbata, *Prochilodus lineatus* (Valenciennes, 1836) (Prochilodontidae); and piauçu, *Leporinus macrocephalus*, Garavello & Britski, 1988 (Anostomidae) four months old were stocked in a pond and monitored for two years. Five specimens of each species were examined monthly (March 2000 to February 2002) for the presence of Myxozoa parasites. Immediately after collection, the fish were transported alive to the laboratory where they were killed by transection of the spinal cord, and then measured, weighed and necropsied.

The parasite was identified according to Lom and Arthur (1989), and the measurements from 40 fresh mature spores of different cysts were obtained using a micrometer incorporated into the eyepiece. The dimensions were expressed as the mean ± standard deviation (S.D.). Smears containing free spores were stained with Giemsa's solution and mounted in low viscosity mounting medium (Cytoseal^{TM}) to provide permanent slides (Adriano et al. 2002).

For histological analysis, the cysts were fixed in 10% buffered formalin for 24 h, embedded in paraffin, cut into 4 μm thick sections and stained with hematoxylin and eosin and sirius red (Adriano et al. 2002). For scanning electron microscopy, free spores were deposited on a coverslip coated with poly-L-lysine and fixed for 2 h at room temperature with glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). After washing in the

same buffer, the preparations were dehydrated in ethanol, dried by CO_2 critical point drying, covered with metallic gold and examined in a Joel JMS 35 scanning electron microscope operated at 15 kV. For transmission electron microscopy, the cysts were fixed in 2.5% glutaraldeyde in cacodylate buffer (2 h), post-fixed in 1% OsO_4 (2 h.), dehydrated in increasing concentrations of acetone and embedded in Epon-Araldite resin. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined in a LEO 906 transmission electron microscope operated at 60 kV.

The chemical and physical properties of the pond water including dissolved oxygen levels and temperature were measured daily. Other proprieties, such as alkalinity, pH, NH_3 and hardness, were measured weekly. Pearson's correlation analysis was used to determine whether there was any correlation between the characteristics of the water and the prevalence of the parasite. The procedure Proc Catmod of the SAS (Statistical Analysis System) statistical package (SAS Institute, Inc. 1986) was used to test the effect of season on prevalence of the parasite. The independent variable was season and the response variable was the presence of parasites. The frequency of parasitism was the weight variable.

Results

Of the fish species studied, only specimens of pacu had the parasite. Of the 120 pacu specimens examined, 45 were 5 - 10 cm long, 41 were 10.1 - 20 cm long and 34 were 20.1 - 36 cm long. Ten fish (8.3%) had plasmodia of an unknown *Henneguya* species. Infection was recorded only in specimens ranging in size from 9.5 to 20 cm.

There was no correlation between the prevalence of the parasite and the chemical and physical characteristics of the water such as dissolved oxygen levels ($r = -0.1147$; $p =$

0.5934), alkalinity (r = -0.3051; p = 0.1471), pH (r = 0.0217; p = 0.9197), hardness (r = -0.3085; p = 0.1424), NH_3 (r = 0.3415; p = 0.1024) and temperature (r = -0.0651; p =0.7623). The presence of the parasite was observed only between the spring of 2000 and the winter of 2001. The prevalence in the autumn was of only 9.1% but reached 25% in the winter. In spring and summer, the prevalences were 13.3% and 21.4%, respectively. The differences of prevalence did not vary significantly ($\chi^2 = 0.40$, df = 3, ns: non significant). The intensity of the parasite was low, and ranged from 1 to 5 plasmodia per fish. The specimen with the highest parasitemia was collected in February 2001 and measured 14 cm. No fish mortality was observed during the study.

Description

Henneguya sp. 2 Figs 1-4

Vegetative stages. The plasmodial forms were yellow, round and measured 0.5 - 3 mm. The parasite occurred in the serous membrane of the visceral cavity and in the tunica externa of the swim bladder. The development was asynchronous and the earliest stages were aligned at the periphery of the plasmodium while the mature spores appeared in the central zone (Fig. 1B, C).

Spores. The mature spores had a pyriform body in face view (Figs. 1A, 3E), with a total length of 33.3 ± 1.5 µm; a width of 4.1 ± 0.4 µm, and a body length of 11.4 ± 0.3 µm. In lateral view, the body spores were fusiform in shape, symmetrical and with a thin suture line in the junction of two thin valves. The valve surfaces were smooth and prolonged by a caudal process 24.1 ± 1.5 µm long (Fig. 3E). These caudal processes were linked to each other by an adhesive mass surrounding the spores, which gave the impression that there

was only one caudal process (Fig. 1A, 3E). The polar capsules were elongated, (length 4.0 ± 0.4 μm; width 1.6 ± 0.2 μm). The polar filaments coiled in six or seven turns were arranged perpendicularly to the long axis (Figs. 3D).

Type host: Piaractus mesopotamicus (Pisces: Characidae).

Site of infection: serous membrane of the visceral cavity and tunica externa of the swim bladder.

Prevalence: 10/120 (8.3%) of *P. mesopotamicus* were infected.

Locality: Research and Management of continental fishing Resources CEPTA/IBAMA located in the municipality of Pirassununga, the state of São Paulo, Brazil.

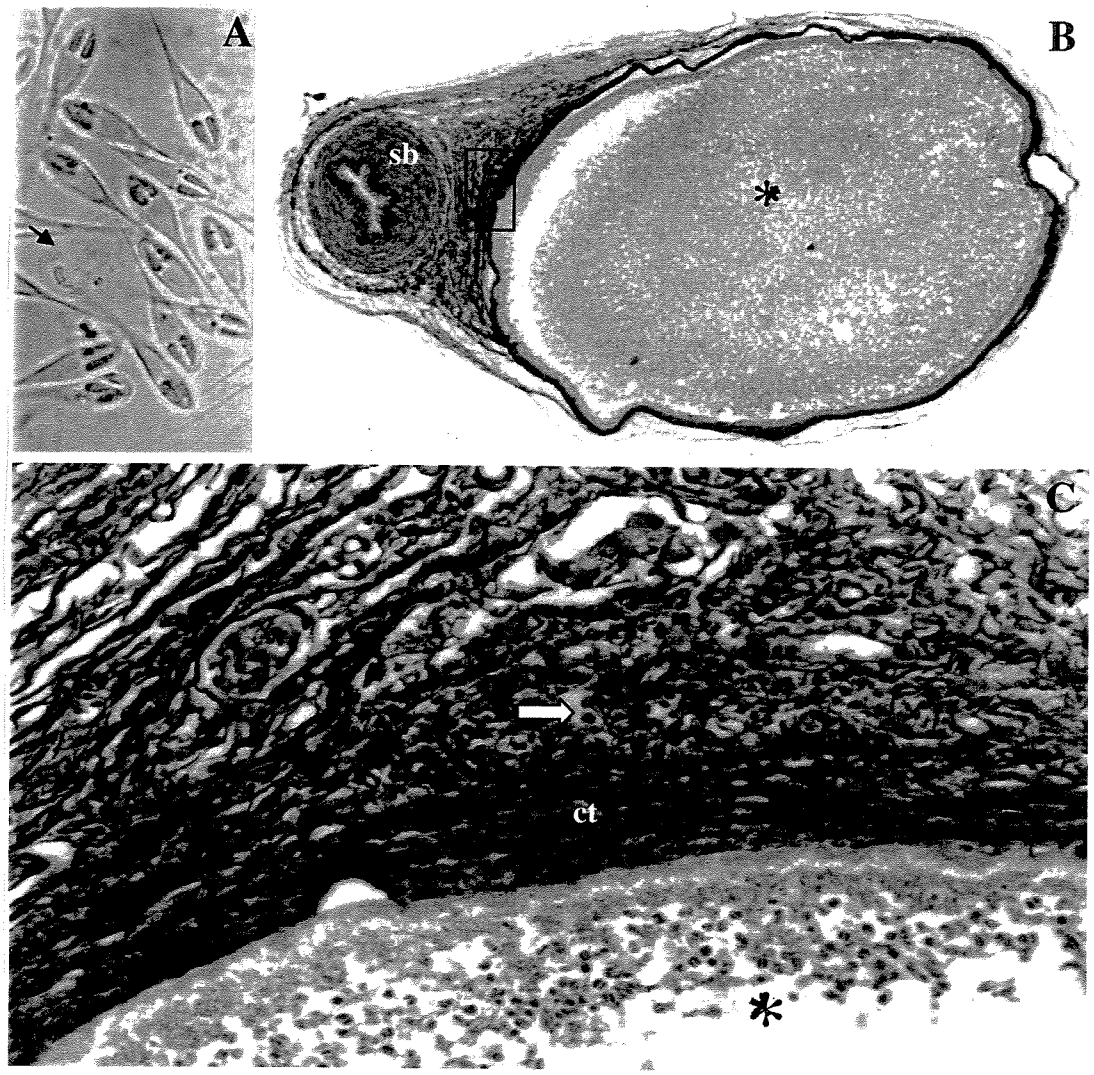

Fig. 1 Light photomicrographs of *Henneguya* sp. 2. **A** Fresh preparation showing mature and one immature (arrow) spores (x3800). (**B-C**) Histological sections of a plasmodium (*) found in the anterior end of the swim bladder (sb). **B** Panel shows thickening of the tunica externa (arrow) (x200). **C** Amplified part of **B**: note the capsule of connective tissue (ct) with collagenous fibres in the region nearest to the parasite and cellular elements typical of a granulomatous reaction in the outermost face (arrow) (x1680).

Histopathological analysis showed that the development of the plasmodia led to the formation of a thick capsule of connective tissue, which contained cellular elements and collagen fibers (Figs. 1B, C). The latter were more frequent in the region closest to the parasite, whereas the cellular elements were more common in the outermost face (Fig. 1C). The development of the parasite in the swim bladder produced thickening of the tunica externa and a granulomatous reaction was observed around the plasmodia. This reaction occurred around all of the plasmodium, but was more evident on the surface adjacent to the tunica interna of the swim bladder (Fig. 1C).

Ultrastructural study revealed a thin layer of fibril close to the plasmodial wall and an outer capsule of connective tissue composed of cross-linked collagen fibres surrounding the plasmodia (Fig. 2A). The wall of the plasmodia consisted of only a single membrane. A thick ectoplasm was present on the internal surface adjacent to the wall. At the interface with the membrane, this ectoplasm consisted of thin granules and no pinocytotic canals were observed. In the subsequent layer, there were large numbers of mitochondria, numerous generative cells and young disporoblastic pansporoblasts in different developmental stages (Figs. 2A, C-E). In advanced stages of maturation, the spores contained valvogenic cells with a large accumulation of electron-dense "valve-forming material" (Figs. 3C, D). The binucleated sporoplasm cell had numerous randomly scattered ribosomes and a few sporoplasmosomes. The polar capsules consisted of a zone dark outer, an intermediate electron-lucent layer and a internal granular content, containing the polar filament with six or seven turns (Figs. 3D). The spores were surrounded by a thin "sheath-like" membrane that involved the valve walls and formed the junction of the two tails (Fig. 3A-C).

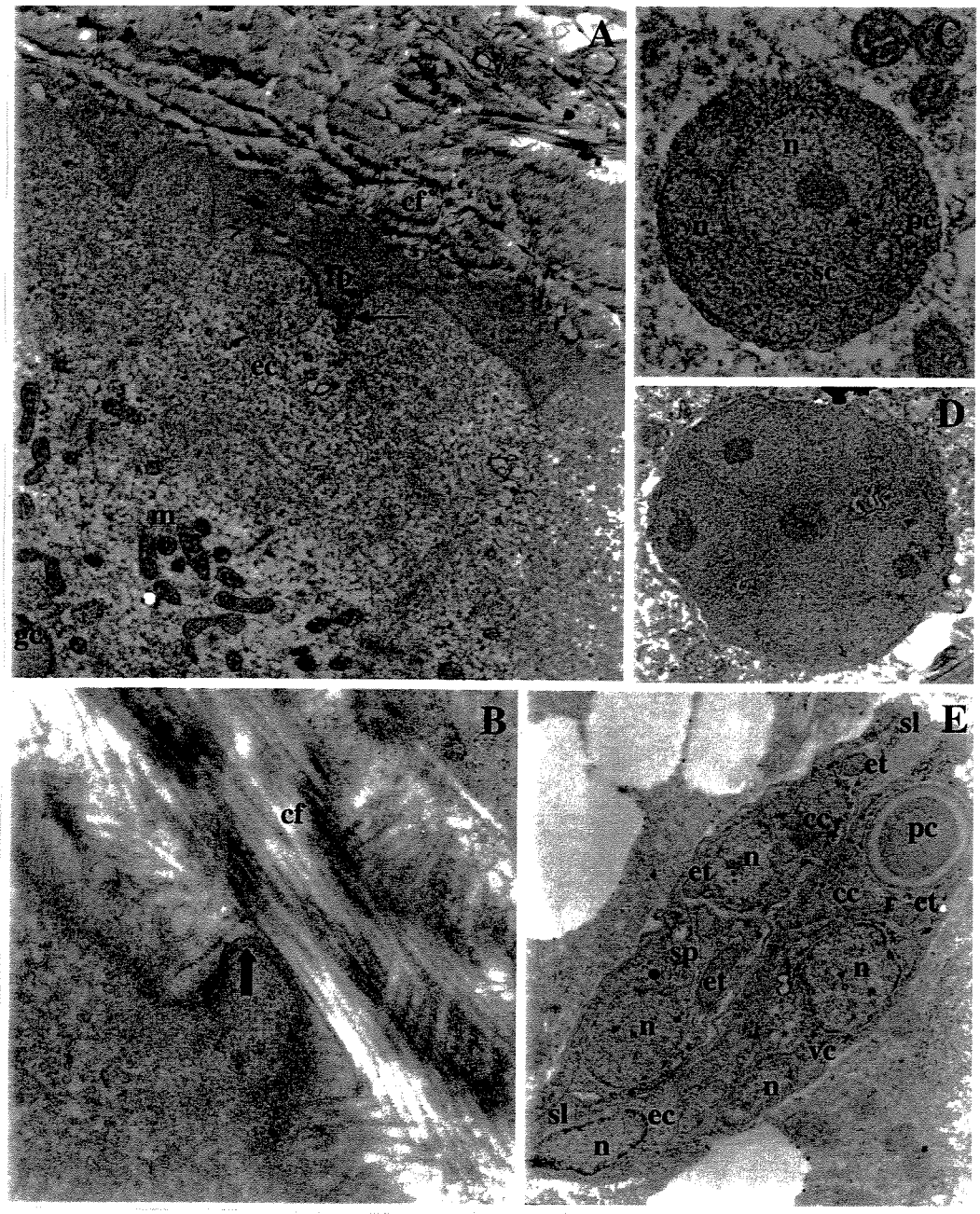

Fig. 2 Electron micrographs of *Henneguya* sp. 2 (**A-B**) section of the host-parasite interface. **A** Plasmodium showing the granular ectoplasm (ec), numerous mitochondria (m), a generative cell (gc), a folded single limiting membrane (arrows) surrounded by a thin layer of fibrils (fb) and network of collagen fibres (cf) (x7430). **B** Collagen fibres (cf) of the connective tissue capsule showing a characteristic orientation. Note the plasmodial wall with a single limiting membrane (arrow) (x34500). (**C-E**) Sporogonic developmental stages. **C** Secondary cell (sc) inside of a primary cell (pc). (x20862). **D** Pansporoblast consisting of undifferentiated cells. (x10431). **E** Pansporoblast with two sporoblasts. Note the envelope cell (ec), the valvogenic cells (vc), the sporoplasm cells (sp), the capsulogenic cells (cc), the nucleus (n), the polar capsule (pc), the external tubule (et) the sutural line (sl), and numerous scattered ribosomes (r) (x13060).

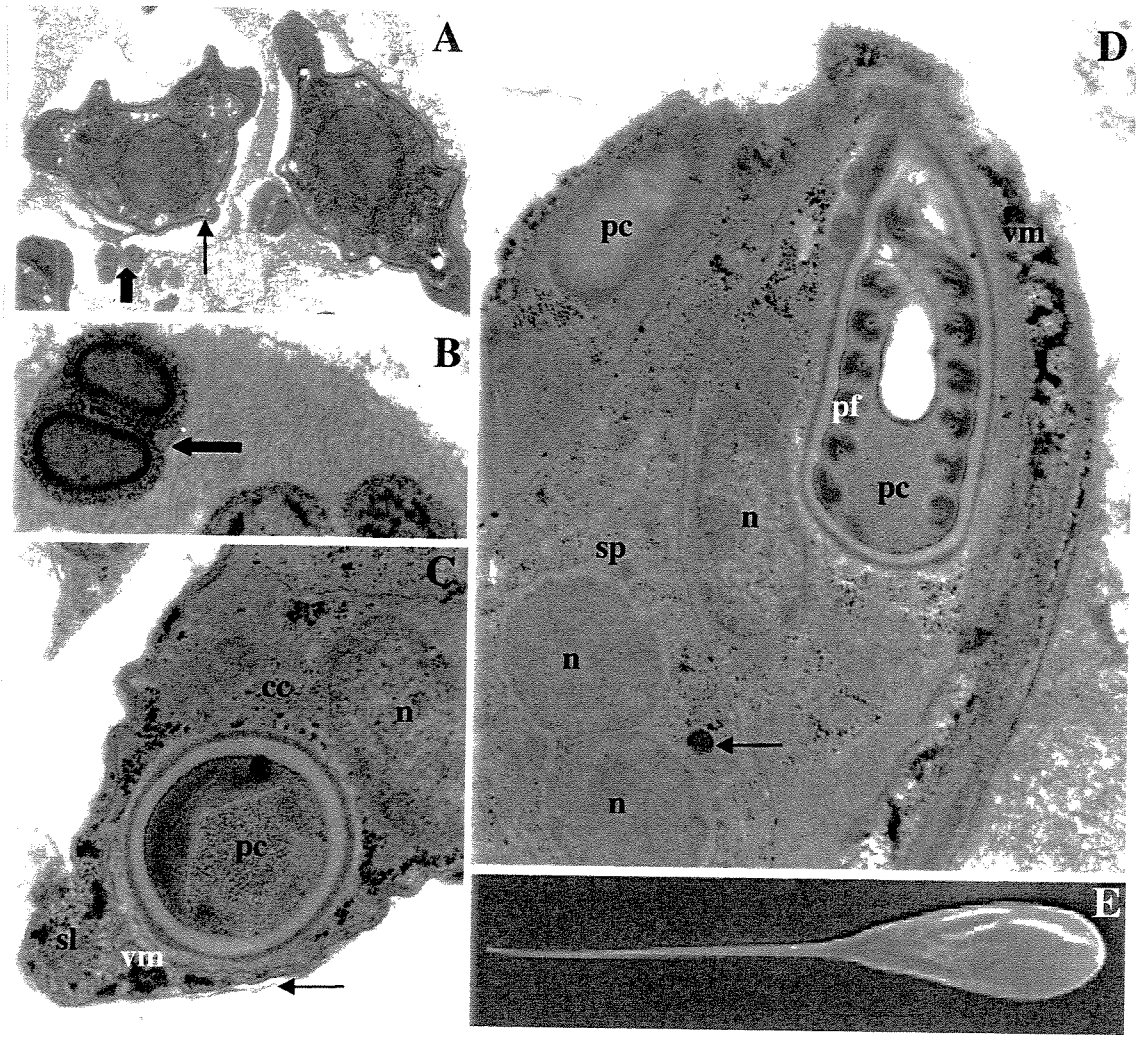

Fig. 3 Electron micrographs of spores of *Henneguya* sp. 2 (**A-D**) section of young spores. **A** Transversal section at the level of the sporoplasm cells (sp). Note the thin, sheath-like membrane surrounding the spores (arrows) (x12230). **B** Detail of transversal sections at the level of the caudal process showing the two tail projections united by a sheath-like membrane (arrows) (x60370). **C** Transversal section at the level of the capsulogenic cell (cc) showing the polar capsule (pc), sutral line (sl), valve-forming material (vm) and the sheath-like membrane surrounding the spores (arrows) (x32340). **D** Longitudinal section of the anterior portion of a young spore showing the capsulogenic cell (cc), polar filament (pf) within the polar capsule (pc), a sporoplasm cell (sp) with two nuclei (n) and a few sporoplasmosomes (arrow) (x24570). **E** Scanning electron image of a mature spore showing the junction of the tail projections. (x9400).

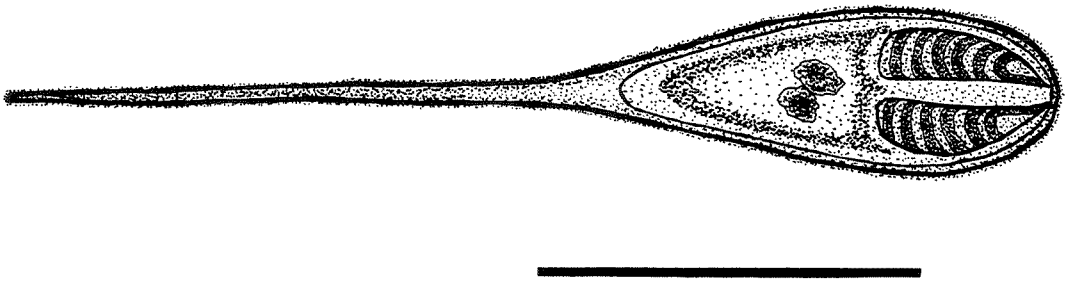

Fig. 4 Schematic representation of mature spores of *Henneguya* sp. 2. Bar = 10 μm.

Discussion

Henneguya sp. 2 was compared with other *Henneguya* species previously reported in South American fishes. Of the 32 *Henneguya* species recorded so far in the continent, only *Henneguya lutzi* and *Henneguya piaractus* have been found in *P. mesopotamicus* and the spores of both differ in shape and size from those of *Henneguya* sp. 2. Of the species described in other South American fish, *Henneguya santae* found in *Tetragnopterus santae* and *Henneguya leporinicola* found in *Leporinus macrocephalus* bear some morphological semblance to *Henneguya* sp. 2, although the first has a smaller caudal process (11.8 μm) and the latter a smaller body spore (7.6 μm) than *Henneguya* sp. 2 (body spore length 11.4 ± 0.3 μm, caudal process length 24.1 ± 1.5 μm). In *Henneguya travassosi* described in *Leporinus copelandi*, the spores are approximately equal in size to those of *Henneguya* sp. 2, but are oval in shape, with plasmodia white occurring in the muscles. *Henneguya*

malabaricus found in *Hoplias malabaricus* and *Henneguya adherens* found in *Acestrorhynchus falcatus* resemble *Henneguya* sp. 2 in spore size and by the presence of a sheath surrounding the spores. However, in both species the spores are ellipsoidal and the plasmodial forms occur in the gills.

The plasmodia of *Henneguya* sp. 2 occurred in the serous membrane of the visceral cavity and in the tunica externa of the terminal region of the swim bladder. The presence of myxosporeans in these tissues has also been reported by others. Molnár et al. (1998) suggested that the site of development of *Myxobolus macroplasmodialis* was the serous membrane of the visceral cavity. According to these authors, the plasmodia occurred free in the visceral cavity of *Salminus maxillosus* (Characidae), but the site of development can has been the serous membrane of the visceral cavity or organs, from which the parasite became detached only at an advanced stage of development. A similar situation was suggested by Adriano et al. (2002) for *Myxobolus porofilus* in the visceral cavity of *Prochilodus lineatus*.

The swim bladder was the site of sporogonic plasmodia or of the extra-sporogonic proliferative stages of myxosporeans whose sporogonic stages occur in other organs (Lom and Dyková 1995). The extra-sporogonic stage of *Sphaerospora renicola* has been implicated as the etiologic agent of swim-bladder inflammation (SBI) in European carp (Dyková and Lom 1988). According to these authors, SBI in the swim bladder wall generates an exsudative-proliferative inflammation, intra and extravascular localization of which is associated with oedema of the connective tissue, massive lymphocytic infiltration, hemorrhaging, and hyperplastic changes in the lamina propria epithelium. To date, the presence of *Henneguya* species infecting the swim bladder is restricted to *Henneguya rhomboides*, found in the swim bladder of *Carassius auratus auratus* in China (Eiras 2002).

The prevalence and intensity of *Henneguya* sp. 2 were low, with only 8.3% of the specimens of *P. mesopotamicus* being infected and the specimen with the highest parasitaemia had only five plasmodia. Other workers have also reported a low intensity and prevalence of myxosporeans in visceral organs. Molnár et al. (1998) recorded a prevalence of 9.7% in *S. maxillosus* parasitized by *M. macroplasmodialis*. Most of the fish examined by these authors had only one plasmodium, with only one specimen harbouring 28 plasmodia. Molnár (2002) found plasmodia of *Myxobolus cyprinicola* in 14.6% of the specimens of *Cyprinus carpio* examined in Europe and the intensity ranged of 1 to 11 plasmodia per specimens. Adriano et al. (2002) reported a prevalence of 3% for *P. lineatus* infected by *M. porofilus* and the fish with the highest parasitemia had only two plasmodia.

There was no correlation between the chemical and physical properties of the water and the prevalence of the parasite. Likewise, there were no significant seasonal differences in the prevalence. However, the occurrence of the parasite was related to the host's size, since only specimens ranging in size from 9.5 to 20 cm were infected. These finding are similar to those of Adriano et al. (2002), who also reported *M. porofilus* only in juveniles of *P. lineatus,* but differ from those of Mitchell (1988) who reported that infection by *Myxobolus muelleri* and *Myxobolus dujardini*, parasites of *Psychochelus oregonenseis*, *P. caurinus* and *Richardsonius blateatus*, was higher in adult fish. Molnár (1998) also recorded the highest prevalence of *Henneguya creplini* in specimens of pikeperch (*Stizostedion lucioperca*) greater than 40 cm in length. The absence of *Henneguya* sp. 2 in very young *P. mesopotamicus* (< 9.5 cm) can be explained by the time required for the appearance of the plasmodia after the initial contact of the fish with the parasite, while the absence of the

parasite in adults (> 20 cm) may indicate that the fish acquire some type of resistance that prevents infection.

The specimens of *P. mesopotamicus* examined were confined to a pond with three other fish species, but *Henneguya* sp. 2 was found only in the pacu, suggesting host specificity.

Histopathological analysis of *Henneguya* sp. 2 showed that development of the parasite in the swim bladder caused thickening of the tunica externa and a granulomatous reaction around the plasmodia. This reaction, which occurred around all of the plasmodium and was more evident on the surface adjacent to the tunica interna of the swim bladder, is common in myxosporeans. Dyková and Lom (1978) reported a granulomatous inflammatory reaction in the gills of *Perca fluviatis* infected with *Henneguya psorospermica*, and Kalantan and Arfin (1991) recorded a granulomatous tissue response to *Myxobolus garrai* found infecting the musculature of *Garra tibanica* from Saudi Arabia. According to Dyková and Lom (1978), the response of fish soft tissues to myxosporidian infections involves displacement, atrophy or hyperplasia of the tissue surrounding the plasmodium during growth and maturation. In more advanced stages, when the cysts are full of mature spores, an inflammatory reaction occurs, resulting in the rapid replacement of the cyst by granulomatous tissue.

Ultrastructural analysis of the capsule of connective tissue surrounding the plasmodia of *Henneguya* sp. 2 revealed a thin layer of fibrils close to the plasmodial wall and an outer layer of cross-liked collagen fibres with a peculiar net-like organization. Current (1979) also observed a capsule of collagen fibres surrounding the plasmodia of *Henneguya adiposa*, a parasite of the adipose fin of *Ictularus punctatus*. However, in this case, the

tissue surrounding the parasite consisted of several layers of parallel collagen fibres, and the collagen immediately adjacent to the plasmodia consisted of randomly oriented fibres.

The capsule of collagen fibres prevented direct contact between the plasmodium wall of *Henneguya* sp. 2 and the host cells. According to Current (1979), the plasmodium wall of *H. adiposa* has a uniform coat and is surrounded by collagen fibres that allow it to take up only interstitial material. A similar situation was described by Current and Janovy (1978) for the intralamellar plasmodia of *Henneguya exilis*, which were covered by a fine granular coat that prevented direct contact between the parasite and the host cells.

No pinocytotic canals were observed in the ectoplasm of the plasmodia of *Henneguya* sp. 2 in contrast to findings for other *Henneguya* species (Current and Janovy 1976, 1978, Current 1979, Rocha et al. 1992, Hallett and Diamant 2001, El-Mansy and Bashtar 2002, Azevedo and Matos 2002, 2003). Current and Janovy (1976) suggested that pinocytotic canals are the principal means of feeding in *H. exilis* and showed that pinocytosis enabled the parasite to take up host cell cytoplasm. According to El-Mansy and Bashtar (2002), the plasmodium wall of myxosporeans is seen to be the organelle responsible to feeding the whole plasmodium and can supply various developing stages with suitable nutrients necessary for growth via the pinocytotic canals which may incorporate host cell cytoplasm through pinocytosis.

The developmental stages of myxosporeans, especially of coelozoic species, are assumed to feed by osmotrophy and by active transport of nutrients (Lom and Dyková 1995). Uspenskaya (1982) investigated myxosporeans with different localizations in fish and recorded all possible types of nutrition in the species investigated, including extracellular digestion of food by enzymes secreted by the parasite, contact or membrane digestion followed by active transport and/or pinocytosis, and phagocytosis followed by

intracellular digestion inside food vacuole. However, as pointed out by Lom and Dyková (1995), our knowledge of myxosporean nutrition is limited to microscopic observations and no experimental study of this aspect has been reported.

Thus, the role of the network of collagen fibres surrounding the plasmodium and the absence of pinocytotic canals linking the ectoplasm of *Henneguya* sp. 2 to the outside in the nutrition of this parasite remains to be clarified.

In conclusion, these results show that although *Henneguya* sp. 2 was unable to incorporate host cell cytoplasm, a characteristic associated with pathogenicity (Current and Janovy 1978, Current 1979, El-Mansy and Bashtar 2002), this species was, nevertheless, able to induce a granulomatous reaction around the plasmodia, indicating that it was pathogenic.

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Capítulo 4

Histology, ultrastructure and prevalence of *Henneguya piaractus* (Myxosporea) infecting the gills of *Piaractus mesopotamicus* (Characidae) cultivated in Brazil

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ABSTRACT: The histopathological and ultrastructural characteristics of *Henneguya piaractus* Martins & Souza, 1997 are reported. *H. piaractus* was found in the gill lamellae of *Piaractus mesopotamicus* (Holmberg, 1887) from a fish farm with a prevalence of 45%. Histological analysis showed that the plasmodia were of the intralamellar type. The development of the plasmodia resulted in marked dilatation of the infected lamellae, with the neighbouring lamellae being displaced laterally. Discreet epithelial hyperplasia was observed, but there was no inflammatory reaction. Ultrastructural analysis showed that the plasmodia had a single, thin wall that was in direct contact with the host cells. Pinocytic canals and points of phagocytosis were observed in the wall. The prevalence of the parasite varied with host size and the smallest prevalence occurred in hosts up to 10 cm long.

KEY WORDS: *Henneguya piaractus* . Myxozoa . *Piaractus mesopotamicus* . histology . ultrastructure . Brazil

INTRODUCTION

Aquaculture is a rapidly developing activity in Brazil. Among the species being considered for fish farming, considerable interest has focused on *Piaractus mesopotamicus* (Holmberg, 1887), a large riverine fish commonly known as pacu. This interest is attributable to the economic importance of this species, its good reproductive performance, and the quality of its flesh (Martins & Souza 1997). In this report, which is part of an ongoing investigation into the characteristics of myxosporean parasites of freshwater fish cultivated in Brazil, we describe the ultrastructural and histological aspects of the *Henneguya piaractus* Martins & Souza, 1997, a parasite of pacu gills. In particular, the interactions between the plasmodium and adjacent cells, the spore's characteristics and the histological alterations in the host in response to infection were examined. Among myxosporeans, the genus *Henneguya* Thélohan, 1892 is the most abundant in South America, with 32 known species (Barassa et al, 2003a). The importance of this genus as a pathogen of wild and cultivated fish has been discussed elsewhere (Dyková & Lom 1978, Current & Janovy 1976, Molnár 1998, Martins et al. 1997, 1999a, Vita et al. 2003). This is the first report of the ultrastructure of this *Henneguya* species.

MATERIALS AND METHODS

Young specimens of four fish species: pacu (*P. mesopotamicus*; Characidae), curimba (*Prochilodus lineatus* (Valenciennes, 1836); Prochilodontidae), matrinxã (*Brycon cephalus* (Gunther, 1869); Characidae) and piauçu (*Leporinus macrocephalus*, Garavello & Britski, 1988; Anostomidae), obtained from breeding programas were maintained together in fish farm conditions in a pond at Center for the Research and Management of Continental fishing Resources – CEPTA/IBAMA located in the municipality of Pirassununga, in São

Paulo state, Brazil and monitored for two years (march 2000 – February 2002). Each month, five specimens of each species were examined for the presence of myxozoan parasites. Immediately after collection, the fish were transported alive to the laboratory where they were killed by transection of the spinal cord, before being measured and necropsied.

The parasite was identified according to Lom and Arthur (1989), and the measurements from 30 fresh mature spores of different plasmodia were obtained using a micrometer incorporated into the microscope eyepiece and were expressed as the mean ± standard deviation (SD). Smears containing free spores were stained with Giemsa's solution and mounted in low viscosity mounting medium (Cytoseal^{TM}) as permanent mounts. For histological analysis, parasitised gills were fixed in 10% buffered formalin for 24 h, embedded in paraffin, cut into 4 μm thick sections, and stained with haematoxylin and eosin and sirius red (Adriano et al. 2002). For ultrastructural analysis, fragments of gills containing plasmodia were fixed in 2.5% glutaraldeyde in cacodylate buffer (2 h), post-fixed in OsO_4 1% (2 h), dehydrated in increasing concentrations of acetone and embedded in Epon-Araldite resin. Ultrathin sections, double stained with uranyl acetate and lead citrate were examined in a LEO 906 electron microscope operated at 60 kV.

The chemical and physical properties of the pond water, including dissolved oxygen levels and temperature, were measured daily. Others proprieties, such as alkalinity, pH, NH_3 and hardness, were measured weekly. Pearson's correlation was used to determine whether there was any correlation between the chemical and physical characteristics of the water and the prevalence of the parasite. The effect of season and host (fish) size on prevalence was tested using the χ^2 test.

RESULTS

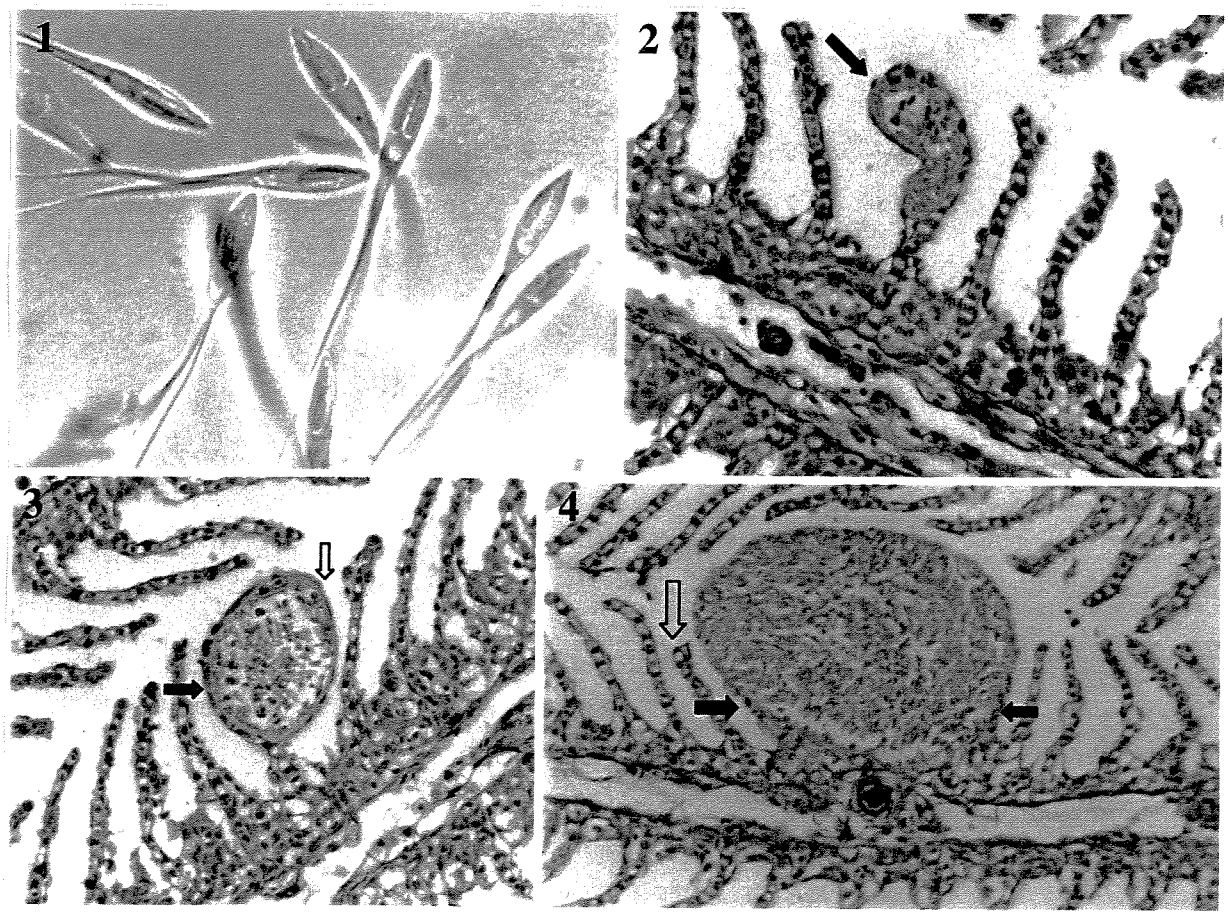
Of the fish species studied, only specimens of pacu had the parasite. Of 120 pacu examined, 45 were 5 - 10 cm long, 41 were 10.1 - 20 cm long and 34 were 20.1 - 36 cm long). Fifty-four fish (45%) had plasmodia of *H. piaractus* in their gill lamellae. The plasmodia were polysporic, white, round or ellipsoidal and measured 25 μm (immature plasmodia) to 2.5 mm (mature plasmodia) in length. Mature spores were elongate, with a total length of 59.6 ± 2.3 μm, a width of 4.1 ± 0.2 μm, and a body length of 12.8 ± 0.7 μm. The valves were extended by a long caudal process (length - 46.4 ± 2.1 μm) (Fig. 1). The polar capsules were elongate (length - 6.5 ± 0.4 μm, width - 1.2 ± 0.2 μm) and the polar filaments were coiled in 8-9 turns aligned perpendicularly to the longitudinal axis of the capsule.

Histological analysis of the gills infected of *P. mesopotamicus* showed that the plasmodia were of the intralamellar type and occurred between the gill lamellar epithelium and the capillary (Figs. 2-4). The parasite produced stretching of the epithelium with accentuated deformation, as well as compression of the capillary and adjacent tissues (Fig. 3). The initial developmental stages of the parasite occurred in different regions of the gill lamellae (basal, medial or distal region) (Figs. 2 and 3). In advanced stages, the plasmodia occupied the entire extent of the gill lamellae and produced marked dilatation and discreet epithelial hyperplasia. The extensive dilatation of infected lamellae caused displacement, deformation and eventually fusion of the neighbouring lamellae (Fig. 4). No inflammatory reaction was observed in the infected gills.

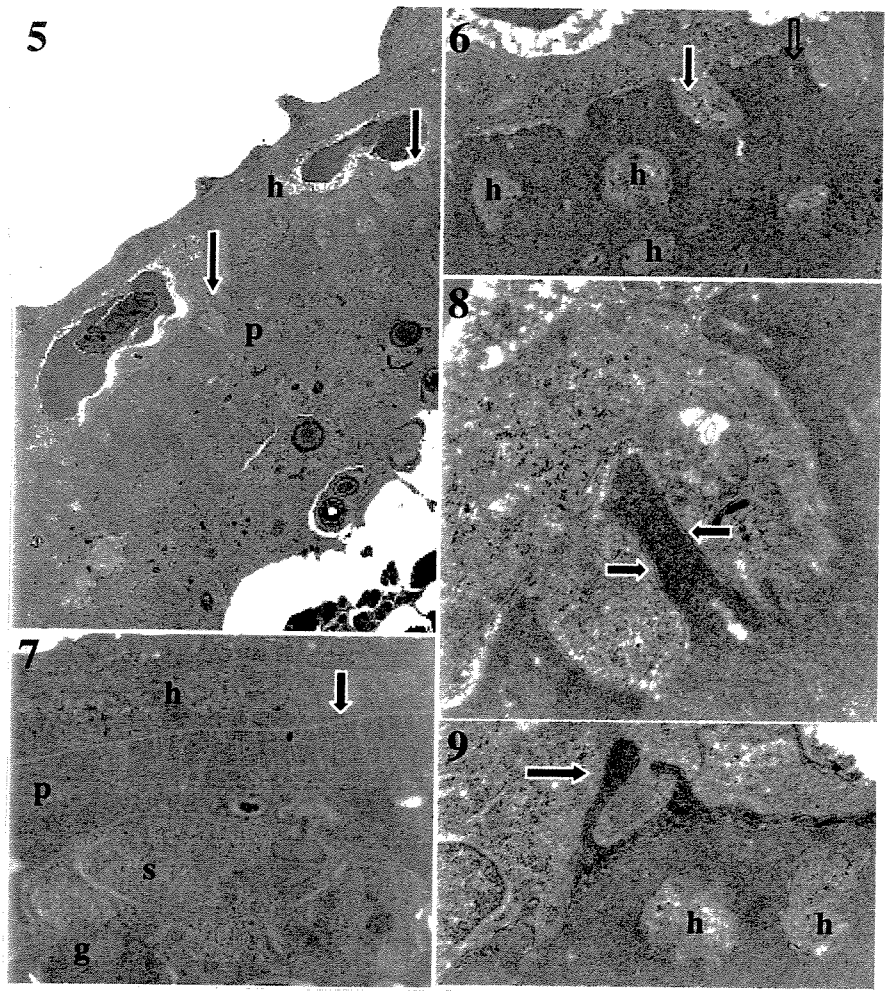
Ultrastructural analysis showed direct contact between the plasmodial wall and the host cells (Figs. 5-9). The plasmodial wall consisted of a single layer (Fig. 8) which was continuous with the pinocytic canals that extended into of the thin, finely granular layer of

the plasmodial ectoplasm (Fig. 7). In addition to the pinocytic canals, several phagocytes points were observed engulfing parts of the host cells (Figs. 5 and 7-9). In some cases, portions of the host cells were seen within large phagosome-like vacuoles in the ectoplasm (Figs. 6 and 9).

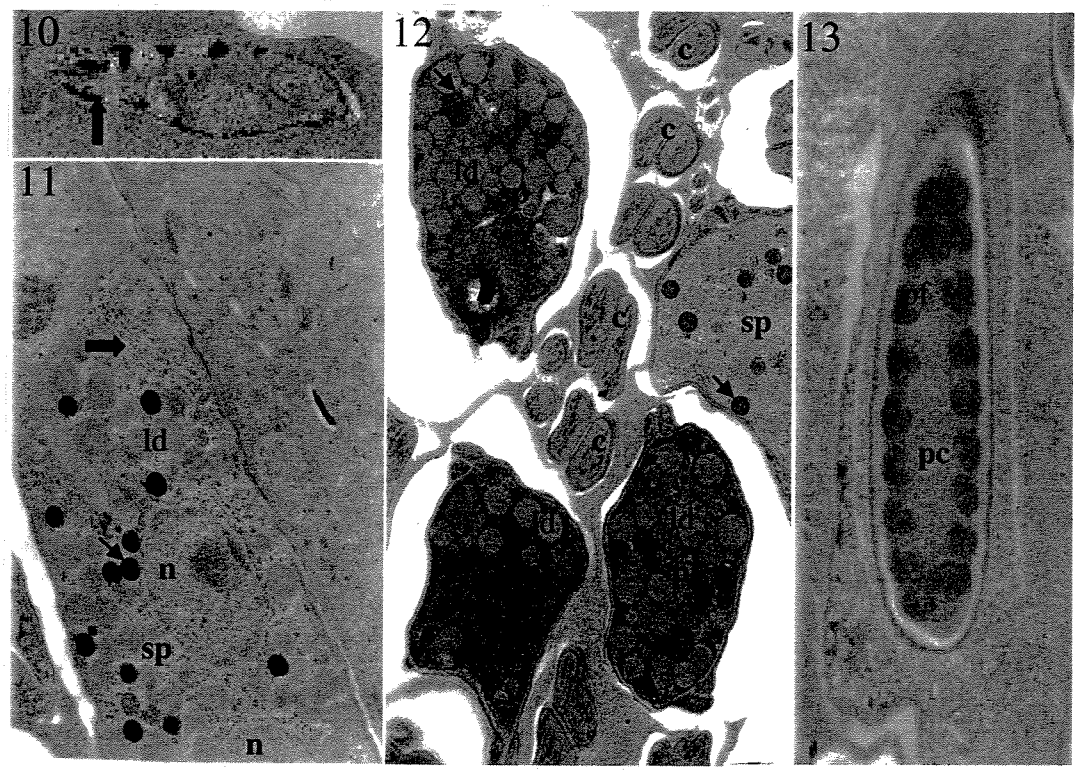
The earliest stages of sporogenesis occurred at the periphery of the endoplasm whereas mature spores were found in the central region (Fig. 7). During the development of the spores, a large accumulation of electron-dense "valve-forming material" was seen throughout the valvogenic cells (Fig. 10). The binucleate sporoplasm contained some dark sporoplasmosomes, numerous ribosomes, and scattered randomly rough endoplasmic reticulum (Fig. 11). Numerous membrane-less spherical bodies of different sizes and moderate electron density were seen in the sporoplasm and were probably lipid droplets (Figs. 11 and 12). The polar capsules were elongated and consisted of a thin, dark wall outer, a less electron dense inner layer, and a dark, granular central area containing the polar filament with its 8-9 coils (Fig. 13).



Figs. 1 – 4. Light photomicrographs of *Henneguya piaractus*. Fig. 1. Fresh mature spores. x3600. Figs. 2-4. Histological sections of gills of *Piaractus mesopotamicus*. Sirius red staining. Fig. 2. Immature plasmodium occupying the apical and median regions of the gill lamella (arrow). x1440. Fig. 3. Young plasmodium in the distal region of the gill lamella. Note the stretching and accentuated deformation of the epithelium (empty arrow) and compression of the capillary and adjacent tissues (black arrow). x1360. Fig. 4. Mature plasmodium occupying the entire length of the gill lamella. Note that the original structure of the attacked gill lamella is no discernible, there was the fusion of the nearest gill lamellae (black arrows) and the neighbouring lamellae has also been pushed laterally (empty arrow). x1120



Figs. 5 – 9. Electron micrographs of the host-parasite interface. Fig. 5. Direct contact between the plasmodium of *Henneguya piaractus* (p) and the host cells (h). Note the points of phagocytosis (arrows). x2720. Fig. 6. Amplified portion of fig. 5 showing pinocytic canals (empty arrow), points of phagocytosis (black arrow) and parts of host cell (h) within the plasmodium. x9750. Fig. 7. Direct contact between the plasmodium (p) and the host cells (h), the presence of numerous pinocytic canals (arrow) and different stages of the life cycle: generative cell (g), and sporogenic stages (s). x9270. Fig. 8. Invaginations showing engulfment of the host cell. Note the contact between the wall of the host cell and that of the parasite (arrows). x21560. Fig. 9. Expanded view of the of the plasmodium wall engulfing part of a host cell (arrow). Note two portions of host cell (h) within the plasmodium. x16000



Figs. 10 – 13. Electron micrographs of spores of *Henneguya piaractus*. Fig. 10. Longitudinal section of a young spore showing the accumulation of valve-forming material (arrow). x13360. Fig. 11. Longitudinal section of a young spore showing the binucleated (n) sporoplasm (sp), sporoplasmosomes (arrowheads), ribosomes and rough endoplasmic reticulum (arrow), and lipid droplets (ld). x12930. Fig. 12. Transversal sections of spores at the level of the sporoplasm (sp) and of the caudal process (c). Note the few dark sporoplasmosomes (arrowheads) and numerous lipid droplets (ld). x10300. Fig. 13. Longitudinal section of the polar capsule (pc) with the polar filament (pf). x15590

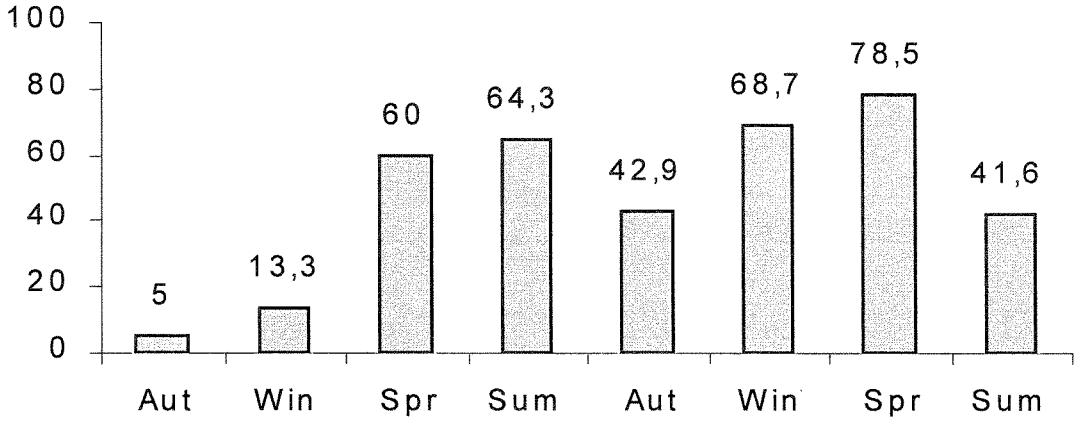


Fig. 14. Prevalence of *Henneguya piaractus* in relation to seasons.

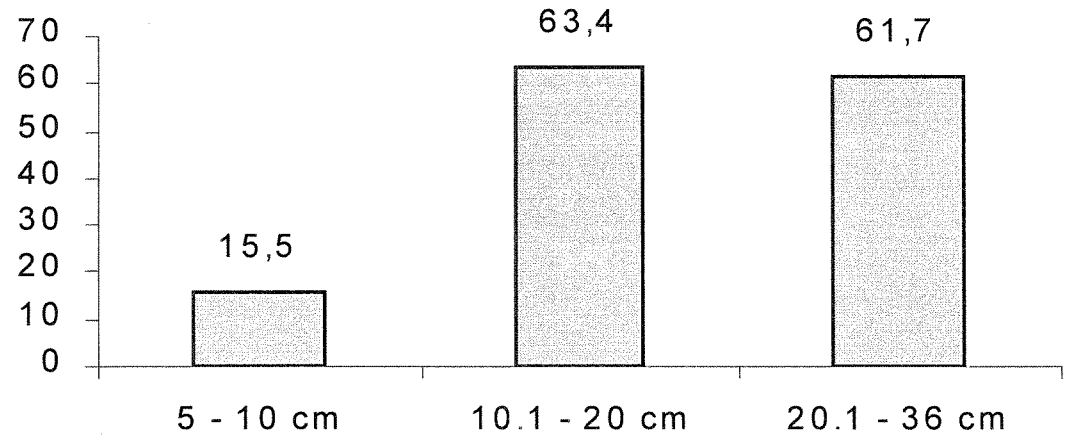


Fig. 15. Prevalence of *Henneguya piaractus* in relation to host size.

There was no correlation between the prevalence of the parasite and the chemical and physical characteristics of the water, such as the dissolved oxygen levels ($r = -0.1821$: $p = 0.3944$), pH ($r = -0.1944$: $p = 0.3627$), NH_3 ($r = 0.0718$: $p = 0.7388$) and temperature ($r = 0.2892$: $p = 0.1705$), but the correlation was significant to the alkalinity ($r = -0.5389$: $p = 0.0066$) and hardness ($r = -0.4964$: $p = 0.0136$). The prevalence of the parasite varied

significantly with the seasons (χ^2 = 32.54, df = 7, $p < 0.05$). The smallest prevalences occurred in the autumn and winter 2000 (prevalences of 5% and 13.3%, respectively), with no significant variation (χ^2 = 6.02, df = 5, ns: non significant) between the spring of 2000 and the summer of 2001 (Fig. 14). The prevalence also varied significantly with host size (χ^2 = 26, df = 2, $p < 0.05$). The smallest prevalence occurred in hosts up to 10 cm long, with no significant difference (χ^2 = 0.015, df = 1, ns) between fish 10.1 - 20 cm long and those > 20 cm long (Fig. 15).

DISCUSSION

Despite the significant difference in prevalence between seasons and in the correlation between the prevalence and the alkalinity and hardness of the water, these results may not represent true seasonal variation of the parasite, but may more likely reflect the time required for infection of the fish and the appearance of plasmodia after the initial contact with the host. Thus, the smallest prevalence of the parasite occurred in the autumn and winter of 2000 (beginning of the study), with no significant variation in the subsequent seasons (spring 2000 to summer 2001). Likewise, the prevalence varied significantly with host size, with the smallest prevalence occurring in fish up to 10 cm long. When these smaller fish were excluded from the analysis, there was no significant variation in prevalence. The correlation between the prevalence of the parasite and the alkalinity and hardness of the water may reflect the normally high concentration of $CaCO_3$ in the water caused by preparation of the pond at beginning of the study, which coincided with the smallest prevalence of the parasite. The greater prevalence of the parasite in larger fish was similar to that of other myxosporean species, such as *Henneguya creplini* infecting the gills

of *Stizostedion lucioperca* (Molnár 1998) and *Myxobolus muelleri* and *Myxobolus dujardini*, parasites of *Psychochelus oregonenseis*, *P. caurinus* and *Richardsonius blateatus* (Mitchell 1988), and may indicate that *P. mesopotamicus* does not acquire immunity to this parasite.

The prevalence of *H. piaractus* observed here (45.8%) was smaller than the 97.3% previously reported for cultivated pacu (Martins & Souza 1997). This difference may reflect the conditions of cultivation or handling of the fishes, but supports the idea that this species is a common parasite of these fish in Brazil. The specimens of *P. mesopotamicus* examined were confined to a pond with three other fish species, but *H. piaractus* was found only in pacu, indicating host specificity. Molnár (1998) suggested that *Henneguya* species may have a relatively strict host specificity. However, *H. piaractus* has been reported to infect pacu, tambaqui (*Colossoma macropomum*), a large characid native to the Amazon river basin, and tambacu, a hybrid of these two species (*P. mesopotamicus* male x *C. macropomum* female), in a fish farm (Martins et al. 1999b). This may indicate that *H. piaractus* has a host specificity restricted to closely related species - pacu and tambaqui are characids that can crossbreed with each other.

Various species of *Henneguya* that parasitise gills are reported to be important pathogens, including *Henneguya exilis* in the channel catfish (Current & Janovy 1976), *Henneguya psorospermica* in *Perca fluviatis* (Dyková & Lom 1978), *Henneguya waltairensis* in *Channa punctatus* (Kalavati & Narasimhamurti, 1985), *Henneguya creplini* in *Stizostedion lucioperca* (Molnár, 1998), *Henneguya curvata* in *Serrasalmus spilopleura* (Barassa et al, 2003a) and *Henneguya chydadea* in *Astyanax altiparanae* (Barassa et al. 2003b).

Henneguya piaractus causes important pathological alterations in the gills of cultivated pacu (Martins et al. 1997), including haemorrhage and severe inflammatory foci in the gill epithelium. Two layers of elongated fibroblast-like cells and a inflammatory mononuclear infiltrate surround the parasite. Infected fish generally remain near the pond banks or congregate near inflowing water. Feeding activity decreases over time, the fish become lethargic and swim erratically with an apparent loss of equilibrium before eventually dying (Martins et al. 1997). As shown here, in advanced stages of infection, the plasmodium occupies the entire gill lamella and causes marked dilatation and discreet epithelial hyperplasia. The extensive dilatation of the infected lamellae, pushs the neighbouring lamellae sideways and produce deformation and, eventually, fusion. In a massive infection, these alterations may partially compromise the gill functions by reducing the epithelial area and by compressing the blood capillaries.

Ultrastructural analysis showed that sporogenesis in *H. piaractus* following the general pattern of other *Henneguya* species (Current 1979, El-Mansy & Bashtar 2002, Azevedo & Matos 2002, 2003, Vita et al. 2003). However, numerous spherical lipid droplets were seen immersed in the sporoplasm cells of *H. piaractus*. Carbohydrates, in the form of glycogen, are a common energy reserve in sporoplasm of different myxosporeans (Desser & Paterson 1978, Rocha et al. 1992, Azevedo et al. 2002). The finding of lipid droplets in the sporoplasm of *H. piaractus* suggests that lipids can also be used as an energy reserve in some myxosporeans. This conclusion agrees with Redondo et al. (2003) who reported the occurrence of lipid reserves in *Enteromyxum scophthalmi*, a myxosporean parasite of *Scophthalmus maximus* in Europe.

The plasmodial wall of *H. piaractus* consisted of a single membrane, as in other myxosporean species (Current & Janovy 1978, Current 1979, Dohole et al. 2002), although

double membrane have been reported in some cases (Current & Janovy 1976, Desser & Paterson 1978, Rocha et al. 1992, Casal et al. 2002, El-Mansy & Bashtar 2002). The plasmodial membrane of *H. piaractus* was in direct contact with the host cells and had pinocytic canals that extended into the thin plasmodial ectoplasm. Pinocytic canals have been observed in the plasmodial walls of several *Henneguya* species (Current & Janovy 1976, 1978, Current 1979, Rocha et al. 1992, El-Mansy & Bashtar 2002, Azevedo & Matos 2002, 2003). There is strong evidence that pinocytosis enables the plasmodium of *Henneguya exilis* to take of host cells' cytoplasm, as well as interstitial material adhering to the plasmodial wall, and this activity may contribute to the extreme pathogenicity of the interlamellar form of this parasite. (Current & Janovy 1976). The plasmodial wall of myxosporeans is responsible for feeding the plasmodium and can supply the developing stages with the nutrients necessary for growth via the pinocytic canals which can take up host cell cytoplasm through pinocytosis (El-Mansy & Bashtar 2002). In addition to pinocytic canals, the plasmodial wall of *H. piaractus* also contained several points of phagocytosis which engulfed parts of the host cells. Thus, this species can obtain food by pinocytosis and phagocytosis. The latter means of obtainig food has been reported in other myxosporeans. Phagocytosis followed by intracellular digestion within a food vacuole was observed in *Kudoa quadratum* (Uspenskaya 1982). The phagocytosis of chondrocytes was observed in *Myxobolus cerebralis,* and extrasporogonic stages of some *Sphaerospora* are known to phagocytise erythrocytes (Lom & Dyková 1995).

The plasmodia of *H. piaractus* has no coat nor were they surrounded by a capsule of collagen fibres that could prevent direct contact between the host cells and the plasmodial surface. A similar situation has been described for the interlamellar plasmodia of *Henneguya exilis,* with points of the plasmodial surface being in direct contact with the host

cells. At these points, the host cells' cytoplasm appeared to be passing into pinocytic canals of the plasmodial wall (Current & Janovy 1976). The plasmodial wall of *Henneguya adiposa* has a uniform coat and is surrounded by collagen fibres which prevent direct contact between the parasite and host cells and only allow the uptake of interstitial material (Current 1979).

Although no important inflammatory response was observed in the fish examined here, the alterations in the structures of infected and neighbouring gill lamellae, the direct contact between the plasmodial wall and the host cells, and the occurrence of pinocytic canals and points of phagocytosis corroborated the potentially pathogenicity of this parasite reported by Martins et al. (1997).

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Capítulo 5

Myxobolus sp. 1, a new myxosporean in *Piaractus mesopotamicus* (Pisces: Characidae) cultivated in Brazil: taxonomy and host- parasite relationship

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Key words: Myxosporea, *Myxobolus* sp. 1, *Piaractus mesopotamicus*, Characidae, histology, ultrastructure

Abstract. The characteristics of *Myxobolus* sp. 1 and its relationship to the host (*Piaractus mesopotamicus*) are described based on light and electron microscopy and histological observations. Polysporic plasmodia measuring 20 μm to 2.1 mm in size were found in 63.3 % of the *P. mesopotamicus* examined. The parasite was found in the gall bladder, urinary bladder, gills, spleen, fins, head surface, liver and heart. Generative cells and disporoblastic pansporoblasts occurred along the periphery of the plasmodia, and mature spores were found in the internal region. The spores were pyriform in frontal view, with a total length of 10.0 ± 0.6 μm and a width of 5.1 ± 0.3 μm (mean ± SD). The spore wall was smooth with

sutural folds. The polar capsules were elongated and pyriform shape, and were equal in size (length-5.7 ± 03 μm, width-1.7 ± 0.2 μm), with the anterior ends close to each other. The polar filaments were tightly coiled in 8-9 turns perpendicular to the axis of the capsule. The plasmodia were surrounded by a collagen capsule. The parasite caused important damage only in the gills, where development occurred in the wall of gill filament arterioles; a mild macrophage infiltrate was also observed. In advanced developmental stages, the plasmodia caused deformation of the arteriole structure, with a reduction and, in some cases, obstruction of the lumen. The parasite was found throughout the period studied and its prevalence was unaffected by host size, season or water properties.

INTRODUCTION

Piaractus mesopotamicus (Holmberg, 1887) is an omnivorous characid popularly known as pacu. This fish attains a large size (easily reaching 12 kg) and is economically one of the most important fish species in Brazil. The high reproductive capacity, rapid growth and widespread commercial acceptance of *P. mesopotamicus* have made it one of the most widely cultivated species by fish farms in Brazil.

To date, *Henneguya lutzi* Cunha & Fonseca, 1918, *Myxobolus colossomatis* Molnár & Békési, 1993 and *Henneguya piaractus* Martins & Souza, 1997 have been found parasitising *P. mesopotamicus*. More recently, Adriano et al. (2003) reported *H. piaractus*, *Henneguya* sp., *M. colossomatis* and *Myxobolus* sp. infecting pacu from the Pantanal in Brazil. In this report, which is part of a survey of myxosporean parasites on fish farms, we describe an ultrastructural and histological analysis of a new *Myxobolus* parasite of pacu.

MATERIALS AND METHODS

Young specimens of four fish species obtained by artificial reproduction, namely pacu (*P. mesopotamicus*; Characidae), curimba (*Prochilodus lineatus* (Valenciennes, 1836); Prochilodontidae), matrinxã (*Brycon cephalus* (Gunther, 1869); Characidae) and piauçu (*Leporinus macrocephalus*, Garavello & Britski, 1988; Anostomidae), were released in a pond at the Center for the Research and Management of Continental Fishing Resources - CEPTA/IBAMA Pirassununga, state of São Paulo, Brazil, and monitored for two years. Five specimens of each species were examined monthly for the presence of myxosporeans from March 2000 to February 2002. Immediately after collection, the fishes were transported alive to the laboratory where they were killed by transection of the spinal cord, and then measured and necropsied.

The parasite was identified according to Lom and Arthur (1989), and the measurements from 43 fresh mature spores of different plasmodia obtained from several specimens of *P. mesopotamicus* were measured with a micrometer incorporated into the microscope eyepiece. The dimensions were expressed as the mean ± standard deviation (SD). Smears containing spores free were stained with Giemsa's solution and mounted in low viscosity mounting medium (Cytoseal^{TM}) as permanent slides. For histological analysis, fragments of infected organs were fixed in 10% buffered formalin for 24 h, embedded in paraffin, cut into sections 4 µm thick and stained with haematoxylin or eosin and Sirius Red (Adriano et al. 2002). For scanning electron microscopy, free spores were deposited on a coverslip coated with poly-L-lysine and fixed for 2 h at room temperature with glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After washing in the same buffer, the preparations were dehydrated in ethanol, critical point dried in CO_2, covered with

metallic gold, and examined in a Joel JMS 35 microscope operated at 15 kV. For transmission electron microscopy, plasmodia were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h, washed in glucose-saline solution for 2 h, and post-fixed in OsO_4, all done at 4^{o} C. After dehydration in an acetone series, the material was embedded in Epon-Araldite resin. Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined in LEO 906 electron microscope operated at 60 kV.

The chemical and physical properties of the pond water, including dissolved oxygen levels and temperature, were measured daily. Other properties, such as alkalinity, pH, NH_3 and hardness, were measured weekly. Pearson's correlation was used to determine whether there was any correlation between the chemical and physical characteristics of the water and the prevalence of the parasite. The influence of host (fish) size and season on the prevalence of the parasite was tested using the χ^2 test.

RESULTS

Of the four fish species studied, only specimens of *P. mesopotamicus* had plasmodia of an unknown *Myxobolus* species. Of 120 pacu examined, 45 were 5-10 cm long, 41 were 10.1-20 cm long and 34 were 20.1-36 cm long). Seventy-six fish (63.3%) had the parasite. Parasite plasmodia were found in several organs, and the prevalence in each organ was: gall bladder - 41.6%, urinary bladder - 26.6%, gills - 25%, spleen - 14.1%, fin - 5.8%, head surface - 5%, liver - 2.5% and heart - 2.5%. Parasite spores were found in melanomacrophage centres in the kidney and, less frequently, in the spleen.

There was no correlation between the prevalence of the parasite and the chemical and physical characteristics of the water, such as dissolved oxygen levels ($r = 0.2660$; $p =$

0.1986), alkalinity (r = 0.1001; p = 0.6339), pH (r = 0.0435; p = 0.8402), hardness (r = -0.1001; p = 0.6417), NH_3 (r = -0.0349; p = 0.8713) and temperature (r = -0.1775; p = 0.4066).

The parasite was found throughout the study and its occurrence did not vary significantly with the seasons (χ^2 = 2.86, df = 7, ns: non significant). The highest prevalence (73.3%) was in the summers of 2000 and 2001, while the lowest (53.3%) was in the autumn and spring of 2001. In the autumn and spring of 2000, the prevalence was 66.6%, and in the winter of 2000 and 2001, the prevalence was 60%. The prevalence was of 62.2% in fish up to 10 cm long, 55.6% in fish 10.1 – 20 cm long, and 61.9% in fish 20.1 - 36 cm long. These differences were not significantly (χ^2 = 1.64, df = 2, ns).

Species description

***Myxobolus* sp. 1** **Figs. 1-4**

The polysporic plasmodia were 20 μm to 2.1 mm in size (Figs. 1B-F, 2A-D). Ultrastructural analysis revealed the presence of different sporogenic stages, such as generative cells and disporoblastic pansporoblasts, along the periphery of the plasmodia and mature spores in the internal region (Fig. 3A). Fresh, mature spores were pyriform in frontal view, with the anterior end more slender than the posterior end (Figs. 1A, 3F, 4A, B), and had a total length of 10.0 ± 0.6 μm and a width of 5.1 ± 0.3 μm. The spore wall was smooth with sutural folds (Fig. 3F). In lateral view, the spores were symmetric, convex and had a conspicuous sutural line (Fig. 3E). The polar capsules were elongated and pyriform in shape, and were equal in size (length-5.7±03 μm, width-1.7±0.2 μm), with the anterior ends close to each other. The polar filaments were tightly coiled in 8-9 turns

perpendicular to the axis of the capsule (Fig. 3D, 4). The sporoplasm was binucleated, with small dark sporoplasmosomes (Fig. 3B).

Type host: Piaractus mesopotamicus Holmberg, 1887 (Characidae).

Site of infection: gall bladder, urinary bladder, gills, spleen, fins, head surface, liver and heart.

Prevalence: 76/120 (63.3%) of *P. mesopotamicus* were infected.

Locality: Center for the Research and Management of Continental Fishing Resources (CEPTA/IBAMA), Pirassununga, state of São Paulo, Brazil.

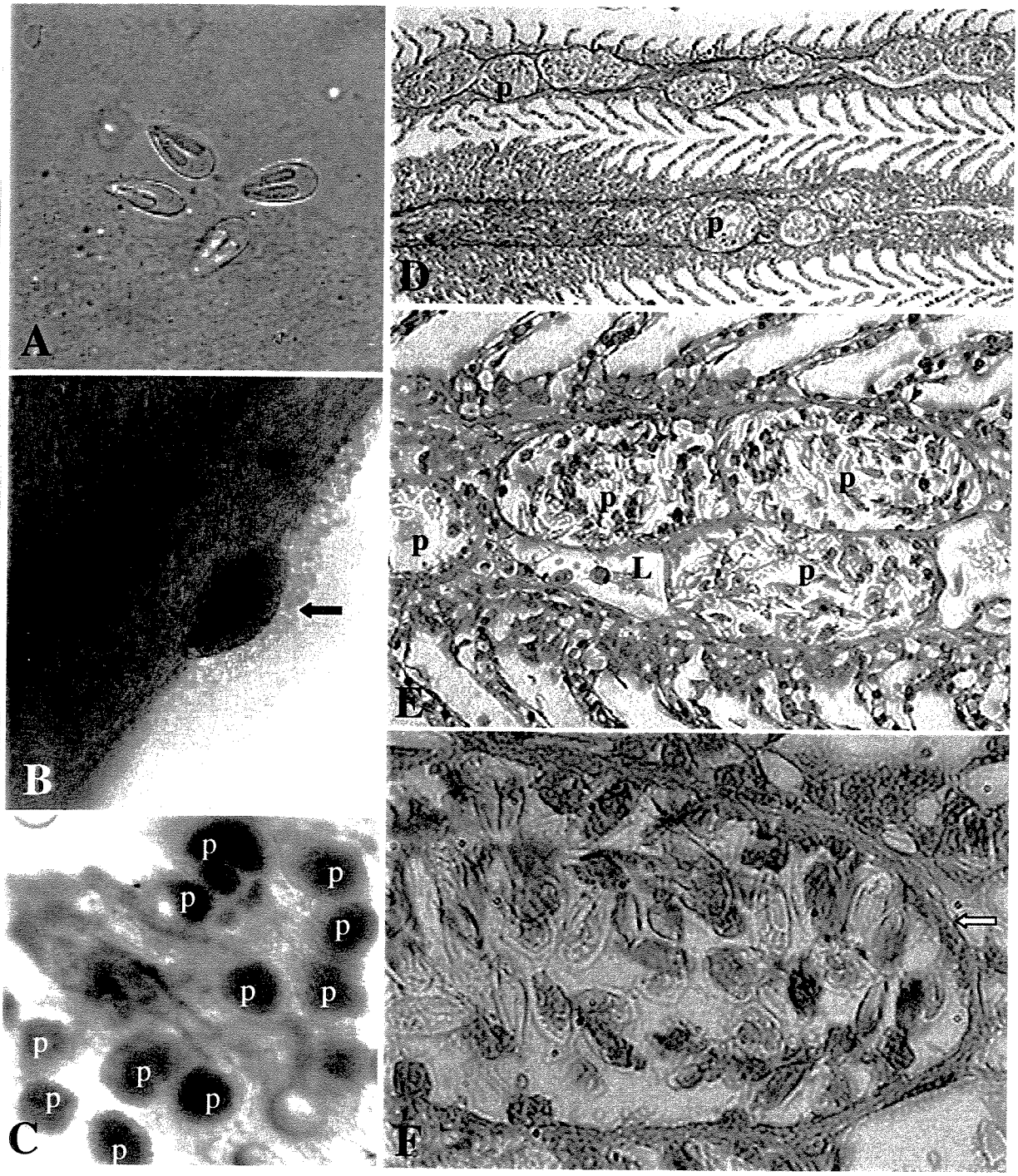

Fig. 1: Light photomicrographs of *Myxobolus* sp. 1. A: Fresh mature spores. 3700x. B-C: Plasmodia of *Myxobolus* sp. 1 in fresh preparations. B: In the gall bladder (arrow). 350x. C: In the urinary bladder (p). 350x. D-F: Histological sections of gills of *Piaractus mesopotamicus* infected with *Myxobolus* sp. 1. Sirius red staining. D: Plasmodium in the arterioles wall of the gill filament (p). 260x. E. Agglomerate of plasmodia (p) producing obstruction of the arteriole lumen (L). 1960x. F. Amplified portion of E: note the capsule of connective tissue (arrow). 3800x

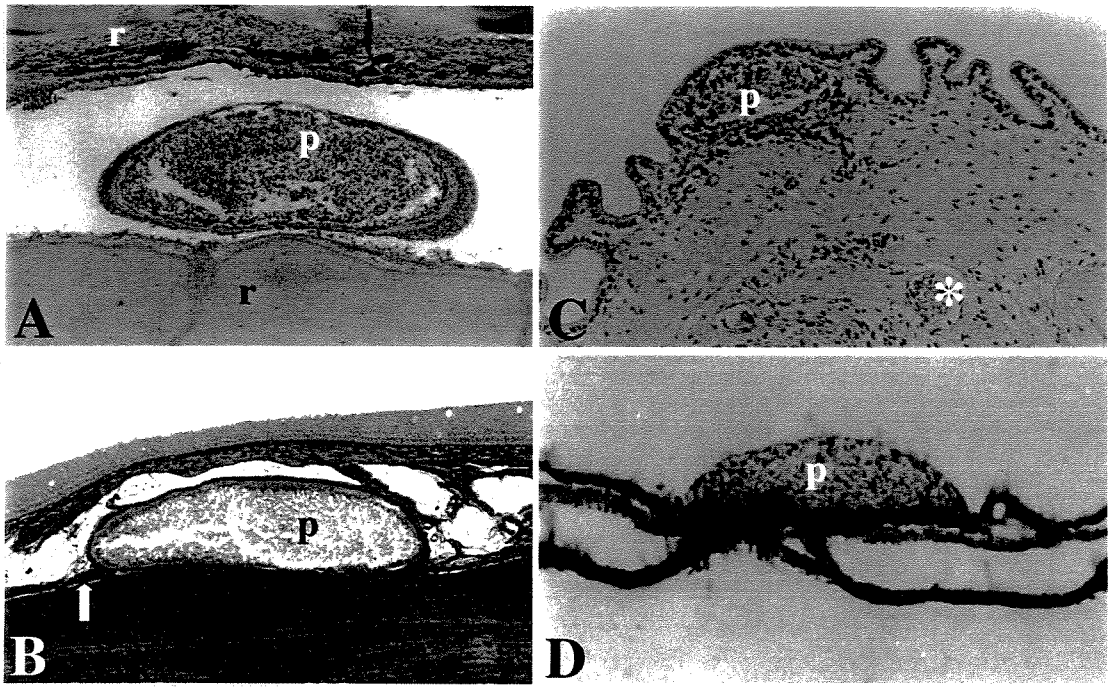

Fig. 2: Histological sections of several organs infected by *Myxobolus* sp. 1. A: longitudinal section of fin showing a plasmodium (p) in connective tissue between the rays (r). 200x. B: Transversal section of operculum showing a plasmodium (p) deep within the subcutaneous tissue, near the periosteum (arrow). 200x. C: Sections of urinary bladder showing plasmodia (p) in the subepithelial connective tissue and in the middle layer (*). 260x. D: Section of gall bladder showing a plasmodium (p) in the serous capsule. 700x.

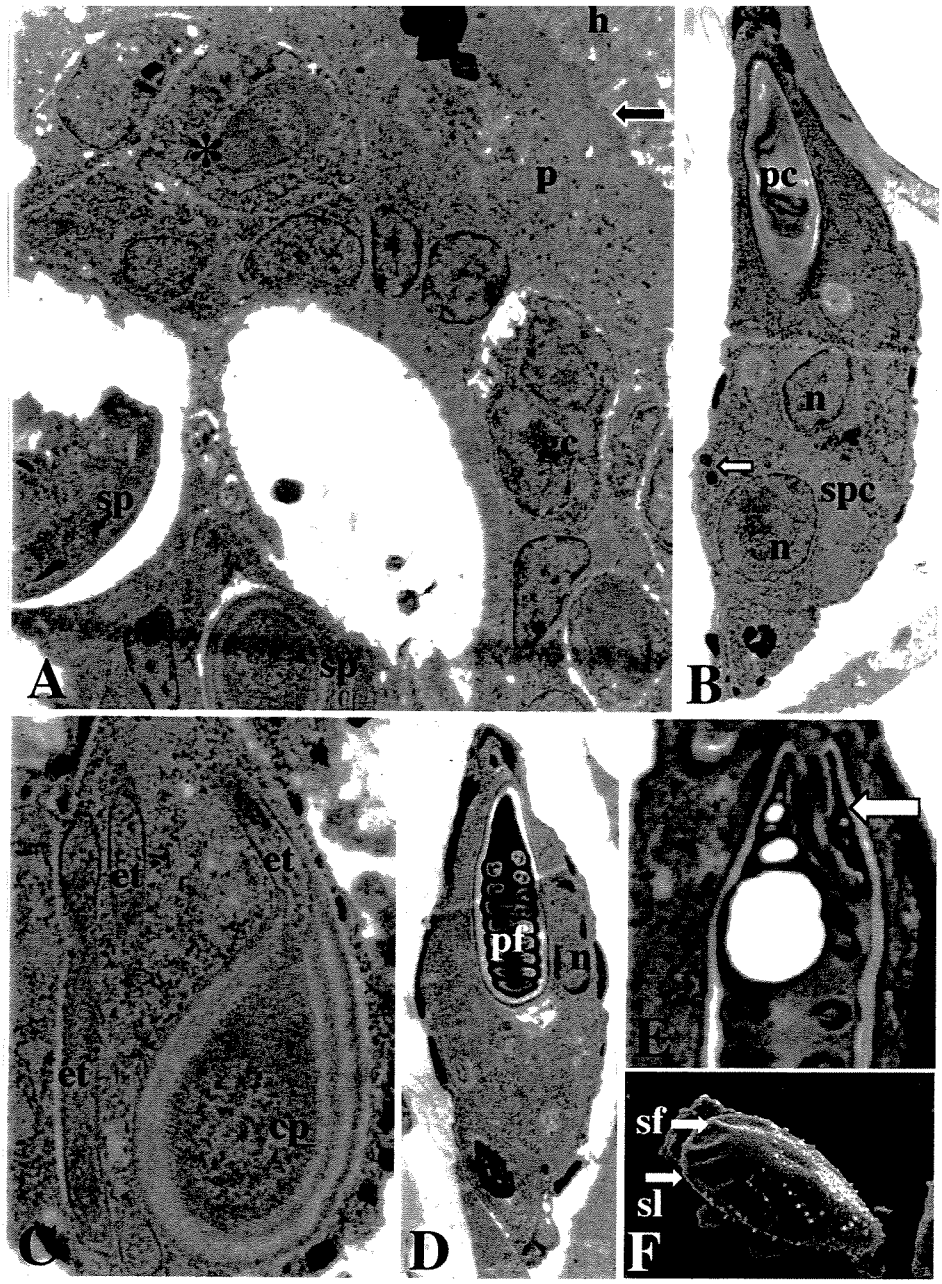

Fig. 3: Electron micrographs of *Myxobolus* sp. 1. A: Section of a plasmodium (p) showing the plasmodial wall (arrow), host tissue - connective tissue capsule (h), disporoblastic pansporoblast (*), generative cells (gc) and fragments of young spores (sp). 7970x. B: Longitudinal section of a young spore showing the polar capsule (pc), sporoplasm cell (spc) with two nuclei (n) and a few sporoplasmosomes (arrow). 10133x. C: Early capsulogenic stage with the capsular primordium (cp) attached to the external tube (et). Note also two longitudinal sections of the external tube. 12926x. D: Longitudinal section of a nearly mature spore showing the polar filament (pf) within the polar capsule and the nucleus (n) of the capsulogenic cell. 7546x. E: Longitudinal section showing details of the anterior end of a polar capsule (arrow). 15920x. F: Scanning electron image of a mature spore showing the sutural folds (sf) and the suture line (sl). 5550x.

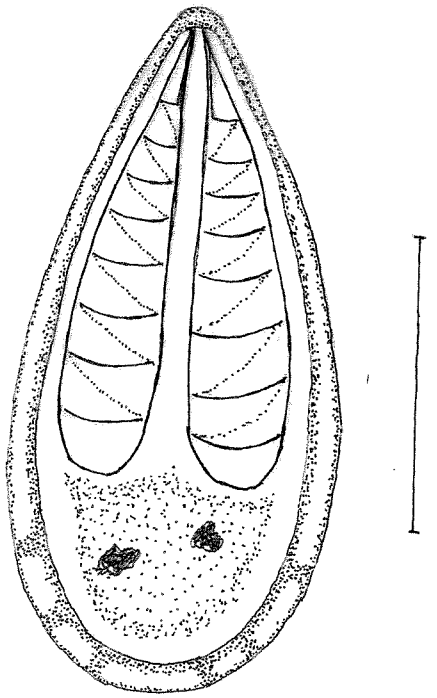

Fig. 4 Schematic representation of mature spores of *Myxobolus* sp. 1. Bar = 5 μm.

Histological analysis showed that at all sites of infection the plasmodia were surrounded by a collagen capsule (Fig. 1F). In the gills, the plasmodia developed in the adventitia of arterioles in the gill filaments, and a mild macrophage infiltrate was observed. In advanced developmental stages, the plasmodia deformed the wall of the arterioles, compressing them in the direction of the lumen, thereby diminishing and, in some cases, obstructing the lumen of the arterioles (Fig. 1D-F). In the gall bladder, the plasmodia appeared externally in the serous capsule (Figs. 1B, 2D), while in the urinary bladder, the parasite developed in the middle layer and in the subepithelial connective tissue (Fig. 2C). In the fins, the parasite developed in connective tissue between the rays (Fig. 2A). In the head, the plasmodia were located deep within the subcutaneous tissue, near the periosteum (Fig. 2B), and in the

spleen they occurred in the fibrous capsule. The prevalence of the parasite in the heart and in the liver was low (2.5%) and plasmodia were seen only in fresh preparations.

DISCUSSION

Myxobolus sp. 1 was compared with other *Myxobolus* spp. parasites of South American fish. The spores of *Myxobolus* sp. 1 resembled those of other South American *Myxobolus* (*M. inaequus* Kent & Hoffman, 1984, *M. cunhai* Penido et al. 1927, microspores of *M. serrasalmi* Walliker, 1969, and *M. maculatus* Casal et al. 2002) in shape, but only the spores of *M. cunhai* and the microspores of *M. serrasalmi* were similar in size. However, *M. cunhai* was related in a host of the family Pimelodidae and their spores differed from those of *Myxobolus* sp. 1 by unequal size of the polar capsules (Gioia and Cordeiro 1996). In contrast to *Myxobolus* sp. 1, the plasmodia of *M. serrasalmi* had spores of two distinct shapes and sizes – macrospores with an oval shape and microspores with a pyriform shape.

The existence of macro and microspores in the plasmodia of *M. serrasalmi* may have resulted from the fusion of two neighbouring plasmodia of different species (Molnár and Békési 1992). However, since plasmodia of *M. serrasalmi* were found in the spleen and kidney (Walliker 1969), it seems improbable that this fusion of plasmodia involved different species in these two organs simultaneously. According to Walliker (1969), all the plasmodia of *M. serrasalmi* contained dense patches of dark brown pigment. However, similar patches were observed scattered throughout uninfected spleen tissue, and macro and microspores were frequently present in and around immature plasmodia. In addition, plasmodia in the kidney lacked a wall. Based on this description, we believe that the structures reported by Walliker (1969) were not myxosporean plasmodia, but were

agglomerations of spores from two different species of *Myxobolus* within melanomacrophage centres.

The melanomacrophage centres, also known as macrophage aggregates, are distinctive groupings of pigment-containing cells in the tissues of heterothermic vertebrates. In fish, these centres are normally located in the stroma of the haemopoietic tissue of the spleen and kidney (Agius and Roberts 2003), and play an important part in the host's defense reactions (Dyková 1984, Agius and Roberts 2003). Infections by different species of the genus *Myxobolus* manifested themselves by the appearance of spores in melanomacrophage centres or in aggregates of melanomacrophage in the kidney, spleen and hepatopancreas (Dyková 1984). Melanomacrophages can attach to large myxosporean spores and transport them to melanomacrophage centres where the spores are encapsulated by fibroblasts and eventually destroyed (Dyková 1984). Thus, spores of different species can accumulate in these centres. If the description by Walliker (1969) represents melanomacrophage centres, then the macro and microspores represent the spores of two species distinct whose site of development is unknown. In this context, the spores of *Myxobolus* sp. 1 are very similar in size and shape to the microspores of *M. serrasalmi*. However, the anterior end of the spores of *M. serrasalmi* is more pointed that in *Myxobolus* sp. 1. In addition, the spores of *Myxobolus* sp. 1 are slightly larger than in *M. serrasalmi* and the polar capsules are proportionally larger in *M. serrasalmi*. Thus, we suggest that *Myxobolus* sp. 1 is a new species.

Myxobolus sp. 1 occurred throughout the period of this study and there was no correlation between the chemical and physical proprieties of the water and the prevalence of the parasite. Likewise, the prevalence of the parasite did not vary significantly with the seasons or the host size. Thus, seem that the life cycle of this parasite was unaffected by

environmental conditions and the development of the host, in contrast to *Myxobolus muelleri* and *Myxobolus dujardini*, parasites of *Psychochelus oregonenseis*, *P. caurinus* and *Richardsonius blateatus*, for which the prevalence is greater in larger specimens (Mitchell 1988), and *Myxobolus porofilus*, a parasite of *P. lineatus*, which was reported only in young specimens (Adriano et al. 2002).

In this study, the specimens of *P. mesopotamicus* examined were confined to a pond with three other fish species, but *Myxobolus* sp. 1 was found only in pacu, which suggested host specificity. Similar host specificity has been reported for *M. porofilus* infecting *P. lineatus* maintained under the same conditions (Adriano et al. 2002). According to Molnár et al. (1998), although little is known about the host specificity of *Myxobolus* species, the number of species with a large host range is low and most species appear to be strictly host-specific or capable of developing only in closely related fishes.

The histological analysis showed that the development of the parasite was not organ-specific, but the plasmodia of *Myxobolus* sp. 1 were always found in connective tissue (wall of the arterioles of the gill filaments, serous capsule of the gall bladder, middle layer and subepithelial connective tissue of the urinary bladder, connective tissue between the rays of the fins, subcutaneous tissue of the head surface and fibrous capsule spleen). A similar non-specificity of organs was reported for *M. colossomatis* parasitizing *Colossoma macropomum*, a large characid from the Amazon river basin (Molnár and Békési 1992).

Of the organs parasitized by *Myxobolus* sp. 1, the parasite caused greatest damage in the gills since the development of the plasmodia reduced the vessel lumen and, in some cases completely obstructed the lumen of the gill filament arterioles. Thus, a high parasite load could compromise the blood circulation and, consequently gill functions.

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5-CONCLUSÕES GERAIS

1- Das quatro espécies de peixes consideradas neste trabalho, apenas exemplares de *Piaractus mesopotamicus* e *Prochilodus lineatus* estavam infectados por mixosporídeos.

2- Duas novas espécies de mixosporídeos: *Myxobolus porofilus* parasitando a cavidade visceral e *Henneguya* sp. 1 infectando o epitélio interlamelar e intralamelar das brânquias foram encontradas em *P. lineatus.*

3- *Myxobolus porofilus* foi encontrado apenas em *P. lineatus* jovens.

4- *Henneguya* sp. 1 produziu hiperplasia de células epiteliais e deformação das estruturas das lamelas infectadas e vizinhas.

5- *Henneguya* sp. 1 foi encontrado durante todo o período do estudo, e sua prevalência não sofreu alterações no decorrer de nossas observações.

6- Três espécies de mixosporídeos foram encontradas em *P. mesopotamicus*: *Henneguya piaractus* infectando as lamelas branquiais, *Henneguya* sp. 2 encontrado na serosa que reveste a cavidade visceral e na túnica externa da bexiga natatória e *Myxobolus* sp. 1 parasitando vesícula biliar, bexiga urinária, brânquias, baço, superfície da cabeça, fígado e coração.

7- *Henneguya* sp. 2 e *Myxobolus* sp. 1 encontrados parasitando pacu são duas novas espécies de mixosporídeos.

8- Os plasmódios de *H. piaractus* são do tipo intralamelar e seu desenvolvimento produziu alterações nas estruturas das lamelas infectadas e vizinhas.

9- A análise dos plasmódios de *H. piaractus* ao microscópio eletrônico de transmissão, mostrou que além da ocorrência de canais de pinocitose, pontos de fagocitose foram observados na interface parasito-hospedeiro.

10- A prevalência de *H. piaractus* foi maior nos pacus com comprimento total acima de 10 cm, *Henneguya* sp 2 foi encontrado parasitando apenas pacus jovens, com comprimento total variando de 9,5 a 20 cm, enquanto em *Myxobolus* sp. 1, o parasito foi encontrado durante todo o período do estudo, sem variação na prevalência.

11- *Myxobolus* sp. 1 não apresentou especificidade para órgãos, mas seu desenvolvimento ocorreu sempre em tecido conjuntivo.

12- *Myxobolus* sp. 1 produziu alterações nos vasos dos filamentos branquiais, diminuindo o lúmen dos mesmos.

13- Os exemplares das quatro espécies de peixes estudadas foram mantidos juntos em um mesmo viveiro durante todo o período do estudo, entretanto, das 5 espécies de mixosporídeos encontradas, nenhuma infectou mais que uma espécie de hospedeiro.

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ANEXO: Dados referentes aos aspectos físicos e químicos da água do viveiro durante o período do estudo.

Mês	Características físicas e químicas (Média ± D. P.)					
	Temp.	O. D.	pH	Dur.	Alc.	NH_3
M	27±1,2	5,4±1	6,7±0,2	17±1,1	22±3	0,13±0,005
A	26,4±1,6	5,9±1	6,5±0,5	13±0,8	17±0,5	0,12±0,005
M	21,7±2,2	5,8±1	6,6±0,1	12±0,9	15±0,8	0,1±0,008
J	20,3±0,8	5,8±0,6	6,4±0,09	7±0,8	12±0,9	0,1±0,009
J	19,7±1,9	6,7±1	6,6±0,3	5±1,5	9±0,5	0,1±0,008
A	21,1±1,3	6±0,5	6,6±0,1	6±0,5	10±0,5	0,13±0,3
S	23,6±2,4	6,2±0,5	6,4±0,2	7±1,1	10±1,7	0,27±0,02
O	26±1,1	5,3±0,5	6,4±0,05	5±0,5	9±0,5	0,12±0,01
N	29,7±1,4	4,5±0,4	6,5±0,05	3±1,1	9±1,1	0,15±0,01
D	27,2±2,3	4,3±0,7	6,2±0,1	3±0,6	8±2,1	0,21±0,05
J	27,7±0,9	4,5±0,5	6,1±0,1	4±0,5	5±0,5	0,2±0,02
F	27,3±0,7	3±0,6	5,9±0,07	4±0,6	7±0,7	0,21±0,007
M	27,5±0,8	3±0,2	6±0,1	4±0,7	12±1,3	0,13±0,007
A	26,4±1,2	4,2±0,2	5,9±0,2	4±0,5	4±0,5	0,11±0,009
M	21,7±1,9	5,8±0,9	6,5±0,09	4±0,9	5±0,9	0,15±0,01
J	20,3±2,4	6,5±06	6,2±0,3	5±1,8	5±1,5	0,05±0,01
J	19,7±1,2	7,1±0,8	7,1±0,07	7±0,6	9±0,7	0,06±0,005
A	21,1±1,2	6,5±0,3	6,7±0,09	7±0,8	10±1,2	0,06±0,005
S	23,6±2,1	5,7±0,4	6,2±0,1	5±0,8	4±0,6	0,06±0,008
O	26,1±0,7	5,3±0,3	6,1±0,05	5±0,8	7±1,9	0,06±0,005
N	29,7±0,6	5,4±0,5	6,4±0,1	5±1	8±0,9	0,05±0,005
D	27,2±0,8	5,5±0,4	6,7±0,08	5±0,7	9±0,5	0,1±0,007
J	28,1±1,1	5,8±0,5	6,5±0,1	6±0,5	12±1,2	0,07±0,005
F	27,9±0,9	5,5±0,4	6,8±0,3	5±0,6	10±1,7	0,05±0,005